Regulation of Food Intake in Adults with and without Obesity: The Role of the Gastrointestinal Tract and Gut-Brain Axis

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Abstract

Obesity is a complex global health issue affecting a significant portion of the population. In the UK, it is estimated that approximately 1 in 4 adults and 1 in 5 children aged 10 to 11 years are living with obesity. Impairment in food intake regulation, including hunger and satiety sensations, are key factors contributing to overeating and weight gain, particularly in individuals with obesity. While various mechanisms may explain these alterations, such as altered appetite and satiety regulators, accelerated gastric emptying (GE), and heightened brain responses to food cues and reward, it remains inconclusive whether these mechanisms are altered in people living with obesity compared to normal-weight (NW) adults.

There are various approaches to studying food regulation, each offering unique insights into the complex mechanisms that control appetite, satiety, and food intake. Non-invasive imaging, particularly magnetic resonance imaging (MRI), provides a powerful tool for investigating the physiological mechanisms underlaying the regulation of food intake. The work in this PhD thesis aims to combine physiological measurements obtained by using MRI with behavioural assessments (i.e., subjective satiety rating), to provide a more comprehensive understanding of appetite control in NW adults and alterations associated with obesity. The work in this thesis included a functional neuroimaging meta-analysis, and three eating behaviour intervention studies, two of which used MRI techniques.

A functional neuroimaging meta-analysis was performed to identify brain areas associated with changes in appetite and satiety regulators in NW and Obese adults. The caudate nucleus and hypothalamus were identified as key areas associated with satiety regulators in NW participants. However, conclusive findings for Obese participants were limited due to the small number of studies conducted in this area.

An MRI study was conducted to investigate the effect of a standard meal on gastrointestinal (GI) responses. The study found that GI responses including gastric content volume (GCV), GE rate, small bowel water content (SBWC), and superior mesenteric artery (SMA) blood flow, and appetite and satiety

regulators were not significantly altered by obesity following the meal. However, Obese participants showed lower satiety subjective rating, and higher insulin and triglyceride levels compared to NW participants.

Different macronutrients play distinct roles in influencing feelings of fullness and satiety, and their impact on the satiety sensation can be a valuable strategy for weight loss. In a pilot MRI study combining gut and brain imaging, responses to a high-fat (HF) emulsion drink and a carbohydrate drink that is matched in caloric content, volume, and viscosity were assessed in NW and Obese participants. Data collection in this study was significantly impacted by the COVID pandemic; therefore, findings from this work are focused on GI responses. The results suggest that the HF drink might induce higher GCV, SBWC, SMA blood flow, and subjective satiety ratings when compared to an iso-caloric, and iso-viscous high carbohydrate drink (HC) in both NW and Obese.

The final study investigated the satiating effect of acute high protein consumption compared to high carbohydrate in NW and Obese participants using ad libitum meal intake and subjective satiety ratings. This study found no significant differences in ad libitum energy intake, subjective satiety, or energy intake between the drinks in either NW or Obese participants.

This research integrated different approaches to measuring the regulation of food intake and alterations in obesity. This holistic approach facilitates a comprehension understanding of the mechanisms governing food regulation, including the impact of macronutrient composition, hormonal influences, gastrointestinal responses, neural signalling, and eating behaviours. While the studies in the thesis did not reveal significant differences in certain aspects of appetite regulation between NW and Obese, including macronutrient compositions, they did highlight several areas requiring further investigations. The complicated nature of obesity and appetite regulation necessitates continued research to better understand these complex mechanisms and inform strategies for obesity management and prevention.

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Publications and Presentation arising from the thesis

Publication

Althubeati, S., Avery, A., Tench, C.R., Lobo, D.N., Salter, A. and Eldeghaidy, S., 2022. Mapping brain activity of gut-brain signaling to appetite and satiety in healthy adults: A systematic review and functional neuroimaging meta-analysis. Neuroscience & Biobehavioral Reviews, 136, p.104603.

Oral presentations

- **The British Feeding and Drinking Group (BFDG) 46th Annual Meeting:** Althubeati, S., Avery, A., Tench, C.R., Lobo, D.N., Salter, A. and Eldeghaidy, S., 2022. Functional neuroimaging meta-analysis to map brain activity of gut-brain signalling to appetite and satiety in healthy adults. Appetite, 179, p.106213.
- **The 2023 Annual Meeting of the Surgical Research Society (SRS):** Althubeati, S., Simpson, E.J., Bush, D., Hoad, C., Elgdeghaidy, S., Gowland, P., Macdonald, I.A. and Lobo, D.N., 2023. O032 Gastrointestinal and satiety responses to oral feeding in participants with obesity compared with those of healthy weight: an MRI study. British Journal of Surgery, 110(Supplement_3), pp.znad101-032.

Poster of distinction presentation

- **The 43rd ESPEN Congress:** Althubeati, S., Avery, A., Lobo, D.N., Salter, A. and Eldeghaidy, S., 2021. A neuroimaging meta-analysis to identify brain areas associated with satiety and appetite regulators. Clinical Nutrition ESPEN, 46, p.S582.

Candidate Statement

All work of this PhD thesis was conducted by the candidate unless otherwise stated.

Abbreviations

In line with current best practice, first person language ‘people (adults) living with obesity’ has been used in the text. However, in the studies, to assist ease of comparison with other related studies, Obese participants is the language that has been used.

ABC-Analysis of Brain Coordinates

ACC- Anterior cingulate cortex

AgRP- Agouti-related protein

ALE- Activation likelihood estimation

ANOVA- Analysis of variance

AP- Area postrema

ARC- Arcuate nucleus

ASL- Arterial spin labelling

AUC- Area under the curve

BES- Binge eating scale

BMI- Body mass index

BOLD- Blood Oxygenation Level Dependent

CART- Cocaine- and amphetamine-regulated transcript

CBF- Cerebral blood flow

CSF- Cerebrospinal fluid

CCK- Cholecystokinin

CNS- Central nervous system

CoEQ- Control of Eating Questionnaire

CSS- Composite satiety score

DE- Dextrose equivalent

DEBQ- Dutch Eating Behaviour Questionnaire

DTE- Desire to eat

EAT- Eating Attitude Test

FDR- False Discovery Rate

FFAs- Free fatty acids

FID- Free induction decay

fMRI- Functional magnetic resonance imaging

FWE- Family-wise error

FWHM- Full width at half maximum

GCV- Gastric content volume

GE- Gastric emptying

GHS- Growth hormone secretagogue

GI- Gastrointestinal

GLP-1- Glucagon-like peptide-1

GLP-2- Glucagon-like peptide-2

HASTE- Half-Fourier Acquisition Single-shot Turbo spin Echo

HC- High carbohydrate

HF- High fat

HP- High protein

HPMC- Hydroxypropyl methylcellulose

IQR- Interquartile range

LBG- Locust bean gum

LH- lateral hypothalamus

MIP- Maximum intensity projections

MNI- Montreal neurological institute

MRI- Magnetic resonance imaging

NPY- Neuropeptide Y

NTS- Nucleus of the solitary tract

NW- Normal weight

OFC- Orbitofrontal cortex

OXM- Oxyntomodulin

PET- Position emission tomography

PFS- Prospective food intake

POMC- Pro-opiomelanocortin

PPU- Peripheral pulse-oximeter unit

PrefQuest- Food preference questionnaire

PVN- Paraventricular nucleus

PYY- Peptide tyrosine tyrosine

RF- Radiofrequency

ROI- Region of interest

rs-fMRI- Resting state functional magnetic resonance imaging

SBWC- Small bowel water content

SMA- superior mesenteric artery

SPMIC – Sir Peter Mansfield Magnetic Resonance Imaging Centre

SQ- Satiety quotient

T_{50}- Half emptying time of gastric content

TE- Echo time

TFEQ- Three Factor Eating Questionnaire

TR- Repetition time

VAS- Visual analogue scale

VOI- Volume of interest

VTA- Ventral tegmental area

α-MSH- Alpha-melanocyte stimulating hormone

Table of content

1 Introduction

Obesity is a global health epidemic that has risen dramatically in recent decades. According to the World Health Organisation (WHO), the global prevalence of obesity nearly tripled from 1975 to 2016, with around 650 million are affected by obesity in 2016 (WHO, 2021). In the UK, the prevalence of obesity is also increasing. The 2021 Health Survey for England estimated that 25.9% of adults in England are obese, and a further 37.9% are overweight but not obese (House of Commons Library, 2023). Obesity is characterised by an excess accumulation of body fat, and it is commonly classified using body mass index (BMI), which is a measure of an individual's body fat based on their height and weight. The WHO and many health organisations use BMI to categorise individuals into different classes of obesity. Adults with BMI $\geq$ 30 kg/m^2 can be classified as living with obesity, whereas 25 kg/m^2 $\leq$ BMI $\geq$ 30 kg/m^2 is overweight, and BMI 18.5 to 24.9kg/m^2 is considered normal-weight (NW) (WHO, 2021). It is also important to note, there are also other methods to assess obesity including waist circumference. Generally, a waist circumference of 35 inches (88 cm) or more for women and 40 inches (102 cm) or more for men is considered indicative of abdominal obesity. Also, body composition analysis can be used to assess obesity. This includes using dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), and skinfold thickness measurements to assess the distribution of body fat and lean mass.

Obesity is associated with several adverse health outcomes, including an increased risk of chronic diseases such as diabetes, cardiovascular disease, and certain cancers (WHO, 2021). Causes of obesity are multifactorial, and it often results from the interplay of various genetic, environmental, behavioural, and metabolic factors. Genetic factors can play a significant role in an individual's susceptibility to obesity. Certain genes may influence metabolism, appetite regulation and the way the body stores, and utilises fat (Krude and Grüters, 2000, Locke et al., 2015). Calorie -dense diets high, such as high-fat foods and sugary beverages can also contribute to weight gain. Hormonal changes, such as insulin

resistance and leptin resistance, can disrupt appetite regulation and contribute to obesity (Considine et al., 1996, Ginsberg, 2000, Zyoud et al., 2022).

While obesity is caused by multiple factors, one of the critical elements that contributes to its development and persistence is alterations in the regulation of food intake. Hunger, satiation and satiety are cycle stages of food intake (Blundell and Halford, 1994). Appetite is defined as the desire to consume food (Heisler and Lam, 2017). Satiation is the process that results in meal termination and the disappearance of hunger (Blundell and Halford, 1994), while satiety refers to the time period between the termination of one meal and the beginning of the next (Strubbe and Woods, 2004, Cummings and Overduin, 2007). The sensation of fullness and appetite involves a complex physiological and psychological process, influenced by an interplay between various factors, including gastrointestinal (GI) responses, neural pathways, environmental cues, and individual behaviours (Dalton et al., 2013). A summary of the factors that affect the onset, timing, and length of satiety feelings are summarised in the satiety cascades which was developed by Blundell et al. (1987) and consequently updated and amended by Blundell et al. (2010) and Tremblay and Bellisle (2015). It is a theoretical framework that explains factors influencing satiety including the physiological processes, the psychological experiences, and instantaneous/immediate behaviours necessary for the eating process (figure 1.1) (Blundell et al., 2010). Particularly in relation to meals, hunger is a well-known early 'signal' or state that initiates the eating process, whereas the release of satiety signals after food intake contributes to ending the eating event. The hunger signals that originate from the stomach send electrical signals via the vagus nerve related to feelings of emptiness or fullness, reinforced by metabolic states such as blood glucose levels (hypoglycemia) and hormone secretions such as ghrelin. Sensory and cognitive processes lead to expectations about meals with expected pleasure and reward, which help define overall meal quality and amount. The stomach and intestine distention and osmotic load provide post-ingestive information that gives feedback on the amount of food consumed. Gut peptide hormones such as PYY, GLP-1 and CCK released after food intake modulate medium-term satiety metabolically by inhibiting food intake (Van Kleef et al., 2012). Long-term satiety is controlled by nutrient oxidation in the

liver during the post-absorptive phase as well as the blood concentrations of amino acid, insulin, and glucose. More details about the long-term regulation are explained in section 2.1.1. The signals relating to sensory and metabolic satiety, as well as those relating to hedonic and homeostatic appetite control, are all integrated by the brain (Figure 1.1).

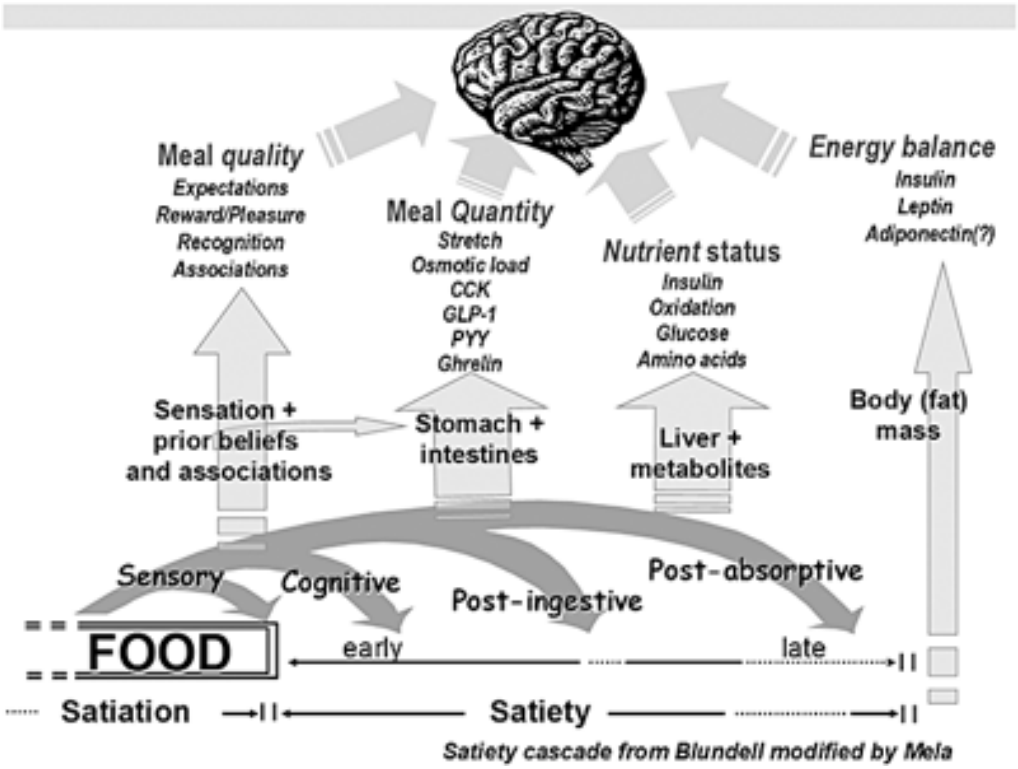


Figure 1.1. Satiety cascade. Figure reproduced from (Blundell et al., 2010).

Previous studies have shown that some people with obesity report a dysfunction in the link between their feelings of hunger and fullness and their eating habits (Barkeling et al., 2007, Sumithran et al., 2011). This may be related to the impaired regulation of food intake mechanisms in people with obesity. Some studies have shown lower postprandial responses of circulating satiety regulators/gut hormones, and higher concentrations of appetite regulators, in people with obesity after a standardised drink, compared with NW participants (Meyer-Gerspach et al., 2014). However, others have found no differences in the appetite and satiety regulators between NW and people with obesity after a standard liquid or breakfast meal intakes (Carroll et al., 2007, Yang et al., 2009). Regulators can be defined as factors that regulate hunger, satiation, and fullness feelings. Satiety regulators could be referred to gut peptide hormones such as

PYY, GLP-1 CCK, insulin, and leptin, which released after food intake, and blood glucose levels (hyperglycaemia) that modulate satiety metabolically by inhibiting food intake. In contrast, appetite regulators refer to hormone secretions of ghrelin which rise before meal ingestion and decrease afterwards to stimulate hunger/appetite and food intake. Additionally, the metabolic state of blood glucose levels (hypoglycaemia) is related to appetite regulators as a drop in blood glucose may stimulate appetite and food intake (Wyatt et al., 2021). More details about appetite and satiety regulators are discussed in section 2.3.

With regards to the function of the GI tract, previous research has reported that the rate of gastric emptying (GE) is more accelerated in people with obesity compared with NW adults (Mora et al., 2005), leading to increased hunger sensation. However, these results conflict with the findings of Meyer-Gerspach et al. (2014) who showed that GE rate was delayed in people with obesity after eating both solid and liquid meals, compared with the NW group. Other studies have shown no differences in GE between people with obesity and NW participants after eating a standardised semi-solid meal (Pironi et al., 1993, Flint et al., 2007).

Neuronal signalling in response to food intake is a critical aspect of understanding how the brain regulates eating behavior and how alterations in this signalling can contribute to the development of obesity. Findings from neuroimaging studies showed that obesity is associated with increased activation in reward-related brain regions in response to high-calorie and palatable foods (Makaronidis and Batterham, 2018). This heightened reward response can lead to overeating. However, a systematic review by Morys et al. (2020) revealed that there is little evidence to support differences between people with obesity and NW adults in brain responses of inhibitory control and reward areas to food picture cues, and such differences might be mediated by factors that are often not considered, such as food cravings, food restraint, self-control, or age.

The conflicting nature of the evidence described above indicate the need for further study to understand changes in satiety and appetite processing in people with obesity. Therefore, the work in this PhD thesis aims to better understand

the appetite and satiety mechanisms in normal-weight adults and alterations in people living with obesity. This will be extended to investigate whether there are differential responses to specific macronutrients between these groups.

1.1 Thesis Overview

All the work described in this thesis was conducted by the author, except where specifically credited to collaborators, at the Sir Peter Mansfield Imaging Centre (SPMIC), and the School of Biosciences, University of Nottingham from 2019 to 2023. The research described in this thesis is original, unless otherwise stated. The layout and content of the thesis chapters are as follows:

Chapter 2 introduces the physiological mechanisms underlying the regulation of food intake. The bidirectional interactions between the brain and the gut and the role of the GI tract in controlling food consumption are outlined. Key methods and approaches that are used in studying the regulation of food intake are highlighted. This includes appetite/satiety rating scales, ad libitum meal intake, GI responses, neuronal signalling to food intake, as well as eating behavioural questionnaires.

Chapter 3 gives a brief description of the principles of MRI and image formation. Additionally, this chapter covers briefly different MRI contrasts.

Chapter 4 employs functional neuroimaging meta-analysis to identify brain areas associated with appetite and satiety regulations in both normal-weight individuals and those with obesity.

Chapter 5 presents an MRI study to explore the gastrointestinal (GI) tract responses to a standard pasta meal in NW and alterations with obesity. The following parameters were measured: gastric content volume (GCV), GE, small bowel water content (SBWC), superior mesenteric artery (SMA) blood flow, subjective satiety, as well as plasma concentrations of appetite and satiety regulators/hormones in normal-weight participants and those living with obesity.

Chapter 6 demonstrates an MRI study to assess the interactions between the gut and brain after consuming a high fat emulsion and an isoenergic, isovolumic,

and isoviscous carbohydrate drink in normal-weight participants and those living with obesity. The chapter describes developing work conducted to optimise the fat emulsion. This is followed by presenting the results from GI responses (GCV, GE, SBWC, and SMA blood flow), and subjective satiety rating after consuming the drinks.

Chapter 7 describes an eating behaviour study in NW and individuals with obesity to measure the satiating effect of protein, which has been suggested to be the most satiating macronutrient. The study explores the impact of acute consumption of high-protein drink on satiety responses assessed by ad libitum meal intake and subjective satiety compared to high-carbohydrate drink (of similar energy content, volume, and viscosity).

Chapter 8 summarises the main findings of the thesis, outlines the advantages and limitations of the thesis, and provides recommendations for future research.

2 Regulation of food intake

The regulation of food intake is a complex process that involves a coordination between the central nervous system (CNS) and the GI tract. The bidirectional interactions between the brain and the gut are vital for ensuring that the body receives the necessary nutrients and maintains energy balance. It influences the sensation of hunger and satiety, ultimately controlling food consumption. The first section of this chapter gives an overview on the physiological mechanisms underlying the regulation of food intake. This includes how the brain and gut communicate to regulate food intake (gut-brain axis). The role of GI tract to regulate food intake is highlighted. The second section outlines key methods and approaches used in studying the regulation of food intake. This includes appetite/satiety rating scales, ad libitum meal intake, GI responses, and neuronal signalling to food intake, as well as eating behavioural questionnaires.

2.1 Regulation of food intake by the CNS

The CNS plays a central role in regulating food intake and appetite. It integrates information from various sources, including peripheral signals from the GI system and adipose tissue as well as sensory inputs, to coordinate a complex network of neural pathways that impact feeding behaviour. The coordination of food intake involves a dynamic interaction between two distinct but interconnected systems: the homeostatic system and the hedonic system. The homeostatic system plays a crucial role in maintaining energy balance and ensuring that the body's physiological needs for nutrients and energy are met (Lutter and Nestler, 2009). The hedonic system, often referred to as the reward system, plays a significant role in influencing food intake and eating behaviours (Batterham et al., 2007, Lutter and Nestler, 2009). Understanding the interplay between the hedonic and homeostatic pathways in food intake is crucial for addressing issues related to overeating, and obesity. This section gives an overview of the homeostatic and hedonic systems. Figure 2.1 illustrates brain areas associated with homeostatic and hedonic pathways for regulation of food intake.

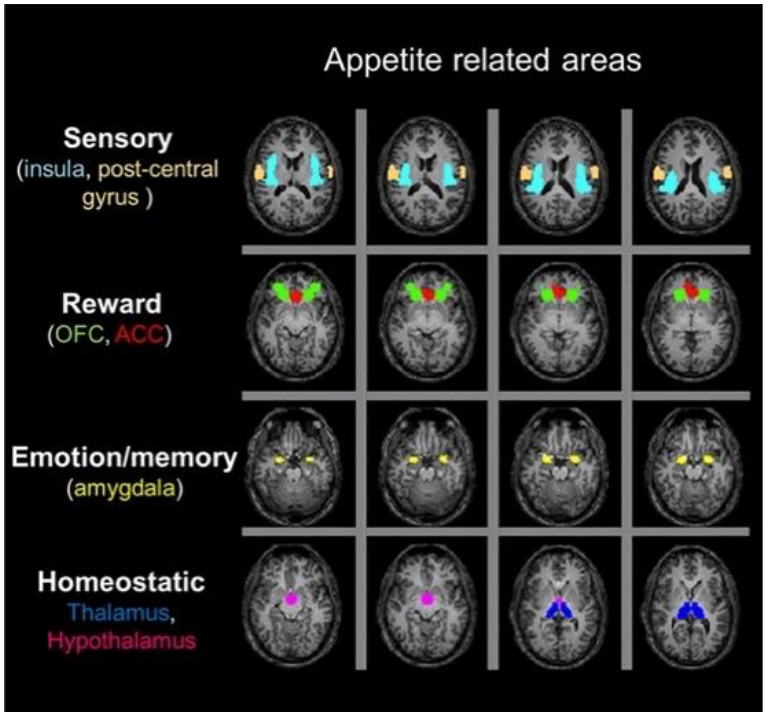


Figure 2.1. Brain areas associated with haemostatic and hedonic pathways for food intake regulation. Figure reproduced from Francis and Eldeghaidy (2015).

2.1.1 Homeostatic system

The homeostatic system involves bidirectional interaction between the GI system and the brain (gut-brain axis). This bidirectional connection allows the gut to transmit important information to the brain regarding the presence of nutrients, the degree of fullness, and other signals related to digestion. In turn, the brain conveys this information to make decisions about hunger, satiety, and meal initiation or termination. When the gut senses a lack of nutrients or a drop in energy levels, it releases a gut peptide called ghrelin (Nakazato et al., 2001) and sends signals to the brain, which may trigger feelings of hunger and the motivation to eat. Conversely, when the gut senses that sufficient nutrients have been consumed, it signals to the brain that it's time to stop eating, leading to feelings of fullness and satisfaction. This is controlled through the enteroendocrine cells within the GI tract which stimulate the release satiety hormones such as glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), and cholecystokinin (CCK) (Adrian et al., 1985b, Le Quellec et al., 1992). The above signals for food intake typically convey a short-term regulation of food intake.

For the long-term regulation, insulin and leptin are two primary regulators/hormones for the long-term process to control food intake, which are produced from the pancreas and adipose tissue, respectively. They act as adiposity signals that provide negative feedback signals for energy balance to reduce food intake (Benoit et al., 2004, Niswender et al., 2004). Additionally, they positively correlate with body fat mass, and send signals to the brain about the status of energy stores. More information about the short- and long-term hormones are provided in the section 2.3.1 and 2.3.2.

Both short-term and long-term peripheral signals travel from the GI tract, pancreas, or adipose tissues to the CNS via vagal afferent nerve signalling, or directly via blood circulation (Cummings and Overduin, 2007, Ahima and Antwi, 2008). The arcuate nucleus (ARC) in the hypothalamus sense and respond to the peripheral signals by releasing modulated neuropeptide in two neuronal populations with different effects on food intake (Schwartz et al., 2000, Chaudhri et al., 2008). The ARC responds to the peripheral signals (such as leptin and ghrelin) by releasing modulated neuropeptide in two neuronal populations with different effects on food intake (Schwartz et al., 2000). Neurons in the medial part of the ARC (orexigenic neurons) express the Agouti-related protein (AgRP) and neuropeptide Y (NPY) in response to the ghrelin hormone, which increase appetite, hunger, and food intake (Broberger et al., 1998, Hahn et al., 1998, Bewick et al., 2005). In respond to the short- and long-term satiety hormones, neurons located in the lateral ARC are anorexic, and they express cocaine- and amphetamine-regulated transcript (CART) and alpha-melanocyte stimulating hormone (α-MSH) driven by pro-opiomelanocortin (POMC), which suppress appetite, and hunger and food intake (Elias et al., 1998, Batterham et al., 2002, Jobst et al., 2004). The balance between these two types of neurons ‘appetite suppressants’ and ‘appetite stimulators’ is crucial in controlling food intake. Neurons in the ACR have additional connections to the hypothalamic paraventricular nucleus (PVN) (Schwartz et al., 2000, Cone et al., 2001, Campos et al., 2022) which sends signals to higher reward areas in the brain, such as the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), nucleus accumbens and amygdala (Schwartz et al., 2000). These brain areas interact with other areas related to executive functioning, such as the dorsolateral

prefrontal cortex and ventral medial prefrontal cortex, to direct eating behaviour (Schwartz et al., 2000). The ARC also connects with the lateral hypothalamus (LH), which is associated with hunger and feeding initiation. Figure 2.2 illustrates the neuronal pathway of the homeostatic system and shows the connection between peripheral satiety signals.

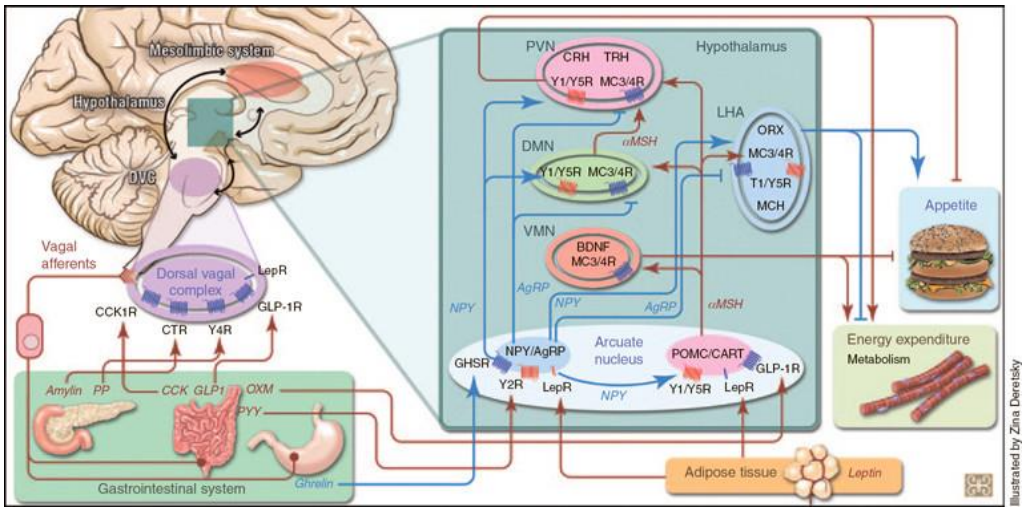


Figure 2.2. The neuronal pathway of the homeostatic system shows the connection between peripheral signals to stimulate or suppress food intake. These signals can include appetite signalling through ghrelin gut hormones and satiety signalling through hormones such as glucagon-like peptide 1 (GLP-1), peptide tyrosine tyrosine (PYY), leptin, pancreatic polypeptide (PP), oxyntomodulin (OXM) and Cholecystokinin (CCK). Figure reproduced from Kim et al. (2013).

2.1.2 Hedonic system

Food intake is a rewarding and pleasurable experience which can override the homeostatic regulation of eating (Batterham et al., 2007, Lutter and Nestler, 2009). When individuals eat foods that are rich in taste, texture, and sensory attributes, that they find pleasurable, the reward and sensory systems are activated. Sensory information related to the food stimuli activate primary sensory areas to identifies and creates the perceptual experience of food (anterior insula and frontal operculum, and primary somatosensory cortex). The rewarding attributes of food stimuli also triggers the release of neurotransmitter dopamine and dopamine 2 from ventral tegmental area (VTA) of the midbrain. Neurons in the VTA project to various areas of the brain, including the nucleus accumbens, prefrontal cortex, and amygdala. These brain-areas assess the

rewarding aspects of the food, its hedonic value, and its potential pleasurable effects. This could lead to reinforcing the desire to continue eating such foods (Schwartz et al., 2000, Campos et al., 2022). For example, food rich in sugar (sweet) and fat are highly associated with reward, and have frequently been attributed to the hedonistic features of eating in both rodents and humans (Berridge, 1991).

Hedonic regulation can override the homeostatic route by boosting the desire to eat foods that are highly appealing and pleasant during times of relative energy excess. Dysregulation of the hedonic system can contribute to overeating and obesity. Individuals may consume calorie-dense, highly palatable foods driven by the desire for rewarding experiences, even when they are not hungry. Understanding the influence of the hedonic system on food intake is essential for addressing issues related to overconsumption and obesity. The integration of sensory and reward signals in the brain can direct behaviour and eventually food intake (Schwartz et al., 2000, Campos et al., 2022).

2.2 Regulation of food intake by the gastrointestinal tract

The GI tract plays a critical role in regulating food intake by digesting and absorbing nutrients, releasing hormones that signal hunger and satiety, and providing sensory input to the brain. This section gives an overview of how food is digested and processed in the stomach. The role of gut hormones in regulating appetite and satiety sensations is then discussed.

2.2.1 Food digestion and gastric emptying

Food digestion is a complex process that begins in the mouth and continues through the GI tract, involving various organs, enzymes, and mechanical actions (Patricia and Dhamoon, 2019). The first step in food digestion is the cephalic phase responses which are physiological responses to food cues such as sight, thought, smell, and taste of food (Powley, 1977, Teff, 2000). The role of cephalic responses is to prepare for optimal nutrients digestion and absorption (Powley, 1977, Powley and Berthoud, 1985, Nederkoorn et al., 2000, Teff, 2000). Cephalic phase responses include increased salivation, gastric motility, and pancreatic (pancreatic polypeptide, glucagon, leptin, and insulin), and gastric

(ghrelin) endocrine secretions, and bile secretions (Powley, 1977, Richardson and Feldman, 1986, Soucy and Leblanc, 1998, Mattes, 2005). Cephalic phase responses also include other non-secretory responses such as changes in respiratory quotient (McGregor and Lee, 1998), changes in cardiac measures (Nederkoorn et al., 2000), increased blood pressure (Nederkoorn et al., 2000), postprandial thermogenesis (LeBlanc and Cabanac, 1989, LeBlanc, 2000), and gastric motor activity (Mattes, 1997, Nederkoorn et al., 2000). The stimulation of cephalic responses may have a role in regulating food intake, appetite, and physiological responses. For example, a previous study by Cecil et al. (1998) has shown that oral administration has delayed gastric emptying more than gastric infusion and significantly affect satiety ratings. Additionally, they showed that a significant correlation between gastric contents and hunger ($r=-0.98$) as well as fullness ($r=0.98$) after the oral administration. This suggests that the orosensory simulation induced greater effect to reduce food intake by delaying gastric emptying.

In addition to the cephalic responses, the GI tract produces and secretes digestive enzymes to break down lipids, proteins, and carbohydrates to complete the process of digestion and, subsequently, the absorption of nutrients (MacFarlane, 2018, Patricia and Dhamoon, 2019). Salivary amylase in the mouth starts to breakdown carbohydrate and after that the digestion continues in the small intestine by pancreatic amylase. Hydrochloric acid and pepsin in the stomach break down protein when a bolus of food particles enters the stomach. Then, digestion of protein continues in the small intestine by proteolytic enzymes including trypsin, chymotrypsin, elastase and carboxypeptidase which break down protein into oligopeptides. For lipids, the primary digestion begins in the small intestine by (1) pancreatic lipase that breakdown triglycerides into 2 monoglycerides and fatty acids, (2) pancreatic phospholipase that breakdown phospholipids into head groups and fatty acids, and (3) pancreatic cholesterol esterase that breakdown cholesterol esters into component cholesterol and fatty acid.

The stomach is a crucial organ in the digestive system, playing a central role in food digestion and the initial breakdown of ingested food. GE is a specific step in this process that occurs in the stomach and involves the release of partially digested food into the small intestine. This section gives an overview of the physiological mechanism of GE and the impact of the GE rate on the regulation of food intake. Below is an overview of the anatomy of the stomach and its function in food digestion.

The stomach is divided into four functional parts (Soybel, 2005). The first part, the fundus, fills with air, the second part, the body, stores food, the third part, the antrum, mixes food with digestive juice and creates fluid motion, and the fourth part, the pylorus, controls the food particles movement into the duodenum. Figure 2.3 shows a schematic diagram of a stomach anatomy. The stomach can also be divided into two parts based on the pattern of gastric movement: the proximal stomach and the distal stomach (Figure 2.3) (Soybel, 2005). The fundus and the proximal part of the corpus make up the proximal stomach while the distal part of the corpus and the antrum make up the distal stomach.

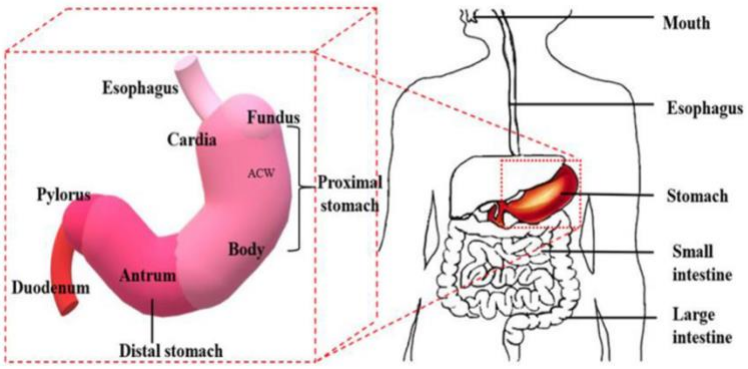


Figure 2.3. A schematic diagram of stomach anatomy and the human digestive tract. Figure reproduced from Liu et al. (2021).

2.2.1.1 The process of gastric emptying

Gastric emptying is the process by which the contents of the stomach are moved into the duodenum (Cifuentes and Acosta, 2022). This is accomplished by four mechanisms/phases: (1) tonic contractions, (2) peristaltic contractions, (3) retropulsion, and (4) emptying (Soybel, 2005, Bellmann et al., 2016) (Figure 2.4). Following food ingestion, the contents of the proximal stomach are forced

towards the distal stomach by tonic contractions. After that, the stomach surface contracts and the peristaltic waves reach the pylorus to increase the width and the indentations, frequently virtually obstructs the antral lumen. Pylorus contraction causes the sphincter to narrow, which dramatically reduces the pyloric opening when the peristaltic wave arrives. Retropulsion is the process by which the chyme is pumped back into the stomach. At this stage, food and gastric juice are thoroughly combined and emulsified, which causes crushing and rubbing between stomach walls and/or food particles. The food particles first appear to be suspended due to a process that involves repeated advances, grinding, and retreats as well as the work of acids and enzymes. Lastly, the emptying process begins when the pylorus partially opens. Fluid and small particles (between 1 and 2 mm) move continually from the stomach to the duodenum while bigger indigestible particles are held in the stomach.

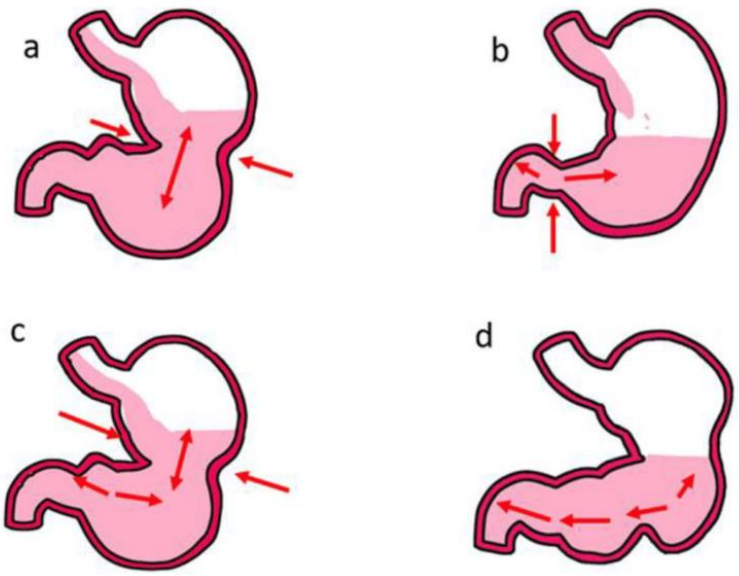


Figure 2.4. The gastric emptying process. (a) Tonic contractions, (b) peristaltic contractions, (c) retropulsion, and (d) emptying. Figure reproduced from Liu et al. (2021).

2.2.1.2 Impact of gastric emptying rate on food intake regulation

GE influences subsequent food and caloric intake. A delay in GE leads to greater satiety and lower caloric intake (Halawi et al., 2017), whilst rapid GE leads to lower satiety and higher subsequent caloric intake (Gonzalez-Izundegui et al., 2021). The rate of GE is mediated by different mechanisms including biological

factors. For example, age, sex, and gut hormones showed to have a significant effect on the GE rate. It has been suggested that elderly people have a delayed GE rate compared to young people (Brogna et al., 2006). While aging was suggested to delay GE rate, a meta-analysis by Bonner et al. (2015) conducted on 49 studies including participants from 28 weeks' gestation to adulthood demonstrated that age did not significantly affect the GE rate. Females have been shown to have a slower GE rate compared with males during specific times across the life-course. In comparison with males and postmenopausal females, it has been suggested that premenopausal females have a slower GE rate (Wang et al., 2015). This could be attributed to the effect of oestrogen during the luteal phase of the menstrual cycle (Wang et al., 2015). Gut hormones such as GLP-1 and CCK decrease GE by relaxing the proximal stomach to increase stomach capacity (Rigaud et al., 1995, Schirra et al., 2006, Schirra et al., 2009) and inhibit gastric acid secretion (Layer et al., 1995, Wettergren et al., 1997, Marciani et al., 2001). These effects are dependent on stimulating vagal afferents, which inhibit reflex vagal motor pathways (Ritter and Ladenheim, 1985, Moran et al., 1994, Li et al., 1997, Tolessa et al., 1998, Tolessa et al., 2001, Näslund et al., 2002).

The rate of GE is also mediated by the ingested food properties, including energy density, and viscosity. Increasing energy density and/or viscosity of a meal has been demonstrated to delay the GE rate (Mazzawi et al., 2019, Campos et al., 2022). Using MRI, Camps et al. (2016) demonstrated that energy density and viscosity of the ingested meal have a significant impact on GE rate in normal-weight participants. Comparing GE rate following consumption of 500 ml of 4 dairy-based shakes, with different energy density (100 kcal compared to 500 kcal) and viscosity (thin and thick), showed both the energy load and the viscosity impacted the GE rate. However, increasing the energy load was shown to be the most significant factor.

Another aspect affecting the GE rate is the separation of solid and liquid within the stomach, which is known as sieving. The sieving process happens as liquids are emptied from the stomach more quickly than solids (Hinder and Kelly, 1977, Meyer et al., 1979). This usually happens as larger solid particles of mixed solid/liquid meals are held for a longer time in the stomach to undergo grinding

to reduce their size before they may be emptied after a lag phase. It has been suggested that blending or homogenising a mixed solid/liquid meal could prolong satiety, as shown by a previous study that found that, in comparison to an unhomogenised meal, a mixed solid/liquid, fat- and vegetable-rich, 2573-kJ meal that was homogenised delayed GE and enhanced satiety (Santangelo et al., 1998). Another study conducted by Marciani et al. (2012) measured the GE rate of roasted chicken with vegetables and a glass of water compared to the same meal blended into soup. The gastric volume of the soup meal decreased more slowly and linearly compared to the solid/liquid meal, which had a faster emptying rate. When compared to the solid/liquid meal (77 ± 6 min), the soup meal tended to take longer to reach half-gastric emptying (92 ± 7 min) ($P = 0.06$). The soup meal was also associated with greater gallbladder contraction compared to the solid/liquid meal, suggesting more CCK secretions happen with the soup meal.

2.2.2 Mesenteric blood flow

The small intestine is the primary site for nutrient digestion and absorption. The digestion of the chyme released from the stomach continues in the small intestine. As the chyme moves to the small intestine, blood flow through the capillaries that supply the gut wall increases to facilitate food digestion, which can be measured as a rise in the blood flow through the superior mesenteric artery (SMA) (Jeays et al., 2007). The SMA is a major supplier of blood to various sections of the GI tract, including the duodenum, the small intestine, and the transverse colon (Jäger et al., 1986, Moneta et al., 1988). Blood flow in the SMA reflects absorptive, secretory, and motor functions in the splanchnic organs. Each of these activities rises after mealtime and subsequently causes a significant rise in splanchnic blood flow (Guyton, 2006). Blood flow in the SMA increases, reaching its peak around 30 to 45 minutes after eating, and returning to baseline levels after approximately 90 minutes (Batton et al., 1983, Sieber et al., 1991, Spencer et al., 2000). However, these changes in the SMA blood flow are influenced by various factors, such as meal composition and caloric content of the meal (Moneta et al., 1988, Sieber et al., 1991, Sieber, 1992, Sidery and Macdonald, 1994, Parker et al., 1995, Jeays et al., 2007), as well as the rate of GE (Sidery et al., 1994). Previous studies have shown that the peak blood flow

in the SMA is positively correlated with the energy content of a meal (Sidery and Macdonald, 1994, Parker et al., 1995, Someya, 2007). The mesenteric circulation supplies oxygen and nutrients to the small intestine, eliminates waste products, and transports absorbed substances away from it (Granger et al., 2011). Studying the blood flow in SMA in this thesis may offer valuable information for examining the digestive processes in both health and illness, which could have a role in food intake regulation.

2.2.3 Small bowel water content

The amount of water present in the small intestine, "small bowel water content"(SBWC), is another factor that plays a vital role in food intake regulation. On a daily basis, around 9 litres of fluid enter the small intestine, with 7 to 8 liters being reabsorbed, while only 1 to 1.5 liters progress into the colon (Volk and Lacy, 2017). Following food consumption, the presence of plasma PYY in the bloodstream facilitates the absorption of fluids and nutrients across the intestinal mucosal surface, resulting in a reduction of SBWC (Savage et al., 1987, Benelam, 2009). Consequently, this leads to the activation of the ileal brake mechanism and the release of satiety hormones (Moneta et al., 1988, Sieber et al., 1989). The ileal brake is defined as an inhibitory mechanism that controls the transit time of food moving through the small intestine to optimise nutrient absorption and digestion (Jeays et al., 2007). A previous study by Read (1992) demonstrated that nutrient infusion into the ileum leads to reduced duodenal and antral peristaltic contractions. However, no alteration was found in the transit time of the small bowel when nutrients were infused into the colon. The ileal brake occurs following the stimulation of enteroendocrine cells and mucosal afferent neurons. This system is controlled by PYY, GLP-1, and CCK. Ileal brake, is also influenced by GE rate, caloric load, and food composition (De Graaf et al., 2004).

2.3 Appetite and satiety regulators

2.3.1 Gut hormones

Ghrelin

Ghrelin is a 28 amino acid peptide hormone released from the gastric fundus A cells in the stomach and is currently considered the only known orexigenic

peptide hormone (Nakazato et al., 2001). In contrast to the other peptide hormones, ghrelin rises before meals ingestion and decreases afterwards, to stimulate hunger/appetite and food intake (Nakazato et al., 2001). Suppression of postprandial secretions of ghrelin is proportional to calorie content (Callahan et al., 2004). Lowest levels of ghrelin can be detected within 20-60 minutes postprandial, depending on a meal composition (Bowen et al., 2006, Carroll et al., 2007, Yang et al., 2009). Ghrelin can increase gastric acid secretion and accelerate GE of solids and liquids (Matsuda et al., 1999, Asakawa et al., 2003, Murray et al., 2005) by binding directly to the ghrelin receptor 'growth hormone secretagogue (GHS)' in the stomach, or through mediation by vagal nerve (Levin et al., 2006).

Peptide tyrosine tyrosine

PYY is a peptide hormone released from luminal cells of the ileum in the small intestine (Adrian et al., 1985b, Grandt et al., 1994). PYY can inhibit gastric acid secretions (Adrian et al., 1985a), and secretion of electrolytes and fluid in the small bowel (Savage et al., 1987). Additionally, it can act as an ileal brake which decreases motility of gastric and intestinal transit (Beglinger et al., 2001). All these mechanisms combined lead to satiety feeling. PYY is found endogenously in two forms: PYY_{1-36} (36 amino acid peptide) and PYY_{3-36} (34 amino acid peptide) (Grandt et al., 1994). The bioactive form of PYY is PYY_{3-36}, which is produced by cleavage of N terminals Tyr-Pro dipeptide from PYY1-36, by dipeptidyl-peptidase 4 (Eberlein et al., 1989, Grandt et al., 1994, Ritter, 2010). PYY performs its function by acting on the Y receptors (Y1, Y2, Y4, Y5, and Y6) (Grandt et al., 1994, Dumont et al., 1995). Endogenous PYY levels reach their lowest point during fasting and rise after eating. This increase is in proportion to calorie intake and with protein and carbohydrate rich meals (Adrian et al., 1985b, Batterham et al., 2003, Batterham et al., 2006). Postprandial PYY peaks within 30-90 minutes after food intake and is proportional to type (i.e., carbohydrate, protein, fat content) and volume of food (le Roux et al., 2006, Essah et al., 2007, Yang et al., 2009).

Cholecystokinin

CCK is a 33-amino acid peptide hormone released postprandially from luminal cells in the duodenum and jejunum (Mutt and Jorpes, 1968, Murphy and Bloom, 2006). There are two types of CCK receptors present in the peripheral tissues and the CNS: CCK1 and CCK2 (Moran and Kinzig, 2004). The forms of circulating CCK are many, ranging in chain length from eight to eighty-three amino acids. CCK-8, CCK-22, CCK-33, and CCK-58 are the major forms of CCK circulating in the blood, all having the same blinding power (attribute) (Cantor and Rehfeld, 1989, Ji et al., 1997, Luo et al., 2014). CCK1 receptors are present within CNS in areas that are involved in food intake regulation, i.e. the dorsomedial hypothalamus, the area postrema (AP) and the nucleus of the solitary tract (NTS) (Moran et al., 1986).

The release of CCK into plasma is greatly stimulated by the consumption of fat, protein, and their final digestion products, but the influence of carbohydrate consumption on CCK release is modest (Liddle et al., 1985). CCK levels rise over 10-30 minutes after food ingestion and activates the release of bile acids from the gallbladder and pancreatic enzymes, which leads to digestion of fat and protein (Liddle et al., 1985). However, CCK has a short half-life, lasting only a few minutes after the consumption of a meal (Geary, 2004). The release of CCK and signalling via CCK receptors mediate stimulation of PYY release and inhibition of ghrelin which moderates food intake and appetite (Degen et al., 2007). CCK directly activates the vagal afferents, which inhibit GE and gastric secretions (Fried et al., 1991). In animal models, peripheral administration of CCK reduces GE through interactions of a gut-brain mechanism involving oxytocin (Olson et al., 1992, Higham et al., 1997, Goyal et al., 2019). Postprandial CCK concentrations inhibits human GE by inducing pylorus contraction and antrum and proximal stomach relaxation (Liddle et al., 1986). Thus, the collective actions of CCK contributes to the feeling of fullness and satiety.

Glucagon-like peptide-1

GLP-1 is an incretin 30-amino acid peptide hormone produced by the splitting of the preproglucagon precursor molecules (Dhanvantari et al., 1996). GLP-1 is produced from luminal cells in the ileum in response to meal intake (Holst et al

2007). Receptors of GLP-1 are distributed in areas involved in food intake regulation, including islets of pancreas, the brain, and the whole GI tract (Nauck et al., 1993, Willms et al., 1996). GLP-1 performs its action through GLP-1 receptors in the NTS and AP of the brainstem, superior optic nuclei and ARC of hypothalamus, PVN, and vagus (Chaudhri et al., 2006a, Parkinson et al., 2009). GLP-1 is released 10-15 minutes postprandially and its blood levels peaks between 30-60 minutes (Flint et al., 1998) with levels remain elevated for at least 180 minutes following meals (Verdich et al., 2001a). Therefore, it plays a role in satiation during and after meal intake. Human and animal studies showed that infusions of GLP-1 induce subjective satiety and reduce food intake (Melhorn et al., 2014). GLP-1 is also an incretin hormone that stimulates the release of insulin and inhibits the production of endogenous glucose and the glucagon release (Willms et al., 1996). Regardless of the meal type, continuous administration of GLP-1 slows the rate of GE and enhance distal gastric stagnation (Näslund et al., 2004). Exogenous GLP-1 has been shown to enhance pyloric tone, reduce vestibular and duodenal motility and relax the proximal stomach during fasting and feeding states (Delgado-Aros et al., 2002, Suganuma et al., 2020). Furthermore, GLP-1 induces increased satiety and supresses pancreatic secretions (Schirra et al., 2006, Punjabi et al., 2011).

Glucagon-like peptide-2 (GLP-2)

GLP-2 is produced by modifying the splicing of pre-proglucagon and its main function is associated with trophic effects on the intestinal mucosa. GLP-2 is a 33 amino acid peptide hormone, co-secreted with GLP-1 from luminal cells in the ileum (Orskov et al., 1986). Some studies have found that GLP-2 could delay the GE of liquids in NW participants (Nagell et al., 2004, Berg et al., 2014) and reduce gastric acid production (Meier et al., 2006). GLP-2 acts on the GLP-2 receptors which are localised in subepithelial myofibroblasts, intestinal syncytium of cells located underneath the epithelium, enteroendocrine cells and enteric neurons (Yusta et al., 2000, Bjerknes and Cheng, 2001, Ørskov et al., 2005, Guan et al., 2006).

Amylin

Amylin is a 37 amino acid peptide hormone which is released into the plasma with insulin in response to food intake. It performs its action through stimulating histamine H1 receptors (Mollet et al., 2001). Amylin directly activates some brain areas that have satiating functions, including the AP and possibly the VTA. Furthermore, amylin has been shown to promote activation of other satiating hormones such as CCK, reduce gastric secretion, delay GE, reduce food intake and postprandial glucose elevation (Lutz et al., 1995, Lutz, 2006).

Oxyntomodulin (OXM)

OXM is a 37-amino acid peptide hormone produced from the splitting of preproglucagon precursor molecules (Le Quellec et al., 1992). OXM is released into the plasma postprandially from luminal cells, in response to caloric intake (Le Quellec et al., 1992). Although it has low affinity to this receptor family, OXM mediates its effects through GLP-1 receptor (Baggio et al., 2004, Lebrun et al., 2006). It also decreases secretion of gastric acid, delays GE of the stomach and reduces food intake (Schjoldager et al., 1989). Intravenous administration of OXM in humans has been shown to increase energy expenditure, supress ghrelin secretion (Chaudhri et al., 2006a, Chaudhri et al., 2006b, Wynne et al., 2006) and decrease appetite and food intake (Cohen et al., 2003, Wynne et al., 2006). Furthermore, OXM induces weight loss in both NW and people with obesity (Cohen et al., 2003, Wynne et al., 2006).

Glucagon

Glucagon is a 29-amino acid peptide hormone released from alpha cells in the pancreas (Jones et al., 2012a). Glucagon performs its action through the glucagon receptor which is located in cells in the kidney, liver, and other tissues (Svoboda et al., 1994). It is secreted into the portal vein in response to hypoglycaemia and increases blood glucose concentrations and energy expenditure (Jones et al., 2012a). Administration of glucagon intravenously has been shown to reduce the intake of food by delaying GE rate (Geary et al., 1992).

Pancreatic polypeptide (PP)

PP is a 36-amino acid peptide hormone released postprandially by pancreatic islet PP cells under vagal control, in response to calorie intake (Larsson et al., 1975, Adrian et al., 1976, Schwartz et al., 1978). Similar to PYY, PP performs its function by acting on the Y receptor family, specifically the Y4 receptor in the ARC of the hypothalamus (Chaudhri et al., 2006a). PP has a role in regulating pancreatic secretion and gastric and gallbladder motility (Adrian et al., 1979). PP induces a delay in GE, inhibits pancreatic enzymes and relaxes the gallbladder (Asakawa et al., 2003). Intravenous administration of PP increases energy expenditure and decreases GE and food intake (Clark et al., 1984).

2.3.2 Associations between gut hormones, reported appetite and measured food intake

Previous studies have measured the association between postprandial concentrations of endogenously released gut hormones, reported appetite and subsequent food intake. A study conducted by Bowen et al. (2006) administered four different types of liquid preloads (50 g of whey, soy, gluten or glucose) and measured association between postprandial concentrations of gut hormones and ad libitum energy intake (3 hours after test meals) in NW and overweight participants. They found that ad libitum energy intake was predicted by glucose (P=0.012) and ghrelin (P=0.039), and inversely predicted by CCK (P=0.056) and insulin (P=0.001) regardless of meal type. This accounted for 5.3% of the variance in ad libitum energy intake. However, other two studies found no association between gut hormones and subsequent ad libitum energy intake. A study carried out by Gibbons et al. (2016) in 16 participants with obesity showed no correlation between postprandial concentrations of CCK following high-carbohydrate (83.6% carbohydrate, 3.2 % fat & 13.2 % protein) or high-fat (39% carbohydrate, 50.3 % fat & 11.7 % protein) meals and ad libitum energy intake, which was administered 3 hours after the test meals. Another study by Hengist et al. (2023) found that subsequent ad libitum energy intake after low-fat (LF, 75% carbohydrate and 10% fat) and low-carbohydrate (LC, 10% carbohydrate and 75% fat) diets did not correlate with mean postprandial PYY (LC diet: P=0.19, LF diet:P=0.98), GLP-1 (LC diet: P=0.68, LF diet: P=0.60), GIP (LC diet: P=0.73, LF diet: P=0.34), active ghrelin (LC diet: P=0.41, LF diet:

P=0.76), total ghrelin (LC diet: P=0.19, LF diet: P=0.76), or leptin (LC diet: P=0.77, LF diet: P=0.27) in 20 NW participants. From the above studies, it seems that there are conflicting results for the association between endogenous concentrations of gut hormones and subsequent energy intake.

Some previous research has demonstrated that gut hormones administered exogenously affect appetite and subsequent food intake. For example, a study conducted by Batterham et al. (2003) reported that intravenous PYY (2 nmol per square meter of body-surface area) decreased ad libitum energy intake ($P<0.001$) two hours after the infusion in both NW and participants with obesity. Another study by Wren et al. (2001) reported that intravenous infusion (5pmol/kg/min) increased the VS hunger scores ($P<0.05$) and ad libitum energy intake ($P<0.001$) four hours after the infusion.

2.3.3 Other appetite and satiety regulators

Blood glucose

Glucose plays a crucial role in regulating energy consumption (Nakrani et al., 2023). Following digestion, carbohydrates are broken down into simple sugars like glucose, proteins are converted to amino acids, and lipids are broken down into fatty acids and glycerol. The body uses these smaller molecules for energy, growth, and repair when they are taken up by the bloodstream (Alberts, 2017).

Blood glucose levels rise following a meal, which activates the pancreas to secrete more insulin, which leads to glucose being stored as glycogen in the liver (Nakrani et al., 2023). When blood glucose levels decline several hours after a meal, the liver subsequently releases glucose back into the blood, reducing fluctuations in blood glucose. A drop in blood glucose may stimulate appetite and food intake (Wyatt et al., 2021) with hypoglycaemia having been reported to increase hunger feelings and the GE rate (Mayer, 1955, Schvarcz et al., 1995, Campfield et al., 1996). Blood glucose concentrations may affect hypothalamic satiety centres including the ventromedial and arcuate hypothalamic nuclei, which have important roles in glucose metabolism (Gao and Horvath, 2008). Glucose performs its action on appetite and satiety through glucoreceptors in the hypothalamic nuclei (Ritter et al., 1981, Kumar, 1999).

Insulin

Insulin is a 51 amino acid peptide hormone that is released from the beta cells of Langerhans in the pancreas. The main function of insulin is to maintain normal blood glucose levels by promoting glucose uptake by cellular tissues, regulating the metabolism of carbohydrates, lipid, and protein, and performing mitogenic effects on cell division and growth (Wilcox, 2005).

Insulin mediates appetite-suppressing effect by acting on insulin receptors in the ARC of the hypothalamus (Havrankova et al., 1978, Houten et al., 1979). In humans, intranasal infusion of insulin decreased fMRI signals in the hypothalamus (Opstal et al., 2017), which is the main area for haemostatic control, and regulates the dopaminergic reward system (Kullmann et al., 2013, Tiedemann et al., 2017, Thanarajah et al., 2019).

Leptin

Leptin is a 16 kD adipokine and acts via the leptin receptor (Chen et al., 1996). It is synthesised and secreted by white adipose tissue. Normal concentrations of leptin decrease the drive to eat and promote energy expenditure via its role in autonomic output and several neuroendocrine axes. Conversely, decreased concentrations of leptin reduces energy utilization, increases the drive to eat and leads to behavioural adaptations to the reduced energy stores (Ahima et al., 1996, Myers et al., 2009). Leptin also plays a major role in regulating metabolism by modifying the secretion of insulin, lipid metabolism, and the synthesis of hepatic glucose (Kulkarni et al., 1997, Liang and Tall, 2001, Minokoshi et al., 2002, Pocai et al., 2005, Nogueiras et al., 2007). In addition, leptin may delay GE through interacting with the CCK and the vagus nerve (Asakawa et al., 1999). Accelerated GE has been found with diminished levels of leptin (Asakawa et al., 1999).

Free fatty acids (FFAs)

FFAs are as species of lipid released during the lipolysis process from adipose tissue and other cell types. FFAs perform active roles in several biological processes in addition to their primary involvement in energy delivery and as structural elements of membranes. FFAs can have an impact on endothelial cells' (Frommer et al., 2015), adipocytes' (Schaeffler et al., 2009), or macrophages' (Håversen et al., 2009) gene expression. FFAs can also affect chemokine and

cytokine production (Frommer et al., 2015, Honda et al., 2015, Hung et al., 2015), adhesion molecule gene expression (Miles et al., 2001, Livingstone et al., 2014), and the emergence of pro-inflammatory and inflammation-promoting lipid-derived substances (Miles et al., 2001, Serhan et al., 2008, Livingstone et al., 2014, Hung et al., 2015).

It has been suggested that FFAs are involved in the regulation of energy haemostasis by stimulating secretions of insulin, GLP-1 and glucagon, and reducing intestinal motility through expression of FFA receptors (FFAR) in the pancreas and intestine (Hara et al., 2014).

Triglycerides

Triglycerides are the predominant dietary lipid in fats and oils, whether sourced from plants or animals (Lichtenstein, 2013). They are made up of three fatty acid molecules linked to a glycerol molecule. In the human body, they are the primary storage source of lipid and energy. They are predominantly synthesised through the glycerol phosphate pathway (Viecili et al., 2017). The liver and adipose tissue are the two primary locations for endogenous triglyceride production (Bayly, 2014). The proximal gastrointestinal tract hydrolyses triacylglycerols into fatty acids and monoacylglycerols that can influence GI functions, hormone release, and satiety (Armand et al., 1999). Obesity or hypertriglyceridemia are metabolic problems that can result from an imbalance in this mechanism (Viecili et al., 2017).

Fasting concentrations of triglycerides positively correlate with visceral fat area (Marston et al., 2019, Sukkriang et al., 2021). During prolonged fasting and starvation, adipose tissue mobilises triglycerides, which increase concentrations of FFAs in the blood circulation (Becker, 2001). FFAs in the blood are quickly absorbed by the liver during a fast or starvation and then return to the blood as triglycerides (Palmer et al., 1978, Guiducci et al., 2006).

Elevated triglyceride levels could induce leptin resistance, which increases the desire to eat and reduces calorie expenditures unrelated to energy need, may have developed as a signal to the brain that a person is starving. Indeed, it is believed that elevated triglycerides could impair the transport of leptin to the blood-brain barrier, causing peripheral resistance (Banks et al., 2004). In

addition, elevated blood triglycerides are frequently associated with insulin resistance as a clinical indicator of the metabolic syndrome (Grundy, 1999, Ma et al., 2020).

Table 2.1. The origin, major receptor and functions of gut hormones and appetite and satiety regulators involved in the regulation process of food intake.

Gut hormone	**Site of secretion**	**Main receptors**	**Effect on food intake**	**Other functions**
Ghrelin	A cells in the gastric fundus	GHS receptor	Increase	• Stimulate secretion of growth hormones • Stimulate gastric motility (emptying)
Peptide tyrosine tyrpsine (PYY)	Luminal cells in ileum	Y2 receptor	Decrease	• Activate the ileal brake to delay motility of gastric and small intestine • Reduce gastric acid secretions
Cholecystokinin (CCK)	Luminal cells in duodenum and jejunum	CCK 1 receptor	Decrease	• Inhibit gastric emptying and gastric secretions • Induces pylorus contraction, and antrum and proximal stomach relaxation • Increase gallbladder contractions • Increase pancreas secretions
Glucagon-like-peptide 1 (GLP-1)	Luminal cells in ileum	GLP-1 receptor	Decrease	• Stimulate secretion of insulin • Reduce gastric emptying • Supresses pancreatic secretions
Glucagon-like-peptide 1 (GLP-2)	Luminal cells in ileum	GLP-2 receptor	Decrease	• Reduce gastric emptying of liquid • trophic effects on intestinal mucosa
Amylin	Pancreas beta cells	Histamine H1 receptor	Decrease	• Reduce gastric emptying • Reduce gastric acid secretion • Increase levels of postprandial glucose

Oxyntomodulin (OXM)	Luminal cells in ileum	GLP-1 receptor	Decrease	• Reduce gastric emptying • Reduce gastric acid secretion
Glucagon	Pancreas alpha cells	Glucagon receptor	Decrease	• Increase levels of blood glucose • Increase energy expenditure
Pancreatic polypeptide (PP)	Pancreatic islet PP cells	Y4 receptor	Decrease	• Delay gastric emptying rate • Inhibition of pancreatic enzymes • Relaxation of the gallbladder
Insulin	Langerhans in the pancreas	Insulin receptor	Decrease	• Maintain normal blood glucose levels • Affect brain areas of haemostatic and reward mechanisms
Blood glucose	Bloodstream after food intake	Glucoreptor	Decrease	• Affecting hypothalamic satiety centres • Hypoglycemia could activate hunger and GE rate
Leptin	Adipose tissue	Leptin receptor	Decrease	• Interact with the CCK and vagus nerve to delay gastric emptying • Modifying secretion of insulin, synthesis of hepatic glucose, and lipid metabolism
Triglycerides	Liver and adipose tissues	-	Fasting triglyceridemia increases food intake	• Inducing leptin resistance which lead to satiety, and calorie expenditure reduction
Free fatty acids (FFAs)	Released during fat lipolysis of adipose tissues	FFAR	Decrease	• Stimulating secretions of insulin, GLP-1, and glucagon, and reducing intestinal motility through expression of FFAR

2.4 Effect of macronutrient compositions on food intake regulation

Macronutrients have been suggested to exert different effects on appetite and satiety sensations, even when they contain the same number of calories (Holt et al., 1995, Bludell et al., 1996). Evidence suggests that there is a hierarchy in

satiating effect, with protein being more satiating than carbohydrate, and carbohydrate being more satiating than fat (Westerterp-Plantenga et al., 2006, Veldhorst et al., 2008, Johnstone, 2013). Studies have shown that individuals who follow a high-protein diet, about 30% of total energy from protein, tend to feel fuller and eat less compared to those who have a normal protein intake, about 18% of the total energy intake (Moran et al., 2005, Leidy et al., 2007). Additionally, an increased protein intake has been proposed to help in weight maintenance. In a randomized controlled trial involving 256 adults with overweight or obesity who were following a weight loss program, it was observed that weight regain was more pronounced in individuals who adhered to a low-protein diet (10–15% of total energy from protein) compared to those who followed a high protein diet (23–28% of total energy from protein) (Aller et al., 2014). Previous studies comparing protein, carbohydrate and fat suggest that protein is more satiating than carbohydrate and fat; however, there is inconsistency in findings.

The inconsistency in findings regarding the satiating effects of protein, carbohydrates, and fats highlights the need for a deeper understanding of how macronutrients influence food intake regulation. A crucial step in understanding how macronutrients influence appetite and eating behaviour is employing appropriate methodologies. The next section presents the methodologies commonly used to measure food intake regulation, which is the groundwork for the work presented in this thesis.

2.5 Effect of taste on food intake regulation

A person's choice of food is a dynamic process that evolves over the course of their life and is impacted by a variety of environmental and personal factors (Barclay and Brand-Miller, 2011). A food product's likelihood of being purchased and consumed by consumers is influenced by a variety of factors, including personal preferences, cultural, environmental, and contextual factors as well as the food's sensory qualities (Furst et al., 1996, Drewnowski et al., 1997, Olsen et al., 2012). Previous studies on food choice have indicated that people rank taste as one of the most significant considerations when choosing what to eat (Lennernäs et al., 1997, Glanz et al., 1998, Biloukha and Utermohlen,

2001). A study conducted by Kourouniotis et al. (2016) on 1306 university students reported that 82% of participants regarded taste as very important when choosing food. Participants who rated taste as very important factor also tended to consume less fruit (P= 0.03) and vegetables (P=0.05) and had a lower quality diet (P=0.001). Additionally, participants had a considerably higher likelihood of consuming foods high in salt, sugar, and fat such as fruit juice, soft drinks, cakes and puddings, sweet pastries, pizza, hot chips, and potato chips, takeout meals, pizza, chocolate, and confectionery (P=0.001). A higher intake of salt, fat and sugar has been connected to an imbalance in adult energy (Grimes et al., 2013) which is a major factor contributing to the rising rates of overweight and obesity globally (Hill et al., 2000, Bermudez and Gao, 2011, Grimes et al., 2013).

2.6 Approaches to measure regulation of food intake

The focus of this thesis is to gain insights into how food intake is regulated in adults with and without obesity and to ascertain whether there are differences in response to different macronutrients. Measuring the regulation of food intake involves assessing the various factors that influence eating behaviour including appetite and satiety. This section summarises key approaches used in measuring the regulation of food intake. This includes appetite/satiety rating scales, ad libitum meal intake, GI responses, and neuronal signalling to food intake, as well as eating behaviour questionnaires.

2.6.1 Appetite Rating Scales

Appetite rating scales are used to assess and quantify an individual's subjective feelings of hunger, fullness, and desire to eat. These scales are valuable in studying appetite regulation, understanding eating behaviours, and evaluating the effectiveness of dietary interventions. Appetite and satiety ratings can be measured through visual analogue scales (VAS), which were devised and validated by Blundell et al. (1987) to rate the subjective feelings of desire to eat, prospective food intake, fullness, and hunger. VAS is straightforward and commonly used tool to assess appetite and satiety sensations, and used in research and clinical settings to regularly evaluate subjective feelings (Stubbs et al., 2000). It is commonly represented as 100 mm horizontal lines that are connected at both edges by opposing descriptors, such as "extremely hungry" at one end and "completely full" at the other (Hill and Blundell, 1982). Participants self-report their level of hunger and fullness by placing a mark on a horizontal line to indicate the strength of a subjective sensation or a certain state at a specific time, allowing the sensation to be measured and quantified. Interpretation of the VAS scale is typically clear-cut as the descriptive terms are already presented at the end of each line (Stubbs et al., 2000). A variety of questions about satiety are commonly asked in VAS which comprise the following four main domains: desire to eat (DTE), hunger, fullness, and prospective food intake (PFI).

The pen and paper (P&P) format of the VAS scale are used in the interventional studies of this thesis. Previous studies used a composite satiety score to create

an overall satiety score (Van Can et al., 2014). In the composite satiety score, multiple factors or variables related to satiety and measured by the VAS are combined. In this thesis, participants were asked to report their appetite and satiety feeling by answering VAS questionnaire of DTE, hunger, fullness, and PFI (Appendix 10.1). In addition, a composite satiety score (CSS) was calculated using the following equation (Hansen et al., 2018).

$$\text{CSS (mm)} = \frac{[\text{fullness} + (100-\text{DTE}) + (100-\text{hunger}) + (100-\text{PFI})]\ \text{mm}}{4}$$

The satiety domain was not included in the VAS as may be confused with the fullness domain. Hence, the satiety domain was not included in the calculation of the CSS. In Chapters 5, 6, and 7 of this thesis, the VAS scale (DTE, hunger, fullness and PFI domains) and CSS were applied to measure appetite and satiety responses.

2.6.2 Satiety quotient

Satiety quotient (SQ) is another tool used in appetite studies to quantify and compare satiety effects of meals or foods relative to their energy content. The tool was developed by Green et al. (1997). It aims to provide a numerical value that reflects how well a particular food or meal satisfies hunger and reduces appetite.

SQ is typically calculated for each VAS domain (DTE, hunger, fullness, and PFI) by dividing changes in the satiety VAS scales by the energy content of a meal. The following equation is used to measure SQ for each VAS domain (Drapeau et al., 2007, Drapeau et al., 2013)

$$\text{VAS(mm/100 kcal)} = \frac{[\text{rating pre-eating episode} - \text{rating post-eating episode}]\ \text{mm}}{[\text{energy intake of eating episode kcal}]\ \text{x}\ 100}$$

The possible range of SQ is between -20.5 and 20.5 where a higher SQ for each VAS domain represents higher appetite/satiety responses and lower values represents weaker responses (Drapeau et al., 2013). The mean of the four SQ is used to determine satiety phenotypes of participants. This typically refers to individual variations in experiencing satiety/fullness sensation after eating. Some individuals may find certain foods more filling, while others may not experience the same level of satiety from those foods. If the mean SQ is ≤ 8

mm/100 kcal then the person is classified as having a low satiety and if the mean SQ is ≥ 8 mm/100 kcal, then they would be classified as having a high satiety (Drapeau et al., 2013). Low phenotypes refer to individuals who struggle to accurately identify their hunger sensations before or after a meal (Drapeau et al., 2013). Thus, SQ measurements allow the identification of factors that contribute to individual differences in satiety and to potentially develop more effective dietary interventions to promote satiety and manage appetite. In this thesis, SQ was used in Chapter 7 to compare satiety between high-protein and high-carbohydrate drinks.

2.6.3 Ad Libitum meal intake

Ad libitum meal intake refers to a method of food consumption in which individuals are allowed to eat freely and without imposed portion control or specific restrictions. When participants are given ad libitum access to food, they can consume as much, or as little, as they want until they feel satisfied or full. An ad libitum meal intake is used to quantify satiety sensation by recording the amount and energy consumed to satiation after a test preload has been consumed for a particular period of time (Blundell et al., 2010). This approach has been shown to have a good degree of intra-individual reproducibility in normal-weight adults (Arvaniti et al., 2000, Gregersen et al., 2008, Nair et al., 2008) and those with obesity (Lara et al., 2010, Horner et al., 2014b). In this thesis, the ad libitum lunch meal was used in the Chapter 7 to compare satiety responses between high-protein and high-carbohydrate drinks.

2.6.4 Gastrointestinal responses to food intake

Alongside the subjective ratings of appetite and satiety, there is a need to measure more objective physiological responses that relate to satiety and appetite and their subjective ratings (De Graaf et al., 2004). Measuring gastrointestinal responses to food intake involves assessing various physiological and biochemical changes that occur in the digestive system in response to eating. These measurements give insights into how the body processes food, absorbs nutrients, and regulates appetite. Below are some common methods for measuring GI responses to food intake.

2.6.4.1 Gut hormones

Section 2.3.1 demonstrated the relationships between gut hormones and their role in appetite/satiety sensation. Studying gut hormones as an objective biomarkers of appetite regulation can provide valuable insights into how these hormones influence hunger, satiety, and overall appetite. These data help in the understanding of the mechanisms involved in appetite control and may have implications for managing conditions like obesity and eating disorders. Satiety/appetite research studies measuring gut hormones typically involve collecting blood samples from participants before and after an intervention, including a test meal and/or dietary changes. Changes in hormone levels in response to the interventions or across groups (for example, NW vs. Obese) are then assessed. The most used gut hormones used to assess satiety are CCK, PYY, ghrelin, and GLP-1 which are usually measured during fasting and postprandially (Delzenne et al., 2010, Yeomans et al., 2016).

Although, these hormones are considered the gold standard for assessing satiety, they have some limitations. Gut hormones degrade quickly, thus blood samples need to be mixed with enzyme inhibitor to prevent degradation (Yi et al., 2015, Malandrino and Smith, 2018). Measuring gut hormones is expensive and invasive compared with the VAS scale. However, combining gut hormone measurements with other physiological and behavioural assessments can provide a more comprehensive understanding of appetite control.

2.6.4.2 Gastric emptying

Measuring GE is important for understanding satiety sensations. Several methods are available to assess GE, including gamma camera scintigraphy, isotope respiration test (breath test), and magnetic resonances imaging (MRI). These methods are briefly described below.

Scintigraphy

Scintigraphy is a commonly used, and highly accurate, method to measure GE. In this procedure, a small amount of a radioactive substance (usually technetium-99m sulfur colloid) is mixed with a solid or liquid meal. The individual then undergoes imaging using a gamma camera at specific time intervals to track the movement of the radioactive meal through the digestive

tract. The rate at which the radioactive meal leaves the stomach provides information about GE. Scintigraphy is considered a gold standard method for measuring GE. It is commonly used and highly accurate method. While it provides a direct measurement for GE, it does involve radiation exposure (Schwizer et al., 2003). Scintigraphy has been widely used to assess GE in NW and Obese participants after solid, semi-solid, and liquid meal meals (Feinle et al., 1999, Buchholz et al., 2013, Gonzalez-Izundegui et al., 2021).

Breath tests

Breath Tests are validated, reliable, and non-invasive test tool for measuring GE rates of solids and liquids without radiation exposure. They assess GE by measuring the appearance of specific gases in the breath. The individual consumes a meal or drink containing a non-absorbable, stable isotope-labelled substrate (e.g., ^{13}C-labelled octanoic acid). As the labelled substrate is digested and absorbed, it is metabolised in the liver and exhaled in the breath as labelled CO_2. The rate of appearance of labelled CO_2 in the exhaled breath in the breath correlates with GE. Although the technique is non-invasive, it is an indirect measurement of GE, and highly time consuming. Similar to the scintigraphy, breath tests have been widely used NW and Obese participants after solid, semi-solid, and liquid meal meals (Mora et al., 2005, Horner et al., 2014a).

Magnetic resonance imaging (MRI)

The use MRI in measuring GE and GI responses has increased in the past decade. MRI provides detailed and direct assessment of the GE (Carbone et al., 2010, Fruehauf et al., 2011). It can capture dynamic changes during the process of GE, making it suitable for studying the process under various conditions. MRI does not require radiation exposure or invasive procedures like scintigraphy, making it a safe option for repeated measurements. MRI has been validated against the gold standard methods of measuring GE (scintigraphy) in healthy participants after both liquid and solid meals (Kunz et al., 1999, Teramoto et al., 2012, Khalaf et al., 2020a) and also in patients with gastroparesis (Hayakawa et al., 2017), diabetes (Parkman et al., 2010), and dysphagia (Menys et al., 2017). Despite the great benefits of MRI to assess GE, it is expensive and may not be readily available in all clinical or research settings. Also, MRI might not be

suitable for individuals who are claustrophobic, or have MRI contraindications (i.e., pacemaker, metallic implants). Due to the relevance of MRI to the work conducted in this thesis, the principle of MRI is covered in next chapter.

2.6.5 Mesenteric blood flow

Blood flow in the SMA can be assessed using a diagnostic method known as Duplex ultrasonography. This approach combines conventional ultrasound imaging with Doppler ultrasound to measure the velocity and direction of blood flow in the SMA. It is a non-invasive and widely used technique for examining blood flow in various arteries and has been applied in the assessment of SMA blood flow (Ritenour, 1990, Perko, 2001). The measurement of SMA blood flow can also be performed using phase contrast MRI, a method that allows for quantitative imaging of flowing fluids. This technique relies on the principle that spins moving along a magnetic field gradient produce a phase shift that is proportionate to their velocity. Several studies have used phase-contrast MRI to evaluate changes in SMA blood flow, both during fasting and following food intake (Chowdhury et al., 2016, Terlouw et al., 2022). For example, a study conducted by Totman et al. (2009) measured SMA blood flow following an oral glucose tolerance test by phase contrast MRI in nine NW participants. After the oral glucose tolerance test, they observed a significant increase in SMA blood flow from baseline levels of 2.3 ml/s to 12.9 ml/s, with participant-specific peak times ranging from 22 to 51 minutes.

2.6.6 Small bowel water content

Small bowel water content (SBWC) represents free fluids within the small bowel (Hoad et al., 2007). SBWC is a useful biomarker that shows how secretion and absorption are balanced in health and disease, as well as how treatments are working. The visualisation and quantification of water content in the digestive system provide valuable insights into understanding the mechanisms involved in food regulation (Dellschaft et al., 2022). Previous research indicates that the balance of absorption and secretions (salivary, gastric, pancreatic, biliary, and intestinal) in response to luminal contents, as well as the underlying motility patterns—whether fed or fasting—as well as the velocity of material delivery from the stomach, will all influence SBWC (Dellschaft et al., 2022). Hence, studying the SBWC is a novel measure of GI function and pathology.

Before the development of MRI, it was difficult to measure SBWC. Previously, a variety of techniques, including radioisotopic imaging, intubation, and aspiration, have been used to conduct comprehensive research on the factors affecting pancreatico-biliary secretion and gastric emptying, which ultimately input into the small intestine (Sha, 2000, Chowdhury and Forsmark, 2003, Van Den Abeele et al., 2017). MRI is a very useful tool to measure SBWC due to its non-ionizing, non-invasive, and good sensitivity to mobile water (Dellschaft et al., 2022). Initially, echo-planar magnetic resonance imaging sequence was used to measure SBWC (Hykin et al., 1994, Adkin et al., 1995). Since the early work, modifications to coil design, sequence design, and field strength have permitted major improvements in image quality. Hoad et al. (2007) developed and validated a new technique that measures free water in the small bowel using a strongly T2 weighted single-shot fast spin echo (RARE) sequence, which is the same sequence used for biliary and pancreatic ducts, showing fluid-containing hollow organs as bright areas in MRI images (Adkin et al., 1995, Schiller et al., 2005). However, some free water in the small intestine cannot be observed with this MRI sequence. This includes free water that is trapped in mucus or in food matrices.

Quantification of SBWC in response to the consumption of liquid and solid meal has been demonstrated in several studies involving both normal weight/healthy participants (Marciani et al., 2010, Marciani et al., 2012) and individuals with altered appetite as described in Khalaf et al. (2020b). For more comprehensive information readers are referred to Dellschaft et al. (2022).

2.6.7 Neuronal signalling to food intake

Recent advances in neuroimaging techniques have enabled new avenues to study food regulation and neuronal signalling that influences appetite and satiety in health and disease. Among these techniques, functional MRI **(**fMRI) and positron emission tomography (PET) are the two most frequently employed methods to study neuronal signalling responses to food intake and reward. fMRI is a non-invasive imaging method that is widely used to measure changes in blood flow and oxygenation in the brain. PET is used to assess metabolic activity in various brain regions through the injection of a radioactive tracer into the bloodstream, which allows tracking glucose or other substances uptake in the

brain. There are different approaches to measure neural response to food intake. Due to the relevance of this thesis approaches used in fMRI are outlined below.

In a typical food-related fMRI study, brain activity is measured as individuals are exposed to visual or sensory (smell or taste) cues for a period of time, which is then followed by a rest period. This design is commonly referred to as "block design" and is form of task-fMRI. fMRI is highly effective in identifying brain regions responsible for food reward and pleasure (Batterham et al., 2007, Farooqi et al., 2007), as well as for examining how cues associated with food decision-making and dietary selections influence brain activity (Wang et al., 2016). Additionally, fMRI enables the evaluation of changes in brain activity before (fasted state) and after (fed state) meals, offering valuable insights into the regulation of appetite and feelings of fullness.

One way of assessing these changes is by measuring cerebral blood flow (CBF), typically by using arterial spin labelling (ASL) technique (Page et al., 2009, Lennerz et al., 2013, Eldeghaidy et al., 2016). CBF-fMRI studies enable the evaluation of the impact of physiological states by measuring the absolute CBF at different time points, which can then be compared with a baseline CBF measure. In addition, changes in resting state fMRI network can also be evaluated at different hunger and satiety levels (Liu et al., 2000, Smeets et al., 2005) or across different groups (for example NW vs. Obese).

2.6.8 Questionnaires and surveys to assess eating behaviour data

2.6.8.1 Binge eating scale (BES)

The BES is based on 16 questions developed by Gormally et al. (1982) to evaluate the severity of binge eating traits with evaluation of the affective, behavioural and cognitive manifestations. Binge eating refers to compulsive eating episodes which are composed mainly of two elements: loss of control of overeating, and eating large and extreme amounts of food in a short period (American Psychiatric Association, 2013). The questionnaire includes the following domains: eight questions that reflect behavioural symptoms (such as consuming big amounts of food or eating quickly) and eight questions on accompanying feelings and cognitions (such as the worry of not being able to stop eating). Responses for each item have a range between 0 to 3 where 0

indicates no serious symptoms of BE while 3 indicates severe BE symptoms (Escrivá-Martínez et al., 2019).

Total scores for this questionnaire ranged from 0 to 46. A score of ≤17 suggests the absence of binge eating disorders, 18 -26 moderate disorders of binge eating and ≥27 indicates severe disorders (Marcus et al., 1988). BES has high specificity and sensitivity to differentiate between normal and compulsive eaters in non-clinical and clinical populations (Freitas et al., 2006, Grupski et al., 2013, Duarte et al., 2015). This scale was used for studies conducted in this thesis (Chapters 6 and 7).

2.6.8.2 Control of eating questionnaire (CoEQ)

CoEQ is a questionnaire developed by Dalton et al. (2015) with 21-items to measure experiences during the past seven days using the following domains: sweet craving (items 3, 13, 14 and 15), craving control (items 9, 10, 11, 12 and 19), savory craving (items 4, 16, 17 and 18), and positive mood (items 5,6, 7 and 8) (Dalton et al., 2017). These items are evaluated using 100-mm visual analogue scales, with higher scores in the domains representing greater characteristics. Items 1 and 2 in the questionnaire measure general appetite feelings. Items 20 and 21 are measuring individual's control level over resisting a nominated, craved food item. These four items in the questionnaire are not included in the calculation of subscales.

2.6.8.3 Depression scale

Depression scale is commonly used to measure depression in clinical and research settings (Beck et al., 1961). It is a 21-item self-reported questionnaire that measure depressive symptoms according to Diagnostic and Statistical Manual for Mental Disorders (American Psychiatric Association, 2013). Items are added together to generate an overall score with a higher score indicating greater levels of depression.

2.6.8.4 Dutch eating behaviour questionnaire (DEBQ)

DEBQ is a 33-items questionnaire developed by Van Strien et al. (1986) that assess different types of eating attitudes that may contribute to emotional, restraint and external eating, and weight gain (Brunault et al., 2015). It is a gold standard tool to assess the cognitive, behavioural, and emotional dimensions of

eating behaviour with good reliability and validity (Dutton and Dovey, 2016, Arhire et al., 2021, de Carvalho et al., 2023). The response ranges from 1 (never) to 5 (very often) for each item in the questionnaire. Higher scores indicate greater alignment with each of the eating behaviours.

2.6.8.5 Eating attitude test (EAT)

EAT is the most standardised measure of eating disorder symptoms and concerns. However, it does not give a specific diagnosis of an eating disorder. It is a 26-item test which examines three aspects: dietary (13 items that assess the avoidance of high caloric foods and concern with becoming slimmer), bulimia and food preoccupation (6 items that assess thoughts on food and bulimia), and oral control (7 items that assess the perceived pressure from others to gain weight and self-control eating) (Garner and Garfinkel, 1979, Garner et al., 1982). Six responses are presented for each item (except for item 26): always (3 score), usually (2 score), often (1 score), sometimes (0 score), rarely (0 score), or never (0 score). Item-26 has a different scoring system with zero scores for always, usually, and often and 1 score for sometimes, 2 scores for rarely and 3 scores for never. A large body of literature has been published on its application across a wide variety of age groups and cultures (Garfinkel and Newman, 2001). EAT possesses great psychometric properties including excellent sensitivity and specificity with high validity and test-retest reliability (Garner et al., 1982, Mintz and O'Halloran, 2000, Lee et al., 2002).

2.6.8.6 Food preference questionnaire (PrefQuest)

PrefQuest, a questionnaire developed by Deglaire et al. (2012) with four scales for evaluating recalled preferences for the tastes of sweet, salt, fat and sweet, and fat and salt in adults. It is a web-based questionnaire with 83 items in total, with subscales measuring a person's preference for sweetness (21 items), saltiness (11 items), and fattiness subscales (20 items refer to fattiness and sweetness and 31 items refer to fattiness and saltiness). Each item is represented on a 9-point rating scale, with higher scores indicating higher preference to a food item.

2.6.8.7 Three-factor eating questionnaire (TFEQ)

The TFEQ is a 51-item questionnaire developed by Stunkard and Messick (1985) that assesses eating behaviour across three dimensions: cognitive restraint of eating (21 items, factor 1), disinhibition (16 items, factor 2) and susceptibility to hunger (14 items, factor 3). Cognitive restraint is defined as a conscious control of eating to promote weight loss or control body weight (score ranging from 0 to 21). Disinhibition is the overconsumption of eating in response to different stimuli such as emotional stress, which is accompanied by a lack of food intake control (score ranging from 0 to 16). Susceptibility to hunger is defined as eating in response to hunger feelings and perceptions (score ranging from 0 to 14) (Löffler et al., 2015). This questionnaire has been validated for normal-weight people, people with obesity, the general adult population (Hyland et al., 1989), and different ethnic populations (Bardone-Cone and Boyd, 2007). All scales demonstrated adequate test–retest reliability (Laessle et al., 1989). All TFEQ items are assigned either 0 or 1 points. Higher scores in the domains represent greater characteristics.

2.6.8.8 Power of food scale (PFS)

The PFS is a 21-item scale developed by Cappelleri et al. (2009) with good validity and reliability that assesses the psychological impact of an environment with an abundancy of ‘palatable' foods (Lowe et al., 2009, Andreeva et al., 2019, Torelli et al., 2022). The scale assesses appetite for palatable food but not consumption at three food proximity levels. First, food available level which assess general food related thoughts. Second, food physically present level which measures a person's desire for foods that are physically present in front of them. The last level is food tasted which assesses the desire for and enjoyment of food when first tasted.

2.6.8.9 Intake24 online dietary recall survey

This survey is an open source self-completed computerised system (https://intake24.co.uk/) designed and validated by Newcastle University (Intake24, 2023) based on multiple-pass 24-hour recall (Raper et al., 2004). It includes a database of more than 2500 foods linked to food composition codes (Roe et al., 2015). It was originally designed for participants aged between 11-24 years and subsequently extended for the general adult aged 11-88 years

(Rowland et al., 2018). The system allows participants to record all drink and food ingested with a series of pictures to help for estimating portion sizes. Participants also could verify their responses at each step. The system will ask some questions if some food items considered to be missing such as ‘do you have butter on toast’. This method was used to obtain 3-days dietary intake as well as quantifying energy intake for the rest of study days for the Chapter 7.

To conclude, approaches to measuring food intake regulations, there are a variety of methods available to measure appetite, satiety, and food intake. In choosing the ones to be used in trials undertaken in this thesis, a combination of factors, including cost, invasiveness, and facilities available, were all taken into account.

3 MRI Theory and Image Processing

MRI is widely recognised as one of the most powerful imaging techniques for measuring the structural and functional aspects of the GI tract and brain, and their responses to food consumption and regulation of food intake. MRI was used extensively in the studies conducted within this thesis. The following section gives a brief description of the principles of MRI. For more details the reader is referred to McRobbie (2003).

3.1 Basic principles of MRI

MRI is a non-invasive imaging technique that generates detailed three-dimensional detailed anatomical images of the internal structures of the body. It relies on the principles of nuclear magnetic resonance, which involves the interaction of certain atomic nuclei with strong magnetic fields and radiofrequency (RF) pulses (McRobbie, 2003). In MRI, hydrogen nuclei (protons), are primarily used because their abundance in the body and the high-water content of tissues. Protons also have a magnetic property referred to as "spin". These spins behave like tiny magnets.

MRI machines generate strong, static magnetic fields (B_0) using superconducting magnets. When a person is placed in the external magnetic field of the MRI machine, the protons “spin” around their axis and align themselves with the direction of the magnetic field (i.e., parallel to external magnetic field B_0, longitudinal plane), with a net magnetisation M_0, as shown in Figure 3.1. The protons also process, or spin, around the B_0 field at a characteristic frequency known as the "Larmor frequency". The Larmor frequency is directly proportional to the strength of the magnetic field and the gyromagnetic ratio of the ^1H for protons.

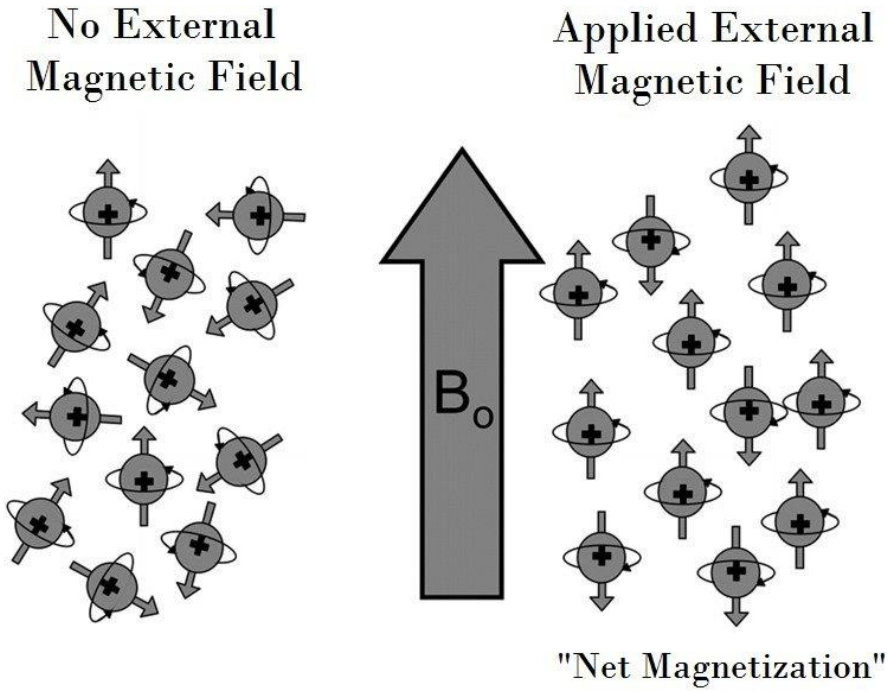


Figure 3.1. The process of protons in the absence of the magnetic field (the left side, protons randomly oriented), and when a strong magnetic field B_0 is applied (the right side, protons aligned parallel to the magnetic field). Figure reproduced from Caligari Conti (2016).

To manipulate these aligned protons, RF coils within the MRI machine emit short bursts of RF energy at "Lamour frequency". These RF pulses are applied at an angle relative to the magnetic field, typically 90 degrees, and are used to excite the protons (Figure 3.2), causing them to temporarily deviate from their aligned positions (i.e., to move away from the main magnetic field, B_0). Because the RF pulse matches the resonant frequency of the protons in the magnetic field, it causes the protons to flip into the transverse plane (My). This phenomenon is known as "nuclear magnetic resonance". When the RF pulse is switched off, the excited protons gradually realign themselves to their original aligned state, parallel to the main field B_0 (Figure 3.2). During the return of the proton's equilibrium alignment, protons emit RF signals.

The process of returning to equilibrium is termed relaxation, and the process involves two relaxation times. T1 also known as spin-lattice relaxation or longitudinal relaxation time, and it is the time taken for the protons' longitudinal

magnetisation (Mz) to recover to its equilibrium value (M_0). The other relaxation time is known as T2, or spin-spin relaxation, also known as transverse magnetisation. This is the time takes for the protons' transverse magnetisation (My) to decay. As the protons relax and return to equilibrium, they emit a signal known as Free Induction Decay (FID), which contains information about the characteristics of the tissue being imaged, such as its proton density, T1 and T2 relaxation times, and other tissue properties. The FID signal is detected by the MRI machine's receiver coil. The detected signal is then processed and used to create the final MRI image. Figure 3.2 gives an illustration of the excitation and relaxation process of the protons.

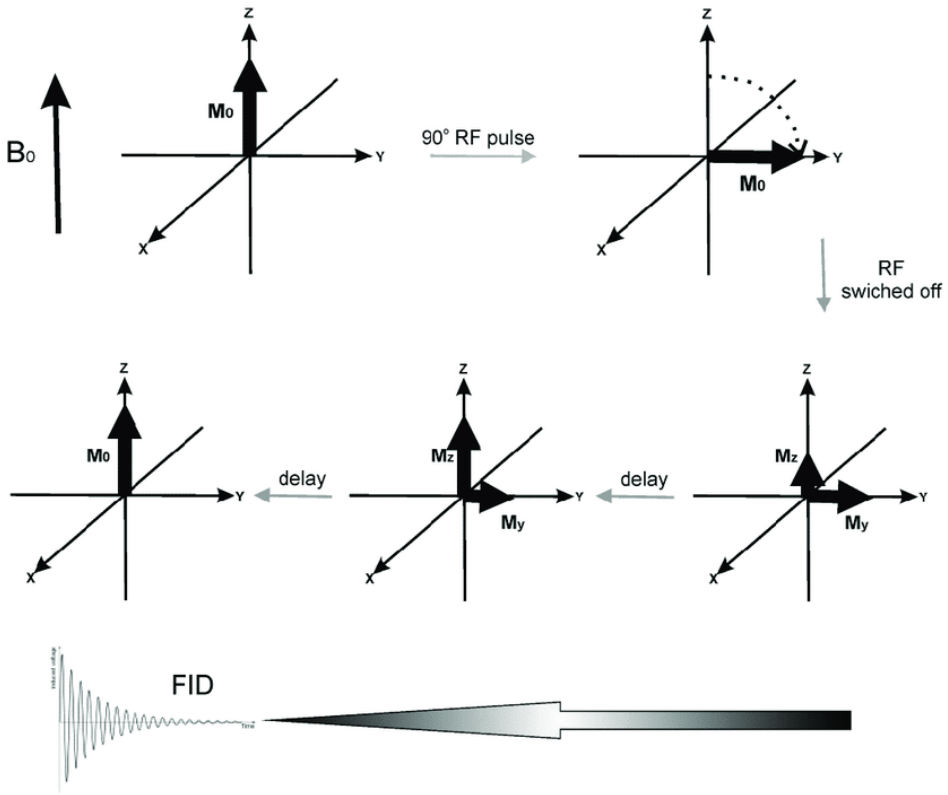


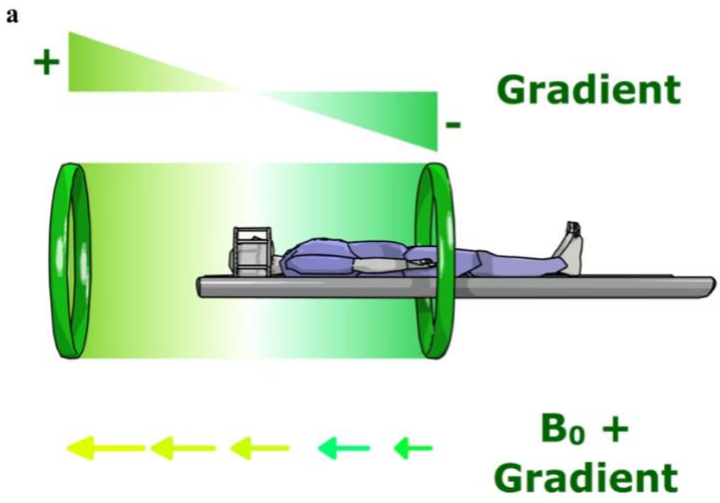
Figure 3.2. The diagram illustrating the main concept of MRI. Upper section: The magnetisation (M_0) is rotated to the transverse plane by the radiofrequency (RF) pulse. Lower section: Following the switching off the RF pulse, the protons slowly reach equilibrium, and emit a signal known as Free Induction Decay (FID) that is detected by the MRI machine's receiver coil and contains information about the characteristics of the tissue being imaged. Figure reproduced from Dulińska-Litewka et al. (2019).

3.2 Image formation

Gradient coils within the MRI machine generate additional, spatially varying magnetic fields. These gradient fields are used to encode spatial information into the MR signals. There are three types of gradient fields: slice selection (z-axis), phase encoding (y-axis), and frequency encoding (x-axis).

Slice selection gradient (Gz) is applied along the z-axis (the direction of the main magnetic field, B_0). It creates variations in the magnetic field strength along the z-axis and is used to select a specific slice or section of the body for imaging. By applying Gz, MRI can capture images of individual slices within the body. *Phase encoding gradient (Gy)* is applied along the y-axis (perpendicular to both the z-axis and the main magnetic field B_0), and it creates variations in the magnetic field to encode spatial information along the y-axis. Gy determines the position of data in the phase-encoding direction and helps create the two-dimensional image. *Frequency encoding gradient (Gx)* creates variations in the magnetic field strength along the x-axis and is used to encode spatial information along this axis. It determines the frequency of the signals collected and helps create the two-dimensional image (along with the Gy).

The coordinated application of these gradient coils during the MRI scan allows the collection of data from different spatial positions within the body. By changing the strengths and timing of the gradient fields, the MRI machine can encode the spatial location of signals and create images with specific contrasts and spatial resolutions.



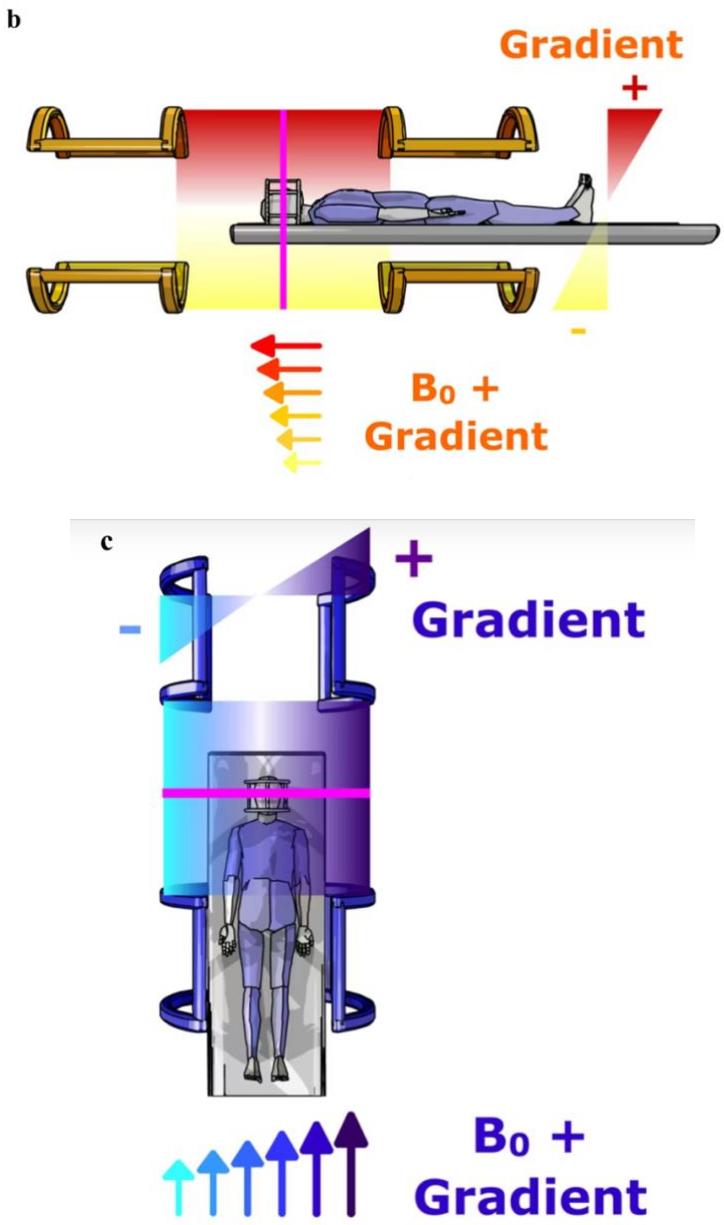


Figure 3.3. Representation of gradient fields, applied in MRI scanner, with illustration of the direction of each gradient: (a) slice selection (G_z), (b) phase encoding (G_x), and (c) frequency encoding (G_y). Figure adapted from IMAIOS (2023).

3.3 T1-weighted and T2-weighted contrasts

Different tissues in the body have varying relaxation times (T1 and T2), resulting in variations in signal intensity and contrast in the MRI images. These differences in contrast are essential for identifying and distinguishing different tissues. Figure 3.4 illustrates the variation in T1 and T2 relaxation times across

different tissue type (fat, muscles, and fluid). Various MRI pulse sequences are used to highlight specific tissue properties and create different types of images. In T1-weighted contrast, tissues with longer T1 times, such as fat, appear bright, while tissues with shorter T1 times, like muscle, appear darker. In T2-weighted contrast, tissues with longer T2 times, such as cerebrospinal fluid (CSF), appear bright, while tissues with shorter T2 times, such as bone, appear darker. Figure 3.5 show an example of abdominal image illustrating the difference appearance of fat and water in T1-weight and T2-weights MRI scans.

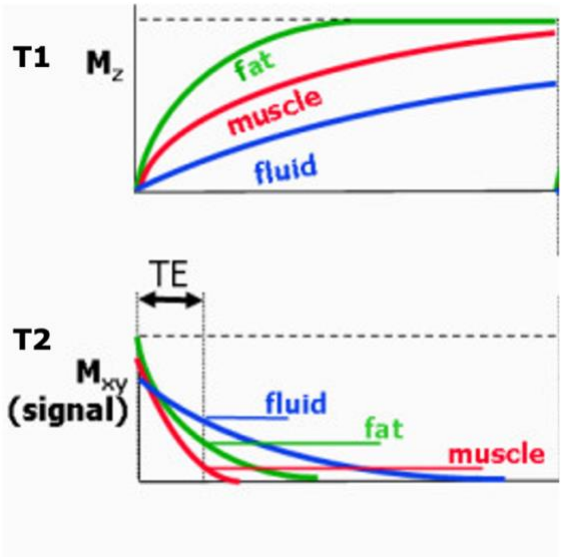


Figure 3.4. T1 and T2 relaxation times for fat, fluid, and muscle. Figure adapted from Ridgway (2010).

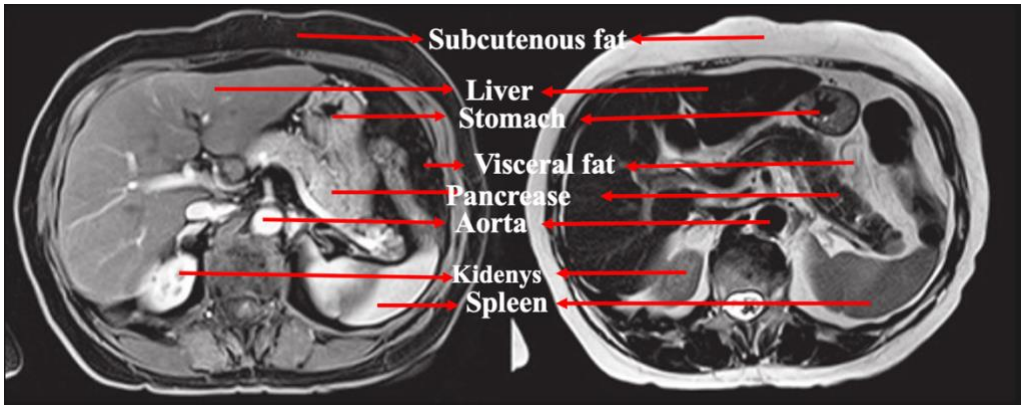


Figure 3.5. Examples of T1 and T2 weighted scans illustrating abdominal MRI image of T2-weighted (left side) and T1-weighted contrast (right side). Figure adapted from Santos et al. (2017).

Contrast enhancement of MRI images strongly depends on echo time (TE) and repetition time (TR). TE controls the timing of signal acquisition after the application of the RF pulse. It is adjusted in MRI sequences to emphasise specific tissue properties and create contrast in the resulting images. TR is the time between the applications of the RF excitation pulse to the application of the next pulse. It also influences the appearance of MRI images and is tailored to the tissue to be examined. Adjusting the TE and TR in MRI sequences can influence image/tissue contrast. Longer TE values enhance T2 contrast, while shorter TE values emphasize T1 contrast. Longer TR values can increase T1 contrast.

In this thesis, MRI was employed extensively to investigate and understand the GI responses to food consumption and the regulation of food intake in normal-weight participants and individuals with obesity. This is demonstrated in Chapter 5 and Chapter 6. Chapter 5 assesses the GI responses to a standard pasta meal, while Chapter 6 uses advances in MRI to assess food intake to different macronutrients.

4 Mapping brain activity of gut-brain signalling to appetite and satiety in people with and without obesity: A systematic review and functional neuroimaging meta-analysis

Understanding how neurohormonal gut-brain signalling regulates appetite and satiety is vital for the development of therapies for obesity and altered eating behavior. However, reported brain areas associated with appetite or satiety regulators show inconsistency across functional neuroimaging studies. The work in this chapter aimed to systematically assess the convergence of brain regions modulated by appetite and satiety regulators, and to generate quantitative brain activation maps of neurohormonal gut-brain signalling in normal-weight adults that can be used to define alterations with eating behaviour.

The work detailed in this chapter was published in Neuroscience & Biobehavioral Reviews. It was also presented as an oral presentation at the 46th British Feeding and Drinking Group, and as a poster of distinction at the 43rd ESPEN Congress.

Althubeati, S., Avery, A., Tench, C.R., Lobo, D.N., Salter, A. and Eldeghaidy, S., 2022. Mapping brain activity of gut-brain signaling to appetite and satiety in healthy adults: A systematic review and functional neuroimaging meta-analysis. Neuroscience & Biobehavioral Reviews, 136, p.104603.

The British Feeding and Drinking Group (BFDG) 46th Annual Meeting: Althubeati, S., Avery, A., Tench, C.R., Lobo, D.N., Salter, A. and Eldeghaidy, S., 2022. Functional neuroimaging meta-analysis to map brain activity of gut-brain signalling to appetite and satiety in healthy adults. Appetite, 179, p.106213.

The 43rd ESPEN Congress: Althubeati, S., Avery, A., Lobo, D.N., Salter, A. and Eldeghaidy, S., 2021. A neuroimaging meta-analysis to identify brain areas associated with satiety and appetite regulators. Clinical Nutrition ESPEN, 46, p.S582.

4.1 Introduction

Recent advances in non-invasive neuroimaging techniques have allowed the study of neurohormonal gut-brain signalling pathways that modulate appetite in health and disease (Gibson et al., 2010). A number of studies report attenuation in appetite-related brain areas including the insula, amygdala, OFC, ventral striatum, ACC, caudate, parahippocampal cortex, thalamus, and hypothalamus, when humans are fed, compared with when they are hungry (Jakobsdottir S, 2012, Sun et al., 2014). In contrast, studies investigating endogenously released appetite/satiety regulators, following food ingestion, or exogenously administered hormones with brain responses conducted on NW adults show inconsistency in the reported brain areas. For instance, some studies demonstrate increased activation in the insula with increasing concentrations of satiety regulators (Batterham et al., 2007, Schilling et al., 2014) while others found decreased activation in the insula with decreasing concentrations of satiety regulators (Spetter et al., 2014). There are different reasons for these discrepancies, including different designs (endogenously released *vs*. exogenous administration) or different paradigm/stimulation (no-task 'rest', visual-task "food images", taste-task) used during brain imaging examination. In people living with obesity, functional neuroimaging studies have shown that the brain activity in these people is significantly different from that in NW adults in several brain regions. Brain regions implicated in food reward responses have greater activation in these people paired with hypoactivity in areas associated with homeostatic satiety (Rothemund et al., 2007, Stoeckel et al., 2008, Szalay et al., 2012). Hence, separating brain data from participants living with obesity from NW participants is essential for an accurate understanding of brain activation associated with appetite and satiety processing.

By pooling data from published work on the interplay between appetite and satiety regulators and the brain in modulating appetite, a more accurate picture of regional brain activation associated with appetite and satiety processing can be established. Coordinate-based neuroimaging meta-analyses including activation likelihood estimation (ALE) methodology (Turkeltaub et al., 2002) and Analysis of Brain Coordinates (ABC) (Tench et al., 2021) allow the

identification of consistent brain activations across studies. These techniques use coordinates in standard anatomical space reported by neuroimaging studies to assess the agreement, or overlap, in activation patterns and infer a quantitative brain map of the overlapped regions (Muller et al., 2018, Tench et al., 2021). Thus, by using these methods a quantitative brain activation map of the neurohormonal gut-brain interactions could be generated.

At the time of writing this thesis, there was only one published systematic review that investigated the effects of appetite/satiety regulators on brain regions involved in appetite and satiety (Zanchi et al., 2017). However, neuroimaging data in this previous review were combined from adults with normal-weight and those with obesity and did not conduct functional neuroimaging meta-analysis to quantitively determine the concurrence/overlap of brain areas activated in response to appetite and satiety regulators across studies.

4.2 Aims and Hypothesis

Primary aim

To provide a comprehensive analysis of the functional neuroimaging literature on brain areas associated with changes in appetite and satiety regulators in NW and Obese adults.

Secondary aim

To produce a quantitative brain activation map of the neurohormonal gut-brain interactions, using coordinate-based neuroimaging meta-analysis.

Hypothesis

The hypothalamus, insula and caudate nucleus are key brain areas regulating appetite and satiety and are positively associated by appetite regulators and negatively by satiety regulators in NW adults. Such regulation is disrupted in adults with obesity.

4.3 Material and methods

4.3.1 Systematic review of the literature

A comprehensive search was carried out in the MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) databases between November 2019 and January 2021 to identify relevant studies using keywords from functional neuroimaging techniques and appetite and satiety responses including terms related to satiety and/or appetite regulators. In addition, a manual searching process was used to find relevant studies in the reference lists of all included studies.

4.3.2 Search strategy

The full search strategy is described below in Table 4.1. This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009). The protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/) with registration number CRD42020223921. Searches were restricted to human studies published in the English language but not restricted to publication dates. After duplicates were removed, all records were screened for title and abstract. The remaining publications were then reviewed independently for eligibility based on full texts by three authors using the set criteria. The eligibility criteria were based on the PICO (Population-Intervention-Comparator-Outcomes) model, summarised in Table 4.2.

Table 4.1. Search Strategy used in the systematic review.

1. "gut peptide" [MeSH terms]
2. "gut peptides" [MeSH terms]
3. "gut hormone" or "gastrointestinal hormone" [MeSH terms]
4. " gut hormones" or "gastrointestinal hormones" [MeSH terms]
5. "peptide YY " [MeSH terms]
6. "PYY" [MeSH terms]
7. "ghrelin" [MeSH terms]
8. "cholecystokinin" [MeSH terms]
9. "CCK" [MeSH terms]

10. "glucagon like peptide 1" [MeSH terms]

11. "GLP-1" [MeSH terms]

12. "satiety response" or "satiety" [MesSH terms]

13. "appetite" [MeSH terms]

14. "brain" [MeSH terms]

15. "imaging" [MeSH terms]

16. "neuroimaging" or "functional neuroimaging" or "neuroimaging" [MeSH terms]

17. "MRI" or "nuclear magnetic resonance imaging" [MeSH terms]

18. "fMRI" or "functional magnetic resonance imaging" [MeSH terms]

19. "PET" or "positron emission tomography" [MeSH terms]

20. "food" [MeSH terms]

21. "nutrient" [MeSH terms]

22. "nutrients" [MeSH terms]

23. "food intake" [MeSH terms]

24. "caloric intake" or "nutrient intake" [MeSH terms]

25. "meal" [MeSH terms]

26. "meals" [MeSH terms]

27. "meal intake" [MeSH terms]

28. "drink" [MeSH terms]

29. "drinks" [MeSH terms]

30. #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11

31. #12 or #13

32. #14 or #15 or #16 or #17 or #18 or #19

33. #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29

34. #30 and #31 and #32 and #33

35. #30 and #31 and #32

Table 4.2. Eligibility criteria based on the PICO (Population-Intervention-Comparator-Outcomes) model.

Inclusion criteria	**Population**	• Normal-weight (18.5 to 24.9kg/m^2) or adult humans with obesity (BMI ≥ 30 kg/m^2) between 18-65 years old with no medications that would influence appetite or metabolism
	Intervention	• Acute macronutrients interventions (carbohydrate, protein, or fat) consumed as a drink or a meal or • Exogenous infusion of appetite/satiety regulators • No restrictions were applied on the amount of macronutrients given, the level of hormone infusion, the number of hours fasted, the consumption/infusion and the route of macronutrient ingestion (oral or gastric) or gut hormone infusion (bolus, intravenous or subcutaneous injection)
	Comparator	• No specific comparators with controls such as water, placebo, saline or fasting included. Most studies are "before and after" studies where the participants serve as their own controls
	Outcomes	• Primary outcome: concurrence of brain regions modulated in response to appetite and satiety regulators in NW and Obese • Secondary outcome: quantitative brain-activation maps generated from coordinate based meta-analyses to assess the concurrence of brain regions modulated in response to appetite and satiety regulators
	Study design	• Controlled trials, randomized controlled trials, randomized cross-over design trials, and cohort studies
Exclusion criteria		• Studies that involved participants with gastrointestinal, endocrine, and neurological diseases, or adolescents • Publications with no direct correlation analysis performed between brain responses and satiety/appetite regulators or with long-intervention studies • In-vitro studies • Reviews

4.3.3 Risk-of-Bias Assessment

The quality of the included papers was assessed for potential risk of bias using the Cochrane collaboration to assess the risk of bias in randomised cross-over and randomised controlled trials (ROBINS-I) (Ding et al., 2015, Higgins et al., 2019).

4.3.4 Data extraction

For each study, the following information was extracted: authors, year of publication, total number of participants, participant details [mean age, sex and body mass index (BMI)], time of first brain imaging scan after treatment administration, intervention, administration method (e.g. oral, gastric or intravenous cannula), assessed appetite/satiety regulators, neuroimaging modality and brain stimulation method (e.g. gustatory, visual) and correlation results between brain areas and appetite and satiety regulators. Extracted data were grouped into 1) brain areas correlated positively and/or 2) correlated negatively with appetite regulators, 3) brain areas correlated positively and/or 4) correlated negatively with satiety regulators. In the appetite analysis, data were analysed during the fasting state or for contrast fasted>fed. In the satiety state analysis, data were derived from a direct contrast between fed state versus fasted/hunger state (fed>fasted) or data assessed postprandially within 1.5 hours following the last consumption. Brain areas from each of the sub-grouped data were then pooled and common brain areas across studies were evaluated. To illustrate the concurrence of brain areas generated from the systematic review, anatomically defined masks for overlapped brain areas were generated using WFU PickAtlas toolbox (Maldjian et al., 2003) in SPM software (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The generated masks were displayed using the MRIcroGL software (Rorden et al., 2007) and overlaid on brain template in Montreal Neurological Institute (MNI) space.

4.3.5 Coordinate-based neuroimaging meta-analysis

Neuroimaging studies included in the meta-analysis were pooled from those included in the systematic review. Studies that did not report coordinates for brain activations in response to appetite/satiety regulators in the article or supplementary material were excluded from the meta-analysis. The recently suggested standard protocol for neuroimaging meta-analysis by Eickhoff et al. (2016) was followed and therefore included only neuroimaging studies that

reported brain activation using whole-brain voxel wise analyses (Turkeltaub et al., 2012). In addition, seed-based functional connectivity analysis in rs-fMRI studies were excluded from the meta-analysis, as they usually focus on particular areas in the brain. Coordinates of brain regions that are directly correlated with satiety and appetite regulators were manually extracted. Extracted coordinates were checked and when inconsistencies between the coordinates reported in the original study were identified coordinates were rechecked and corrected. Studies that reported coordinates in Talaraich space (Talairach and Tournoux, 1988) were converted to the standard space of the MNI (Evans et al., 1993) using the icbm2taal algorithm implemented in the Ginger ALE toolbox (Laird et al., 2010).

There are multiple algorithms for performing coordinate-based neuroimaging meta-analysis, each has different empirical parameters and assumptions, and each can produce different results conditional on the assumptions. Therefore, to obtain robust results of brain areas associated with appetite and satiety regulators, two different tools were employed in the current neuroimaging meta-analysis: the ALE and ABC meta-analysis methods. The next section describes in the ALE and ABC analyses conducted in this chapter.

4.3.5.1 Activation likelihood estimation meta-analysis

The ALE-approach is the most popular method of performing neuroimaging meta-analysis. The algorithm takes into account the number of participants in each study to apply a relevant smoothing, resulting in a higher specificity of the actual overlap between studies (Eickhoff et al., 2009). However, in order to produce results that are not overly representative of single studies, it is recommended that at least 17–20 experiments should be included in the analysis. In addition, the ALE algorithm does not allow assessment of the effect sign associated with the coordinates when a decrease/increase brain activity are combined in a single analysis. This recommendation is based on the finding that, from this size on, the average contribution of the most dominant experiment to any above-threshold cluster is less than half, and the average contribution of the two most dominant experiments to any above-threshold cluster is less than 80% when controlling for multiple comparisons using cluster-level family wise error correction (Eickhoff et al., 2016). ALE meta-analyses were performed using

Ginger ALE version 3.0.2 (http://www.brainmap.org/ale). ALE analysis uses the reported activation peaks from the individual studies as a three-dimensional Gaussian probability distribution (kernel) centered at the given coordinates to create a modeled activation (MA) map for each study. Individual MA-maps are then combined to calculate statistical ALE maps and ALE values for each cluster. These calculations are confined to a grey matter mask provided by the Ginger ALE software. The ALE maps indicate areas of the brain where convergence between activation foci is greater than would be expected by chance (i.e., a null distribution of clusters). The recommendations of Eickhoff et al. (2016) for all analyses was followed. The statistical significance threshold of ALE maps was assessed and corrected for multiple comparisons by employing a cluster-level family-wise error (FWE) at $P < 0.05$, following an initial cluster forming threshold of uncorrected $P < 0.001$ Eickhoff et al. (2016). The P-value was calculated for each voxel based on probabilities of reaching an ALE value that differed from that of the corresponding voxel on a null-distribution map, via random permutation. Five-thousand permutations were used to generate the P-values (Laird et al., 2010).

Discriminating between close and distant coordinates was crucial for accurately assessing spatial convergence across studies. A voxel-wise approach was utilised where each study's foci were modelled as Gaussian probability distributions centred on the reported coordinates. The size of the Gaussian kernel was determined based on empirical assessments of spatial uncertainty in neuroimaging, typically ranging between 4- and 20-mm full width at half maximum (FWHM) (smoothing data for each study are shown in Table 4.3). This modelling allows for the assessment of the likelihood that each voxel is activated, accounting for both the precision of coordinate reporting and the spatial resolution of the studies. Overlapping probabilities from these Gaussian kernels were then accumulated across experiments to create a statistical ALE map (Eickhoff et al., 2009, Turkeltaub et al., 2012, Eickhoff et al., 2016). Thresholding this map based on cluster size correction and permutation testing, as described above, ensured that only significant clusters of spatially convergent activity were considered, effectively discriminating between closely located foci and more dispersed activations

The generated meta-analysis maps from the ALE methods were displayed using the MRIcroGL software (Rorden et al., 2007) and overlaid on brain template in MNI space.

4.3.5.2 Analysis of Brain Coordinates

ABC requires a minimum of 5 studies and does not take any account of the sample size in each study. A further difference between the two algorithms is in the thresholding for statistical significance, where ALE uses a cluster level family wise error rate method and ABC directly relates the threshold to the aim of detecting replicated results. ABC methodology (Tench et al., 2021) was performed using the ABC toolbox implemented in the NeuRoi image analysis software (www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx). The algorithm of this recently developed model-based method uses the density of coordinates from independent studies as its statistic and requires only the human grey matter volume (one parameter). Statistical thresholding is performed by requiring a minimum proportion of the studies contributing to a cluster and is generally more conservative than false discovery rate (FDR<0.05). Importantly, this method, in contrast to the ALE-approach, does not require the empirical choice of Gaussian smoothing kernel to extrapolate coordinates to voxel-wise activation maps or the randomization of the coordinates in the empirical space to define the statistical threshold.

4.4 Results

4.4.1 Systematic review

4.4.1.1 Selection and inclusion of studies

Of the 1390 studies identified in the initial search, 81 were selected for full text assessment (Figure 4.1). A total of 31 eligible studies (25 studies with NW participants and 6 studies with Obese participants) were included. Characteristics of the included studies are summarised in Table 4.3. The quality assessment of each paper is shown below in Table 4.4.

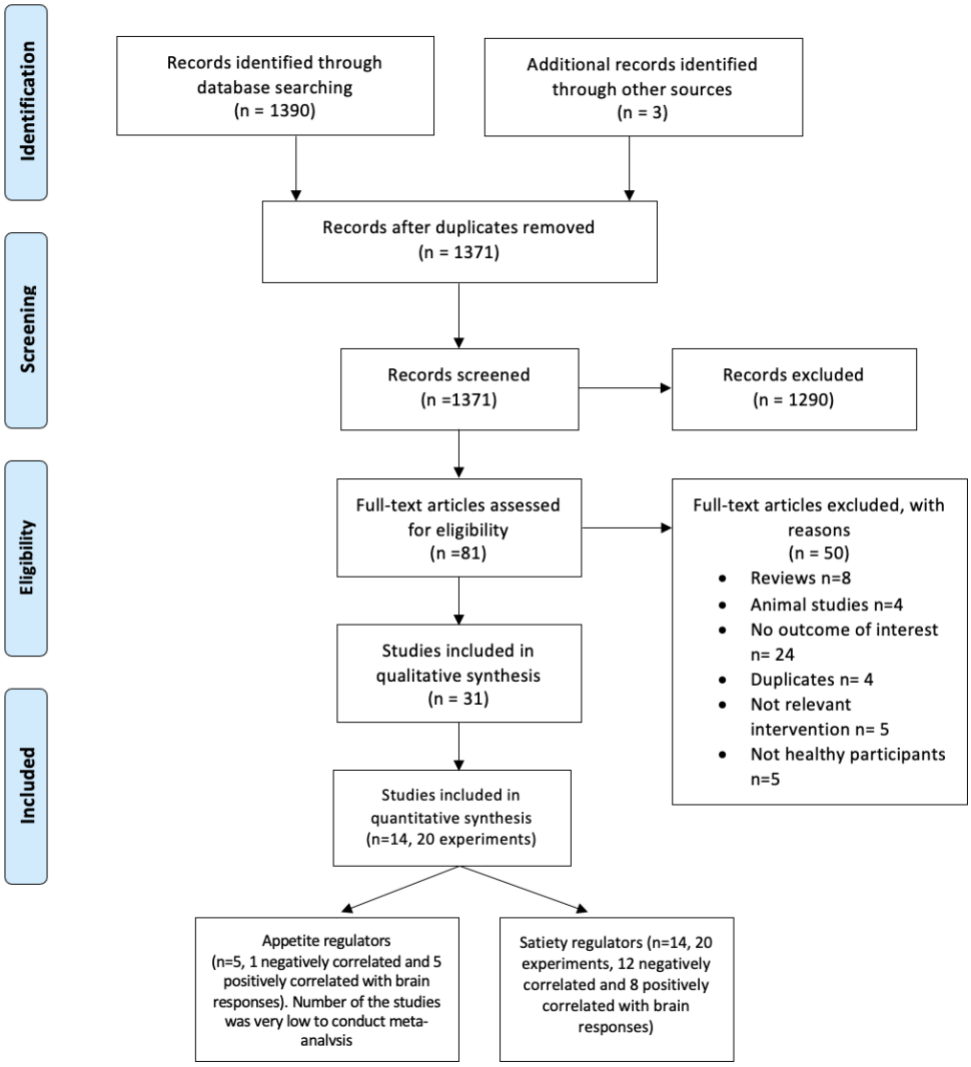


Figure 4.1. PRISMA Diagram

4.4.1.2 Characteristics of included studies

Of the 31 studies included, 17 investigated the effect of endogenously released appetite and satiety regulators and 11 investigated the effect of exogenously administered regulators on brain responses. Twenty-two studies used fMRI, of which eight used food picture task-fMRI (Malik et al., 2008, De Silva et al., 2011, Page et al., 2011, Jakobsdottir et al., 2012, Kroemer et al., 2013, Goldstone et al., 2014, Heni et al., 2015, Dorton et al., 2017), five studies used taste stimuli (Liu et al., 2000, Li et al., 2012, Spetter et al., 2014, Sun et al., 2014, Eldeghaidy et al., 2016), four studies assessed neurological responses across a time course "physiological fMRI design"(Batterham et al., 2007, Lassman et al., 2010, Jones et al., 2012b, Little et al., 2014), four studies used resting state fMRI (Page et al., 2013, Wolnerhanssen et al., 2015, Zhang et al., 2015, Al-Zubaidi et al.,

2019), three studies used ASL (Page et al., 2009, Lennerz et al., 2013, Schilling et al., 2014) and three studies used PET imaging technique (Tataranni et al., 1999, Gautier et al., 2000, Pannacciulli et al., 2007).

Across the included studies, brain responses for the hungry state were assessed following fasting that ranged between 4 and 14 hours, whereas for the satiety state brain responses were assessed within 1.5 hours postprandially. Seven studies administered standard meals with different amounts of protein, fat and fibre, containing ingredients such as soya bean, beef or milkshake (Tataranni et al., 1999, Gautier et al., 2000, Pannacciulli et al., 2007, Jakobsdottir et al., 2012, Spetter et al., 2014, Sun et al., 2014, Al-Zubaidi et al., 2019). Eight studies administered target nutrients such as whey protein solution, glucose drink, soybean oil emulsion or flavoured fat emulsion samples (Liu et al., 2000, Li et al., 2012, Kroemer et al., 2013, Page et al., 2013, Heni et al., 2015, Wolnerhanssen et al., 2015, Eldeghaidy et al., 2016, Dorton et al., 2017). Seventeen studies reported that nutrients were administered orally (Tataranni et al., 1999, Gautier et al., 2000, Liu et al., 2000, Pannacciulli et al., 2007, De Silva et al., 2011, Jakobsdottir et al., 2012, Li et al., 2012, Kroemer et al., 2013, Lennerz et al., 2013, Page et al., 2013, Spetter et al., 2014, Sun et al., 2014, Heni et al., 2015, Zhang et al., 2015, Eldeghaidy et al., 2016, Dorton et al., 2017, Al-Zubaidi et al., 2019), while three studies administered nutrients via the intra-gastric route (Little et al., 2014, Spetter et al., 2014, Wolnerhanssen et al., 2015). Spetter et al. (2014) reported that they administered nutrients by both the nasogastric tube and the oral routes. Eleven studies administered exogenous appetite and satiety regulators including PYY, GLP-1, ghrelin, insulin, glucose and CCK by intravenous (Batterham et al., 2007, Malik et al., 2008, Page et al., 2009, De Silva et al., 2011, Page et al., 2011, Jones et al., 2012b, Goldstone et al., 2014, van Bloemendaal et al., 2014), intranasal (Schilling et al., 2014) or intra-gastric infusion (Lassman et al., 2010, Little et al., 2014). Details of the included studies are provided in Table 4.3.

Table 4.3. Characteristics and main results of the included studies

Reference	Subjects	Mean age (years), gender & BMI (kg/m^2)	Time of brain imaging & type of brain analysis	Intervention	Administration	Hormone investigated	Neuroimaging modality & paradigm	Smoothing and threshold level	Results
		Endogenously released regulators studies							
Al-Zubaidi et al. (2019)	n= 24	- Age: 24.3±1.3 - Sex: All M - BMI: 23.4 ±1.4	- After 20 minutes of the nutrient intake - Whole brain	- 300 ml of glucose (75 g) ingestion - Fasted state	Orally	Glucose Insulin	rs-fMRI	-6 mm FWHM -FDR at P = 0.05	After glucose ingestion relative to fasting (hunger > satiety): - insulin levels → superior frontal gyrus ↓, posterior insula ↓ - glucose → fusiform gyrus ↑
Dorton et al. (2017)	n= 22	- Age: 21.2 ± 2.1 - Sex: 10 M & 12 F - BMI: 22.6 ± 1.9	- After 20 minutes of the nutrient intake - ROI [ventral striatum (nucleus accumbens) and bilateral	- 300 ml of glucose (75 g) ingestion - 300 ml of water - ingestion	Orally	GLP-1 PYY	Task-fMRI (food picture paradigm)	-5 mm FWHM -P < 0.05, corrected for multiple comparisons	After glucose intake: - GLP-1 → the dorsal striatal ↓

			dorsal striatum (caudate/putamen)]						
Eldeghaidy et al. (2016) *	n= 17	- Age: 25 ± 2 - Sex; 11 M & 6 F - BMI: 22.4 ± 0.8	- Immediately - Whole brain	- Two emulsion stimuli; flavoured fat stimulus (FS) & flavoured not fat control stimulus (CS) following: ▪ 250 ml of high fat drink/load (22% fat) ▪ 250 ml of water load	Orally	CCK	Task-fMRI (taste stimuli paradigm)	- 8 mm FWHM -P < 0.05, cluster-level corrected	Responses to the CS and FS after the high fat meal: - CCK → primary somatosensory cortex ↓, amygdala↓, supramarginal gyrus ↓, middle and posterior insula ↓, temporal gyrus ↓, thalamus ↓, cerebellum ↓, operculum ↓
Gautier et al. (2000) *	n= 22 (11 NW & 11 Obese)	- Age: 35 ±8 (NW), 27±5 (Obese) - Sex: All M	- After 25 minutes of the meal intake	- Liquid formula meal (1.5 kcal/ml	Orally	Insulin GLP-1 Leptin FFA	PET	-Smoothing level is not mentioned	After the liquid meal ingestion in NW participants:

		- BMI: ≤25 (NW), ≥ 35 (Obese)	- Whole brain+ ROI (hypothalamus, thalamus, DLPFC, anterior prefrontal cortex, ACC, insular cortex, posterior orbitofrontal cortex, hippocampus/parahippocampal gyrus, caudate ventricle, precuneus, putamen, parietotemporal cortex, occipital cortex,	Ensure-Plus: 15% protein, 53% carb & 32% fat)				-P < 0.005, uncorrected for multiple comparisons	- insulin → posterior OFC ↓, hippocampus/ parahippocampus ↓, putamen ↓, thalamus ↓, precuneus ↑ - FFA → DLPFC ↑ After the liquid meal ingestion in Obese participants: - insulin → posterior OFC ↓, hippocampus/ parahippocampus ↓, precuneus ↓, putamen ↓, thalamus ↓.

			cerebellum & midbrain)						
Heni et al. (2015) *	n=12	- Age: 23±2 - Sex: 6 M & 6 F - BMI: 21.2 ± 1.1	- After 30 minutes of the meal intake - Whole brain	- 300 ml of glucose (75 g) ingestion - 300 ml of water ingestion	Orally	GLP-1	Task-fMRI (food picture paradigm)	-6 mm FWHM -P<0.05, corrected for multiple comparisons	After glucose intake: - insulin→ OFC ↓
Jakobsdottir et al. (2012) *	n= 15	- Age: 23.4 ± 3.5 - Sex: All M - BMI: 22.4 ± 2	- One hour of the meal intake - Whole brain	- Standard meal consisted of 1600 kcal, 15.8 % protein, 44.4% carbohydrate and 39.8% fat	Orally	Glucose Insulin Ghrelin TAG Leptin	Task-fMRI (food picture paradigm)	-6 mm FWHM -FDR at P<0.05	After satiation with the standard meal: - leptin → hippocampus ↓, insula ↓, temporal lobe bilaterally ↓, frontal gyrus ↓
Kroemer et al. (2013) *	n=26	- Age: 24.4 ± 3.4 - Sex: 13 M & 13 F - BMI: 21.1 ± 2	- After 5 minutes of the meal intake - Whole brain	- 300 ml of glucose (75 g) ingestion	Orally	Ghrelin	Task-fMRI (food picture paradigm)	-8 mm FWHM -Whole brain uncorrected P < 0.001/ROIs FWE correction	During fasting: - ghrelin → middle frontal gyrus ↑, midbrain ↑, superior/medial frontal gyrus ↑, inferior frontal gyrus ↑, medial occipital/temporal gyrus ↑, hypothalamus ↑, subthalamic nucleus ↑, fusiform gyrus ↑, thalamus ↑, superior occipital gyrus , inferior frontal gyrus ↑, middle frontal gyrus ↑, pallidum,

									amygdala ↑, inferior frontal gyrus, caudate body ↑, inferior temporal g., fusiform gyrus ↑, middle/superior frontal gyrus ↑, thalamus (anterior nucleus) ↑, medial/superior frontal gyrus, ↑anterior cingulate ↑, postcentral (supramarginal gyrus & rolandic operculum ↑)
Lennerz et al. (2013)	n=12	- Age: 29.1 - Sex: All M - BMI: 32.9	- Four hours of the meal intake - Whole brain + ROIs (ventral striatum, hypothalamus, midbrain)	- Low glycemic meal (37% of predictive glucose) - High glycemic meal (84% of predictive glucose) - Both meals: 500 kcal, ~ 68.9 g carbohydrate, ~13 g fat, ~18 g protein.	Orally	Glucose Insulin	rCBF/ASL	-8 mm FWHM -Whole brain/Bonferroni corrected P < 0.002	High GM vs low GM: - glucose and insulin→ nucleus accumbens ↑
Li et al. (2012) *	n= 14	- Age: 23 - Sex: All M - BMI: 21.2	- After 6 minutes of meal ingestion	- 300 ml of whey protein (257	Orally	GLP-1 Ghrelin Glucose Insulin	Task-fMRI (taste stimuli paradigm)	-8 mm FWHM	After whey protein ingestion: - GLP-1: lateral orbito-frontal cortex↓ - insulin: caudate↓ - CCK; thalamus ↓

			- ROIs (thalamus, hypothalamus, insula, parahippocampal/hippocampal cortex, putamen, caudate OFC & amygdala)	g/L) ingestion - 300 ml of soybean emulsion (11 g/L) - 300 ml of glucose (250 g/L) ingestion - Water: 300 ml		CCK		-P < 0.05, corrected with Monte Carlo simulations	- ghrelin: amygdala ↑ After glucose ingestion: - insulin: thalamus↓, middle insula↓, amygdala↓, lateral OFC ↓ - glucose: thalamus↓ - CCK: caudate ↓ - GLP-1: Latera OFC ↓, middle insula↓ - ghrelin: middle insula ↑, later OFC ↑ After fat ingestion: - CCK: caudate ↓, thalamus ↓ - ghrelin: amygdala ↑, middle insula ↑, lateral OFC ↑
Liu et al. (2000)	n= 21	- Age: 34 ± 3 - Sex: 11 M & 10 F - BMI: not available	- After 10 minutes of meal intake - Whole brain	- 296 ml of dextrose (75 g) - 300 ml of distilled water	Orally	Insulin	rs-fMRI	Smoothing and threshold levels are not mentioned	- Fasting insulin→ hypothalamus ↓, somatosensory cortex ↓, SMA ↓, cerebellum↓, anterior cingulate ↓, OFC ↓
Page et al. (2013) *	n= 20	- Age: 31 ± 7 - Sex: 10 M & 10 F - BMI: 22± 2.5	- After 60 minutes of meal intake - Whole brain+ ROI (hypothalamus)	- 300 ml of glucose (75 g) ingestion - 300 ml of fructose (75 g) ingestion	Orally	GLP-1 PYY Ghrelin	rs-fMRI & fMRI-ASL	-6 mm FWHM -FWE whole- brain correction of P<0.05	After glucose ingestion: - insulin → caudate ↓, putamen ↓

Pannacciulli et al. (2007) *	n= 42	- Age: 31 ± 8 - Sex: 22 M & 20 F - BMI: 31 ± 9	- After 25 minutes of the meal intake - Whole brain	- Standard liquid formula meal (1.5 kcal/ml Ensure-plus, 15% protein, 53% carbohydrate and 32% fat)	Orally	GLP-1	PET	-15 mm FWHM -P ≤ 0.001, uncorrected for multiple comparisons	After the liquid meal ingestion: - GLP-1 → hypothalamus ↑, inferior frontal gyrus ↑, middle frontal gyrus ↑,
Spetter et al. (2014) *	n= 14	- Age: 24.6± 3.8 - Sex: All M - BMI: 22.3± 1.6	- After 5 minutes of the meal intake - Whole brain+ ROI (hippocampus insula, amygdala, midbrain, putamen, caudate, pallidum, nucleus accumbens and	- Oral chocolate milk - Nasogastric chocolate milk infusion per 100ml; 84.6 kcal, 16% protein, 56.7% carbohydrate and 26% fat) - Nasogastric water	Orally & nasogastric tube	Glucose Insulin Ghrelin CCK	Task-fMRI (taste stimuli paradigm)	-8 mm FWHM -FWE whole- brain correction of P<0.05	During nasogastric infusion of chocolate milk: - insulin → middle and posterior Insula ↓, putamen↑

			hypothalamus)						
Sun et al. (2014) *	n= 32	- Age: 25.3 ± 5.6 - Sex: 15 M & 17 F - BMI: 25.3 ± 4.5	- After 30 minutes of the meal intake - ROI (hippocampus insula, amygdala, midbrain, putamen, caudate, pallidum, nucleus accumbens and hypothalamus)	- Milkshake (per 945 ml; 918 kcal, 10.7% protein, 52.4% carbohydrate and 25% fat) during; - Fasting - Satiation with fixed lunch meal (425 kcal for women & 625 kcal for men) and satiation with ad lib lunch meal	Orally	Glucose Insulin Ghrelin TAG	Task-fMRI (taste stimuli paradigm)	-6 mm FWHM -FWE at P<0.05	Responses to milkshake after the fixed meal: - ghrelin: amygdala↑, midbrain↑, insula↑, pallidum↑, hippocampus↑. - triglycerides: midbrain ↓, insula ↓,hippocampus ↓, putamen ↓, pallidum ↓
Tatarannai et al. (1999) *	n= 11	- Age: 34 ± 3 - Sex: All M - BMI: not available	- 25 minutes of the meal intake	- Liquid formula meal (1.5 kcal/ml	Orally	Glucose Insulin Leptin FFA	PET	-20 mm FWHM -P<0.005, uncorrected for	After the liquid meal ingestion: - insulin→ OFC↓, insula ↓. - FFA → OFC ↓, insula ↓, DLPFC ↑

		19±6% body fat	- Whole brain	Ensure-Plus: 15% protein, 53% carb & 32% fat)				multiple comparisons	
Wolnerhanssen et al. (2015) *	n= 12	- Age: 24.8 years - Sex: All M - BMI: 22.9	- After 5 minutes of the meal intake - Whole brain	- 300 ml of glucose (75 g) - 300 ml of fructose (25 g) - 300 ml tap water	Nasogastric tube	Insulin Glucose GLP-1	Rs-fMRI	-5 mm FWHM -P< 0.05, uncorrected	After glucose ingestion relative to placebo: - insulin→ caudate ↑, pallidum ↑, OFC ↑
Zhang et al. (2015)	n=40 (20 NW & 20 Obese)	- Age: 20-28 - Sex: All M - BMI: 21.5 ± 1.4 (NW), 33.6 ± 3.5 (Obese)	- Immediately - ROIs (dACC and precuneus)	- Liquid formula meal (1.5 kcal/ml Ensure-Plus: 15% protein, 53% carb & 32% fat)	Orally	Glucose Insulin	rs-fMRI	-8 mm FWHM -P< 0.05, Monte Carlo corrected	After the liquid meal: - insulin → dACC ↓
		Exogenously administered regulators studies							
Batterham et al. (2007) *	n=8	- Age: 29.6 ± 2.1 - Sex: All M - BMI: 21.7 ± 0.7	- Immediately - Whole brain + ROIs (solitary	- PYY infusion - Placebo (saline) infusion Immediately	Intravenous	Glucose Insulin PYY Ghrelin	Physiological fMRI	-4 mm FWHM -Uncorrected for ghrelin effect & P<0.05 cluster-level corrected	After PYY infusion: • ghrelin: hypothalamus ↑, VTA ↑ & brainstem ↑ • PYY: globus pallidus ↑, middle frontal gyrus ↑, anterior lobe cerebellum ↑, anterior cingulate ↑,

			nucleus and tract, parabrachial nucleus, substantia nigra, nucleus accumbens & hypothalamus)	after infusion				for other hormones effect	inferior parietal lobule ↑, medial superior frontal gyrus ↑, substantia nigra ↑, OFC ↑, peri- aqueductal grey ↑, VTA ↑, precentral gyrus ↑, parabrachial nucleus ↑, insula ↑, putamen ↑, hypothal- amus ↑, superior tem- poral gyrus ↑, middle frontal gyrus ↓, angular gyrus ↓
De Silva et al. (2011) *	n=16	- Age: 29.5 - Sex: 11 M & 5 F - BMI: 22.1	- After 90 minutes of the infusion - ROI (bilaterally amygdala insula, OFC, nucleus accumbens, caudate & putamen)	- -Saline infusion - -Standard breakfast (579 kcal) and then saline infusion, - -0.8 pmol/kg/min of GLP-17-36 amide - 0.3 pmol/kg/min of PYY 3-36	- Orally for the breakfast - Intravenous for hormones	GLP-1 PYY	fMRI- food picture paradigm	-Smoothing level is not mentioned -P<0.05, cluster-level corrected	In response to fed saline state: - PYY infusion → OFC ↓, nucleus accumbens ↓ - GLP-1 infusion→ insula ↓

				- -Combined PYY3-36 & GLP-17-36 amide & (0.3 pmol/kg/min & 0.8 pmol/kg/min respectively)					
Jones et al. (2012) *	n= 20	- Age: 34.1 - Sex:7 M & 5 F - BMI: 25.1	- Immediately - Whole brain	- Fasting state: 1. 1.25 or 5 pmol/kg/min of ghrelin injection 2. intragastric lipid (dodecanoate, C12) + ghrelin - Postprandial state: 1. 0.3 mmol/kg of ghrelin bolus 2. saline	Intravenous	Ghrelin	Physiological fMRI	-Smoothing level is not mentioned -FWE whole- brain correction of P<0.05	Ghrelin (pre-prandial state): - thalamus ↑, hypothalamus (upper) ↑, midbrain, cerebellum↑, medulla and pons regions of the brainstem↑, parahippocampal gyrus (amygdala/ hippocampus) ↑, insula↑, precentral gyrus ↑, postcentral gyrus ↑ Ghrelin (post-prandial state): - thalamus (ventral anterior nucleus) ↓, parahippocampal gyrus (amygdala/ hippocampus) ↓, insula↓, hypothalamus (upper)↓, midbrain and pons regions of the brainstem↓, medulla ↓, postcentral gyrus ↓, cerebellum↓ - precentral gyrus & motor cortex ↑

Lassman et al. (2010) *	n=19	- Age: 37 - Sex:13 M & 6 F - BMI: 25.4	- Immediately - Whole brain	- 250 ml of lipid dodecanoic acid - 250 ml of saline (0.9% control) or - oral CCK receptor anatagonist dexloxiglumide (600 mg), administrated orally 1 hour before the intragastric infusion	Intravenous	CCK	Physiological fMRI	-Smoothing level is not mentioned -Uncorrected P<0.005	- CCK: hypothalamus ↓, medulla ↓, midbrain ↓, precuneus ↓, cerebellum ↓, cingulate gyrus ↓, caudate ↓, thalamus ↓, temporal gyrus ↓
Malik et al. (2008) *	n=21	- Age: 24.1 ± 1.1 - Sex: All M - BMI: 22.3 ± 0.7	- Immediately - Whole brain	- Ghrelin infusion - Placebo (saline) infusion. Two ghrelin infusion (0.5 mg/kg for each time	Intravenous	Insulin Glucose	Task-fMRI (food picture paradigm)	-6 mm FWHM -P< 0.001 uncorrected	• After ghrelin infusion → hippocampus ↑, amygdala ↑, OFC ↑, caudate ↑, pulvinar ↑, VTA ↑, substantia nigra ↑, insula ↑, occipital gyrus↑, fusiform ↑

				for 20 minutes)					
Page et al. (2009) *	n= 9	- Age: 28 ± 5 - Sex: 8 M & 1 F - BMI: 23.6 ± 2	- 30 minutes at the start of hypoglycemic session. - 90 minutes during the session of euglycemic. - Immediately after infusion - Whole brain+ ROI (hypothalamus)	- Euglycemia (2 mU/kg/min of insulin+ 20 % glucose adjusted to achieve euglycemia (plasma glucose= 95 mg/dL) - Hypoglycemia (plasma glucose= 50 mg/dL)	Intravenous	Glucose Insulin	fMRI-ASL	-Smoothing and threshold levels are not mentioned	• Hypoglycemia relative to euglycemia (hypoglycemia > euglycemia): - hypothalamus↑, inferior frontal gyrus ↑, ACC ↑, caudate↑, pars triangularis L ↑, superior temporal gyrus ↑, visual association cortex ↑, putamen ↑, pars opercularis ↓, medial frontal gyrus↓, cerebellum↓
Page et al. (2011) *	n= 21	- Age: 31.4 ± 7.9 - Sex: 12 M & 9 F - BMI: 25.2±4	- Immediately - Whole brain	- Euglycemia (2 mU/kg/min of insulin+ 20 % glucose adjusted to achieve	Intravenous	Insulin Ghrelin Leptin	Task-fMRI- (food picture paradigm)	-6 mm FWHM -FWE whole-brain correction of P<0.05	• Euglycemia relative to hypoglycemia (euglycemia > hypoglycemia): anterior cingulate cortex↑, ventromedial- prefrontal cortex ↑

				euglycemia (plasma glucose= 95 mg/dL) - Hypoglycemia (plasma glucose= 50 mg/dL)					
Goldstone et al. (2014) *	n= 22	- Age: 25.9 ± 1.7 - Sex: 17 M & 5 F - BMI: 23.9 ± 0.6	- After 95 minutes of the meal intake - Whole brain+ ROI (nucleus accumbens, caudate, anterior insula, amygdala, hippocampus, OFC)	- Fasted-saline injection - Fed saline with standard breakfast (730 kcal, 55% CHO, 31% fat & 14% protein) - - Fed ghrelin injection with standard breakfast (730 kcal, 55% CHO, 31% fat &	Intravenous	Glucose Insulin GLP-1 PYY Ghrelin triglycerides	Task-fMRI (food picture paradigm)	-6-mm FWHM -FDR at P<0.05	• Ghrelin → OFC ↑, hippocampus↑

				14% protein)					
Little et al. (2014) *	n=12	- Age: 38 ± 3.4 - Sex: 7 M & 5 F - BMI: 19.7–28.9	- One hour of the meal intake - Whole brain	- 250 ml of glucose (45g) following 2 placebo tablets - 250 ml of glucose (45g) following 600 mg of CCK1 receptor antagonist (dexloxiglumid) - 250 ml of saline (0.9%, control) following 2 placebo tablets	Intravenous & intragastric infusion	Glucose Insulin CCK GLP-1	Physiological fMRI	-4 mm FWHM -FDR at P<0.05	- Glucose vs saline: - glucose: hypothalamus↓, brainstem ↓, medulla↓, pons↓, cerebellum↓ cerebellum anterior ↓, lingual ↓, fusiform↓, thalamus↓ - insulin: hypothalamus↓, brainstem ↓, medulla↓, pons↓, cerebellum↓ cerebellum anterior ↓, lingual ↓, fusiform↓, thalamus↓. - Glucose + dexloxiglumide vs saline: - insulin: cerebellum↓, lingual gyrus ↓, cuneus ↓ - GLP-1: cerebellum ↓, lingual gyrus ↓, cuneus ↓

Schilling et al. (2014) *	n=48	- Age: 23.96 ± 3.4 - Sex: All M - BMI: 20 < BMI < 25	- After 30 minutes of infusion - Whole brain + ROIs (hippocampus, insula, putamen)	- Oral cortisol vs. intranasal insulin - Oral cortisol vs. oral placebo - Oral vs. intranasal placebo. - Intranasal insulin vs. intranasal placebo - Insulin (100 I.E. /ml) & cortisol (30 mg)	Intravenous	GLP-1 Insulin	fMRI-ASL	-12mm FWHM -P= 0.05 corrected for multiple comparisons	• Intranasal insulin infusion → putamen ↑, insula↑, inferior frontal gyrus↑, caudate nucleus↑
van Bloemendaal et al. (2014)	n= 32 (16 NW & 16 Obese)	- Age: 57.8 ± 1.9 (NW), 58 ± 2.1 (Obese) - Sex: 8 M & 8 F for each group - BMI: 23.2 ± 0.4 (NW), 32.6 ± 0.7 (Obese)	- Immediately - ROIs (insula, striatum, amygdala, and OFC)	1. Exenatide, 2. Exenatide together with the GLP-1 receptor antagonist exendin 9-39, or	Intravenous	GLP-1 Insulin	Task-fMRI (food picture paradigm)	-8 mm FWHM -FWE at P<0.05 corrected for multiple comparisons	• Obese healthy: exenatide vs placebo → amygdala ↓, insula ↓ & OFC ↓.

				3. Placebo (Fig. 1A). The participants were blinded to the type of infusions.					

* Indicates included studies in the Activation Likelihood Estimation meta-analysis

ACC, anterior cingulate cortex; ASL, arterial spin labelling; BMI, body mass index; CBF, cerebral blood flow; CCK, cholecystokinin; DLPFC, Dorsolateral prefrontal cortex; Dex, dexloxiglumide; FDR, false discovery rate; FFA, free fatty acids; fMRI, functional magnetic resonance imaging; F, female; FEW, family wise erroe; FWHM, full width at half maximum; GLP-1, Glucagon-like peptide-1; M, male; NGT, nasogastric tube, OFC, orbitofrontal cortex; PET, position emission tomography; PYY, peptide YY; rs-fMRI, resting state fMRI; rCBF, regional cerebral blood flow; ROI, region of interest; SMA, supplementary motor area; VTA, ventral tegmental area.

Table 4.4. Cochrane risk of bias assessment of the included studies in the systematic review.

Author., (year)	Appropriatecross-over design †	Randomized treatment order	Carry-over effect †	Unbiased data †	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other bias
Al-Zubaidi et al. (2019) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Batterham et al. (2007) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
De Silva et al. (2011) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Dorton et al. (2017) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Eldeghaidy et al. (2016) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Low	Low	Low
Gautier et al. (2000) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Low	Low	Low

Heni et al. (2015) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Unclear	Low	Low
Jakobsdottir et al. (2012) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Low	Low	Low
Jones et al. (2012) *	Unclear	Unclear	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Kroemer et al. (2013) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Unclear	Low	Low
Lassman et al. (2010) *	Unclear	Unclear	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Lennerz et al. (2013)	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Low	Low	Low
Li et al. (2012) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Little et al. (2014) *	Unclear	Low	Unclear	Low	Unclear	Unclear	Unclear	Low	Low	Low
Liu et al. (2000) *	Low	Unclear	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Malik et al. (2008) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Low	Low	Low
Page et al. (2009) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low

Page et al. (2011) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Unclear	Low	Low
Page et al. (2013) *	Low	Low	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Pannacciulli et al. (2007) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Unclear	Low	Low
Schilling et al. (2014) *	Unclear	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Spetter et al. (2014) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Low	Low	Low
Sun et al. (2014) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low
Tataranni et al. (1999) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Unclear	Low	Low
van Bloemendaal et al. (2014)	Low	Unclear	Low	Low	Unclear	Low	Unclear	Low	Low	Low
Wolnerhanssen et al. (2015) *	Low	Unclear	Low	Low	Unclear	Unclear	Unclear	Low	Low	Low
Zhang et al. (2015) **	NA	Unclear	NA	NA	Unclear	Unclear	Unclear	Low	Low	Low

ǂDomains specific only for cross-over study design. *Cross-over trials. **Controlled trials. NA; not applicable.

4.4.2 Modulation of brain responses to appetite and satiety regulators

As a first step, the data extracted from the 31 studies were grouped into brain areas that associated: 1) positively and/or 2) negatively with appetite regulators, 3) associated positively and/or 4) negatively with satiety regulators. Data from brain areas from each of the sub-groups were then pooled and common brain areas across studies evaluated. Changes in brain responses from the baseline were not shown in this review as it was not reported of the authors of the studies included. A full list of overlapped brain areas is reported in Table 4.5. Figure 4.2 illustrates the concurrence of key brain areas commonly reported across studies based on the findings from the systematic review for NW adults.

4.4.2.1 Appetite regulators

In NW adults, eight studies reported positive association of ghrelin with concurrence in brain activation (Batterham et al., 2007, Malik et al., 2008, De Silva et al., 2011, Jones et al., 2012b, Li et al., 2012, Kroemer et al., 2013, Goldstone et al., 2014, Sun et al., 2014) mostly found in the amygdala (five studies) (Malik et al., 2008, Jones et al., 2012b, Li et al., 2012, Kroemer et al., 2013, Sun et al., 2014), OFC (five studies) (Malik et al., 2008, De Silva et al., 2011, Li et al., 2012, Goldstone et al., 2014, Sun et al., 2014), insula (four studies) (Malik et al., 2008, Jones et al., 2012b, Li et al., 2012, Sun et al., 2014), and hippocampus (four studies) (Malik et al., 2008, De Silva et al., 2011, Jones et al., 2012b, Goldstone et al., 2014), Figure 4.2A. A single study reported negative association with ghrelin concentrations in the caudate nucleus, hypothalamus, insula, amygdala, hippocampus, and thalamus (Jones et al., 2012b).

Regarding Obese adults, no study examined brain areas associated by appetite regulators in Obese adults.

4.4.2.2 Satiety regulators

In terms of satiety regulation in NW adults, eight studies reported positive correlation in response to satiety regulators with concurrence in the ACC (three studies) (Batterham et al., 2007, Page et al., 2009, Page et al., 2011) and putamen (three studies) (Batterham et al., 2007, Page et al., 2009, Schilling et al., 2014),

Figure 2.2B. Fifteen studies reported attenuation in activity in various brain areas (Tataranni et al., 1999, Gautier et al., 2000, Liu et al., 2000, Page et al., 2009, Lassman et al., 2010, De Silva et al., 2011, Jakobsdottir et al., 2012, Li et al., 2012, Page et al., 2013, Schilling et al., 2014, Spetter et al., 2014, Sun et al., 2014, Heni et al., 2015, Eldeghaidy et al., 2016, Al-Zubaidi et al., 2019). Most studies showed concurrence in the insula (eight studies) (De Silva et al., 2011, Jakobsdottir et al., 2012, Li et al., 2012, Schilling et al., 2014, Spetter et al., 2014, Sun et al., 2014, Eldeghaidy et al., 2016, Al-Zubaidi et al., 2019), hypothalamus (five studies) (Liu et al., 2000, Page et al., 2009, Lassman et al., 2010, Page et al., 2013, Spetter et al., 2014), OFC (four studies) (Gautier et al., 2000, De Silva et al., 2011, Li et al., 2012, Heni et al., 2015), thalamus (four studies) (Gautier et al., 2000, Lassman et al., 2010, Li et al., 2012, Eldeghaidy et al., 2016), putamen (four studies) (Gautier et al., 2000, Page et al., 2013, Spetter et al., 2014, Sun et al., 2014), and caudate nucleus (three studies) (Lassman et al., 2010, Li et al., 2012, Page et al., 2013), Figure 4.2C.

In Obese adults, one study out of the four studies reported positive correlation in response to satiety regulators which was correlated with the nucleus accumbens (Lennerz et al., 2013). Two studies reported negative association in response to satiety regulators in the OFC (Gautier et al., 2000, van Bloemendaal et al., 2014). A single study reported negative correlation in the insula and amygdala (van Bloemendaal et al., 2014), ACC (Zhang et al., 2015) and hippocampus, precuneus, putamen and thalamus (Gautier et al., 2000).

Table 4.5. List of reported brain areas in the systematic review. This list included brain areas correlated with endogenously released or exogenously administered appetite or satiety regulators.

Brain areas	**Number of studies (percentage)**	**Reference**
Brain areas correlated positively with appetite regulators (8 studies)		
Amygdala	5 (62.5%)	Malik et al. (2008); Jones et al. (2012); Kroemer et al. (2013); Li et al. (2012); Sun et al. (2014)

OFC	5 (62.5%)	Malik et al. (2008); De Silva et al. (2011); Li et al. (23); Goldstone et al. (2014); Sun et al. (2014)
Insula	4 (50%)	Malik et al. (2008); Jones et al. (2012); Li et al. (2012); Sun et al. (2014)
Hippocampus	4 (50%)	Malik et al. (2008); De Silva et al. (2011); Jones et al. (2012); Goldstone et al. (2014)
Palldium	3 (37.5%)	Kroemer et al. (2013); Li et al. (2012); Sun et al. (2014)
Midbrain	3 (37.5%)	Jones et al. (2012); Kroemer et al. (2013); Sun et al. (2014)
Hypothalamus	3 (37.5%)	Batterham et al. (2007); Jones et al. (2012); Kroemer et al. (2013)
Fusiform gyrus	2 (25%)	Malik et al. (2008); Kroemer et al. (2013)
Thalamus	2 (25%)	Jones et al. (2012); Kroemer et al. (2013)
Caudate	2 (25%)	Malik et al. (2008); Kroemer et al. (2013)
Brainstem	2 (25%)	Batterham et al. (2007); Jones et al. (2012)
VTA	2 (25%)	Malik et al. (2008); Batterham et al. (2007)
Brain areas correlated positively with satiety regulators (12 studies)		
ACC	3 (25%)	Batterham et al. (2007); Page et al. (2009); Page et al., (2011)
Putamen	3 (25%)	Batterham et al. (14); Page et al. (26); Schilling et al. (29)
Insula	2 (16.6%)	Batterham et al. (2007); Schilling et al. (2014)
Caudate	2 (16.6%)	Page et al. (2009); Schilling et al. (2014)
Hypothalamus	2 (16.6%)	Batterham et al. (2007); Page et al. (2011)
Inferior frontal gyrus	2 (16.6%)	Page et al. (2009); Schilling et al. (2014)
Superior gyrus	2 (16.6%)	Batterham et al. (2007); Page et al. (2009)
Nucleus accumbens	1 (8%)	Lennerz et al. (2013)
OFC	2 (16.6%)	van Bloemendaal et al. (2014); Gautier et al. (2000)

Brain areas negatively correlated with satiety regulators (15 studies)		
Insula	9 (60%)	Al-Zubaidi et al., (2019)*;* De Silva et al., (2011)*;* Eldeghaidy et al., (2016)*;* Jakobsdottir et al., (2012)*;* Li et al., (2012); Schilling et al., (2014); Spetter et al. (2014)*;* Sun et al. (2014); van Bloemendaal et al. (2014)
Hypothalamus	5 (33%)	Lassman et al. (2010); Liu et al. (2000); Page et al. (2009); Spetter et al. (2014)*;* Page et al. (2013)
OFC	4 (26%)	De Silva et al. (2011); Heni et al. (2015); Li et al. (2000)*;* Gautier et al. (2000)
Thalamus	4 (26%)	Eldeghaidy et al. (2016); Lassman et al. (2010); Li et al. (2012*);* Gautier et al. (2000)
Putamen	4 (26%)	Spetter et al. (2014)*;* Page et al. (2013)*;* Sun et al. (2014)*;* Gautier et al. (2000)
Caudate	3 (20%)	Lassman et al. (2010); Li et al. (2000); Page et al., (2013)
Cerebellum	3 (20%)	Eldeghaidy et al. (2016); Lassman et al. (2010); Page et al. (2009)
Temporal gyrus	2 (13%)	Eldeghaidy et al. (2016); Lassman et al. (2010)
ACC	1 (7%)	Zhang et al. (2015)
Amygdala	1 (7%)	van Bloemendaal et al. (2014)
Hippocampus	1 (7%)	Gautier et al. (2000)
Precuneus	1 (7%)	Gautier et al. (2000)

ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; VTA, ventral tegmental area.

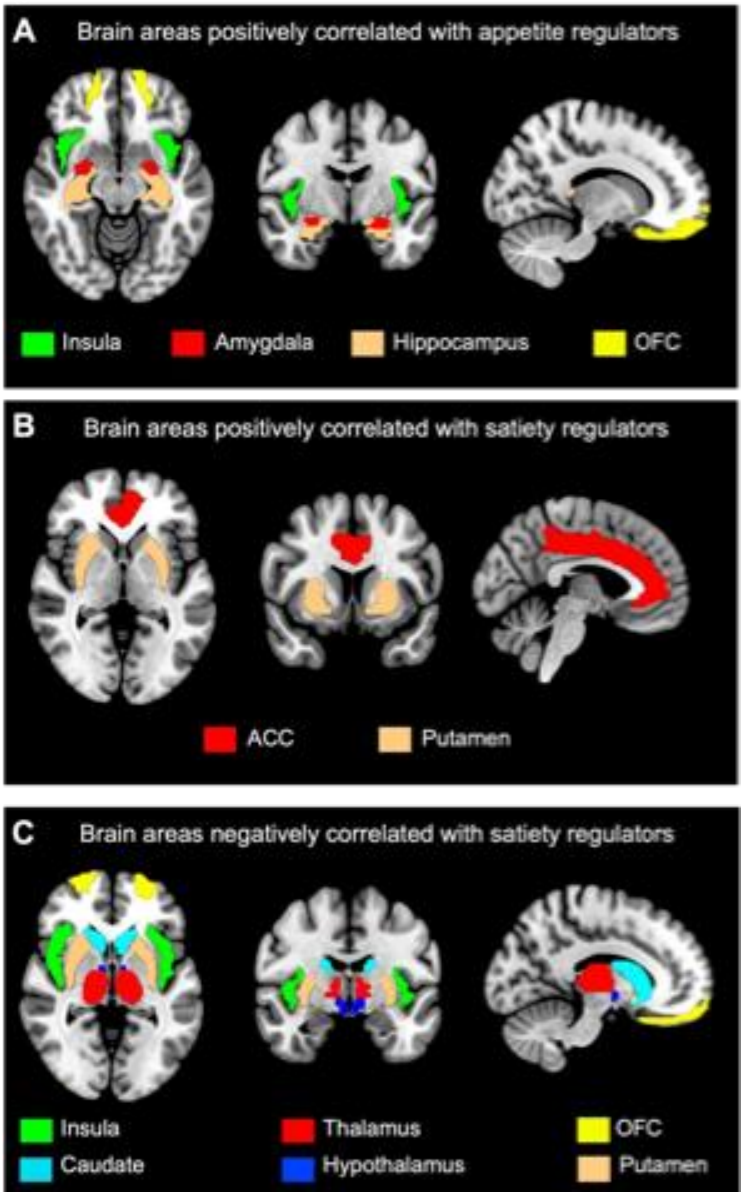


Figure 4.2. Results of the systemic review showing concurrence of key brain areas commonly reported across studies conducted on NW adults. (A) Brain areas positively correlated with appetite regulators, showing the concurrence in the insula, amygdala, hippocampus, and orbitofrontal cortex (OFC). (B) Brain areas positively correlated with satiety regulators showing the concurrence in the anterior cingulate gyrus (ACC) and the putamen. (C) Brain areas negatively correlated with satiety regulators showing the concurrence in the insula, caudate, thalamus, hypothalamus, OFC, and putamen.

4.4.3 Coordinate-based neuroimaging meta-analysis

In a second step, the concurrence/overlap in brain regions activated in response to changes in appetite and satiety regulators in NW adults were examined quantitatively using neuroimaging meta-analysis. In determining the total number of studies required for the coordinate-based meta-analysis in this thesis, the established guidelines for ALE meta-analyses were adhered. The literature recommends a minimum of 17 to 20 experiments to ensure sufficient statistical power and robustness in detecting convergent activation patterns across studies (Muller et al., 2018). After reviewing the available studies, 20 experiments were included in the analysis, aligning with the upper range of this recommendation to maximise the analysis's reliability and validity. Meta-analysis was not conducted for Obese adults because of the low number of studies including this population. Studies were initially grouped following the same methods used for the systemic review: 1) brain areas correlated positively with appetite regulators (4 studies), 2) and/or correlated negatively with appetite regulators (1 study), 3) brain areas correlated positively with satiety regulators (8 studies), 4) and/or correlated negatively with satiety regulators (12 studies). However, due to the small number of studies (less than the 17 required for the ALE analysis), for each of these sub-groups, separate meta-analysis was not performed. Instead, two primary meta-analyses were performed: one for appetite regulators and the other for satiety regulators, each combined across studies reported negative and positive correlation with brain responses.

4.4.3.1 Concurrence of brain area modulated by appetite regulators

Of the five studies eligible for the meta-analysis with appetite regulators, four assessed positive correlation (Malik et al., 2008, Jones et al., 2012b, Kroemer et al., 2013, Goldstone et al., 2014), and a single study assessed negative correlation (Jones et al., 2012b). Due to the low number of investigations, this analysis was not performed.

4.4.3.2 Concurrence of brain area modulated by satiety regulators

In terms of the satiety analysis, the ALE and ABC meta-analyses across 14 independent studies (20 experiments combined across increased/decreased brain activation to satiety regulators) included 212 NW adults and 123 foci provided convergent results, revealing the same cluster (Figures 4.3, and 4.4) in the caudate nucleus. For the ALE-analysis, the caudate cluster was centered at MNI (-10,12,6) and for the ABC analysis at MNI (-12,10,8). Four studies contributed to the caudate cluster in the ALE-analysis, while five studies contributed to the ABC-analysis (Table 4.6). The forest plot of the ABC approach (Figure 4.5) demonstrated that two studies reported positive correlation (increase in caudate activity) while three studies reported negative correlation (decrease in caudate activity) with satiety regulators. In addition, ALE analysis revealed additional cluster in the hypothalamus centered at MNI (2, -4, -12), with five studies contributed to this cluster (Table 4.6, and Figure 4.3).

Table 4.6. Studies and relative foci coordinates in MNI space contributing to the identified clusters in employed meta-analyses on satiety regulators, using the activation likelihood estimation (ALE) method and Analysis of Brain Coordinates (ABC) approach.

ALE	**ABC**	**Coordinates in MNI space**		
		x	y	z
Contributors to cuadate cluster				
Page et al. (2009)	Page et al. (2009)	-10.5	8.2	12.7
Page et al. (2013)	Page et al. (2013)	-10.4	9.9	4.4
Lassman et al. (2010)	Lassman et al. (2010)	-14.1	18.4	5.1
Wolnerhassen et al. (2015)	Wolnerhassen et al. (2015)	-11.3	10.4	10.8
	Little et al. (2014)	-14.1	4.3	4.0
Contributors to hypothalamus cluster				
Lassman et al. (2010)		-9	0.0	-7.7

Batterham et al. (2007)		-6	-11.94	-10.23
Page et al. (2009)		-3	-5.7	-9.8
Pannacciulli et al. (2007)		-4	-4.0	-19.32
Little et al. (2014)		-4	-1.0	-13

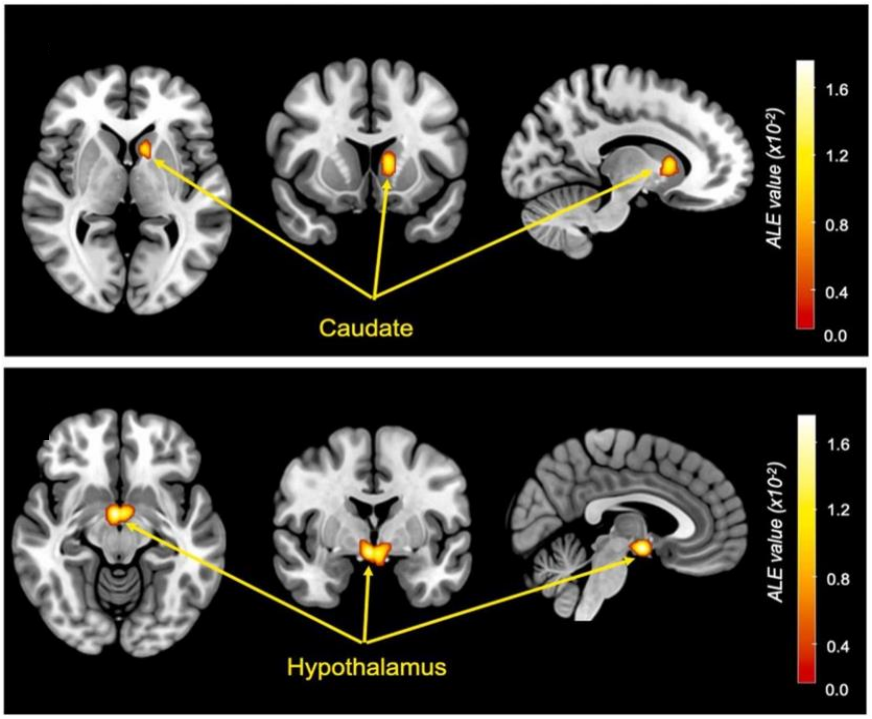


Figure 4.3. Results of the ALE meta-analysis showing convergent clusters with significant ALE values correlated with satiety regulators showing correlation in (A) the caudate nucleus centered at MNI (− 10,12,6), Z = 4.62, ALE value= 1.5 × 10-2, cluster volume= 1000 mm3, and (B) the hypothalamus centered at MNI (2, − 4, − 12), Z = 4.21, ALE value= 1.32 × 10-2, cluster volume= 1728 mm3. Maps are family-wise error (FWE)-corrected for multiple comparisons P < 0.05.

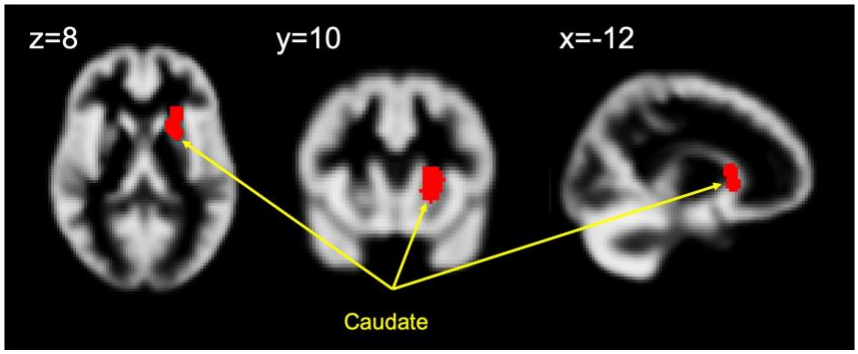


Figure 4.4. Results of the ABC meta-analysis, showing convergent clusters in the caudate nucleus centred at MNI (-12, 10, 8), False Discovery Rate (FDR) corrected for multiple comparisons <0.05.

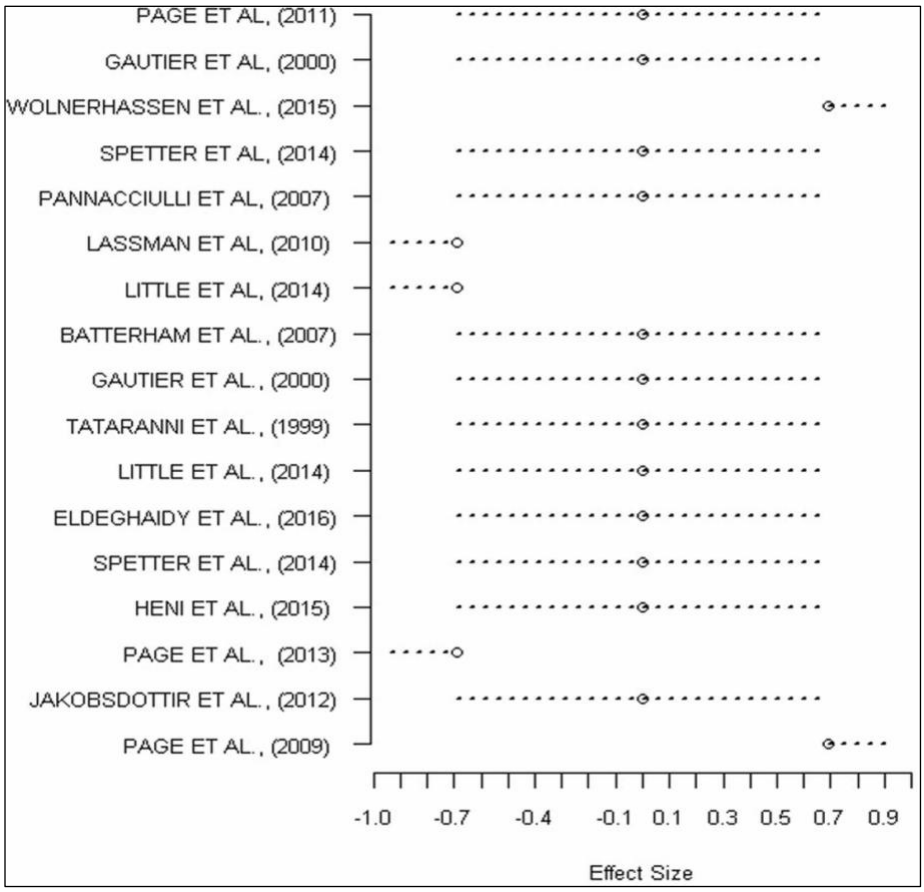


Figure 4.5. The forest plot from the ABC analysis illustrating the effect sign associated with studies contributing to the increased and/or decreased caudate activity in response to satiety regulators.

4.5 Discussion

This systematic review included studies that directly assessed brain activation in response to appetite/satiety regulators endogenously released, following acute food ingestion, and/or exogenously administered regulators in NW and Obese adults. In addition, two different coordinate-based meta-analysis approaches (ALE and ABC) were employed to reveal convergent brain areas of neurohormonal gut-brain signalling to appetite/satiety regulators.

4.5.1 Concurrence of brain area modulated by appetite regulators

The systematic review revealed that the insula was one of the most reported regions across studies in NW adults, with an overlap in 50% of the studies in response to appetite regulators. These results supported the hypothesis and are in agreement with those of the previous systematic review (Zanchi et al., 2017). The insula is an important relay that connects the hypothalamus, OFC, and limbic system. It is often referred to as "ingestive cortex" because it contains primary taste neurons, projecting from the oral cavity (Scott and Plata-Salamán, 1999), as well as primary visceral afferents from the gut (Craig, 2002). The insula encodes multi-modal sensory features of foods (de Araujo et al., 2012), and its activity is modulated by hunger and satiety (de Araujo et al., 2006).

The systemic review also identified the amygdala, and OFC as key brain areas associated with appetite regulators in 62% of the studies in NW adults. The amygdala, and OFC encode motivation value of food cues (Jay et al., 2003). More specifically, the amygdala pass information about sensory cues onto the OFC and has an important role in reward processing (Padoa-Schioppa and Assad, 2008). These results agree with the literature (Zanchi et al., 2017). However, these analyses to assess the overlap quantitively in appetite regulators were not conducted due to the small number of studies identified for the coordinate based meta-analysis. No study examined brain areas associated by appetite regulators in Obese adults.

4.5.2 Concurrence of brain area modulated by satiety regulators

The results from the systematic review and meta-analysis revealed strong concurrence across studies in NW adults and confirms the role of hypothalamus in appetite regulation which support the hypothesis. The hypothalamus is widely recognised as the gatekeeper to control food intake, highly influenced by nutrients, it is physically connected to other areas involved in maintaining homeostatic energy balance and receives projections from the gastrointestinal tract via the brainstem (Blouet and Schwartz, 2010). The results from the systematic review revealed a negative correlation with satiety regulators, which is inconsistent with the previous systematic review that reported associations in opposite directions. These discrepancies might be due to variations in the inclusion criteria. Unlike Zanchi et al. (2017), the work in this chapter did not combine NW and Obese adults or include studies with participants below the age of 18 years. In addition, the current focus was on the neuromodulation of appetite/satiety regulators in response to acute food intake, hence long-duration intervention studies were not included, unlike Zanchi et al. (2017), which may have also led to differences in findings.

The caudate nucleus is associated with perception of food stimuli, reward processing, and cognitive appetite control (Chen and Zeffiro, 2020). The negative correlation of the caudate nucleus with satiety regulators reported in the current review support the study's hypothesis and was confirmed by the ALE and ABC meta-analysis methods. However, it is important to note that the results from the meta-analyses were combined across negatively and positively correlated studies. The relation of caudate nucleus to hunger and satiety is not yet clear, which perhaps explains the findings in this study. While some neuroimaging studies show reduction in caudate activity (Batterham et al., 2007, De Silva et al., 2011) in response to satiety regulators, others show increased activity (Page et al., 2009, Wolnerhanssen et al., 2015). Increases in caudate activity after a meal could reflect top-down attentional control (Balleine et al., 2007), whereas the suppression after meal termination could be due to the dopamine-driven inhibition response (Mehta et al., 2012).

This systematic review found the thalamus to be another key area of the brain involved in correlating satiety regulators in NW adults. Thalamic brain activity has been reported to vary as a function of hunger or satiety (Tataranni et al., 1999), ghrelin application (Higgins et al., 2007) and glucose infusion (Jones et al., 2012b, Little et al., 2014). The thalamus is the gateway to sensory perception, and it plays a major role in integrating proprioceptive information from the gastrointestinal tract (Kelley et al., 2005, Little et al., 2014) through the vagus nerve (Coss-Adame and Rao, 2014). The results from this systematic review demonstrate a negative correlation between thalamic activity with satiety regulators, which may reflect the role of food stimulation in modulating thalamus activity. This could be due to the role of the thalamus in integrating sensory perception (visual and taste cues) or the connection with the vagus nerve which sends information regarding the meal size and physical characteristics. The systematic review also revealed the association of insula with satiety regulators with 53% of the studies reporting decreases in insula activation in response to satiety regulators.

In terms of studies in Obese adults, only four studies were included in this review, and no meta-analysis was performed due to the limited number of the studies. Brain areas including those reported in the NW studies (insula, ACC, OFC, thalamus, putamen, hippocampus, and precuneus) also showed to be associated by satiety regulators. However, due to the limited number of studies and diversity of results, no conclusion can be made in Obese adults regarding the concurrence of brain regions associated by appetite and satiety regulators. Hence, further studies that recruit Obese adults are required to elucidate the altered mechanism of appetite in people with obesity.

4.6 Differences between endogenously released and exogenously administered hormones

The systematic review's findings indicated that certain brain regions were more affected by endogenously generated hormones than by hormones that were given externally. For instance, it demonstrated a greater role of endogenously released

appetite regulators in the amygdala and OFC, and endogenously released satiety regulators in the insula and thalamus modulation. The following statements could be used to explain these observations. The thalamus and insula's involvement are most likely a result of their functions as multimodal food processing regions. The thalamus plays a role in integrating sensory perception (visual and taste cues), which sends information regarding the meal size and physical characteristics (Kelley et al., 2005, Little et al., 2014). The insula is thought of as a multi-modal sensory area encoding food stimulation (de Araujo et al., 2006). The motivation value of food signals is encoded by the amygdala and OFC in relation to their engagement with appetite endogenous regulators ((Gottfried et al., 2003). More specifically, the OFC, which is crucial to reward processing, receives information regarding sensory cues from the amygdala (Padoa-Schioppa and Assad, 2008). These findings could demonstrate that food stimulation has a role in modulating the activity of the above brain regions.

For the exogenously administered hormones, the coordinates meta-analysis shows that exogenously induced satiety hormones modify the hypothalamus and caudate nucleus responses. As previously mentioned, the caudate nucleus is involved in reward processing and cognitive appetite control, while the hypothalamus is thought of as the gatekeeper for controlling food intake (Chen and Zeffiro, 2020). These result demonstrate the effectiveness of exogenously administered appetite/satiety regulators in regulating energy intake in human.

4.7 Strengths and limitations

At the time of writing this thesis, this is the first study to employ functional neuroimaging meta-analysis to quantitatively define the overlap of brain areas associated with satiety regulators across studies. The results from the generated activation maps of the meta-analysis (in our case from a total of 212 participants across the included studies) are more robust than those of any individual imaging study. The generated activation map from the NW adults can be used as a reference or baseline to compare alterations with obesity, or in people with altered eating

behaviour. Another strength is the stringent and well-defined inclusion and exclusion criteria, which enabled an unbiased assessment of the central mechanisms regulating satiety and appetite. The present systematic review provides data and a clear overview of appetite neuroimaging findings in NW adults.

This study has a number of limitations as described below:

- it included a very limited number of studies recruiting adults living with obesity.
- it included relatively small number of appetite/satiety studies in the meta-analysis, a consequence of the strict inclusion selection criteria. This did not allow sub-analyses on appetite/satiety regulators positively/negatively correlated with brain responses to be performed, or to investigate possible differences in neurohormonal gut-brain signalling in response to endogenously released appetite and satiety regulators compared with exogenously infused regulators.
- the small number of studies included in the meta-analysis might be responsible for the absence of other key brain areas related to appetite and satiety regulations including the insula, thalamus and amygdala, OFC and ACC. The location of brain clusters/foci for the OFC and ACC activations varies widely across studies and this might also explain the lack of their concurrence in the analysis.
- Although a strict selection criterion was employed in this study, the presence of individual differences in food preferences is possible.
- There are variations in the imaging modalities (task-based fMRI, resting state fMRI and cerebral blood flow), study designs (endogenously released or exogenous administration), and food-related paradigms/stimulations (i.e., food images or taste stimuli) in the studies included, which might introduce some heterogeneity. While the ideal would be to separate the studies based on stimulation type and/or study design and/or imaging modality and performed separate analyses, this was not possible due to the small number of studies. Despite these variations and drawbacks, the robustness of the

findings is supported by similar results obtained with two different approaches (ALE and ABC), particularly for the caudate convergence. In addition, variations/heterogeneity in the included studies may reduce the sensitivity of the meta-analysis, making it likely to be conservative, rather than causing false positive results/activations.

- Finally, another limitation of this review was that the risk of bias analysis was performed using the Cochrane risk of bias guideline (Higgins et al., 2019). As this tool is not specifically designed for neuroimaging studies, the risk of bias might be inappropriately estimated (Acar et al., 2018).

4.8 Conclusion

This systematic review and quantitative meta-analyses add to the growing body of evidence describing brain areas involved in appetite and satiety processing. Robust evidence was presented from the systematic review and two different coordinate based meta-analysis approaches/methods (ALE and ABC) for the importance of the hypothalamus and caudate nucleus in association with appetite and satiety processing. Although there was a clear correlation between these brain regions and satiety regulators, the results of this study could be affected by additional factors that were not examined, such as psychological disorders like mood disorders or any prior dietary preferences of the participants. No possible conclusion could be made for the adults living with obesity due to the very limited number of studies conducted on these people. Hence, more work is needed to fully elucidate the complex interactions associated with the central regulation of appetite/satiety in adults with normal-weight and those with obesity. This study could help future work to understand the underlying mechanism of appetite regulation in normal-weight participants. Moreover, the results of the meta-analysis can be used for comparison in future studies to define alterations with obesity or altered eating behaviours and develop new treatment strategies. Future research should take advantage of these appetite-regulatory systems pharmacologically which could mimic some of the significant impacts of bariatric surgery.

5 An MRI study to measure the effect of a standard pasta meal on gastrointestinal responses in adults with and without obesity

Advantages in MRI allows exploring the gastrointestinal function, by combining different measurements in a single scan session. The Nottingham GI-MRI research group has developed non-invasive MRI techniques to assess post-prandial GE and SBWC, and SMA blood flow in NW participants. To date, there been no research conducted to assess SBWC and SMA in individuals with obesity after food consumption. This study aims to building on previous work in NW participants to explore for the first-time alterations in GI physiology (GE, SBWC, and SMA) in people with obesity, using advanced MRI techniques.

The work detailed in this chapter was initiated by colleagues in the University of Nottingham Medical School under the direction of Dr Liz Simpson. Approximately 70% of data collection was completed before the author became involved. The author was directly involved in collection of the remaining data and analysis and interpretation of all data is her own work. Data was presented as an oral presentation at the 2023 Annual Meeting of the Surgical Research Society (SRS).

Althubeati, S., Simpson, E.J., Bush, D., Hoad, C., Elgdeghaidy, S., Gowland, P., Macdonald, I.A. and Lobo, D.N., 2023. O032 Gastrointestinal and satiety responses to oral feeding in participants with obesity compared with those of healthy weight: an MRI study. British Journal of Surgery, 110(Supplement_3), pp.znad101-032.

5.1 Introduction

There is conflicting evidence to suggest that obesity is associated with specific changes in the GI tract (Sam et al., 2012). Previous studies have shown that Obese participants have alterations in their GI functions, including altered responses of gut hormones and GE rate (Mora et al., 2005, Meyer-Gerspach et al., 2014). However, other studies showed no differences in GE and gut hormone responses between

Obese and NW participants (Pironi et al., 1993, Carroll et al., 2007, Flint et al., 2007, Yang et al., 2009).

In addition to the above factors, as explained in section 2.4, SBWC and SMA blood flow influence the process of food regulation and might have an impact on how people with obesity regulate food intake. Previous studies have reported that SBWC decreased after both liquid and solid meal intake (Marciani et al., 2010, Marciani et al., 2012) and have showed an increased SMA blood flow after consuming liquid meals in adults with a NW (Moneta et al., 1988, Sieber et al., 1989). Measuring SBWC and SMA in people with obesity can provide insights into how the gastrointestinal system functions in individuals with excess body weight. Understanding these dynamics may contribute to a better understanding of the metabolic and digestive alterations that often accompany obesity.

5.2 Aims and hypothesis

Primary aim

To compare the effect of ingestion of a mixed meal on GE rate between the NW and Obese.

Secondary aim

To compare the effect of ingestion of a mixed meal on GCV, SMA blood flow, SBWC, subjective satiety measured by the VAS, plasma hormone concentrations (insulin, PYY, ghrelin, GLP-1, and GLP-2), blood glucose, triglycerides and FFAs between NW and Obese.

Hypothesis

Obese participants will have lower GCV and faster GE rate, as well as lower SBWC, SMA blood flow, postprandial hormone concentrations, and satiety feelings following the ingestion of a mixed meal compared with NW adults.

5.3 Methods

5.3.1 Study design and ethics

This observational, unblinded study was carried out at Sir Peter Mansfield Imaging Centre (SPMIC) at the University of Nottingham. Ethical approval for the study was obtained from the Medical School Research Ethics Committee at the University of Nottingham, reference A16042015. Registered at https://ClinicalTrials.gov refence NCT03860623.

5.3.2 Eligibility criteria

5.3.2.1 Inclusion criteria

Healthy participants with no history of gastrointestinal motility disorders (e.g., irritable bowel syndrome, gastroesophageal reflux disease, gastroparesis, sphincter of Oddi dysfunction, etc.), diabetes, or previous thoracic or abdominal surgery and were non-smokers were recruited. Participants aged 18–60 years old with a BMI of normal-body weight (BMI 18.5-24.9 kg/m2) or Obese (BMI 30–40 kg/m2) who self-reported stable weight in the previous 3 months. Posters on University Campuses, local community spaces (post offices, libraries, community boards at supermarkets), and local social media platforms (Facebook) were used to advertise the study and recruit participants.

5.3.2.2 Exclusion criteria

Those with acute illness in the preceding 6 weeks, on regular medication, with a history of substance abuse or having factors precluding safe MRI were excluded. Additional exclusion criteria included a history of gastrointestinal motility disorders (e.g. gastroesophageal reflux disease irritable bowel syndrome, gastroparesis, sphincter of Oddi dysfunction, etc.), previous thoracic or abdominal surgery.

5.3.3 Sample size calculation

No previous data were available for GE data after consumption of a solid meal in Obese to inform sample size requirements for this study. Therefore, data from a previous study conducted by Marciani et al. (2013a) on 12 NW participants, who consumed a mixed solid/liquid 542 kcal meal with half emptying time of gastric

content (T_{50}) of 132 ± 26 minutes (mean ± SD), was used to calculate sample size for this study. Employing this in the sample size calculation, 11 participants in each independent group would give the 90% statistical power (α= 0.05) to detect a 29% difference in T_{50} in the Obese group.

5.3.4 Study protocol

Participants were invited to attend 2 visits: a screening visit at the David Greenfield Physiology Unit (DGPU) and a single MRI study visit at the SPMIC as described below. Participants were asked to consume a standard diet for the 3 days immediately before the MRI study visit. This normalised pre-study diet, which was based on foods in their normal diet, was designed to provide 15% of total daily energy intake as protein, 35% as fat and 50% as carbohydrate, calculated from their estimated energy requirements. Participants were also asked to refrain from nicotine, caffeine, and alcohol from 6 pm prior to the study day, and to fast overnight.

Screening visit

Healthy volunteers, who had expressed their interest in the study, were invited to attend a screening visit. If they met the eligibility criteria and were willing to participate, participants signed a consent form, and completed a health questionnaire, an MRI safety questionnaire, and an abnormal scan form. Participants were then asked to come at the DGPU for blood samples to measure their fasting glucose levels, urea and electrolytes, liver function, thyroid function, and full blood count. Additionally, their weight, height, and blood pressure were recorded. Participants were then asked to fill out 4-days food intake diary using household measures. Dietary data were then analysed using the Nutritics software (https://www.nutritics.com/en/).

MRI visit

The GI-MRI protocol used in this study was based on protocol previously developed and used by our GI MRI research group (Hoad et al., 2007, Totman et al., 2009, Chowdhury et al., 2016). On the study day, participants reported at SPMIC at 8:00 and were cannulated by a trained research nurse, using venous cannula retrograde

into a dorsal foot vein, for blood sampling. Baseline measurements (T0) of MRI scans (GCV, SBWC, and SMA blood flows), blood samples (PYY, GLP-1, GLP-2, ghrelin, glucose, and insulin) and satiety VAS questionnaires were collected. Participants were then given a test meal (~500 g portion) with 250 ml tap water, and they were asked to consume the meal within 15 minutes in an upright, seated position outside the scanner. Following ingestion of the test meal (T45), participants were scanned at 30 minutes intervals for 4 hours to assess GCV, SBWC, and SMA blood flow. Satiety VAS questionnaires were collected immediately after eating the pasta meal (T45), and then every 60 minutes (at T105, T165, T225, T285 and T345) throughout the experiment (five hours). During the MRI session, a 0.9% saline infusion was used to maintain the patency of the cannulation line, and the foot with the cannula was placed in a warming box compatible with MRI (air temperature 50-55oC). The warming box was used to collect arterialised-venous blood sampling which is important in measuring blood glucose concentrations. Arterialized venous blood is consisted of pure arterial blood mixed with a particular amount of idealised venous blood (Collis and Neaverson, 1967). Monitoring the arterial blood glucose concentration is necessary to precisely study how glycaemic change affects physiological responses and hormone concentrations (Liu et al., 1992). In addition, sampling from arterialized blood could obtain higher probability of identifying small variations in postprandial GLP-1 availability with interventions (Chen et al., 2018).

5.3.5 Test meal

The test meal was comprised of white pasta, tomato-based pasta sauce, olive oil and cheddar cheese (Astbury et al., 2011, Astbury et al., 2014). Appendix 10.2.3 gives a full description of the pasta meal preparation. The energy and macronutrient composition of the meal is shown in Table 5.1.

Table 5.1. Nutrient composition of the pasta meal.

Nutrient	**Nutrition information per 100 g**
Energy (kcal)	145

Carbohydrate (g)	19.3
Total sugar (g)	2.9
Fat (g)	5.1
Protein (g)	5.7
Fibre (g)	1.7

5.3.6 Blood samples and metabolic assays

Blood samples were drawn via a three-way tap; the first 2 ml were discarded to remove saline from the sampling line. Blood was drawn at baseline (T0), and then at 15 minutes (T60) and 30 minutes (T75) following the ingestion of the test meal. After that, every 30 minutes until 5 hours postprandial (T315). Blood samples were processed as soon as possible after collection (unless stated). Processing of samples were carried out as described below.

- Blood glucose: analysed immediately after drawing using YSI 2300 STATplus (Yellow Springs Instruments) and the remaining blood was dispensed.
- Ghrelin and PYY: 2 ml of blood were added to ethylenediaminetetraacetic acid (EDTA) (purple top) vacutainer™ tubes containing 100μL of Trasylol (aprotinin) and then centrifuged at 3000 g and 4°C for 10 minutes.
- GLP-1: 1 ml of blood was added to EDTA purple vacutainer™ tubes containing 10 μL of dipeptidyl peptidase-4 and then centrifuged at 8000 g and room temperature for 2 minutes.
- Insulin, triglycerides, and GLP-2: 5 ml of blood were added to (gold top) SST™ Vaccutainer™ tubes at baseline and 3 ml were used for subsequent analysis. Samples were left to clot for 15-20 min and then centrifuged at 3000 g and room temperature for 10 minutes. Triglycerides were only measured at baseline fasting state.

- FFA: 1.5 ml of blood were added to a lithium heparin microtube containing 50 µl of Ethylene Glycol Tetraacetic Acid (EGTA)/glutathione and 10µl tetrahydrolipstatin (THL) preservative and then centrifuged at 8000 g and room temperature for 2 minutes. It was only measured at baseline fasting state.

Plasma was then removed and stored in a -80º C freezer for later analysis of the GLP-1, ghrelin, PYY and FFA. Aliquots of serum were stored in the -80º C freezer for later analysis for GLP-2, triglycerides, and insulin. Analysis was carried out by the research technicians (Sally Cordon) based in the Medical School using commercially kits as descried below.

- Insulin: Invitrogen enzyme-linked immunosorbent assay (catalogue no: ELISA KAQ1251) kits.
- Total PYY Merck ELISA kits (catalogue no: EZHPYYT66)
- Total GLP-2: Merck ELISA kits (catalogue no: EZGLP2-37K)
- Active GLP-1: Merck ELISA kits (catalogue no: EGLP-35K)
- Total ghrelin: Invitrogen ELISA kits (catalogue no: BMS2192)
- FFA: WAKO reagents and plate reader assays
- TAG: Horiba Medical reagents and Pentra-400 analyser

5.3.7 Gut imaging protocol and analysis

MRI scanning was carried out using a 3T scanner (Ingenia Philips, the Netherlands). dStream Anterior Coil was used and placed over the participant's abdomen. The participant's foot was placed in a warming box compatible with MRI to allow for blood sampling. The following scan protocols were used to measure GCV, SBWC, and SMA blood flow.

GCV: was collected using a sequence of coronal Half-Fourier Acquisition Single-shot Turbo spin Echo (HASTE) acquiring two stacks of 14 contiguous axial slices with a slice thickness of 10 mm with no gaps between slices and reconstructed in-plane resolution 0.78 mm × 0.78 mm and SENSE of 2.0. The parameters of the

sequence were: flip angle 90, TR=840 ms, TE=60 ms. Data were acquired during 2 breath-hold of 10 s each.

Data were analysed using Medical Imaging processing, analysis, and visualization software (MIPAV, Center for Information Technology, National Institute of Health, Bethesda, Maryland, U.S, https://mipav.cit.nih.gov/). Gastric content MRI images were analysed using the Analyze7.5 through 28 image slices for each time point. Within each image slice, a volume of interest (VOI) was manually drawn around GCV. The GCV was then calculated from the sum of the volumes measured from each image slice. T_{50} was calculated when GCV dropped to 50% of initial postprandial gastric volume (V_0). The rate of GCV T_{50} was defined as the rate of volume change in ml/min at the calculated T_{50} volume (Parker et al., 2016). T_{50} was calculated using the GE modelling by the fitted data using the following equation $V(t)=V\_0f\ (1+kt/t\text{-}empt)\ \exp(\ (-t)\ /t\text{-}empt)+\ (1-\ f\ )\ (1\text{-}Gt)$ (Parker et al., 2016). All data were included for fitting until the volume was less than 100 ml.V_0 is the GCV at time 0, and *f*, *k*, *tempt*, and *G* are fitted models. The parameters V_0, *t_empt*, k, G and f are all variable parameters that are fitted during the modelling process which will be different for each individual emptying curve. In general, they have the following characteristics for the curve.

- V0 is the volume of the meal at Time 0
- *t_empt* is the exponential decay curve time constant (exp = exponential)
- *k* is the parameter which allows some lag time before the decay and allows for the volume to go up - which means that secretions can also be accounted for in the early phase of the emptying
- *G* is the gradient of the linear component of the decay - which tends to be dominant at the end of emptying
- *f* is the fraction of the decay that behaves like an exponential decay with (1-f) being the fraction that behaves like a linear decay.

SBWC: was acquired as previously described (Hoad et al., 2007, Marciani et al., 2013b) using a sequence of coronal single-shot fast spin echo acquiring two stacks of 14 contiguous coronal slices with slice thickness 7 mm with no gaps between slices, reconstructed in-plane resolution 0.78 mm × 0.78 mm and SENSE of 2.2, flip angle 90, TE=400 ms, TR=1728 ms. The data were acquired during 2 breath holds of 24 s each.

Data were analysed using a previously described and validated technique by Hoad et al., (2007) in the Matlab® platform (The Mathworks Inc, Cambridge, UK, https://uk.mathworks.com/). This technique identifies fluid in the MRI scans based on the intensity of CSF, which was used in the analysis to calculate a threshold value. Free fluids within the small bowel above the threshold value were segmented and measured. The segmented data were saved as part of the analysis and volumes of fluid were measured from the segmented data across the small bowel.

SMA blood flow: was collected using a sequence of phase-contrast MRI angiography measurements with 20 cardiac phases acquiring 14 contiguous coronal slices with slice thickness 6 mm with no gaps between slices, reconstructed in-plane resolution 1.17 mm × 1.17 mm, and SENSE of 3, flip angle 25°, TE (shortest), TR (shortest), and velocity encoding 140 cm/s for baseline and 200 cm/s for other time points. The data were acquired during a breath hold of under 20 s and varied in length due to differences in heart rate between participants, as it depends on the cardiac cycle for each participant. An MRI-compatible peripheral pulse-oximeter unit (PPU) was used to allow cardiac gating for the SMA blood flow scan.

Data were analysed using the SEGMENT software (Medviso AB, Lund, Sweden, https://medviso.com/segment/). A region of interest (ROI) was manually drawn around the superior mesenteric artery when it was best defined (much brighter or darker than the grey background). The software determined the velocity, flow rate, and net volume of blood flow.

5.3.8 Statistical analysis

GraphPad Prism 9.5 for Mac OS X was used to analyse the data and make graphs (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Unless stated,

parametric data are expressed as mean and standard deviation (SD), whereas non-parametric data are expressed as the median and interquartile range (IQR). The normality of the data was tested by the Shapiro-Wilk test. To test for any significance of differences between groups in variables measured at a single time point, an unpaired t-test was used for parametric data and the Mann-Whitney U-test for non-parametric data. Two-way analysis of variance (ANOVA) was used to identify differences between groups for repeated measurements, (NW vs. Obese). If significant differences between the groups were detected by the 2-way ANOVA analysis, these were further probed using the Bonferroni post hoc test. Differences were considered significant at $P<0.05$. The total area under the curve (AUC) for postprandial responses was calculated using the trapezoid rule, which is a numerical integration technique to approximate the integral or the area under a curve (Yeh, 2002). The Trapezoidal Rule divides the whole area into smaller trapezoids in order to evaluate the area under the curves instead of using rectangles. This integration estimates the area by using a trapezium to calculate the area under the curve. The postprandial AUC was calculated by the GraphPad Prism for the area under the reading curve down to fasting baseline values (Shiang, 2004). The peaks that were below the baseline value were also considered in the AUC calculation.

To test correlation between GCV and CSS, Pearson test for parametric data and Spearman test for non-parametric data were used. Additionally, a univariate general linear model (multiple linear regression) was calculated using IBM SPSS Statistics (version 28) to evaluate the variance in the CSS determined by the independent variables [GCV and body weight]. First, correlation between CSS (a dependent variable) and GCV (independent variable) was analysed including all participants. Then, correlation between CSS (a dependent variable), GCV (first independent variable) and body weight (second independent variables).

5.4 Results

5.4.1 Participant's descriptive results

Figure 5.1. illustrates the recruitment process. One hundred sixty-five participants contacted the research team and were assessed for eligibility. Thirty-one participants were screened for the study, of which three were excluded after screening and seven withdrew. Twenty male participants were included in the final analysis for the study, 10 NW and 10 Obese. Table 5.2 summarises the demographic characteristics of the participants.

Some data were missing and were not included for the final study analysis for the following reasons. One of the Obese participants had MRI data available only up to 180 minutes because of technical issues with the Magnet at the start of the study day which doesn't allow for final scans. Also, baseline data for SMA blood flow was not collected because of technical issues, hence the SMA data for this participant was not included when calculating the AUC. Another Obese participant had missing SBWC data for the timepoint of 30 minutes because of technical issues. To resolve this issue, average data was calculated for the data at timepoint 0 minutes and timepoint 60 minutes to estimate the missing value. Breathing data for 4 participants (one NW and 3 Obese) was not of sufficient quality to analyse GCV. To remove this effect, GCV was analysed using SBWC MRI images which is less affected by poor breathing.

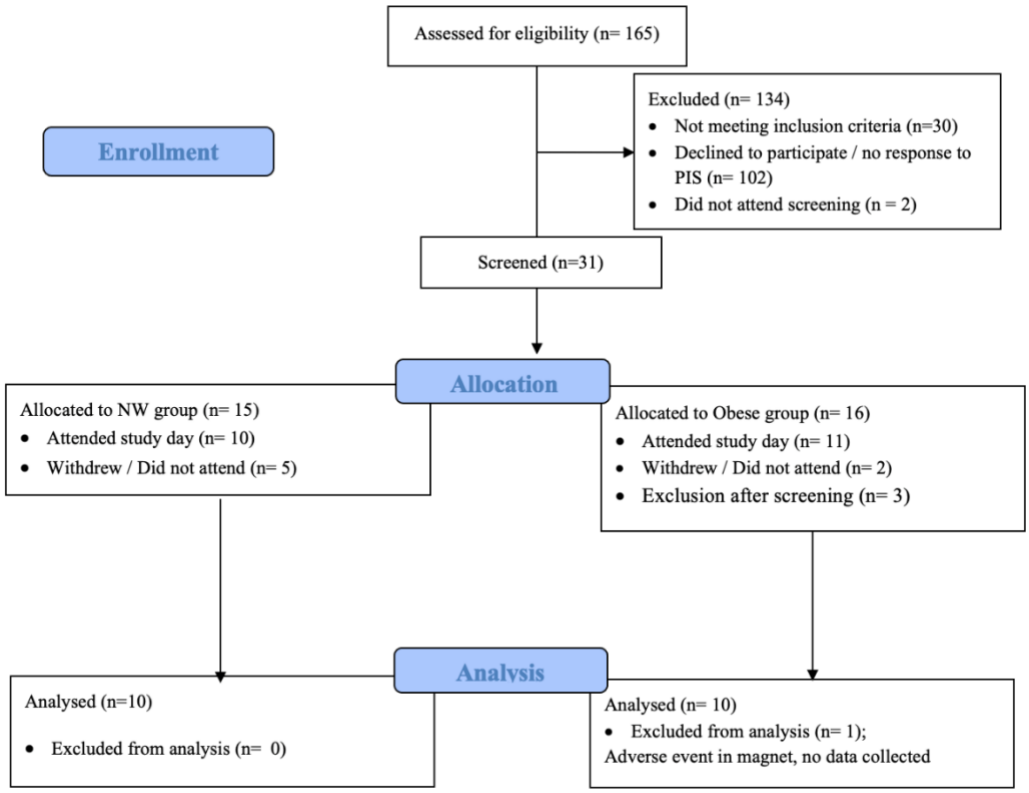


Figure 5.1. Recruitment flow diagram

Table 5.2. Summary demographic characteristics of the participants. Data are presented as the mean ± SD.

	NW group	**Obese group**	**P-value**
Age	37.8 ± 14.2	38.5 ± 13	0.91
Height (cm)	174.7 ± 7.1	176 ± 3.7	0.62
Weight (kg)	72.6 ± 8.2	103.6 ± 7	<0.0001
BMI (kg/m^2)	23.8 ± 1.9	33.6 ± 3.3	<0.0001
Systolic blood pressure (mmHg)	126.7 ± 10.9	134.9 ± 27.3	0.38
Diastolic blood pressure (mmHg)	76 ± 10.2	81 ± 13.4	0.36

5.4.2 Gastric content volume

Examples of stomach MRI images of the GCV in NW and Obese participants at fasting/baseline and after consumption of the test meal are shown in Figures 5.2 and 5.3. The time course values for GCV when fasted and following the pasta meal in each group is shown in Figure 5.4a. There were no significant differences in fasted GCV between the NW and Obese groups (P=0.96, Table 5.3). Gastric volume was significantly increased by eating in both groups (NW: 873% ± 175%, P<0.0001 vs. Obese: 859% ± 156%, P<0.0001). Mean values of GCV peaked at 0 minutes and levels returned to baseline by 240 minutes in both groups (Figure 5.4a). Average T_{50} time were not different between the NW and Obese groups (148 ±16 minutes vs. 170±15 minutes, respectively, P=0.87) with an average T_{50} rate of 1.7 ml/min for the NW group and 1.8 ml/ minute for the Obese group (P=0.89). No significant group × time interactions (P=0.33) or main effect of the group (P=0.26) were found in GCV. A significant main effect of the time was observed (P<0.0001). AUC for postprandial GCV (T0-T275) did not differ between the NW and Obese groups (Table 5.4, P=0.22).

The GCV was correlated with CSS in each group. The results showed a significant positive correlation between GCV and CSS in both groups (CSS: NW: r = 0.65, <0.0001; Obese: r=0.55, P<0.0001; Figures 5.4b). In addition, results from the multiple linear regression model showed that R^2 was equal to 0.30 for relationship between CSS vs. GCV for all participants. However, R^2 improved significantly to 0.44 (P<0.001) for the relationship between CSS vs. GCV vs. body weight. This give indication that 30% of variance in the CSS is explained by GCV and 14% by body weight.

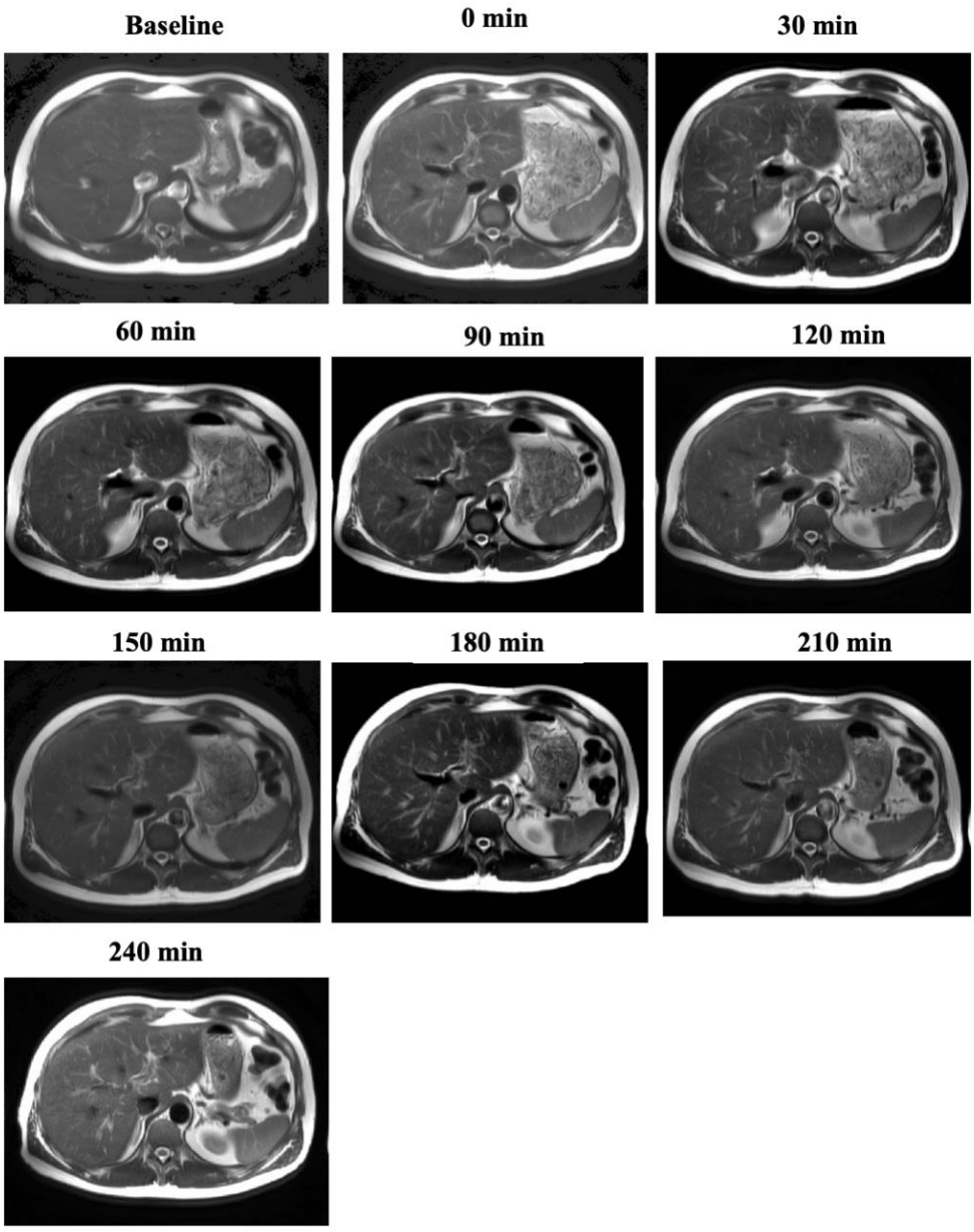


Figure 5.2 . MRI images of stomach of a normal-weight participant at fasting baseline and after consumption of the test meal.

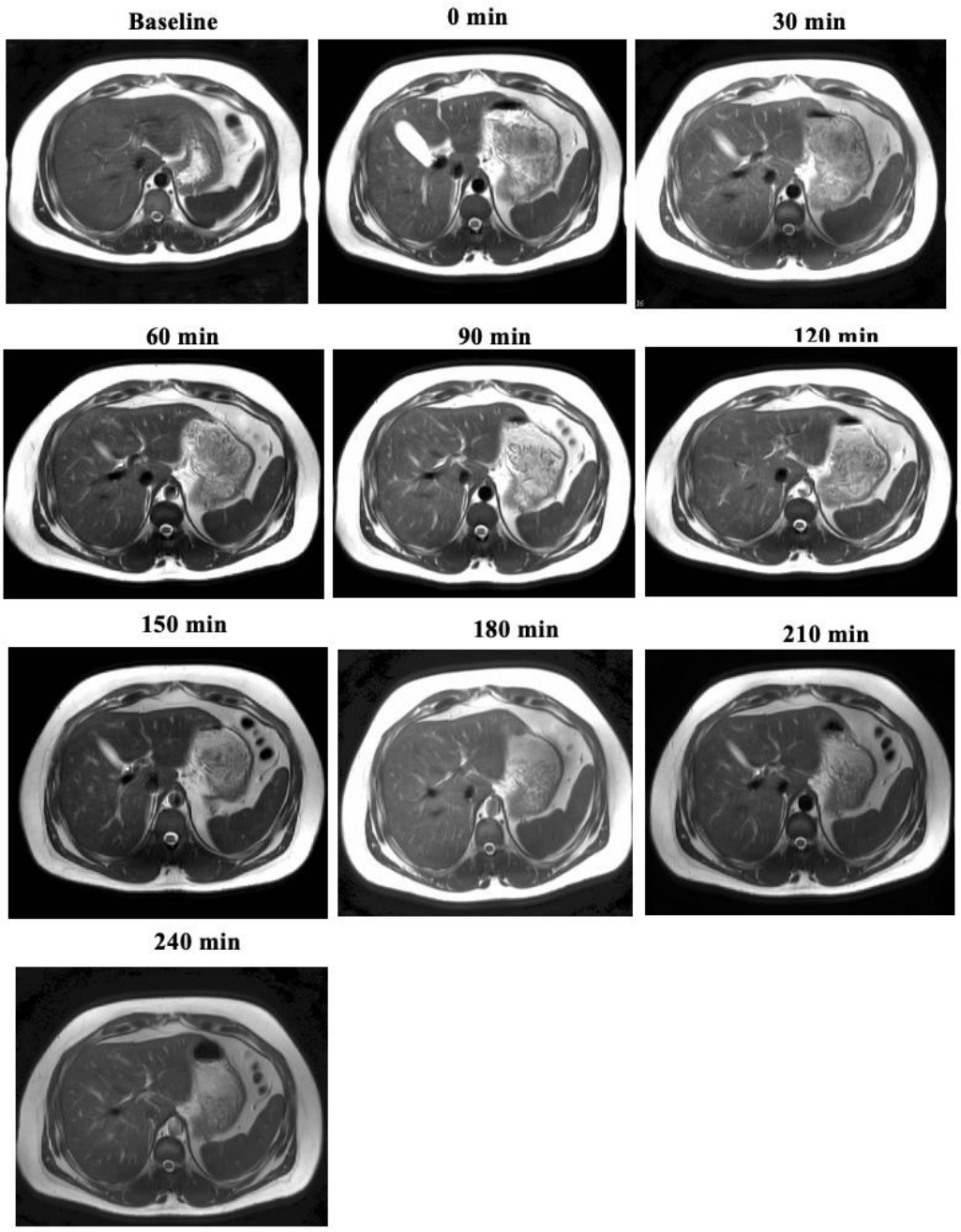


Figure 5.3. MRI images of stomach of a participant with obesity at fasting baseline and after consumption of the test meal.

Table 5.3. Summary of baseline (fasting) characteristics of participants.

	NW group	**Obese group**	**P-value**
MRI measurements			
Fasting GCV (ml) (mean ± SD)	59.2 ± 32.6	58.6 ± 29.5	0.96
Fasting SMA blood flow (ml/s) (mean ± SD)	7.7 ± 2.5	6.5 ± 2.7	0.30
Fasting SBWC (ml) [median (IQR)]	84 (107.3)	118.5 (184.7)	0.35
Satiety VAS scores			
Fasting DTE (mm) (mean ± SD)	57.7 ±29	66.2 ± 14.7	0.20
Fasting hunger (mm) (mean ± SD)	55.9 ± 27.1	64.2 ± 18.8	0.43
Fasting fullness (mm) (mean ± SD)	25.3 ± 26.5	25.6 ± 19.9	0.97
Fasting PFI (mm) (mean ± SD)	60.2 ± 19.2	71.6 ± 16.7	0.17
Fasting CSS (mm) (mean ± SD)	39.1± 22.6	30.9 ± 13.5	0.33
Blood measurements			
Fasting PYY (pg/ml) (mean ± SD)	103 ± 49.6	88 ± 32.4	0.41
Fasting active GLP-1 (pmol/l) (mean ± SD)	7.2 ± 11.5	1.4 ± 2.5	0.15
Fasting GLP-2 (ng/ml) (mean ± SD)	1.82 ± 1	2.10 ± 1.3	0.72
Fasting total ghrelin (pg/ml) (mean ± SD)	3202 ± 1134.6	2857 ± 923.2	0.46
Fasting insulin (mIU/l) (mean ± SD)	12.5 ± 5.8	17.6 ± 11.3	0.21
Fasting blood glucose (mmol/l) (mean ± SD)	4.4 ± 0.9	4.1 ± 0.4	0.41

Fasting triglycerides (mmol/l) (mean ± SD)	0.64 ± 0.17	1.7 ± 0.82	0.0009
Fasting FFAs (mmol/l) (mean ± SD)	0.28 ± 0.16	0.37 ± 0.09	0.11

BMI; body mass index, CSS; combined satiety scores, DTE; desire to eat, FFAs; free fatty acids, GCV; gastric content volume, GLP-1; glucagon-like peptide-1, NW; normal-weight group, PFI; prospective food intake, PYY; peptide YY, SBWC; small bowel water content, SMA; superior mesenteric artery, VAS; visual analogue scale.

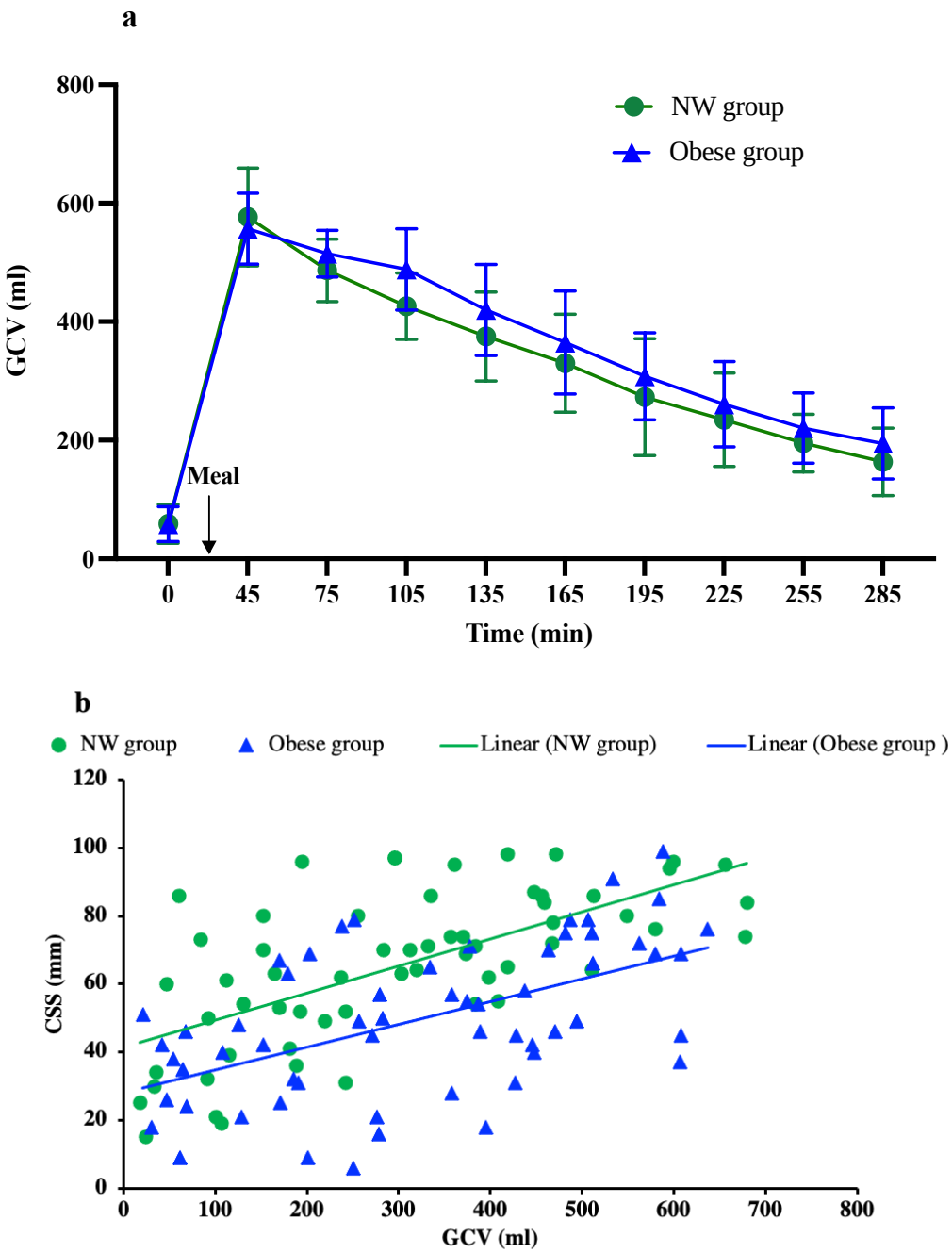


Figure 5.4. (a) The gastric content volume (GCV) over time following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group. (b) Correlation of GCV with composite satiety scores (CSS) in the NW group (r=0.65, R^2=0.43) and the Obese group (r=0.55, R^2=0.30).

Table 5.4. Total area under curve (AUC). Data are presented as the mean± SD.

	NW group	Obese group	P-value
MRI measurements (0-285 min)			
GCV (ml)	2476± 477.8	2734± 444.6	0.22
SMA blood flow (ml/s)	33.3 ± 25.9	50.2 ± 26.9	0.17
SBWC (ml)	772.9± 342.8	927.4 ± 395.6	0.36
Satiety VAS scores (0-345 min)			
PFI (mm)	140± 91.6	99.2± 122.7	0.33
Hunger (mm)	162.1± 79.6	113.8 ± 121.1	0.30
DTE (mm)	155.3± 86.9	122.5± 108.9	0.46
Fullness (mm)	166.1± 112.8	102.7± 120	0.23
CSS (mm)	154.9± 70.9	107.9± 97.3	0.23
Blood measurements (0-315 min)			
Total PYY (pg/ml)	182 ± 314.6	175.4 ± 246.5	0.95
Active GLP-1 (pmol/l)	11.8 ± 80.23	16.2 ± 21.55	0.86
GLP-2 (ng/ml)	2.93 ± 6.8	1.85 ± 7.9	0.75
Total ghrelin (pg/ml)	3375± 7950	2211± 6587	0.73
Insulin (mIU/l)	222.3 ± 104.4	433.2 ± 263.6	0.03
Blood glucose (mmol/l)	6.6 ± 6.8	7.3 ± 4.5	0.79

CSS; combined satiety scores, DTE; desire to eat, GCV; gastric content volume, GLP-1; glucagon-like peptide 1, NW; normal-weight group, PFI; prospective food intake, PYY; peptide tyrosine tyrosine, SBWC; small bowel water content, SMA; superior mesenteric artery, VAS; visual analogue scale.

5.4.3 Superior mesenteric artery blood flow

There were no differences in fasted SMA blood flow between NW and Obese groups (P= 0.30, Table 5.3). The flow in SMA increased significantly by eating in both groups (NW: 121%± 34%; P<0.0001 vs. Obese: 136% % ± 36%, P<0.0001) reaching a peak at 0 minutes for the NW group and 30 minutes for Obese group with no difference between them (P=0.71, Figure 5.6). SMA blood flow dropped to baseline levels by 210 minutes in both groups (Figure 5.5). No significant group × time interactions (P=0.23) or main effect of the group (P=0.70) was shown. A significant main effect of the time was observed (P<0.0001). Postprandial AUC (T0-T275) did not differ between the groups (P=0.17, Table 5.4).

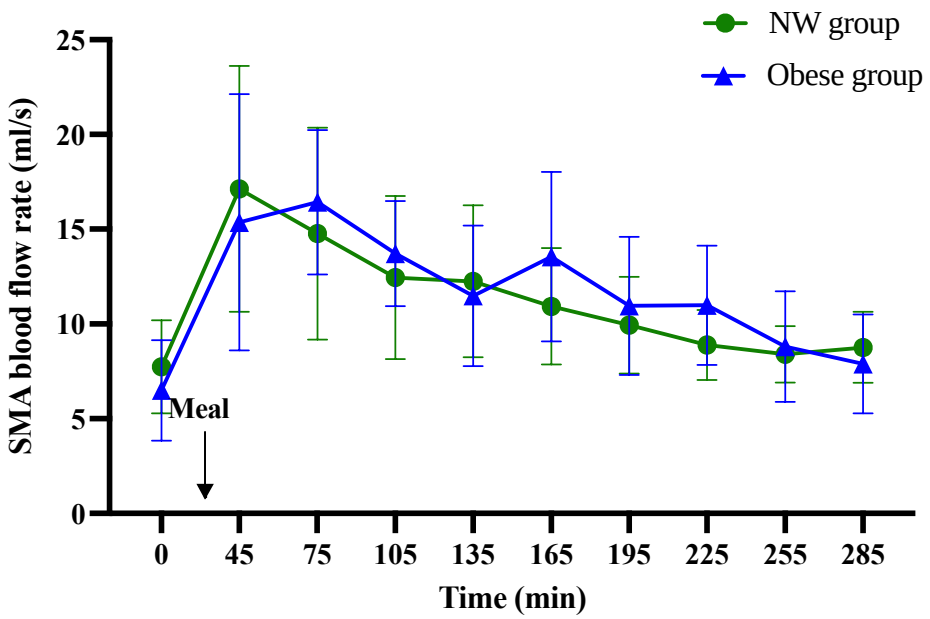


Figure 5.5. Superior mesenteric artery (SMA) blood flow values over time for normal-weight (NW) and Obese participants following the consumption of the pasta meal. Values are presented as the mean and SD, n=10 in each group.

5.4.4 Small bowel water content

There were no differences between the NW and Obese groups in the fasted state (P=0.35, Table 5.3). Highest volumes of SBWC were shown in the fasted state in both groups and then volumes started to decrease after feeding toward lowest values at 150 minutes in both groups (Figure 5.6). No significant group × time interactions (P=0.99) was shown. There was a trend for a group main effect for higher SBWC for the Obese compared with the NW group (P=0.097). A significant main effect of the time was observed (P<0.0001). Total AUC of postprandial SBWC (T0-T275) did not differ significantly between the groups (P=0.37, Table 5.4). Examples of maximum intensity projections (MIP) images of the segmented SBWC of NW and Obese participants at fasting baseline and after consumption of the test meal are shown in Figures 5.7 and 5.8.

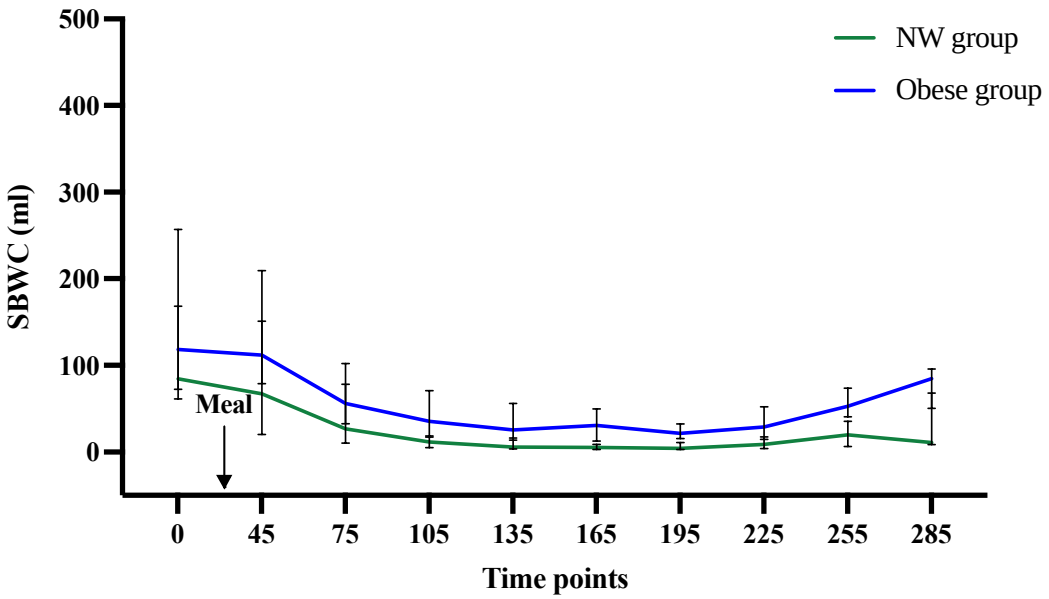


Figure 5.6. Small bowel water content (SBWC) volume over time for normal-weight (NW) and Obese participants following the consumption of the pasta meal. Values are presented as the median and IQR, n=10 in each group.

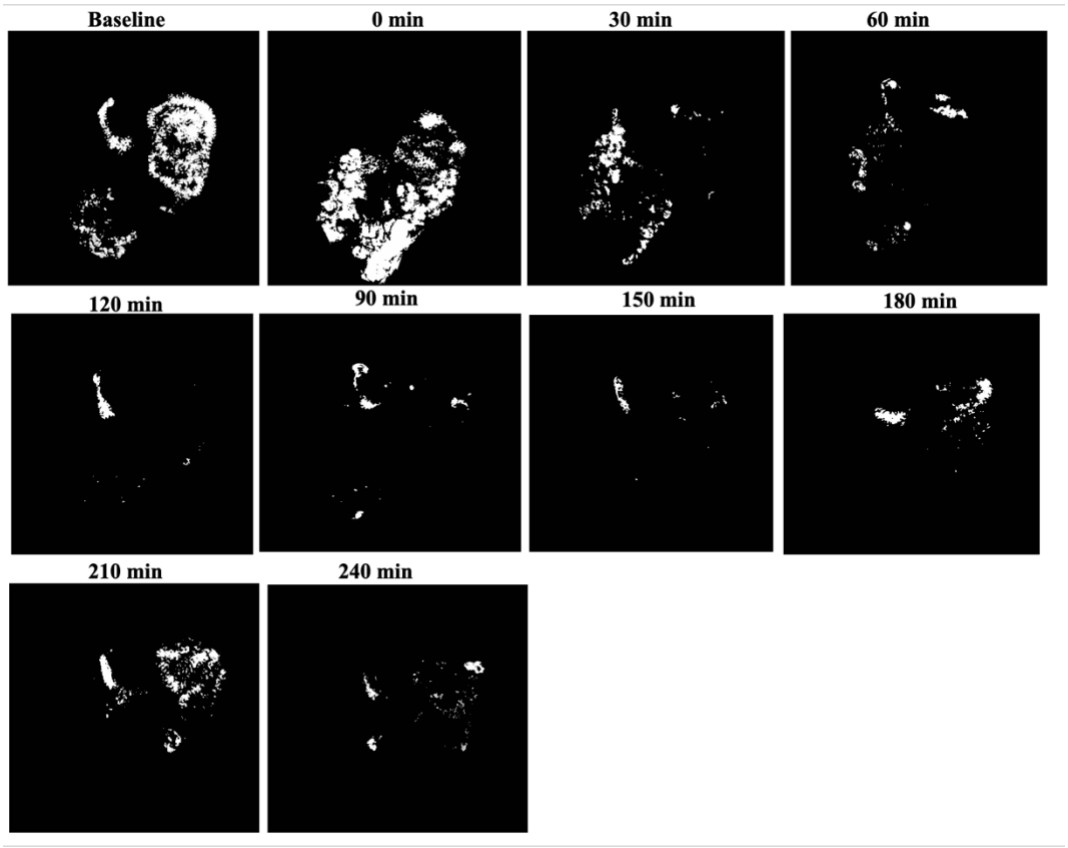


Figure 5.7. Examples of maximum intensity projections (MIP) images of small bowel water content of a normal-weight (NW) participant at fasting/baseline and at different time points following the consumption of the pasta meal.

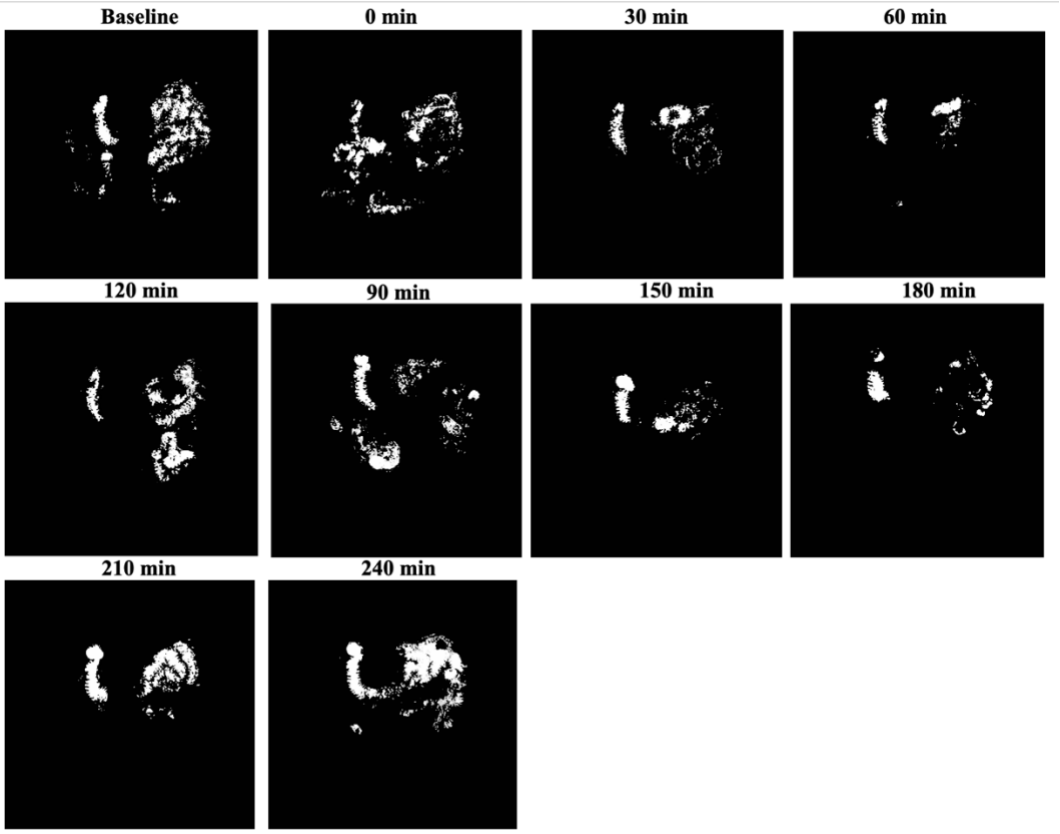


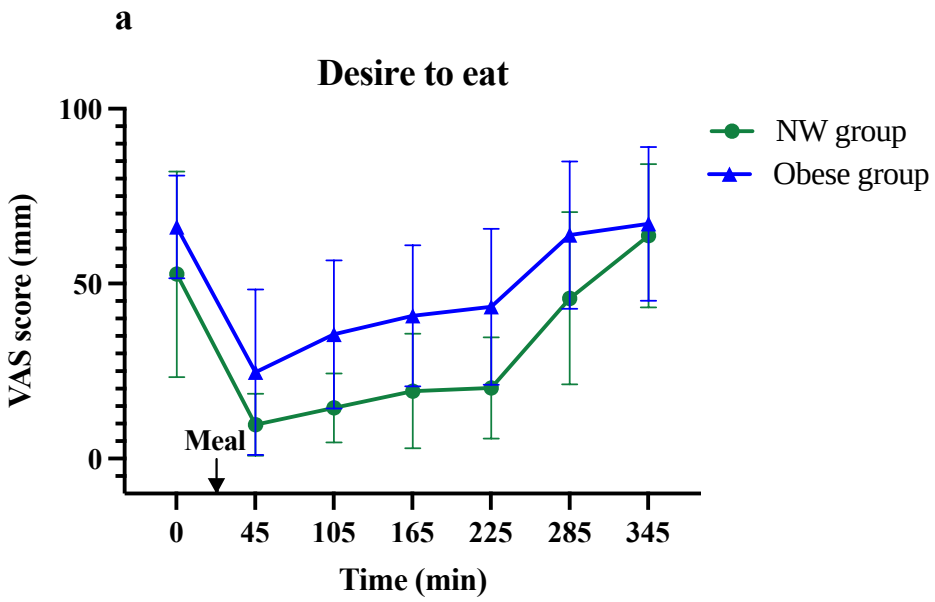
Figure 5.8. Examples of maximum intensity projections (MIP) images of small bowel water content of a participant with obesity at fasting/baseline and at different time points following the consumption of the pasta meal.

5.4.5 Subjective Satiety

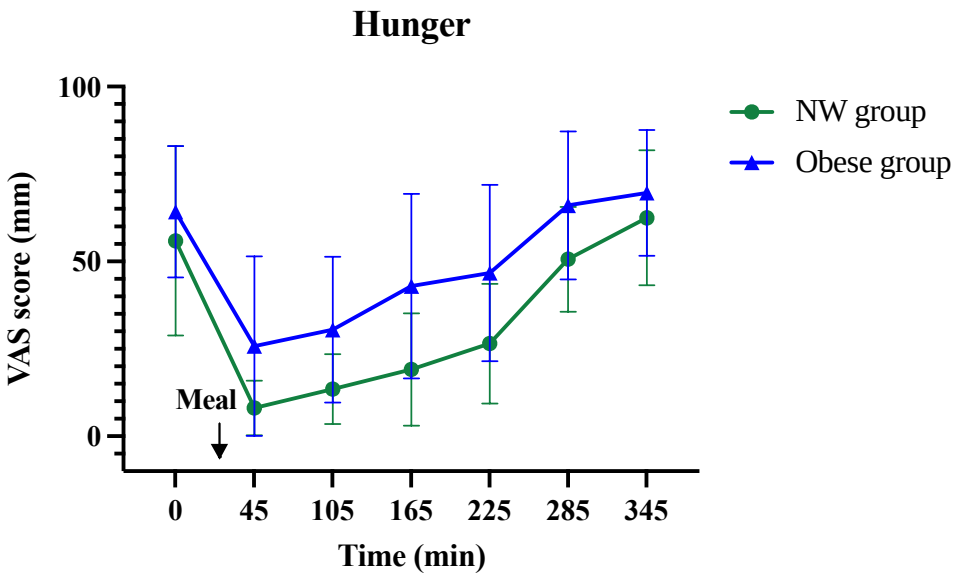
The four domains (DTE, hunger, fullness, and PFI) of the VAS questionnaire to assess feelings of satiety and the CSS when fasted and following the pasta meal in NW group and Obese group are illustrated in Figure 5.9. No differences were found in fasted values between the NW and Obese groups for all VAS domains (DTE, P=0.20; hunger, P=0.43; fullness P=0.97, and PFI, P=0.17) and CSS (P=0.33).

There was no significant group × time interactions for all VAS domains and CSS was shown. However, there was a significant group main effect observed for DTE, hunger, PFI, and CSS. The Obese group had significantly higher scores for the DTE, hunger, and PFI and lower CSS compared to the NW group (group effect: all P=0.02, Figure 5.9a, 5.9b, 5.9d, and 5.9e). For the fullness domain, there was a trend for higher scores for the Obese group compared to the NW group (group main

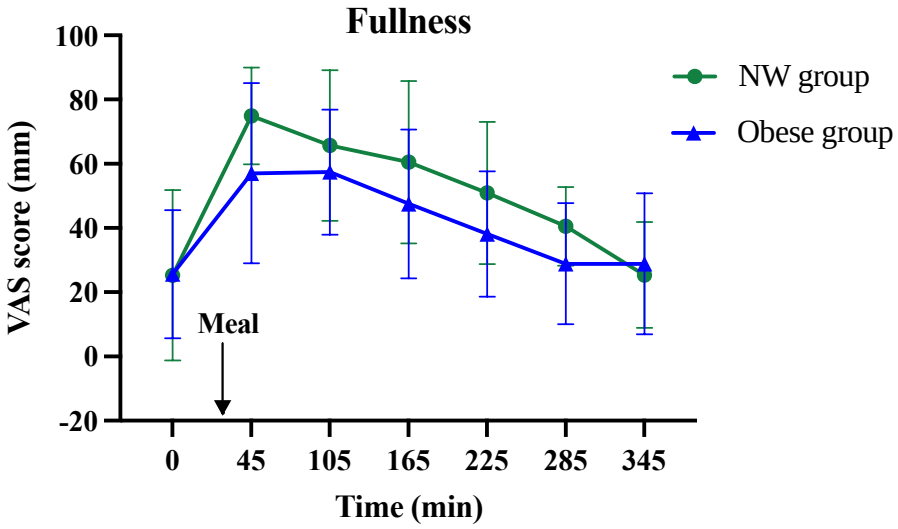
effect: P=0.09, Figure 5.9c). A significant main effect of the time was observed for all VAS domains and CSS (all P<0.0001). Postprandial AUC (T0-T335) did not differ between the groups in all VAS domains (DTE, P=0.46; hunger, P=0.30; fullness P=0.23, and PFI, P=0.33; Table 5.4) and CSS (P=0.23, Table 5.4). For the Bonferroni post hoc test, a significant difference was seen between the NW and Obese groups at 120 minutes for the PFI domain (P=0.04, Figure 5.9d). As expected, all postprandial ratings in both groups of PFI, and DTE decreased significantly after eating, reaching their lowest value at 0 minutes. Ratings of fullness increased after eating, reaching a peak at 0 minutes in both groups.



b


Hunger
100
50
0
VAS score (mm)
Meal
0
45
105
165
225
285
345
Time (min)
NW group
Obese group

c


Fullness
100
80
60
40
20
0
-20
VAS score (mm)
Meal
0
45
105
165
225
285
345
Time (min)
NW group
Obese group

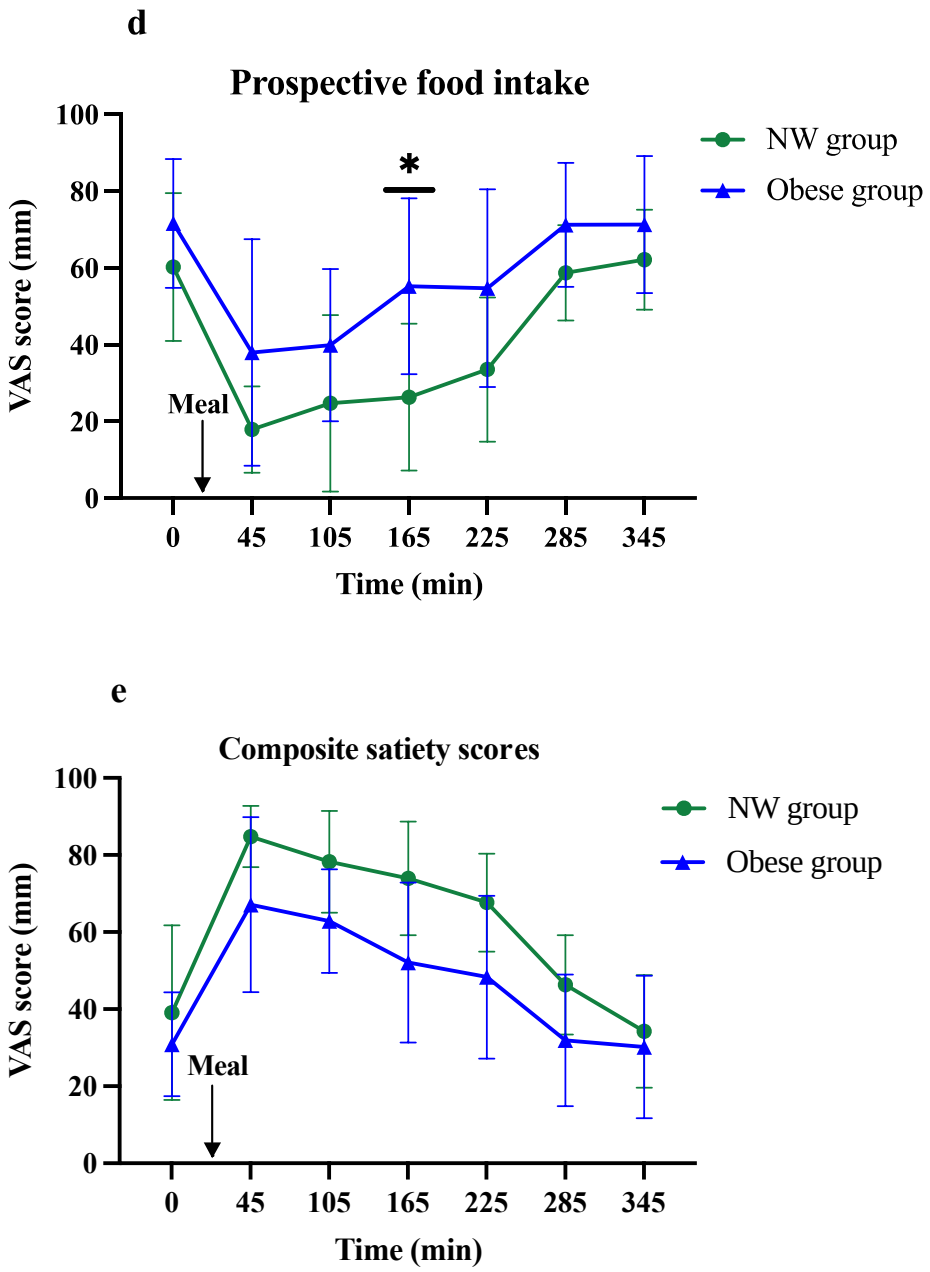


Figure 5.9. Subjective satiety ratings over time using Visual Analogue Scale (VAS) scores of (a) desire to eat, (b) hunger, (c) fullness, (d) prospective food intake, and include composite satiety scores (e) at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group. *Values are significant different between the two groups (P<0.05).

5.4.6 Gut hormones

Total PYY

No differences were seen in fasting PYY concentrations between the NW and Obese groups (P=0.41, Table 5.3). Also, no significant group × time interactions (P=0.91) or the group main effect (P=0.34) was shown. A significant main effect of the time was observed (P=0.0001). As shown in Figure 5.10, no differences were shown in total PYY concentrations between the two groups in response to eating (group effect: P=0.34, Figure 5.10). No differences were shown for total AUC of postprandial (T0-T305) concentrations between the NW and Obese groups (P=0.95, Table 5.4). The time to the peak were 90 minutes for the NW and 30 minutes for the Obese group. Concentrations then return to baseline values by 300 minutes in both groups.

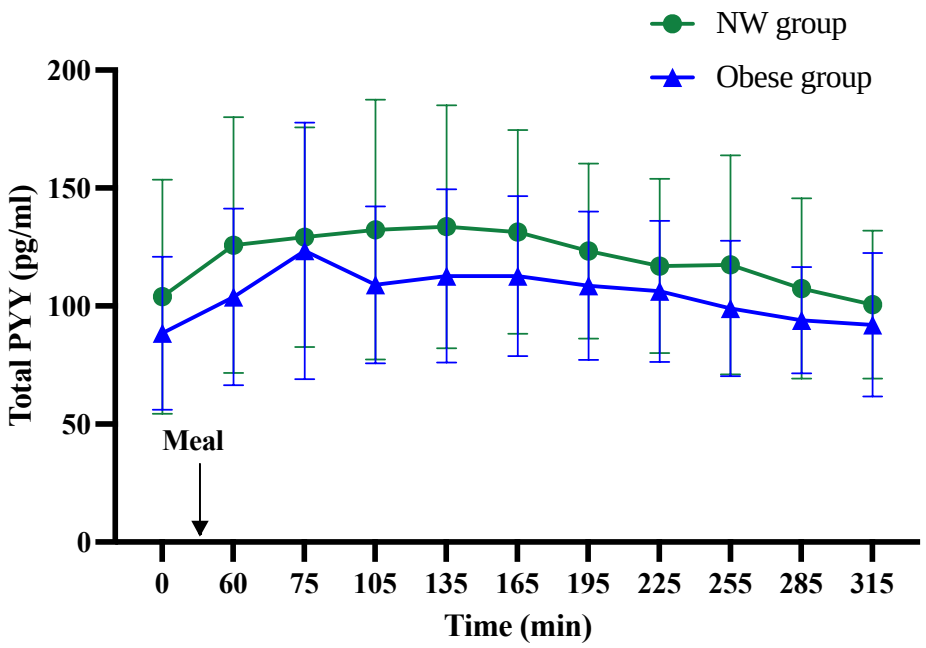


Figure 5.10. Total peptide YY (PYY) levels over time at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group.

Active glucagon-like peptide-1

No differences were seen in fasting GLP-1 concentrations between the NW and Obese groups (P=0.19, Table 5.3). No significant group × time interactions (P=0.30) or the group main effect (P=0.16, Figure 5.11) was observed. A significant main effect of the time was observed (P<0.0001). No differences were also shown for AUC of postprandial concentrations (T0-T305) between the NW and Obese groups (P=0.86, Table 5.4). The NW group had a huge variability in GLP-1 concentrations at fasted and following the consumption of the pasta meal compared with the Obese group. The time to the peak was 90 minutes for the NW group, and 15 minutes for the Obese group. Concentrations then return to baseline values at 300 minutes for the NW group and after 300 minutes for the Obese group.

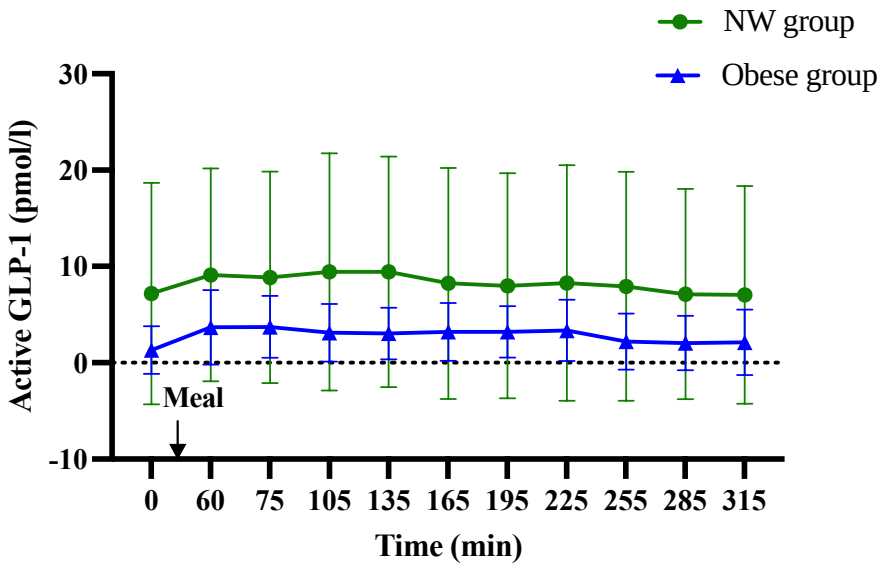


Figure 5.11. Active glucagon-like-peptide-1 (GLP-1) levels over time at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group.

Glucagon-like peptide-2

No differences were seen in fasting GLP-2 concentrations between the NW and Obese groups (P=0.72, Table 5.3). No significant group × time interactions (P=0.58) or the group main effect (P=0.92, Figure 5.12) was found. A significant main effect of the time was observed (P<0.0001). No differences were shown for total AUC for

postprandial concentrations between the NW and Obese groups (P=0.75, Table 5.4). The time to the peak was 60 minutes for both groups. Concentrations then returned to baseline values at 210 minutes in both groups.

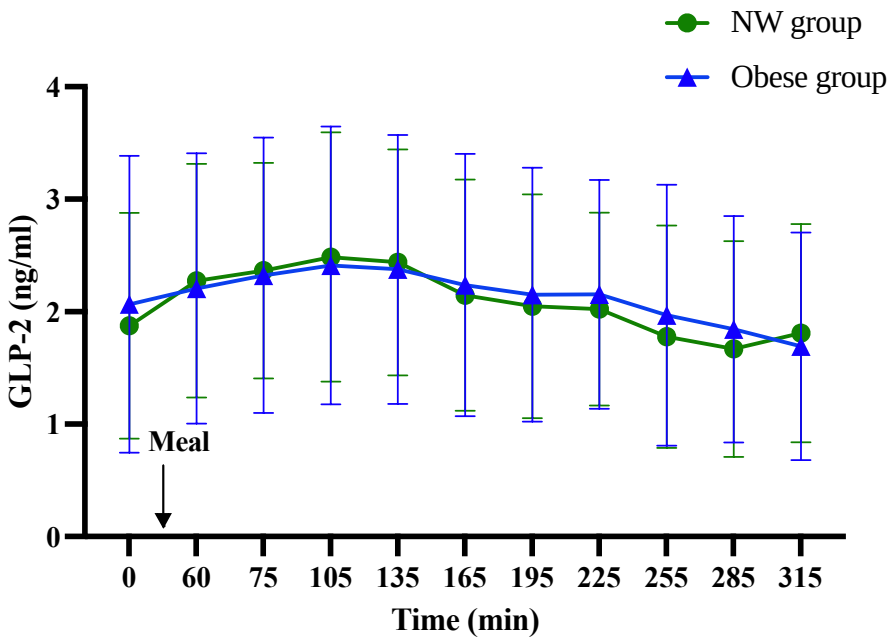


Figure 5.12. Glucagon-like peptide-2 (GLP-2) levels over time at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group.

Total ghrelin

No differences were seen in fasting ghrelin concentrations between the NW and Obese groups (P=0.46, Table 5.3). No significant group× time interactions (P=0.77) or the group main effect (P=0.77, Figure 5.13) was shown. A significant main effect of the time was observed (P<0.0001). No differences were shown for total AUC for postprandial concentrations between the NW and Obese groups (P=0.73, Table 5.4). The time for postprandial nadir (minimum concentrations of ghrelin concentrations) was 60 minutes for the NW group and 90 minutes for the Obese group. Concentrations then return to baseline values at 300 minutes in NW group and 210 minutes in the Obese group

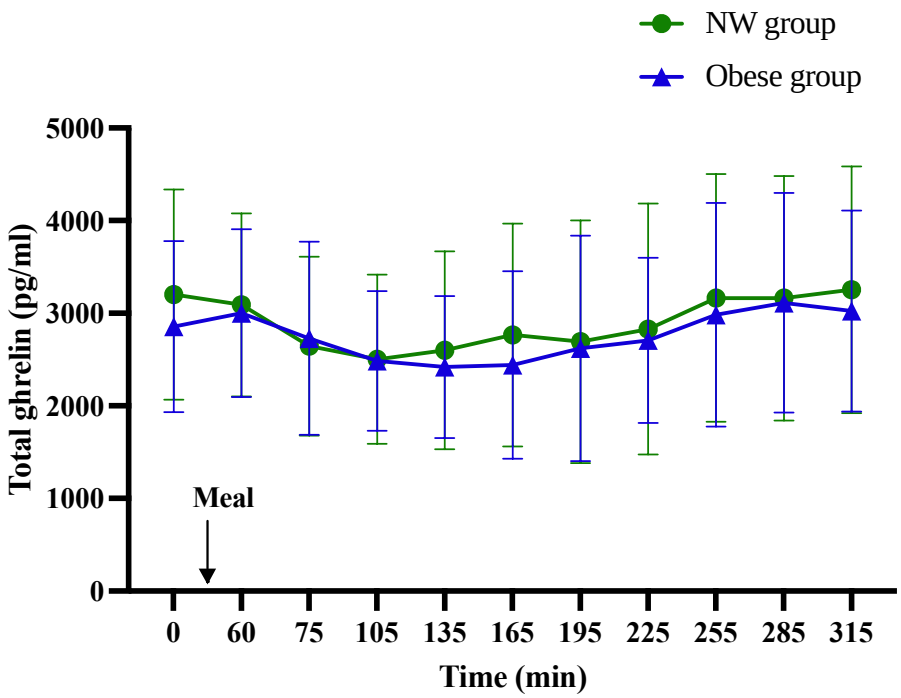


Figure 5.13. Total ghrelin levels over time at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group.

5.4.7 Other blood results

Insulin

No differences were seen in fasting insulin concentrations between the NW and Obese groups (P=0.21, Table 5.3). There was a trend for group × time interactions (P=0.07) and a significant group main effect (P=0.015). In response to eating, the Obese group had higher insulin concentrations compared to the NW group across the 5 hours (Figure 5.14). A significant main effect of the time was observed (P<0.0001). Total AUC for postprandial concentrations was significantly higher for the Obese group than the NW group (P=0.03, Table 5.4). The time to the peak was 30 minutes for both groups. Concentrations then return to baseline values by 300 minutes in both groups.

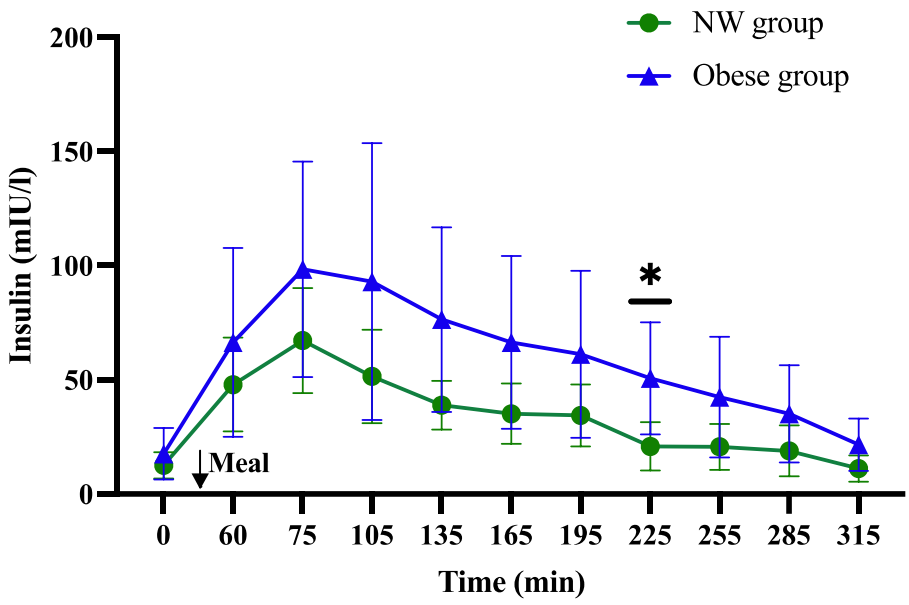


Figure 5.14. Insulin levels over time at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group. *Values are significant different between the two groups (P<0.05).

Blood glucose

No differences were seen in fasting blood glucose concentrations between the NW and Obese groups (P=0.41, Table 5.3). In response to eating, no differences were shown in blood glucose concentrations between the two groups (Figure 5.15). There were no significant group × time interactions (P=0.57) or the group main effect (P=0.47). A significant main effect of the time was observed (P<0.0001).No differences were shown for total AUC for postprandial concentrations between the two groups (Table 5.4, P=0.79). The time to the peak was 30 minutes for the NW group and 60 minutes for the Obese group. Concentrations then return to fasted values at 210 minutes in the NW group and 300 minutes in the Obese group.

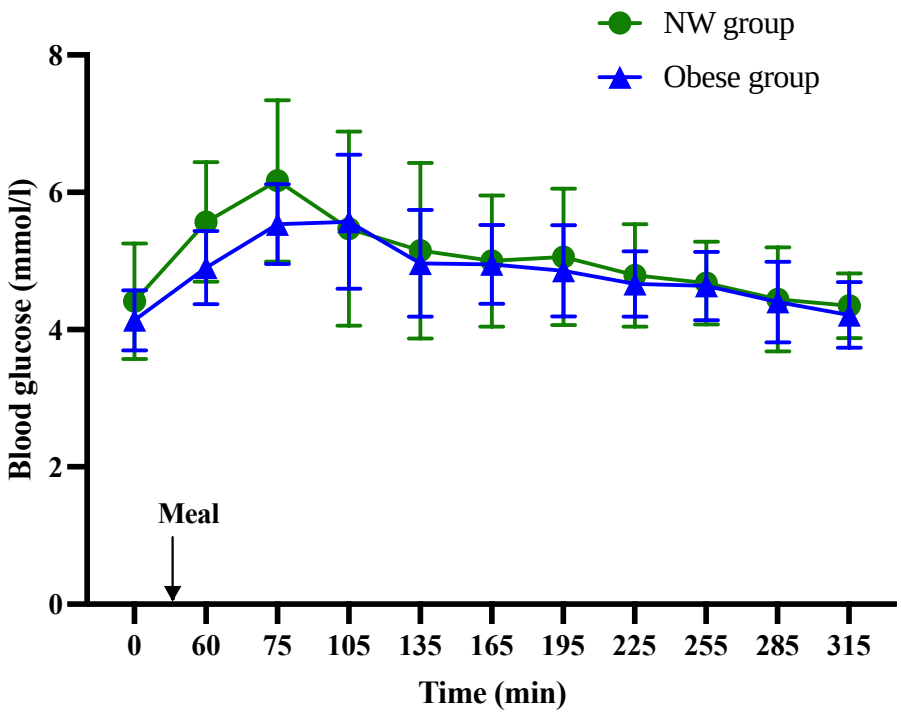


Figure 5.15. Blood glucose levels over time at fasted and following the consumption of the pasta meal in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=10 in each group.

Triglyceride

The triglyceride concentration was measured only in the fasted state. The Obese group had significantly higher concentrations compared with the NW group (P=0.0009, Table 5.3, Figure 5.16).

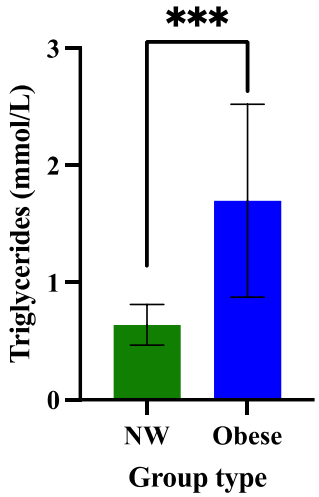


Figure 5.16. Fasting triglyceride concentrations in normal-weight participants (NW group) and Obese participants. Values are presented as the mean and SD, n=10 in each group. *** Values are significant different between the two groups (P<0.0001).

Free fatty acids

Concentrations of FFAs were measured only at the fasting level. There were no differences between the two groups (P=0.11, Table 5.3, Figure 5.17).

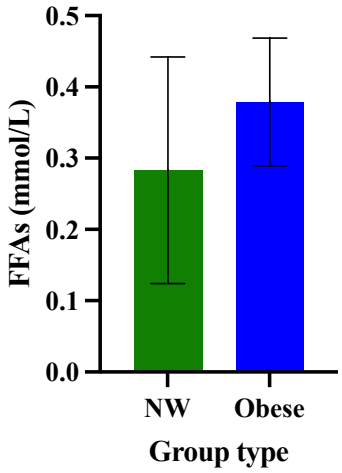


Figure 5.17. Fasted free-fatty acids (FFAs) concentrations in normal-weight participants (NW group) and Obese participants. Values are presented as the mean and SD, n=10 in each group.

5.5 Discussion

This is the first study, to our knowledge, that uses MR methods to measure the GI functions of GCV, GE, SMA blood flow, and SBWC as well as gut hormone release, blood glucose, triglycerides, FFAs, and subjective satiety in NW and Obese groups. The results showed no differences between the NW and Obese groups in terms of GCV, GE, SMA blood flow, and SBWC, as well as all hormones. Except for insulin, and blood glucose. However, subjective satiety ratings were significantly lower in the Obese group compared with the NW group.

GE is controlled by gut hormones and other factors, and it is considered a critical factor in regulating food intake and satiety. Consequently, it may play a role in the pathogenesis of obesity and this study hypothesised that participants with obesity would have a higher GE rate compared to the NW participants, as reported by Mora et al. (2005). However, the observations of Mora et al. (2005) are not unequivocal and the present study adds to previous findings, suggesting no differences in GE rate between NW and Obese participants following food intake were found (Buchholz et al., 2013, Seimon et al., 2013). The present study, and the study of Seimon et al. (2013) included only male participants, while the study of Mora et al. (2005), included both male and female participants which could explain the inconsistency seen in the results.

Investigating SBWC could help to a better understanding of food intake regulation in people with obesity, as it reflects small intestine functions (Dellschaft et al., 2022). Postprandial plasma concentrations of PYY facilitate fluid absorption via the intestinal mucosal surface to increase small intestine transit time, hence reducing SBWC (Savage et al., 1987, Benelam, 2009). In this study, PYY concentrations were increased slightly, while the SBWC decreased after both groups ate the pasta meal. This is consistent with previous studies, in which SBWC decreased after both

liquid and solid meal intake in NW participants (Marciani et al., 2010, Marciani et al., 2012). This is the first study that compared SBWC between NW and Obese participants after feeding, hence, the results of the Obese participants couldn't be compared to the previous studies.

SMA blood flow is known to increase after food intake to aid nutrient absorption. Previous research has shown that gastric distension could stimulate SMA blood flow (Vanis et al., 2010, Vanis et al., 2012). In the present study, SMA blood flow increased significantly after eating in both groups with no differences between them. The lack of differences between groups may be a result of the same energy content being consumed by both groups, as previous studies have suggested that blood flow is positively correlated with energy content (Sidery and Macdonald, 1994, Parker et al., 1995, Someya, 2007). These findings of higher postprandial responses of SMA blood flow support its role in food digestion and absorption (Jeays et al., 2007). Hence, SMA blood flow after eating may be an important marker of digestive processes in both health and illness, which could have a role in food intake regulation.

To date, no study has compared SMA blood flow between NW and Obese participants following food intake. The results of the NW group support those of previous studies, wherein SMA blood flow increased after liquid and solid meals in NW participants (Moneta et al., 1988, Sieber et al., 1991).

Some previous studies have found that circulating postprandial plasma concentrations of PYY and GLP-1 are reduced, while ghrelin is increased in people with obesity, when compared to NW individuals, suggesting that these hormones have a role in the pathophysiology of obesity (Batterham et al., 2003, Meyer-Gerspach et al., 2014). However, GLP-1, PYY and ghrelin were not different in this study between the groups at fasting and in response to the pasta meal intake. The findings of our study are consistent with are those of Adam and Westerterp-Plantenga (2005), who observed no differences between the fasting and postprandial responses of GLP-1 between NW and Obese participants after eating a standard breakfast meal with water. Similarly, a study conducted by Yang et al. (2009) did

not find differences in fasting and postprandial PYY responses between NW and Obese participants following high-carbohydrate (88% carbohydrate, 4% fat and 8% protein) and high-fat (25% carbohydrate, 4% protein and 71% fat) breakfast meals. Moreover, Carroll et al. (2007) reported no differences between NW and Obese participants in their fasting and postprandial responses regarding GLP-1 and ghrelin after a soy liquid meal. These results conflict with another study that showed higher GLP-1 and lower ghrelin responses in the Obese participants compared with NW participants after three protein liquid meals (soy, whey and gluten) (Bowen et al., 2006). However, they reported that Obese participants had higher fasting GLP-1 and lower ghrelin concentrations than NW participants, which might influence the postprandial responses. Of note, test meals varied widely in the previous studies and included liquid, semi-solid and solid meals with different macronutrient compositions, which make for inconsistent results across the studies.

There is a lack of studies investigating the relationship between endogenous GLP-2 and satiety and GE in humans. Unlike GLP-1, GLP-2 has no incretin effect on blood glucose homeostasis and insulin. The main effects of GLP-2 are seen to maintain mucosal morphology, intestinal integrity, and nutrient and energy absorption. Previous studies showed no influence of GLP-2 infusion on GE (Schmidt et al., 2003, Meier et al., 2006), while one study showed it slowed GE (Nagell et al., 2004),

Blood glucose concentrations may have a role in changes to appetite, as a reduction in blood glucose concentrations can increase appetite and initiation of food consumption, while an increase in feelings of satiety has been shown to occur with hyperglycaemia (Mayer, 1955). However, it is difficult to distinguish whether changes which occur to satiety, as a result of elevated blood glucose concentration, stem from hyperglycaemia alone or from other metabolic changes / processes which co-exist, such as endogenous insulin production. Fasting and postprandial concentrations of blood glucose were not different between groups in this study. These results confirm the findings reported by Carroll et al. (2007), which did not

find differences between NW and Obese participants in their postprandial concentrations of blood glucose following a liquid meal.

Previous studies that examined the relationship between circulating insulin concentrations and food intake suggested that insulin has an appetite-suppressing effect on NW participants and, to a lesser extent, Obese participants (Verdich et al., 2001b). As expected, insulin concentrations in the current study were higher in the Obese compared to the NW group. These results are consistent with previous studies, which showed greater increases in circulating insulin concentration in the Obese compared to NW participants after carbohydrate intake (van Vliet et al., 2020, Wikarek et al., 2020). Elevated insulin concentrations seen in the Obese group suggests insulin resistance, which may lead to further weight gain (Verdich et al., 2001b, Zyoud et al., 2022). Insulin resistance is believed to be linked to weight gain by increasing appetite and basal lipolysis process (Girousse et al., 2013), and decreasing the thermic effect of food (Camastra et al., 1999).

The processes involved in feeling sated include behavioural and psychological events, metabolic processes and peripheral physiology, as well as metabolic and neurotransmitter interactions in the brain (Dalton et al., 2013). The present study adds to a previous study conducted by Adam and Westerterp-Plantenga (2005), which observed lower satiety scores in the Obese compared with the NW group following a standard breakfast. Lower satiety scores lead to eating more and, hence, can contribute to the development of obesity. With regard to the correlation between GE and satiety, the present study found fullness and CSS were positively correlated with GCV in both groups, which is consistent with previous studies (Marciani et al., 2015, Gonzalez-Izundegui et al., 2021).

In this study, triglycerides and FFA were measured once during the fasting state and there was no difference in FFA between the groups. Fasting plasma triglyceride was higher in the Obese compared with the NW group, which was expected, as it positively correlates with visceral fat area (Marston et al., 2019, Sukkriang et al., 2021) and is associated with insulin resistance. Elevated triglyceride levels could induce leptin resistance, which increases the desire to eat and reduces calorie

expenditures unrelated to energy need, may have developed as a signal to the brain that a person is starving (Banks et al., 2004). Hence, hypertriglyceridemia seen in the Obese group could explain the lower satiety feelings that they reported compared to the NW group.

5.6 Strength and limitations of the study

This is the first study that used MR methods to measure the GI functions of GCV, GE, SMA blood flow, and SBWC in NW and Obese participants. It included only male participants to remove the effect of oestrogen during the luteal phase of menstrual cycle as it could delay GE rate (Wang et al., 2015). It used identical test meals for both groups, so the results were directly comparable.

The present study has some limitations as listed below.

- Sample size was calculated based on NW participants, which may be underpowered to detect differences between groups.
- It included only male participants; hence, we cannot apply our findings to females.
- Despite being a research strength, the test meal was the same for all groups instead of being adjusted for body weight. Given that breakfast meals account for 18%–20% of daily energy intake (Trumbo et al., 2002), this could cause some participants to overestimate or underestimate their energy requirements. It is important participants are meeting their energy requirement, as this is necessary for basic metabolic processes such hormone and enzyme production, metabolism, and brain function (de Nava and Raja, 2022).

- Total ghrelin concentrations were measured as opposed to the acylated ghrelin form and total PYY concentrations were examined as opposed to $PYY_{3–36}$. This suggests that variations in total PYY and ghrelin and concentrations may not precisely show variations in $PYY_{3–36}$ and active ghrelin and concentrations. Nevertheless, a substantial correlation exists

between acyl- and total ghrelin (Marzullo et al., 2004). Whereas it was previously believed that only the acylated ghrelin was the active form, it is now believed that unacylated ghrelin also applies some biological activities (Broglio et al., 2004, Asakawa et al., 2005), thereby supporting the idea that total ghrelin is more significant overall. Meanwhile, research indicates that the $PYY_{1–36}$ to $PYY_{3–36}$ ratio is comparable in NW and Obese (le Roux et al., 2006).

- *Ad libitum* meal intake was not measured, which could have more objectively measured satiety than through the VAS questionnaire, although the VAS questionnaire has been validated and is commonly used to assess satiety feelings (Flint et al., 2000).

5.7 Conclusion

In conclusion, the present study showed that GI functions, gut hormones, and other satiety regulators (except for insulin and triglycerides) were not altered with obesity. The Obese group had higher insulin and triglycerides concentrations and lower satiety feelings compared to the NW group, which could be factors for weight gain. However, the brain plays a major role in regulating food intake and future studies should include brain measurements in response to food intake to allow better understanding of the interactions between gut and brain to tackle obesity.

6 An MRI pilot study to explore gastrointestinal responses to a high fat emulsion drink in people with and without obesity

The gut-brain axis plays a major role in the regulation of food intake. However, the interaction between the brain and gut to regulate appetite and satiety is not yet fully understood, particularly in obesity. Understanding the physiological mechanisms to regulate appetite and satiety could lead to the development of food products that enhance satiety feeling. The imaging team at the SPMIC have developed an MRI protocol that combines gut and brain imaging in a single MRI scan session. The primary objective of the work described in this chapter was to employ this innovative imaging protocol to investigate satiety and appetite responses in individuals with and without obesity, using a high-fat emulsion. The initial work described in this chapter involves the formulation of high-fat emulsion and control beverages with equivalent caloric and viscosity profiles.

6.1 Introduction

The effect of macronutrients on satiety is an important aspect of nutrition and weight management. Different macronutrients, which include carbohydrates, fats, and proteins, can have distinct impacts on satiety due to their various metabolic and physiological effects (Westerterp-Plantenga et al., 2006, Veldhorst et al., 2008, Johnstone, 2013). The satiety effect of fats versus carbohydrates is a subject of ongoing research and can vary depending on several factors, including meal composition and the type of fats and carbohydrates consumed (Yang et al., 2009, Gibbons et al., 2016). Obesity is generally caused by the overconsumption of high caloric food (Mendoza et al., 2007). Several studies reported that people with obesity highly prefer fatty foods compared with normal-weight participants (Dressler and Smith, 2013). This is mainly driven by the hedonic/reward attributes of fat, which plays as key factor in food choice. Neuroimaging studies have demonstrated that taste and texture of oral fat activate taste and reward-related brain

areas (Rolls and McCabe, 2007, Grabenhorst et al., 2010, Eldeghaidy et al., 2011, Stice et al., 2013). These rewarding attributes of fatty food may lead to overconsumption, and could results in weight gain and, in extreme cases, obesity (Golay and Bobbioni, 1997, Bray et al., 2004). Although fatty foods have high caloric content, they have been suggested to have a relatively low satiety effect compared with matched caloric content from protein and carbohydrate (Blundell and MacDiarmid, 1997).

The impact of carbohydrates on satiety can vary depending on their type. Simple carbohydrates, like sugars and refined grains, can lead to rapid changes in blood sugar levels, potentially causing hunger soon after consumption (Bornet et al., 2007, Maki and Phillips, 2015). The consumption of palatable foods high in refined/simple carbohydrate may also be a strong contributor to overeating. Several animal and human studies have reported the activation of the mesolimbic dopaminergic reward pathway in response to sweet intake and, over time, lead to overeating and obesity (Thornley et al., 2011, Thanarajah and Tittgemeyer, 2020). In contrast, complex carbohydrates, especially those rich in fibre, have a more significant satiating effect. They provide sustained energy and reduce overeating by promoting fullness.

Previous MRI studies have investigated the impact of carbohydrate and fat meals on satiety responses. These studies often focus on understanding the brain responds to different carbohydrate-rich or fat-rich foods and how these responses influence feelings of fullness (Eldeghaidy et al., 2016, Al-Zubaidi et al., 2019). Other studies have assessed the GI responses and subjective ratings of satiety (Goetze et al., 2007, Marciani et al., 2015). This study aimed to use advances in MRI methods to combine brain and gut imaging to explore the interactions between the gut and brain in response in NW and Obese participants to a high fat emulsion drink compared to a high carbohydrate drink, that is matched in caloric content and viscosity. Unfortunately, the Covid-19 pandemic significantly disrupted these research activities and, due to the time restrains for the PhD, data available for inclusion in this chapter is only focused on the GI responses to fat and carbohydrate in NW and

Obese participants. Hence, no brain data is included in this chapter as there was not enough time to acquire the skills needed to analyse the brain data.

6.2 Aims and hypothesis

Primary aim

To compare GCV and the sensation of postprandial fullness (measured by VAS) between NW and Obese participants following the ingestion of high-fat (HF) and high-carbohydrate (HC) drinks.

Secondary aims

- To compare SBWC and SMA blood flow between NW and Obese participants following the consumption of HF and HC drinks.
- To compare eating behaviours measured by questionnaires between NW and Obese participants.

Hypothesis

This study hypothesises that Obese participants will have a lower GCV compared to NW participants following the consumption of HF drink compared to the HC drink.

6.3 Methods

This section starts by describing the development work on designing the fat emulsion and the carbohydrate drinks, followed by the methods used for the "main MRI study".

6.3.1 Development work on designing the study drinks.

Designing the fat emulsion drink.

The initial design of the HF emulsion drink in this study was based on a previous work by Eldeghaidy et al. (2016) and Eldeghaidy et al. (2011) that measured brain responses and gut hormone levels (gut-brain axis) following the consumption of a high fat emulsion in NW adults. In Eldeghaidy et al. (2016), the 250 ml HF drink

was composed of 22% rapeseed oil, 1% sucrose stearate emulsifier (E-473), and mineral water, in addition to flavours and 9% sucrose. The types of the oil and emulsifier were articularly chosen because of their low odour and taste properties (Miettinen et al., 2002, De Araujo and Rolls, 2004), which is an important factor to consider when designing a brain imaging study. The fat level in the emulsion drink was chosen as it is typically found in high-fat food products such as mayonnaise and salad dressing (Hussein et al., 2015, Eldeghaidy et al., 2016).

In the previous studies of Eldeghaidy et al. (2011) and Eldeghaidy et al. (2016), the HF drink was prepared by mixing sucrose stearate, mineral water and oil using a high-shear blender (Silverson) followed by high-pressure homogenizer (at pressures 500 and 50 bar for the first and second stages respectively) to produce a fat emulsion with a small droplet size (0.4-μm) (Hollowood et al., 2008). However, the design of the current study differs from that of Eldeghaidy et al. (2016), as the aim was to combine brain and gut imaging to assess the gastric volume content of the HF drink in NW and Obese participants, in addition to rather than just exploring brain responses of the drinks. Therefore, other considerations in designing the HF drink were taken into account including the GE rate and stability of the HF emulsion. Work by Hussein et al. (2015) demonstrated that a small droplet size (0.4-μm) of 20 % fat emulsion takes a long time (T_{50}: 330 ± 61 minutes) to empty from the stomach compared with a large (6-μm) droplet size (T_{50}: 230 ± 22 minutes). A long scan time was not desirable in this study, as it could be uncomfortable for participants, particularly for the Obese group. Hence, in this study the fat emulsion was designed to generate a large (6-μm) droplet size. Appendix 10.2.4 gives a full description of the fat emulsion preparation.

Designing carbohydrate drink

One of the aims of this study was to assess the effect of macronutrient on the gut-brain axis, by designing a carbohydrate drink that was matched for caloric content, viscosity, and volume to the HF drink. To match the energy content of the HF drink (593 kcal/300 ml), the HC drink was designed to have 0% fat. HC drink was created with 52% (w/w) maltodextrin, with dextrose equivalent (DE) of 18 (Maltosweet

180, Azelis UK) dissolved in mineral water. Maltodextrin polymers were preferable to glucose monosaccharides they have lower osmolarities than the monosaccharide glucose solution (Brouns and Kovacs, 1997). The maltodextrin DE of 18 was chosen as it has moderate sweetness (Saldivar and Perez-Carrillo, 2016, Muhamad et al., 2018).

However, the generated control drink (HC) was found through sensory testing, by the research team, to be perceived as more viscous compared with the HF drink, and as explained in section 2.2.1.2, the physical characteristics of the meal/drink including viscosity play a significant role in satiety feeling/responses. Therefore, as described below, work was done to match the viscosity of the HF and HC drinks.

6.3.2 Viscosity measurement

In collaboration with colleagues from the food structure research group, viscosity measurements of the HC were carried out using a Physica MCR 301 Rheometer (Figure 6.1) at 25°C per second. Drinks samples of 50 ml were prepared and tested for stress viscosity using a concentric cylinder geometry (CC27) for 5 minutes, and viscosity measurements were acquired at 50 logarithmic ramp shear rates ranging from 0.1 to 100 s^{-1} and 100 to 0.1 s^{-1}. Measurements were repeated 3 times, and an average of the replicates was calculated. This yielded a viscosity of 61.4 mPa·s at sheer rate of 50 s^{-1}. This shear rate of 50 s^{-1} has been selected as the matching viscosity point for the tested drinks based on previous studies which suggested 50 s^{-1} represents the shear rate in mouth (Hollowood et al., 2008). To match the viscosity of the HF drink with the HC, five fat emulsion drinks were prepared with different concentrations of the hydroxypropyl methylcellulose (HPMC, Benecel™, K15M, Ashland, IMCD UK Ltd) thickening agent (0.25%, 0.3%, 0.35%, 0.40% and 0.45%). Measurements were repeated 3 times, and an average of the replicates was calculated and plotted against the viscosity measurement of the HC sample. As shown in Figure 6.2, the HF drink with 0.45% HPMC concentration was found to be the most closely match with the HC drink at the 50 s^{-1} shear rate. Hence this HMPC concentration was used in the HF drinks.

Figure 6.1. Anoton Parr, Benelux SC-MCR-301-Rheometer.

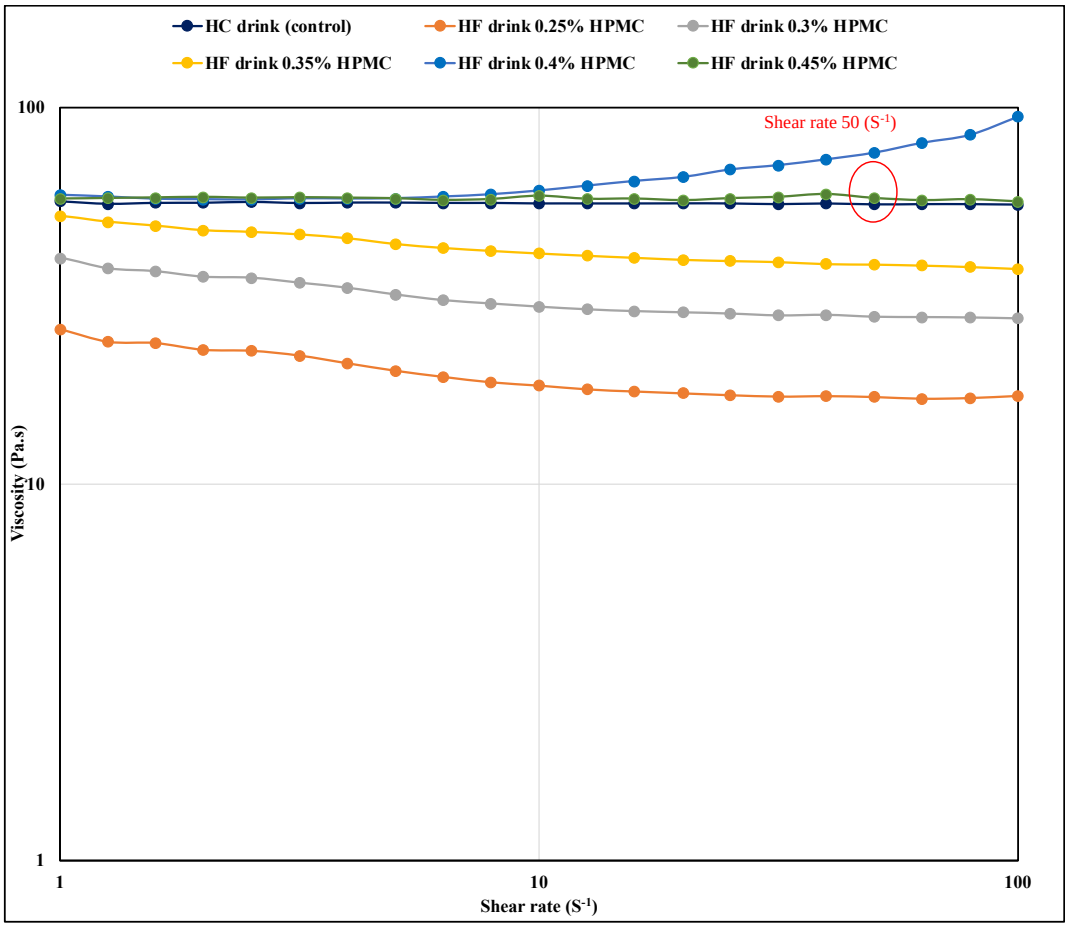


Figure 6.2. Shows rheology data for 5 high-fat (HF) drinks and the carbohydrate drink (HC drink, maltodextrin). The plots show the matched viscosity profile of the HF emulsion with the addition 0.45% HPMC (hydroxypropyl methylcellulose) and the carbohydrate drink (HC drink, maltodextrin).

However, another factor was taken into consideration in designing the fat emulsion which was the intragastric acid stability of fat emulsion. Previous research showed that the stability of fat emulsion may impact GE, hence satiety sensation (Marciani et al., 2008). Emulsions that remain stable in the stomach's acidic environment empty from the stomach more slowly than emulsions that break up and layer, possibly via enhancing the satiety perception and CCK hormonal response (Marciani et al., 2007, Marciani et al., 2008). Another factor may be that water and fat/cream will empty at different rates when separated. Therefore, fat emulsion stability in the gastric phase was assessed using the in vitro digestion model described below.

6.3.3 In vitro digestion assessment for the fat emulsion stability in the gastric phase

In collaboration with colleagues from the in vitro digestion research group, the stability of the fat emulsion in this study was assessed. An in vitro digestion experiment was undertaken to test the stability of the 22% fat emulsion drink in the gastric phase for a period of 24 hours using the fat emulsion used in Eldeghaidy et al. (2016) with the addition of 0.45% HPMC. In addition, we also wanted to assess the stability of HF using emulsifier and thickening agent used in Hussein et al (2015). Therefore, four samples were assessed using two fat emulsifiers (sucrose stearate and Tween 20) and two thickening agents [HPMC and locust bean gum (LBG)]. Table 6.1 shows the composition of 100 g total solution of the four samples used for in vitro digestion experiment. Samples were prepared as described in Appendix 10.2.4 and 10.2.5. The concentration of the LBG (0.5%) was based on the work of Hussein et al (2015), whereas the concentration of HPMC (0.45%) was based on the viscosity matching of the control drink (as described above).

Table 6.1. Composition of 100 g total solution of the four samples used for in vitro digestion experiment.

Fat emulsion sample	Rapeseed oil (g)	Thickening (g)	Water (g)	Emulsifier (g)
Sucrose + HPMC	22	0.45	76.5	1
Sucrose + LBG	22	0.3	76.7	1
Tween + LBG	22	0.3	76.7	1
Tween + HPMC	22	0.45	76.5	1

Preparation of gastric solutions

To simulate the gastric digestion, an in vitro digestion procedure adapted from INFOGEST static in vitro digestion model was used (Brodkorb et al., 2019). For the purposes of this study only the gastric phase was simulated. Firstly, the simulated gastric fluids (SGF) were prepared according to the compositional Table

6.2 by mixing a series of electrolytes solutions which mimics the composition of the physiological fluids.

Table 6.2. Volumes of electrolytes solution of the digestion fluids for a volume of 400 mL diluted with water

			SSF (pH 7)		SGF (pH 3)	
Salt solution added	Stock concentrations		Milliliters of stock added to prepare 0.4 L (1.25×)	Final salt concentration in SSF	Milliliters of stock added to prepare 0.4 L (1.25×)	Final salt concentration in SGF
	(g/L)	(M)	(mL)	(mM)	(mL)	(mM)
KCl	37.3	0.5	15.1	15.1	6.9	6.9
KH2PO4	68	0.5	3.7	3.7	0.9	0.9
NaHCO3a	84	1	6.8	13.6	12.5	25
NaCl	117	2	–	–	11.8	47.2
MgCl2(H2O)6	30.5	0.15	0.5	0.15	0.4	0.12
(NH4)2CO3*	48	0.5	0.06	0.06	0.5	0.5
HCl		6	0.09	1.1	1.3	15.6
CaCl2(H2O)2b	44.1	0.3	0.025	1.5	0.005	0.15

Gastric Phase: The emulsions were mixed with SGF at two ratios in a 50 ml Falcon tube. The first mixing ratio is 1:2 of emulsion to final digestion mixture (emulsion: SGF) where 10 ml of each emulsion is being mixed with 8 ml of SGF added to the rest of digestion components to achieve a total volume of 20 ml as in Table 6.3. The second is 1:4 where 5 ml of each emulsion is mixed with 13 ml of SGF added to the other digestion components to achieve a total volume of 20 ml as in Table 6.4. To each digestion tube, 1.0 mL of porcine pepsin (EC 3.4.23.1) and 5.0 μL of CaCl2 to achieve 2000 U /mL and 0.075 mM, respectively in the final digestion mixture. The pH was then reduced to 3 using 1 M HCl by direct measurement and accordingly distilled water was added to complete the volume to 20 ml and samples were returned to the shaking incubator (150 rpm for 2 h at 37°C).

Table 6.3. Gastric phase 1:2 and 1:4 ratios: duration 120 minutes for each ratio.

Components	**1:2 ratio**	**1:4 ratio**
Liquid food	10 ml	5 ml
Simulated gastric fluid (SGF)	8 ml	13 ml
Pepsin solution	1.00 ml	1.00 ml
0.3 M of $CaCl_2$	5 μl	5 μl
HCl to reach pH at 3.0	0.5000 ml	0.5000 ml
Water	0.4950 ml	0.4950 ml
Total sample to be removed for sampling during gastric stage (ml)	0 ml	0 ml
Final gastric mixture volume	20 ml	20 ml

Stability test

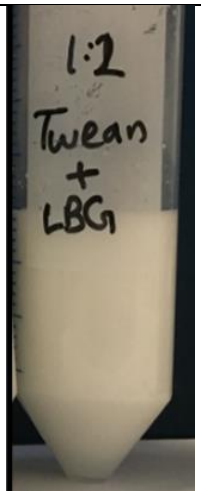
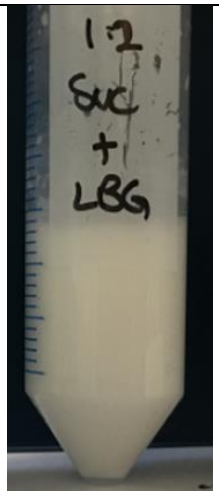
The digested emulsions in each tube at the end of gastric phase were then observed for top layer creaming appearance/separation every 30 minutes for 2 hours and then at 24 hours.

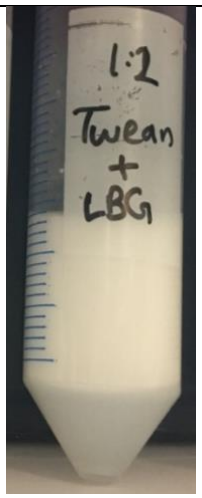
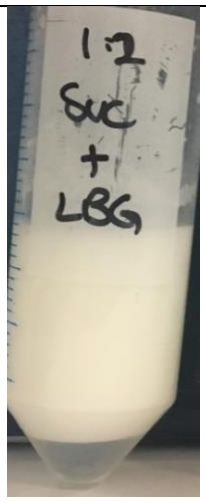
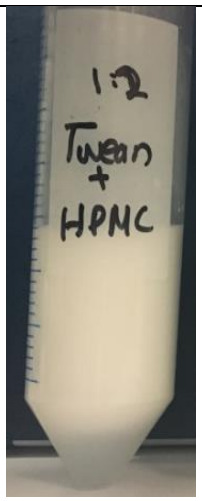
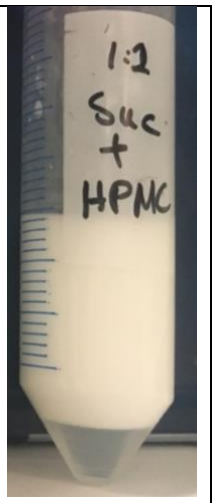
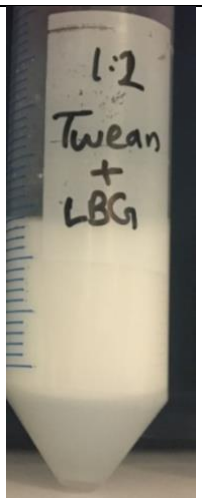
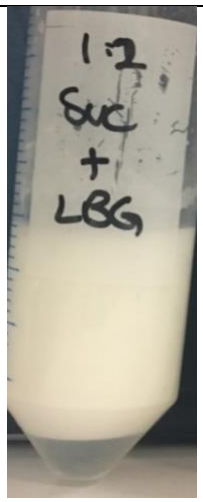
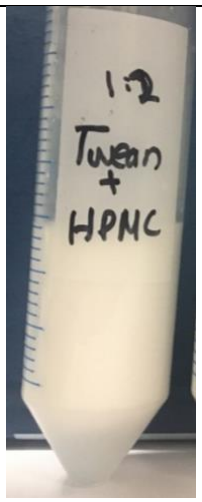
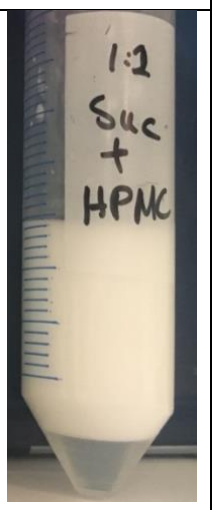
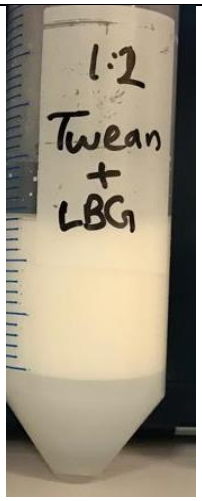
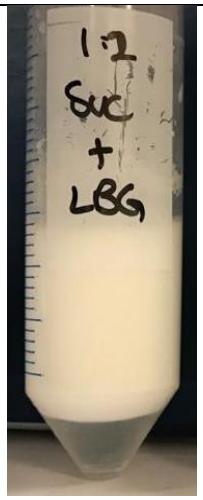
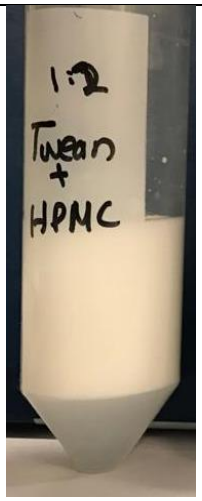
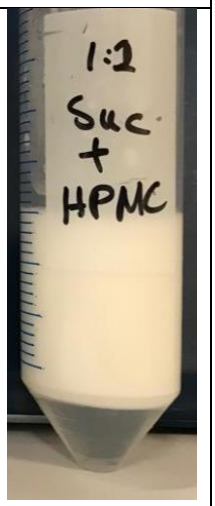
Results

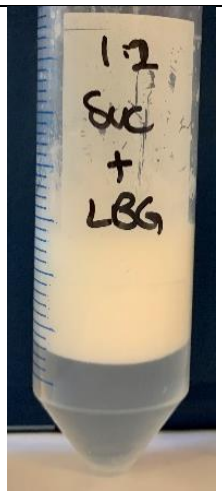
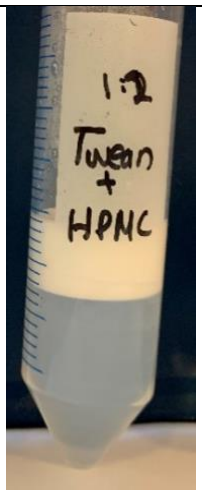
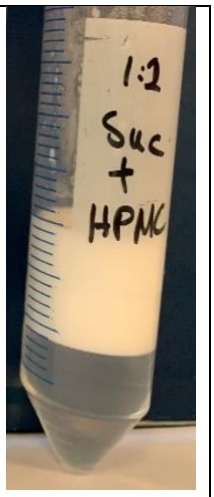
The mixing ratio1:2

Table 6.4 displays the stability of the 4 samples at various time intervals: 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 24 hours. Initially, at 30 minutes, all samples showed stability and there was no appearance of a creaming top layer. The first observation of cream top layer appeared in all samples started at 60 minutes. However, there were variations among the samples. Both Sucrose-LBG and Sucrose-HPMC showed an obvious cream top layer, while Tween-LBG and Tween-HPMC samples displayed partial separation with some appearance of a cream top layer (Table 6.4). The Tween-HPMC showed less creaming in comparison to Tween-LBG. These patterns of separation and creaming remained consistent at 0 minutes and 120 minutes, and even 24 hours.

Table 6.4. Observations of cream top layer separation at 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 24 hours in the 4 digested samples with 1:2 mixing ratio.

Thickening agent		LBG		HPMC	
		Tween	sucrose	Tween	sucrose
Time scale	30 min				

	60 min				
	90 min				
	120 min				

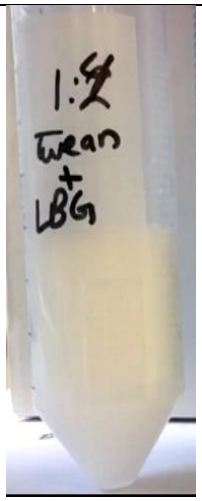
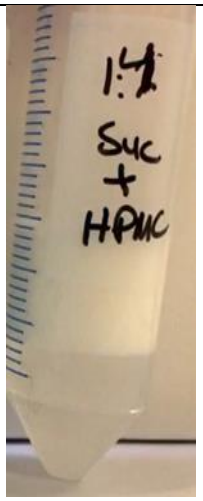
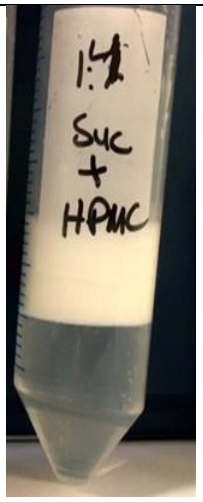
	24 h				

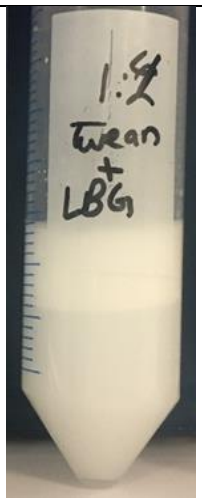
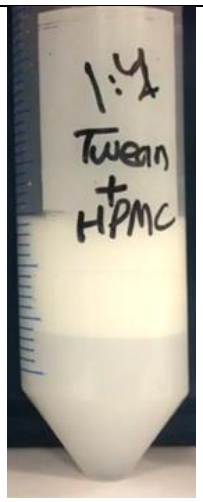
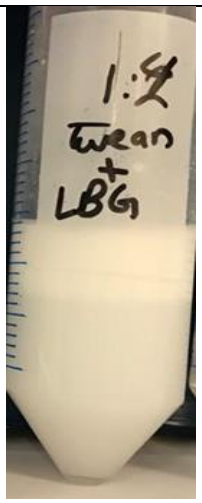
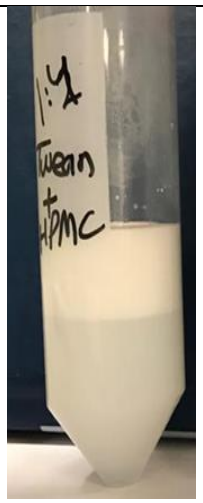
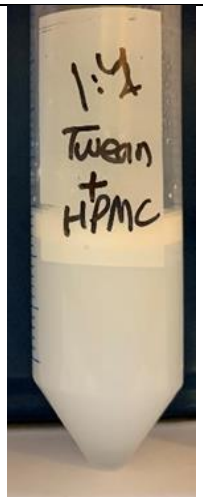
The mixing ratio1:4

Table 6.5 shows the stability of the 4 samples at 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 24 hours. The stability pattern for this ratio follows the same trend as the 1:2 ratio.

Based on the results obtained from the in-vitro testing, it is evident that the fat emulsion containing Tween-HPMC demonstrated the highest level of stability at all timepoints for both the 1:2 and 1:4 mixing ratios. As a result, this emulsion was selected for use in this study.

Table 6.5. Observations of cream top layer separation at 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 24 hours in the 4 digested samples with 1:4 mixing ratio.

Thickening agent		LBG		HPMC	
emulsifier		Tween	sucrose	Tween	sucrose
Time scale	30 min				
	60 min				

	90 min				
	120 min				
	24 h				

6.4 Main “MRI” study data collection

6.4.1 Study design and ethics

Following the development work on the test drinks, a single blinded MRI study was conducted at SPMIC to investigate the effect of fat and carbohydrate macronutrients on satiety in NW and Obese participants. Ethical approval for the study was obtained from the research ethics committee of the Faculty of Medicine and Health Sciences (Sponsor ref: RGS 19047; IRAS ID: 261806) and health research authority (HRA) (IRAS project ID: 261806).

Prior to conducting the pilot MRI study, taste acceptability of the HF and HC drinks were evaluated. The presence of the Tween 20 emulsifier introduced a notable bitter flavour to the fat emulsion. After experimenting with various combinations of flavour compounds, coffee extract (Nielsen Massey Vanillas, Inc, USA) was found the most effective flavour for masking the bitterness associated with the Tween 20 (SIGMA, Aldrich, Gillingham, U.K.) emulsifier. Consequently, this was used to improve the overall taste of the HF emulsion drink. Tween 20 was also added into the HC drink, in order to match the composition of the HF and HC samples. In addition to the coffee extract, taste acceptability of both drinks was enhanced with the addition of 5 artificial sweeteners (Hermesetas, Switzerland). The energy and macronutrient composition of the drinks is shown in Table 6.6.

Table 6.6. Nutrient information per 300 ml of the high fat (HF) emulsion drink and high carbohydrate (HC) drink.

Nutrient	HF drink	HC drink
Energy (kcal)	593.3	586.5
Carbohydrate (g)	0.54	147.3
Of which sugar (g)	0	10.8
Of which fibre (g)	0.84	0
Fat (g)	65.7	0
Protein (g)	0	0

6.4.2 Eligibility criteria

Healthy participants, ranging in age from 18-45 years old with BMI of normal-body weight (BMI 18.5-24.9 kg/m^2) or Obese (BMI 30-40 kg/m^2), were recruited by

advertisement. Four participants with obesity were compared with 4 NW participants. All participants were healthy according to the inclusion criteria explained in section 5.3.3. However, this study had further exclusion criteria, as described below.

- Any reported history of neurological disorders
- Abnormal screening procedures including depression measured by Beck's Depression Inventory (Beck et al., 1961) and eating restriction measured by EAT (Garner and Garfinkel, 1979, Garner et al., 1982).
- Following a medically- or self-prescribed diet during the two weeks prior to the pre-study examination and until the end of the study
- Reported weight loss or gain ≥ 10 % of bodyweight during the six months period before the pre-study examination.
- Pregnancy declared by candidate (Pregnancy tests are also available in the female toilets)
- Left-handed assessed by handedness questionnaire. This is to control for brain's lateralisation effects (activation in one side of the brain) that may show variations between left and right-handed participants.

6.4.3 Sample size calculation

This is a pilot study; hence no power calculation was conducted. However, previous studies have demonstrated significant differences in GE due to meal characteristics using a sample size of 8–12 subjects (Hussein et al., 2015, Marciani et al., 2015), therefore we aimed to recruit 11 participants for this study. This sample size has also been found to be suitable for a pilot brain imaging study assessing the gut-brain axis in response to fat intake (Frank-Podlech et al., 2019). While the original plan was to recruit 11 participants in each group (11 NW and 11 Obese, a total of 22 participants), time constrains led to the inclusion of only 8 participants (4 NW and 4 Obese participants) in this thesis.

6.4.4 Study protocol

Participants were invited to attend a total of 3 visits at the SPMIC: a screening visit, and two MRI study visits (approximately one week apart). On the day prior to each

MRI scan day (visits 2 and 3), participants were instructed to consume a non-fatty meal between 19:00 and 20:00 and subsequently fast, consuming only water. Participants were also advised to refrain from alcohol and strenuous exercise for 24 hours before the MRI visits.

Screening visit

Using online questionnaires, participants were initially screened for MRI eligibility, depression, intake of medication, and general health condition. If participants met the eligibility criteria, they were asked to come to the SPMIC, fasted for at least 12 hours for a blood sample collection to measure their fasting lipid levels and hemoglobin A1c (HbA1c), as well as to measure their weight, height, and blood pressure. Participants were also asked to complete online questionnaires to assess their eating behaviours including TFEQ (Stunkard and Messick, 1985), BES (Gormally et al., 1982), PFS (Cappelleri et al., 2009), PrefQuest (Deglaire et al., 2012), and CoEQ (Dalton et al., 2017).

MRI visit 1

Figure 6.3 shows a schematic diagram of the MRI protocol. Participants were asked to come in the morning (7.30 am) to the SPMIC, fasted for at least 12 hours, to be scanned using the 3T wide-bore Philips Ingenia MRI scanner. Baseline/fasted scans (T0) were collected to measure brain (cerebral blood flow, resting-state fMRI, and visual-task fMRI) and gut (GCV, SBWC, and SMA blood flows) measurements. Participants were then given 300 ml of either the HF or HC drinks to consume outside the scanner within 15 minutes in an upright seated position. Following the consumption of the test drinks (T60), participants were scanned up to 120 mins post-ingestion. Gastrointestinal responses were collected at the following time points: GCV and SBWC at T0, T60, T100, T150 and T180 minutes and SMA blood flow at T0, T40, T70, T90, T110, T140 and T160 minutes.

Brain scans were also collected at T0, T60, T80, T100, and T130. Satiety questionnaires were measured using the VAS scale at T0, T60, T120, and T180 minutes, and blood samples were collected at timepoints T0, T60, T90, T110, T130, T160 and T180 minutes to measure satiety and appetite regulators.

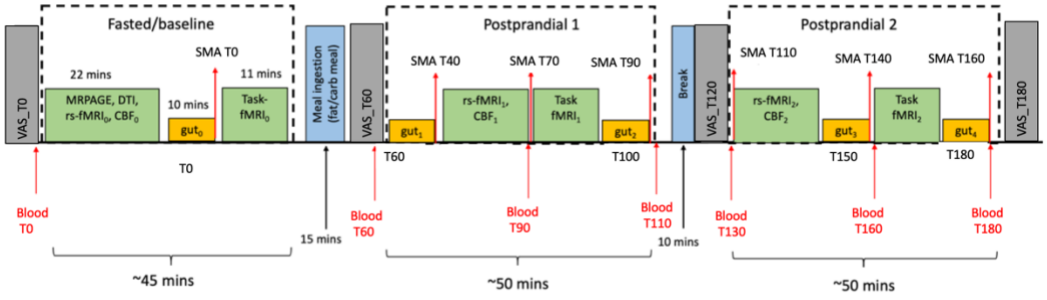


Figure 6.3. MRI study protocol.

MRI visit 2

At least a week after the MRI visit 1, participants were invited to undergo a second MRI scan visit, that was identical to MRI visit 1, but with the different drink. Participants were blinded to the drinks.

6.4.5 Gut imaging protocol

GCV: was measured using a sequence of coronal HASTE acquiring 28 contiguous axial slices with a slice thickness of 7 mm with a 1 mm gap between slices and reconstructed in-plane resolution 0.78 mm × 0.78 mm and compressed SENSE of 3. The parameters of the sequence were optimised by Dr Caroline Hoad using flip angle 90, TR=707 milliseconds, TE=40 milliseconds. The data were acquired during 1 breath-hold of 20 s.

SBWC: was acquired as previously described (Hoad et al., 2007, Marciani et al., 2013b) using a sequence of coronal single-shot fast spin echo acquiring two stacks of 14 contiguous coronal slices with slice thickness 7 mm with no gaps between slices, reconstructed in-plane resolution 0.78 mm × 0.78 mm and SENSE of 2.2, flip angle 90, TE=400 ms, TR=1728 ms. The data were acquired during 2 breath holds of 24 s each.

SMA blood flow: was collected using a sequence of phase-contrast MRI angiography with 20 cardiac phases acquiring 14 contiguous coronal slices with slice thickness 6 mm with no gaps between slices, reconstructed in-plane resolution 1.17 mm × 1.17 mm, and SENSE of 3, flip angle 25°, TE (shortest), TR (shortest),

and velocity encoding 140 cm/s for baseline and 200 cm/s for other time points. The data were acquired during a breath hold of under 20 s and varied in length due to differences in heart rate between participants as it depends on the cardiac cycle for each participant. An MRI-compatible PPU was used to allow cardiac gating for the SMA blood flow scan.

6.4.6 Data analysis

The MRI analysis methods for GCV, SBWC and SMA blood flow were carried out as described previously in section 5.3.7. However, as the GCV was collected for only four timepoints, T_{50} and GE rate was not calculated in this study due to the small number of points.

6.4.7 Statistical analysis

Type of data presented, and the normality test was conducted as explained in section 5.3.8. To assess the effect of HF and HC macronutrient on GI responses regardless of body weight, data from both NW and Obese participants were combined. A two-way ANOVA for repeated measures was used to analyse the data, with the type of drink and time as the main effects of interactions. If the two-way ANOVA revealed significant differences between the drinks, further analysis was conducted using the Bonferroni post hoc test. The total AUC for postprandial responses following each drink was calculated using the trapezoid rule (Section 5.3.8). The AUC was calculated from 0-180 minutes by the GraphPad Prism for the area under the reading curve down to fasting baseline values. The peaks that were below the baseline value were also considered in the AUC calculation. An unpaired-t-test or Mann-Whitney test was used to test differences between drinks (HF vs. HC) in baseline (fasting) levels and total AUC.

Data analysis was also conducted for each participant group (NW vs. Obese) and for each type of drink (HF vs. HC) was also conducted. However, statistical analysis was deemed inappropriate and would not yield valid results due to the small number of participants included in each group (n=4). Therefore, instead, a simple descriptive analysis is presented (mean and SD, or median and IQR). The next section describes the results of this study. Data form the combined participant's

groups, after each drink, is first presented, followed by descriptive results for each NW and Obese group following the HF and HC drinks.

6.5 Results

6.5.1.1 Participant's demographic characteristics

Ninety-five participants contacted the research team and were assessed for eligibility. Twenty-two participants were screened for the study, of which 4 NW and 4 Obese participants were included in this Chapter (Figure 6.4). Table 6.7 summarises the demographic characteristics of the participants.

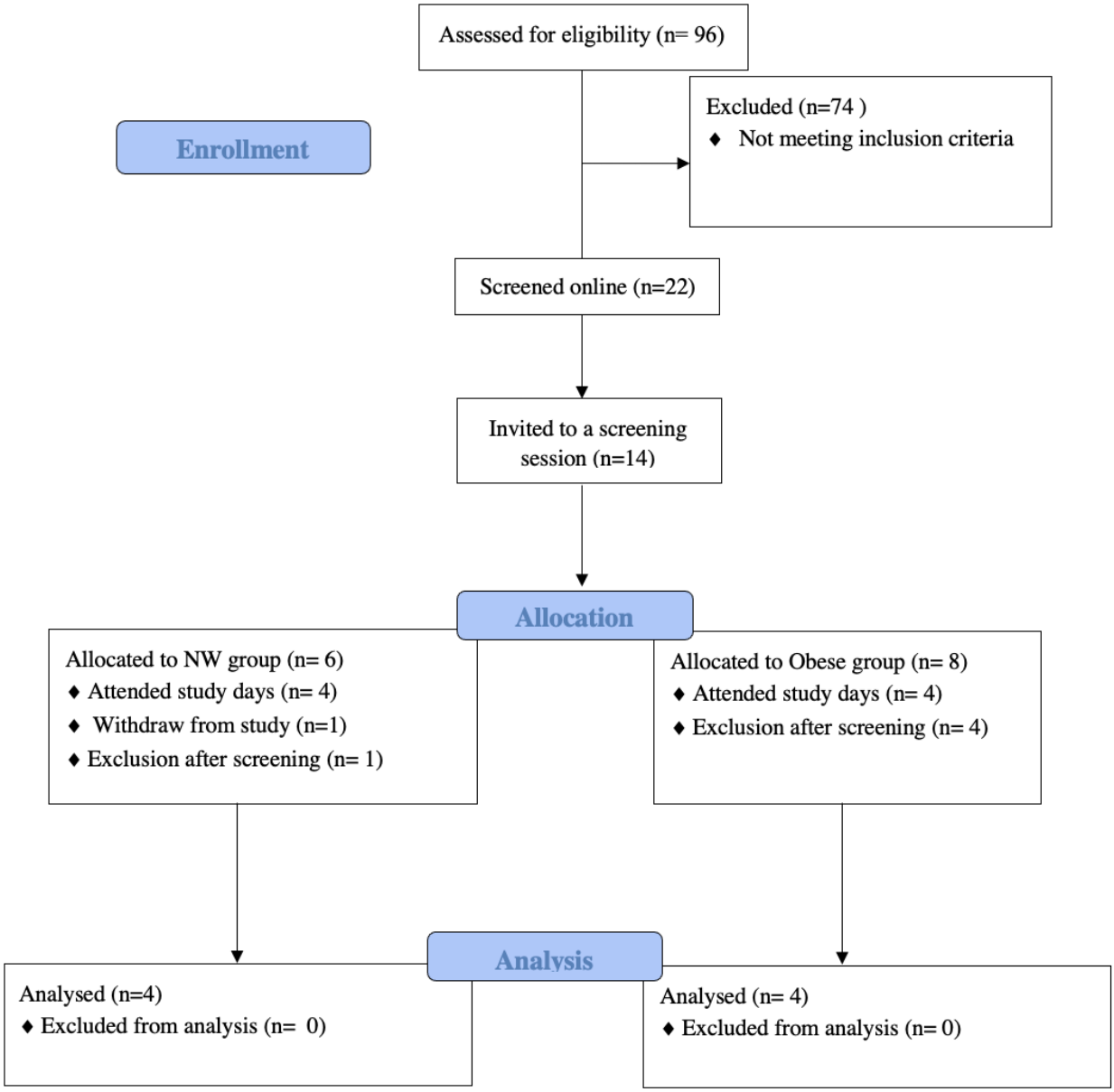


Figure 6.4. Recruitment flow diagram

Table 6.7. Summary of demographic characteristics of participants. Data are presented as the mean ± SD.

	NW group	**Obese group**
Age (year)	29.5 ± 5.9	35.2 ± 4.9
Gender	All female	2 female & 2 males
BMI (kg/m^2)	22.3 ± 1.1	31.6 ± 0.96

6.5.1.2 Gastric content volume

Good quality images of the HF and HC drinks in the stomach were obtained. Figure 6.5 illustrates an example of gastric scans form a NW participant, whereas Figure 6.6 shows scans from an individual with obesity. Images from the HF drinks show a homogeneous emulsion drink, indicating the stability of fat emulsion in the stomach.

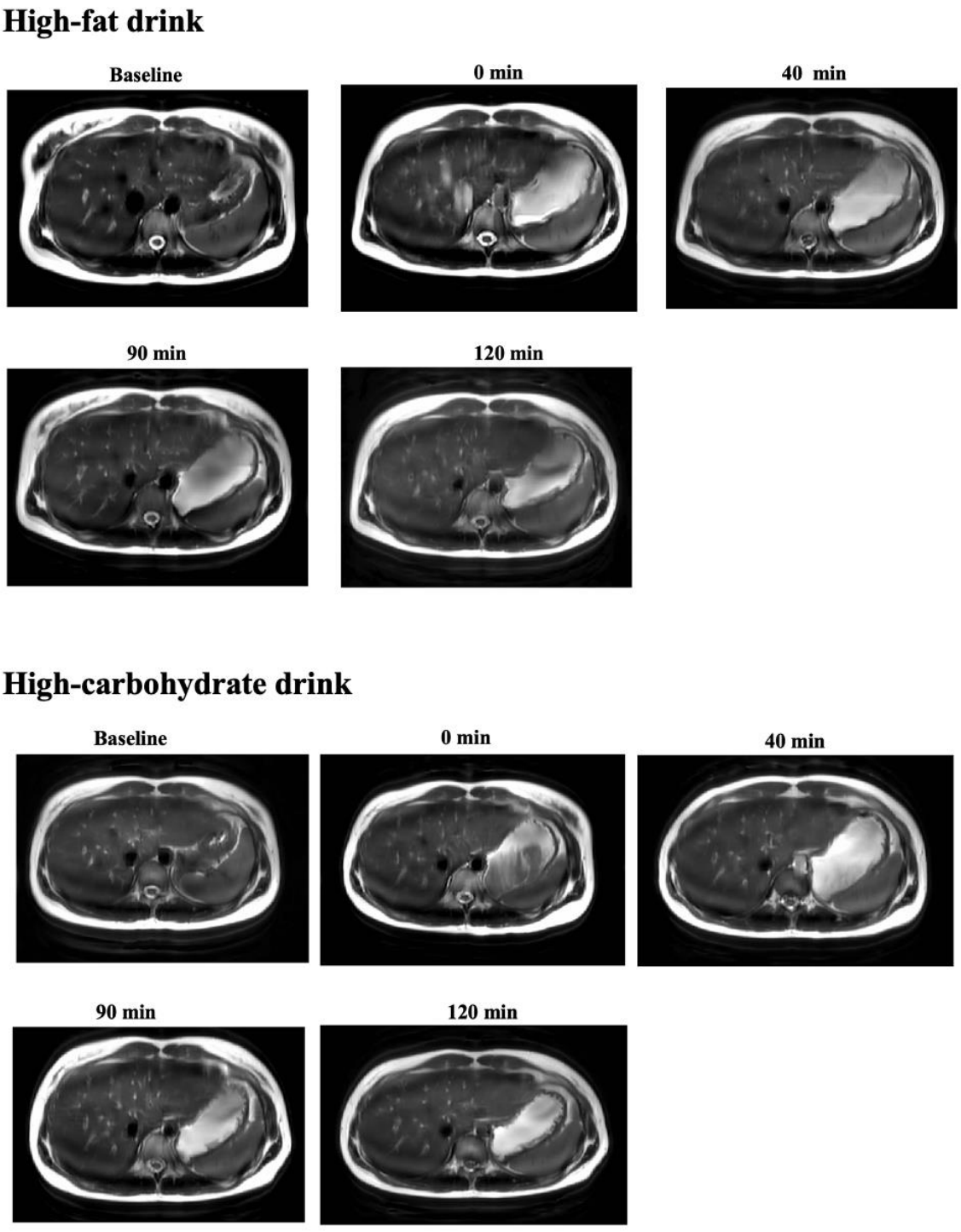


Figure 6.5. Examples of MRI scans of a participant with normal-weight showing the stomach at fasting/baseline and at different time points following the consumption of the high-fat and high-carbohydrate drinks.

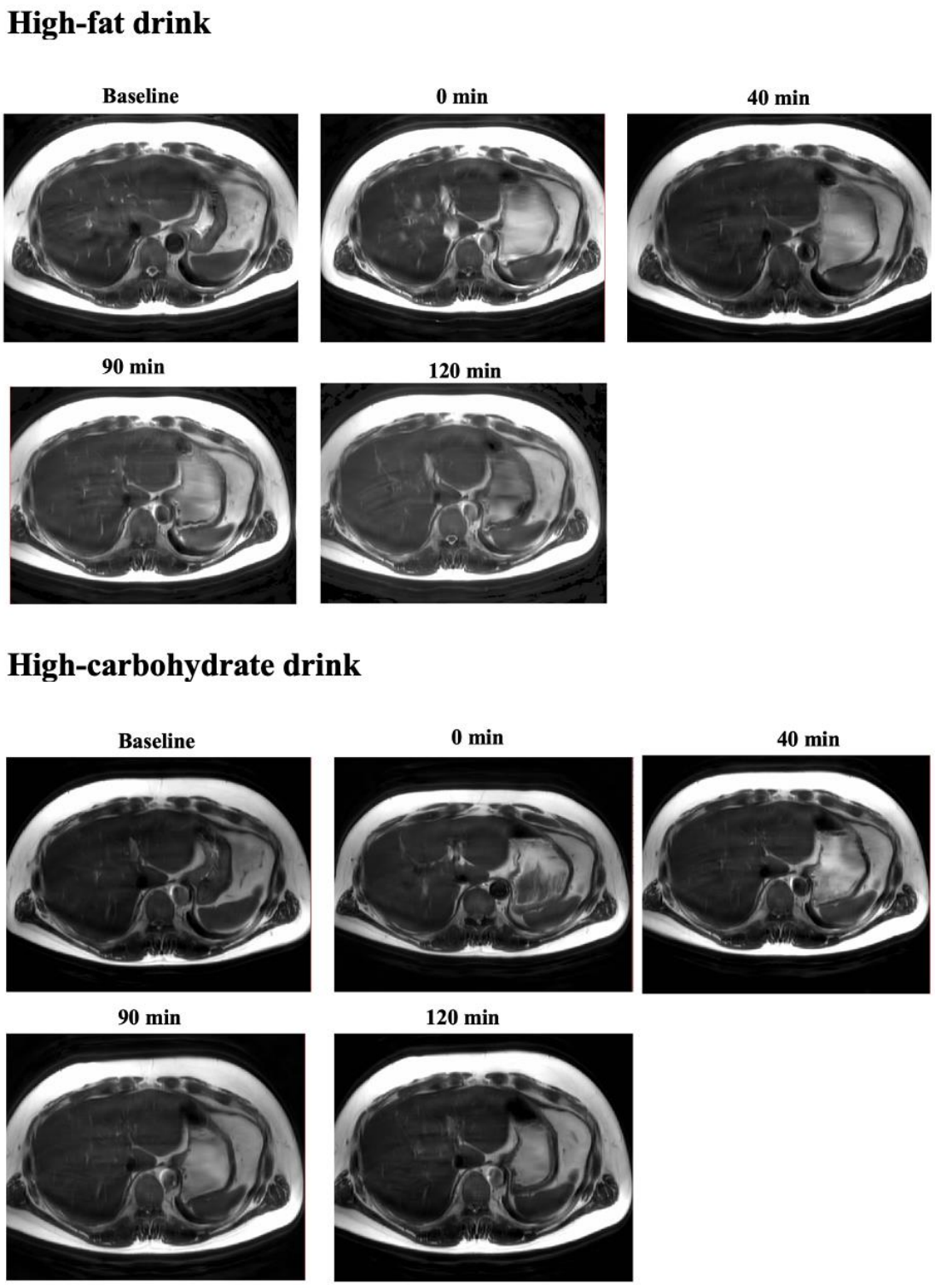


Figure 6.6. Examples of MRI images of a participant with obesity showing the stomach at fasting/baseline and at different time points following the consumption of the high-fat and high-carbohydrate drinks.

Figure 6.7 shows the time courses of the median GCV of the HF and HC drinks across the combined NW and Obese groups. At baseline (fasted), GCV did not show significant different between the HF and HC drinks (P= 0.93, Table 6.8). However, as expected, GCV decreased with time for both drinks. While there is a suggestion of higher GCV values following the HF drink (Figure 6.7), this did not reach statistical significance (drink effect, P= 0.11; drink × time interactions, P=0.1). AUC for postprandial GCV did not differ between the HF and HC drinks (Table 6.9).

Table 6.8. Bassline (fasting) levels of gastric content volume (GCV) at the high-fat (HF) and high-carbohydrate (HC) drinks. Data are presented as the median and (IQR).

Fasting levels		**HF drink**	**HC drink**	**P-value**
Combined participant groups				
GCV (ml)		40 (24)	33 (70)	0.93
Separate participant groups				
GCV (ml)	NW group	40.5 (83.5)	40 (80.5)	Not applicable
	Obese group	34 (24)	33 (70.3)	

6.5.2

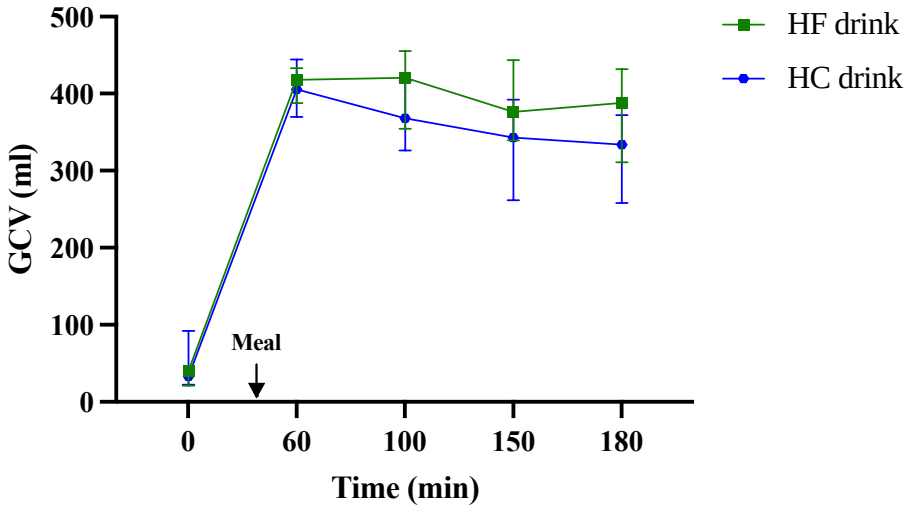


Figure 6.7. The gastric content volume (GCV) over time following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks for combined participant groups. Values are presented as the median and IQR, n=8 for each drink.

Table 6.9. Total area under curve (AUC) following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks across the combined groups. Data are presented as the mean± SD.

	HF drink	**HC drink**	**P-value**
MRI measurements			
GCV (ml) from 0-150 min	1254 ± 188.5	1096 ± 216.9	0.14
SMA blood flow (ml/s) from 0-160 min	57.54 ± 24	40.98 ± 30.75	0.25
SBWC (ml) from 0-150 min	1089 ± 632.4	189.3 ± 293.3	0.0026
Satiety VAS scores from 0-180 min			
DTE (mm)	18.03 ± 58.32	27.27 ± 62.45	0.76
Hunger (mm)	21.11 ± 60.2	18.4 ± 72.9	0.93
PFI (mm)	11.5 ± 38.5	15.5 ± 67.4	0.88
Fullness (mm)	24.2 ± 68.5	25.9 ± 65.9	0.95
CSS (mm)	8.7 ± 43.2	17.8 ± 55.3	0.72

CSS, combined satiety scores; DTE, desire to eat; GCV, gastric content volume; PFI, prospective food intake; SBWC, small bowel water content; SMA, superior mesenteric artery; VAS, visual analogue scale.

As a second step, we visually assessed the effect of each macronutrient on the GCV for the NW and Obese groups individually. Figure 6.8 show the time courses of the mean GCV of the HF and HC drinks in the NW and Obese groups. No differences were observed between the drinks or across the groups at baseline (fasted) (Table 6.8). However, as expected, GCV decreased with time for both drinks in the NW and Obese groups. At 120 minutes, both the NW and Obese groups had a similar GCV after the HF drink (379 ± 32 ml vs. 374 ± 44 ml respectively). In terms of the HC drink, GCV showed a lower level in the Obese group following the HC drink

compared to the NW group (Figure 6.8). Table 6.8 illustrates the CCV values for both the fasted state combined across the NW and Obese groups and separated by group.

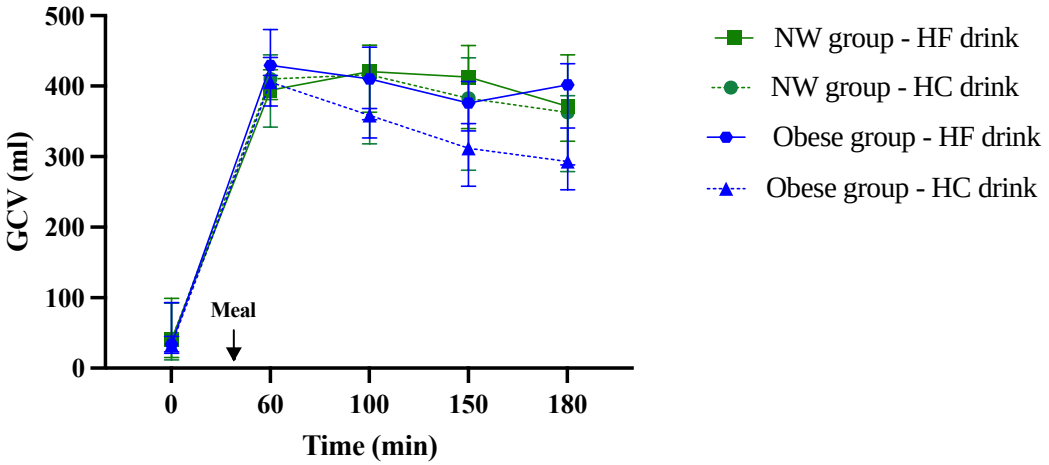


Figure 6.8. The gastric content volume (GCV) over time following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks in normal-weight (NW) and Obese participants. Values are presented as the median and IQR, n=4 in each group.

1.1.1.1 Superior mesenteric artery blood flow

Figure 6.9 shows the time courses of the mean SMA blood flow following the HF and HC drinks. At baseline SMA blood flow, no significant difference between the HF and HC drinks (P = 0.69, Table 6.9) was found. However, the SMA flow rate increased immediately after both drinks, with the highest value at 30 minutes for the HF drink, whereas the HC drink showed a decrease in SMA flow levels (Figure 6.9). Although no significant differences were found using the two-way ANOVA (drink effect P = 0.18; drink × time interactions, P = 0.28), there was a higher pattern of SMA flow rate following the consumption of the HF drink compared to the HC drink (Figure 6.9). Interestingly, rates of blood flow at 70 minutes post-drink were similar for the HF and HC drinks (13.11 ± 2.3 vs. 13.7 ± 5.9 ml/s, respectively). However, while the blood flow increased following the HF drink at 100 and 120 minutes, it decreased following the HC drink (Figure 6.9).

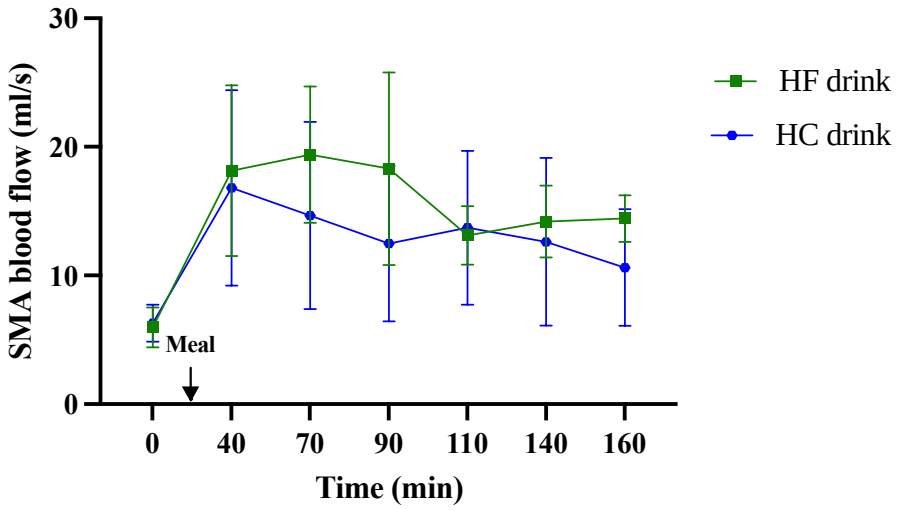


Figure 6.9. Superior mesenteric artery (SMA) blood flow values over time following the consumption of high-fat (HF) and high-carbohydrate (HC) drinks for combined participant groups. Values are presented as the mean and SD, n=8 for each drink.

Table 6.10. Bassline (fasting) levels of superior mesenteric artery (SMA) blood flow at the high-fat (HF) and high-carbohydrate (HC) drinks.

Fasting levels	**HF drink**	**HC drink**	**P-value**
Combined participant groups			
SMA blood flow rate (ml/s)	5.9 ±1.6	6.3 ± 1.4	0.69

(mean ± SD)				
Separate participant groups				
SMA blood flow rate (ml/s) [median (IQR)]	NW group	6.3 (3.6)	6.4 (3.7)	Not applicable
	Obese group	5.5 (2.7)	5.8 (0.2)	

6.5.3

Figure 6.10 displays the time courses of the mean SMA blood flow following the HF and HC drinks in the NW and Obese groups. Interestingly, the SMA in the Obese group showed highest blood flow immediately after consumption of the HF drink, which may have contributed to the increase in SMA observed in Figure 6.10. Table 6.8 provides an overview of the SMA values for both the fasted state combined across NW and Obese groups and separated by group.

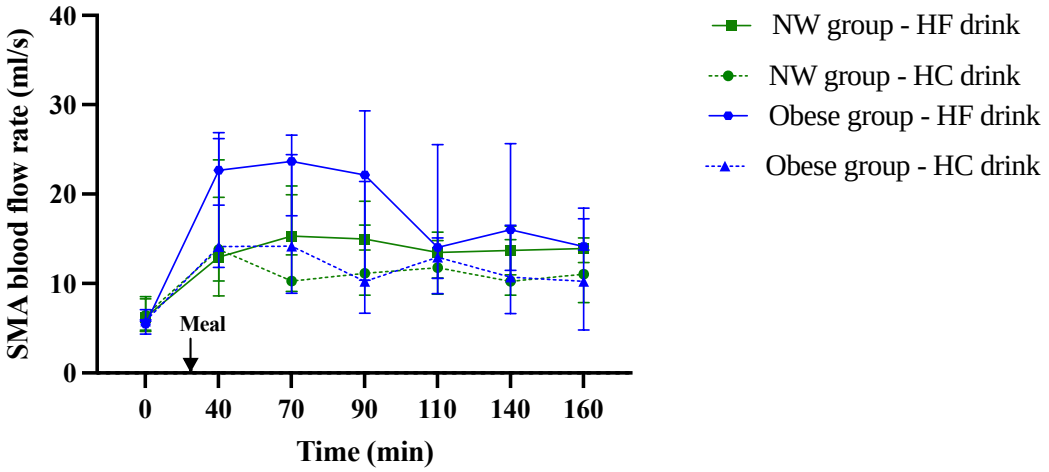


Figure 6.10. Superior mesenteric artery (SMA) blood flow values over time for normal-weight (NW) and Obese participants following the consumption of high-fat (HF) and

high-carbohydrate (HC) drinks in the combined groups. Values are presented as the median and IQR, n=4 in each group.

6.5.3.1 Small bowel water content

Figure 6.11 displays the time courses of the mean SBWC following each drink. At baseline (fasted), there was a trend for lower SBWC levels for the HF drink compared to the HC drink (P=0.139, Table 6.11). However, SBWC increased immediately after the consumption of the HF drink and continued to rise until 120 minutes post-drink (Figure 6.11). In contrast, SBWC decreased immediately after the consumption of the HC drink and remained lower until 120 minutes post-drink (Figure 6.11).

Table 6.11. Bassline (fasting) levels of small bowel water content (SBWC) at the high-fat (HF) and high-carbohydrate (HC) drinks.

Fasting levels		**HF drink**	**HC drink**	**P-value**
Combined participant groups				
SBWC (ml) (mean ± SD)		83.8 ± 52.5	146.1 ± 97.4	0.139
Separate participant groups				
SBWC (ml) [median (IQR)]	NW group	65.5 (110)	102.5 (198.8)	Not applicable
	Obese group	67 (85.25)	245 (183)	

The two-way ANOVA test revealed a significant difference in the SBWC between the HF and HC drinks (drink effect, P<0.0001, Figure 6.11). In addition, there was also a significant drink × time interaction (P<0.0001). The AUC for postprandial SBWC was significantly higher for the HF drink compared to the HC drink (P = 0.0026, Table 6.9). Post-hoc analysis showed significantly higher SBWC levels at 0 minutes (P=0.01), 40 minutes (P= 0.002), 90 minutes (P=0.012), and 120 minutes (P=0.0005) following the consumption of the HF drink compared to the HC drink (Figure 6.11).

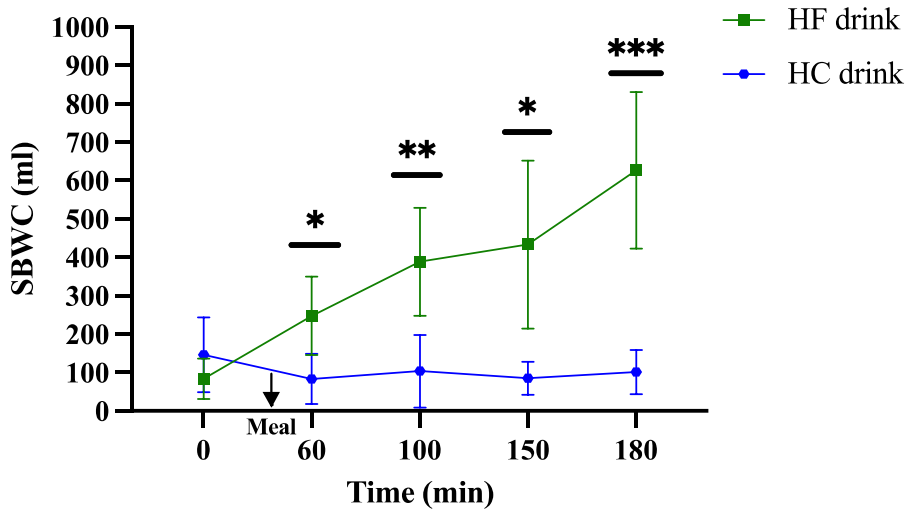


Figure 6.11. Small bowel water content (SBWC) volume over time following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks for combined participant groups. Values are presented as the mean and SD, n=8 for each drink.

Figure 6.12 displays the time courses of the mean SBWC following each drink in the NW and Obese groups. No differences were observed at baseline across study groups except for the Obese group at the HC drink visit which had a slightly higher levels than other groups (Figure 6.13). While the SBWC was shown to increase linearly in both groups following the consumption of the HF drink, Obese group seems to show higher SBWC volumes at 120 minutes post-HF drink. Examples of MIP images of the segmented SBWC of NW and Obese participants at baseline and after consumption of the drinks are shown in Figures 6.13 and 6.14. Table 6.11

shows the SBWC values for both the fasted state combined across the NW and Obese groups and separated by group.

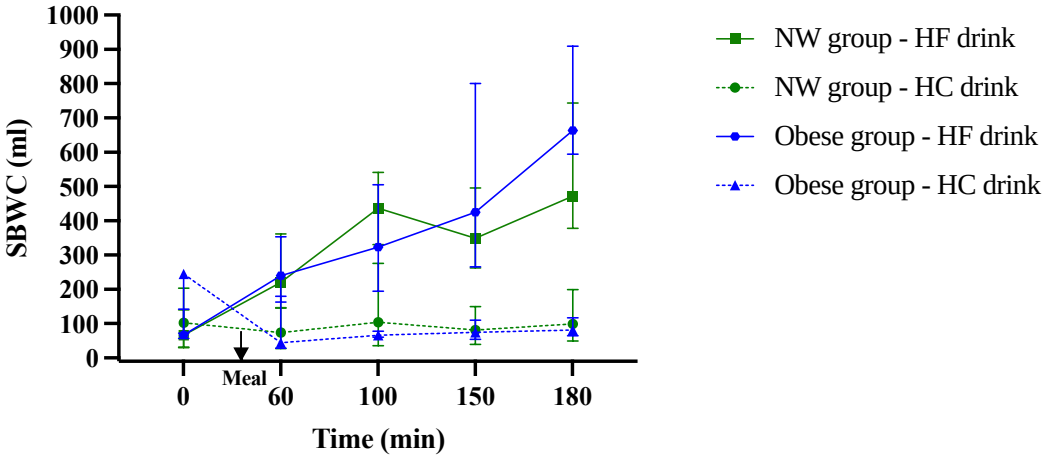


Figure 6.12. Small bowel water content (SBWC) volume over time for normal-weight (NW) and Obese participants following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks. Values are presented as the median and IQR, n=4 in each group.

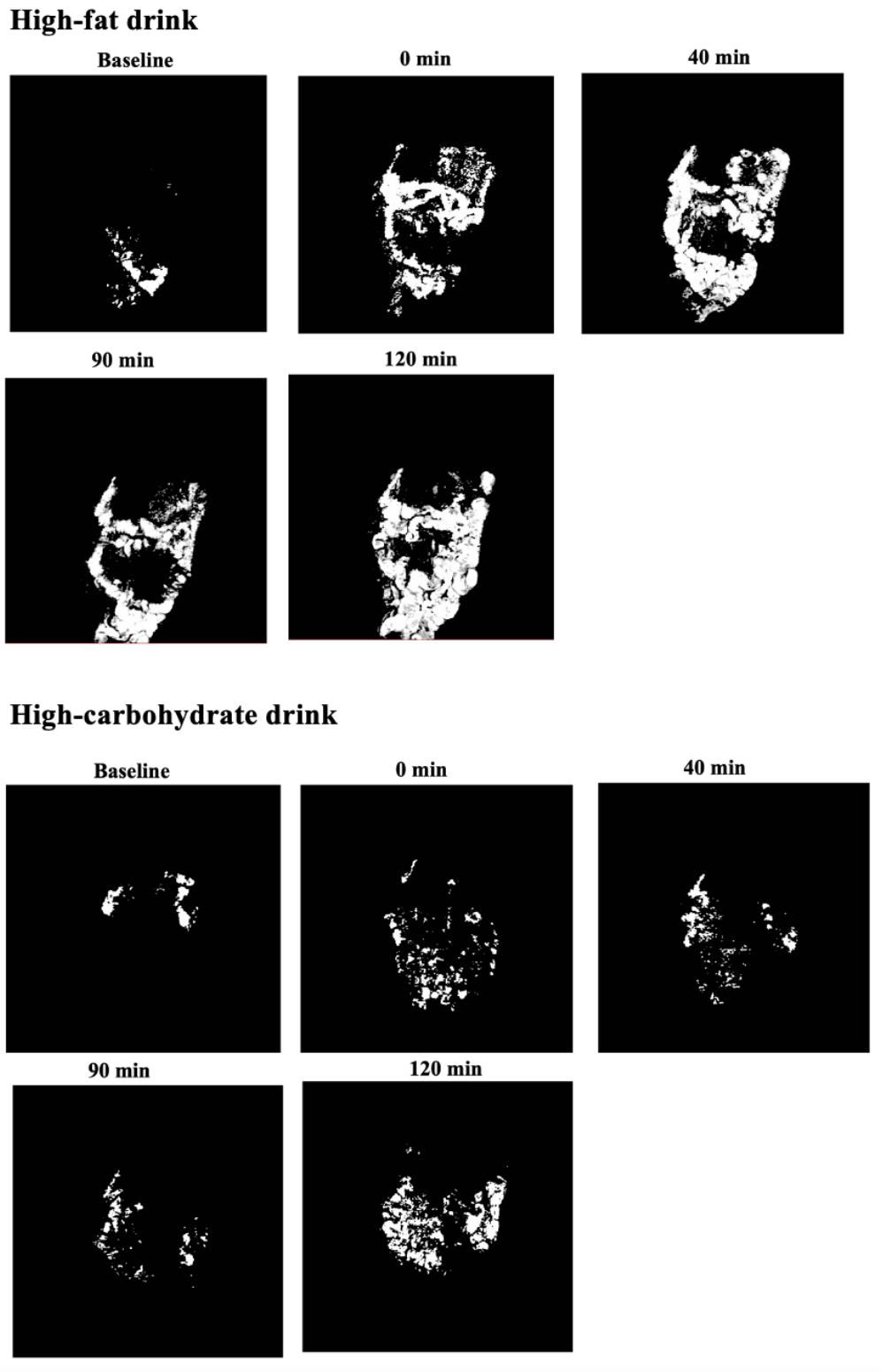


Figure 6.13. Examples of maximum intensity projections (MIP) images of small bowel water content of a normal-weight (NW) participant at fasting/baseline and at different time points following the consumption of the high-fat and high-carbohydrate drinks.

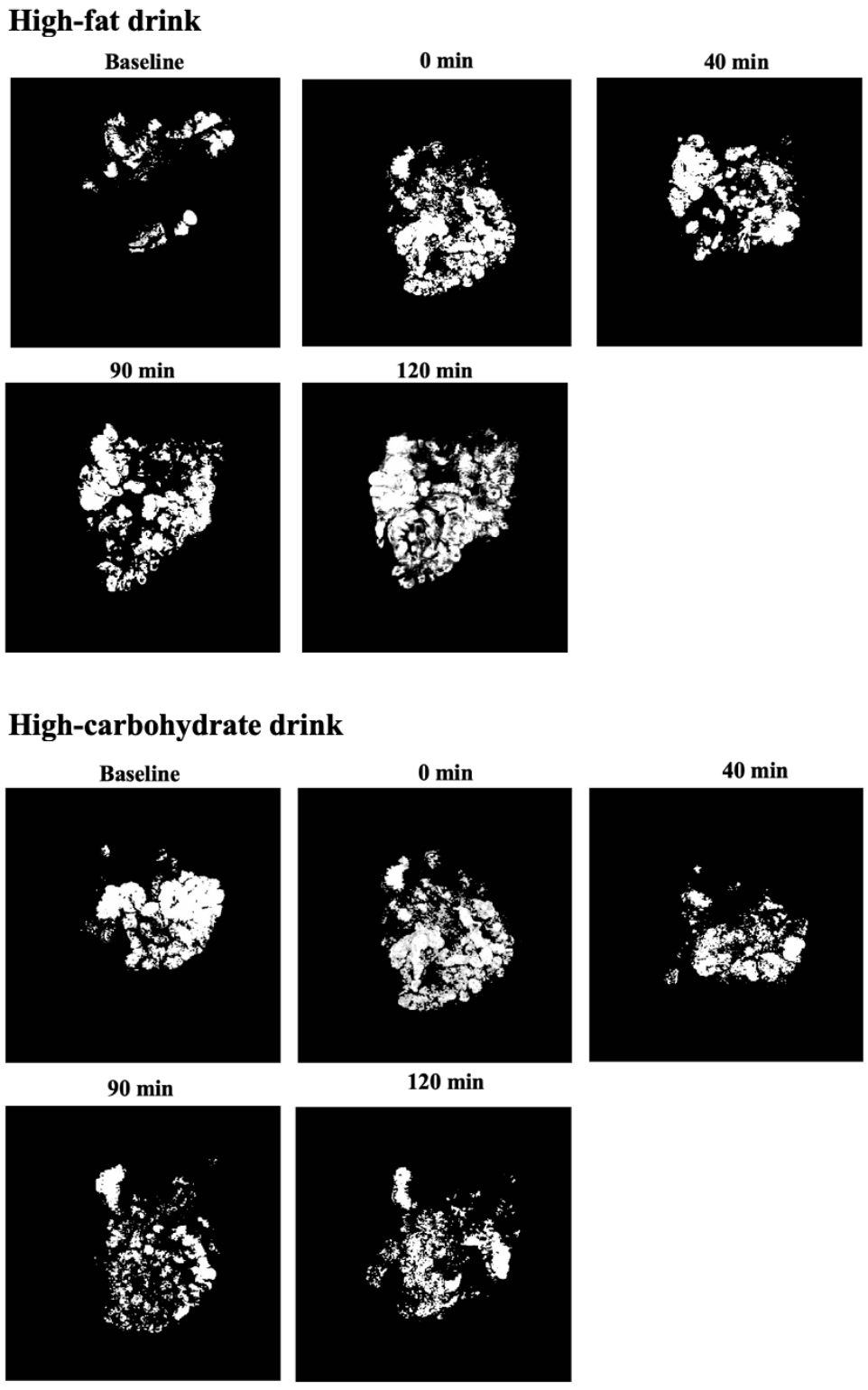


Figure 6.14. Examples of maximum intensity projections (MIP) images of small bowel water content of a participant with obesity at fasting/ baseline and at different time points following the consumption of the high-fat and high-carbohydrate drink.

6.5.3.2 Subjective satiety

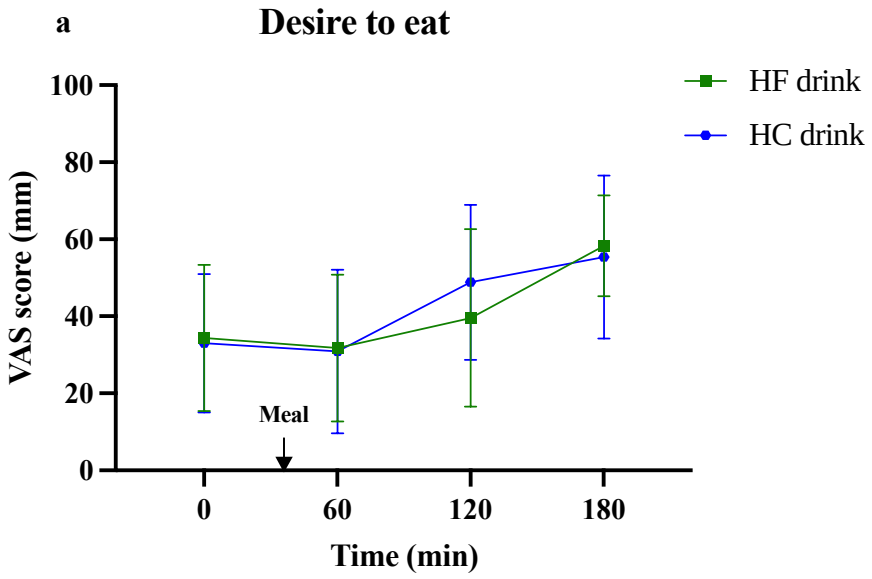
No differences in VAS ratings (desire to eat, hunger, fullness, prospective food) or CSS were found between the HF and HC drinks at baseline/fasted (Table 6.12) or following the drinks, as illustrated in Figures 6.15a, 6.15b, 6.15c, 6.15e.

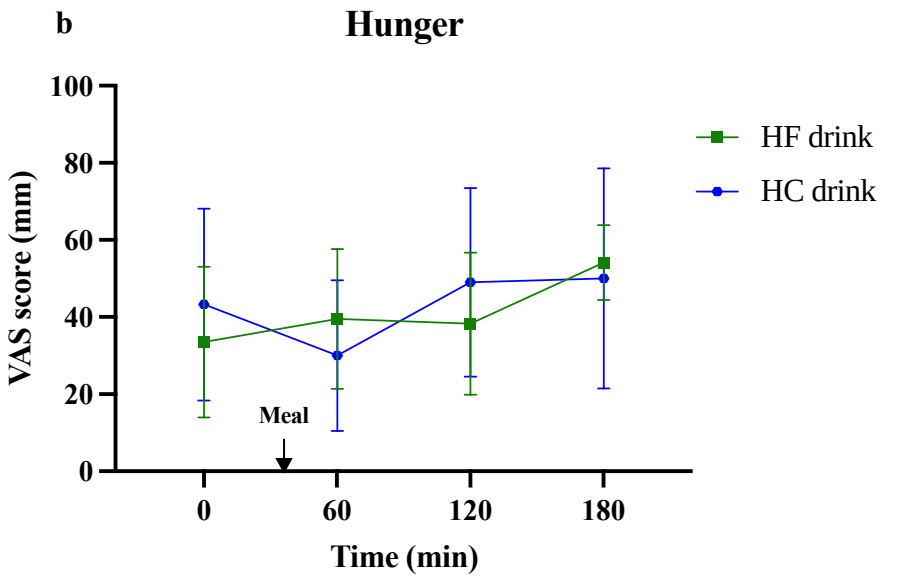
Table 6.12. Bassline (fasting) levels of the visual analogue scale (VAS) domains and composite satiety scores (CSS).

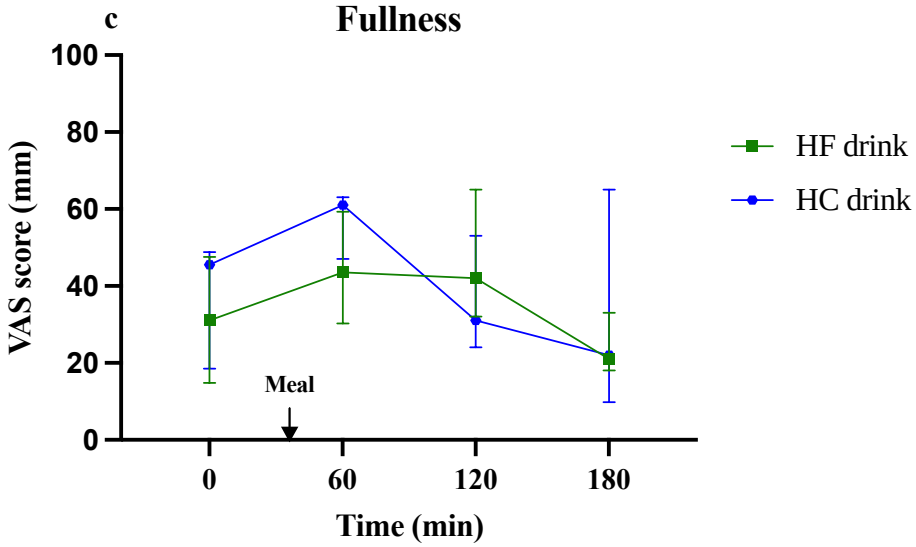
Fasting levels of VAS domains		**HF**	**HC**	**P-value**
Combined participant groups				
DTE (mm) (mean ± SD)		18.03 ± 58.32	27.27 ± 62.45	0.90
Hunger (mm) (mean ± SD)		21.11 ± 60.2	18.4 ± 72.9	0.39
PFI (mm) (mean ± SD)		11.5 ± 38.5	15.5 ± 67.4	0.31
Fullness (mm) (mean ± SD)		24.2 ± 68.5	25.9 ± 65.9	0.50
CSS (mm) (mean ± SD)		8.7 ± 43.2	17.8 ± 55.3	0.48
Fasting levels for each participant group				
DTE (mm) (mean ± SD)	NW group	48.5± 15.2	45.8 ± 15.6	Not applicable
	Obese group	20.3± 8.8	31.8 ± 33.8	
Hunger (mm) [median (IQR)]	NW group	47.5 (37.25)	57 (17)	
	Obese group	24.5 (22.25)	24 (54.5)	
PFI (mm) [median (IQR)]	NW group	45 (19.5)	46.5 (28.75)	
	Obese group	37.5 (39.75)	48 (3.5)	
Fullness (mm) (mean ± SD)	NW group	31.3 ±13.9	38 ± 27.4	

	Obese group	36.3 ± 29.8	39.5 ± 14.6	
CSS (mm) (mean ± SD)	NW group	48.3 ± 10.2	47 ± 15.4	
	Obese group	62.3 ± 15.2	57.8 ± 18.3	

CSS, combined satiety scores; DTE, desire to eat; GCV, gastric content volume; PFI, prospective food intake.




b
Hunger
100
80
60
40
20
0
VAS score (mm)
Meal
0
60
120
180
Time (min)
HF drink
HC drink


c
Fullness
100
80
60
40
20
0
VAS score (mm)
Meal
0
60
120
180
Time (min)
HF drink
HC drink

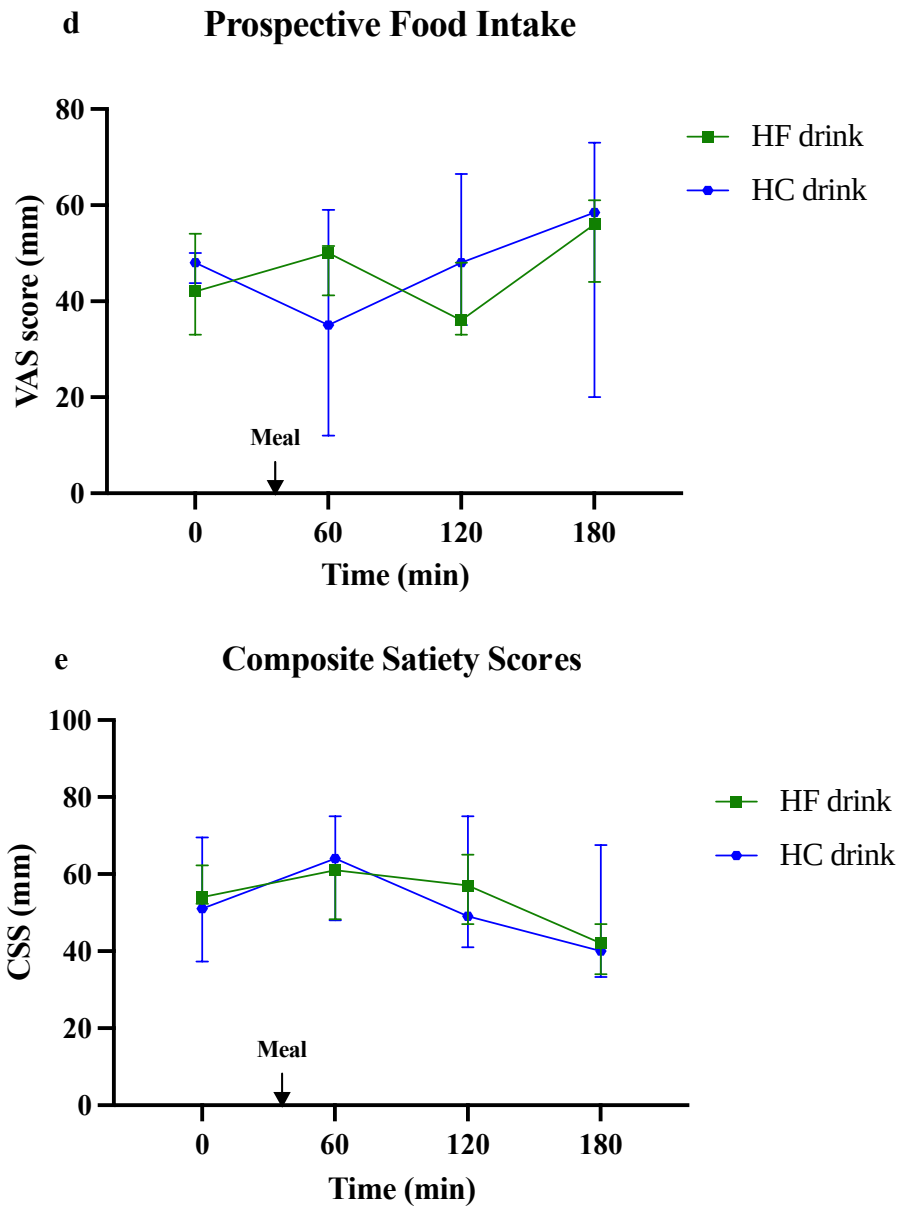
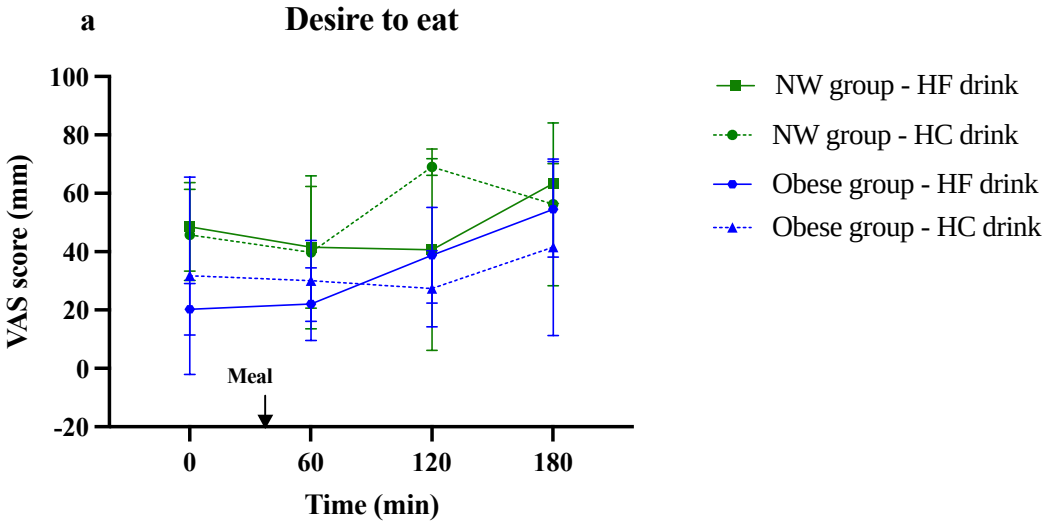
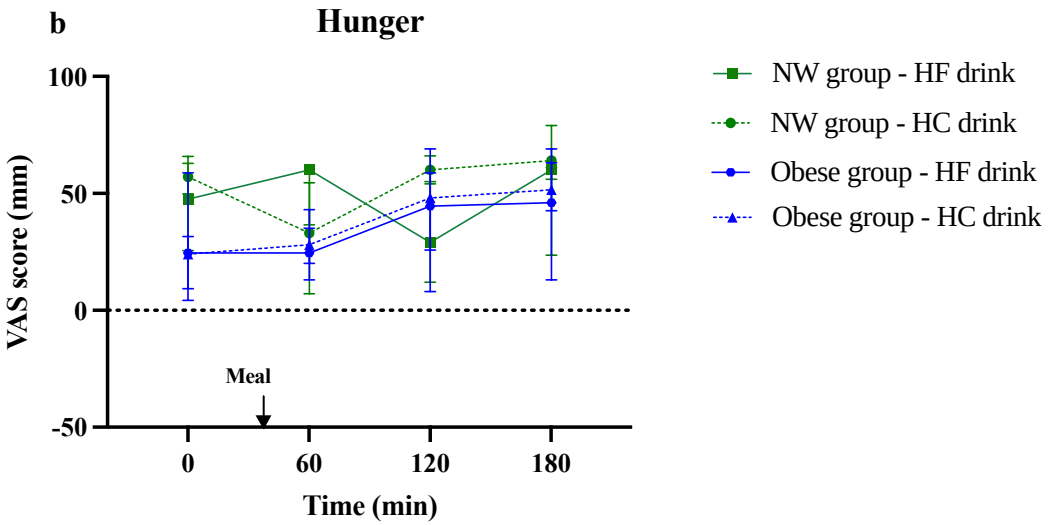
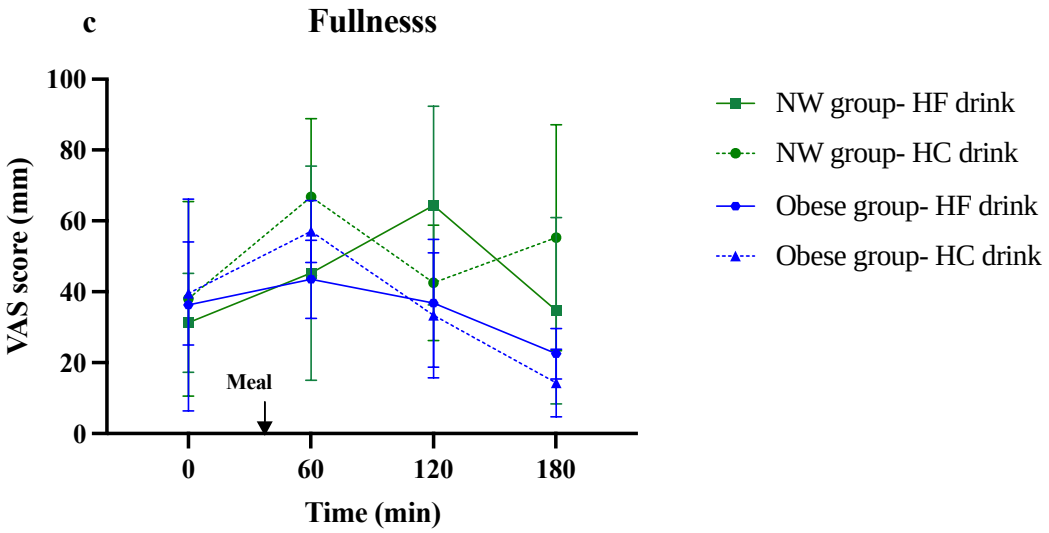


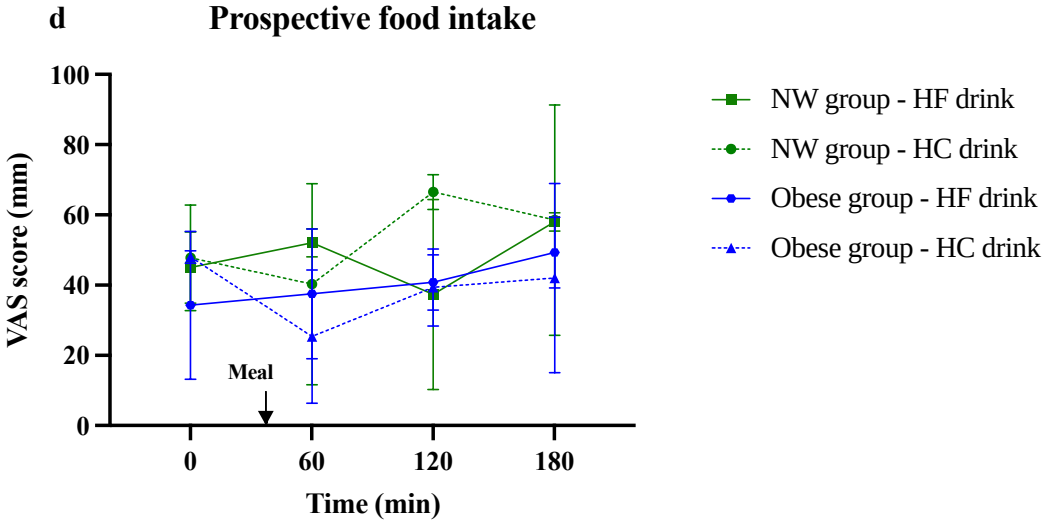
Figure 6.15. Subjective satiety ratings over time using Visual Analogue Scale (VAS) scores of (a) desire to eat, (b) hunger, (c) fullness, (d) prospective food intake, and (e) composite satiety scores at fasted and following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks for combined groups. Values are presented as the mean and SD for desire to eat and hunger, median and IQR for the others, n=8 for each drink.

In terms of each group, there was a pattern toward higher feelings reported by the NW group for desire to eat, hunger, fullness, and prospective food intake following the HC drink compared to the Obese group (Figure 6.16a, 6.16b, and 6.16c). Additionally, CSS was lower for the NW group compared with the Obese group after the HC drinks (Figure 6.16e). No differences were visually observed in fullness ratings between groups and drinks (Figure 6.16d).




b
Hunger
100
50
0
-50
VAS score (mm)
Meal
0
60
120
180
Time (min)
NW group - HF drink
NW group - HC drink
Obese group - HF drink
Obese group - HC drink


c
Fullnesss
100
80
60
40
20
0
VAS score (mm)
Meal
0
60
120
180
Time (min)
NW group- HF drink
NW group- HC drink
Obese group- HF drink
Obese group- HC drink


d
Prospective food intake
100
80
60
40
20
0
VAS score (mm)
Meal
0
60
120
180
Time (min)
NW group - HF drink
NW group - HC drink
Obese group - HF drink
Obese group - HC drink

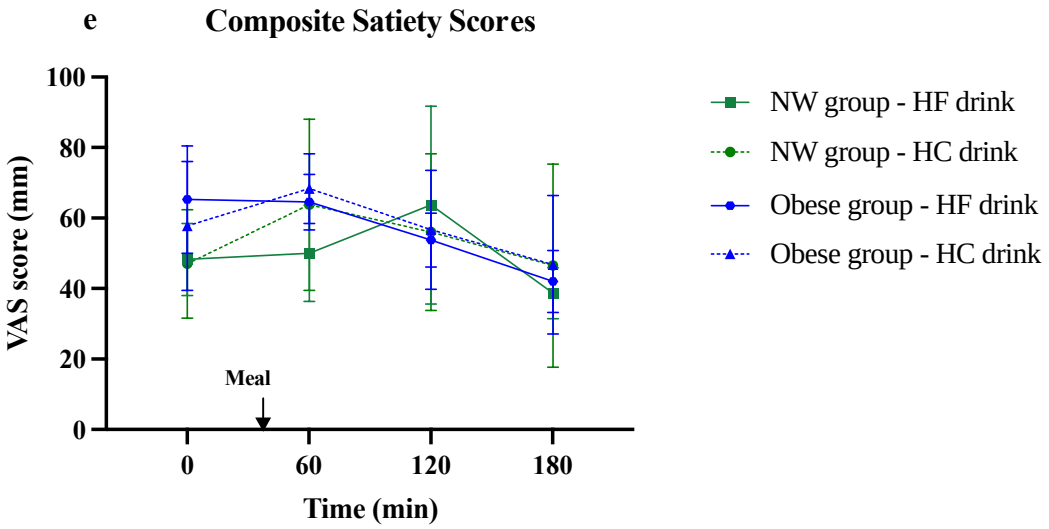


Figure 6.16. Subjective satiety ratings over time using Visual Analogue Scale (VAS) scores of (a) desire to eat, (b) hunger, (c) fullness, (d) prospective food intake, and (e) composite satiety scores at fasted and following the consumption of the high-fat (HF) and high-carbohydrate (HC) drinks in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, except for hunger, which is presented as the median and IQR, n=4 in each group.

6.5.4 Eating behaviour questionnaires

6.5.4.1 Three-eating factor questionnaire

Four domains were measured in the TFE questionnaire: restriction, disinhibition, and hunger. As expected, there is a trend for higher scores seen for the Obese group compared with the NW group in all domains and aggregate scores (Figure 6.17).

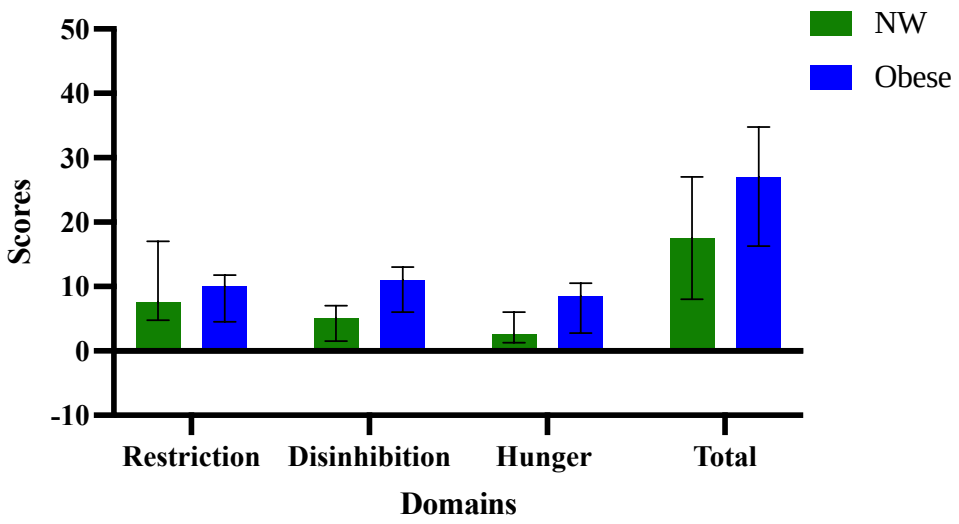


Figure 6.17. Three-factor eating (TFE) scores in normal-weight (NW) and Obese participants. Values are presented as the median and IQR, n=4 in each group.

6.5.4.2 Control of eating questionnaire

Four domains were measured in this questionnaire: craving control, craving for sweets, craving for savoury and positive mood. The data for this questionnaire was available for analysis from 3 NW and 3 Obese participants. As expected, scores for craving control were higher in the Obese group compared with the NW group (Figure 6.18). Unexpectedly, scores for higher positive mood were seen in the NW group compared with the Obese group. For other domains, no differences appear to find between groups.

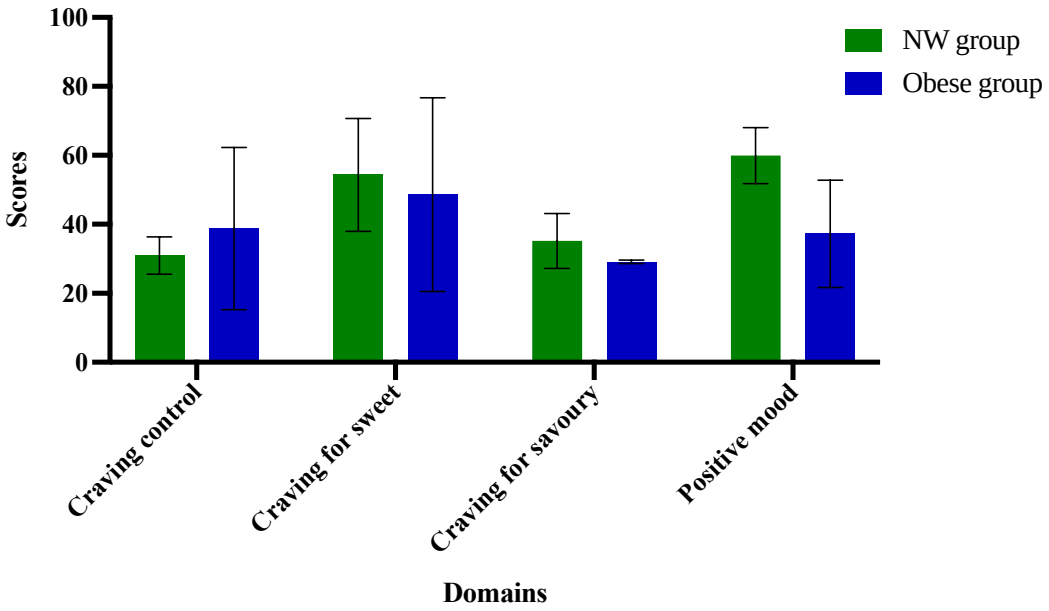


Figure 6.18. Control of eating questionnaire (CoEQ) scores in normal-weight (NW) and 2 Obese participants. Values are presented as the mean and SD, n= 3 for NW and n=2 for Obese.

6.5.4.3 Binge eating scale

The data for this questionnaire was available for analysis from 3 NW and 3 Obese participants. Surprisingly, the NW group had a higher score than the Obese group (Figure 6.19), though there were very large inter-individual variations.

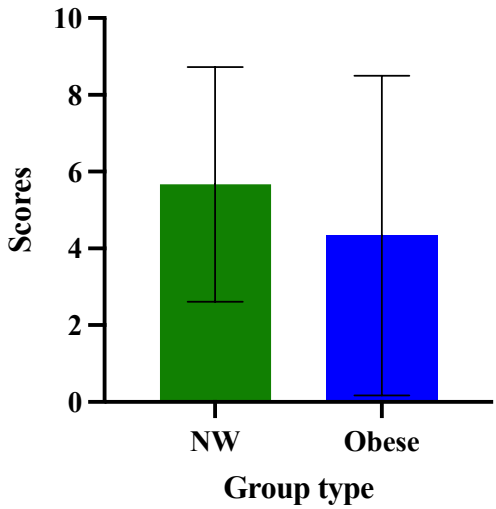


Figure 6.19. Total scores for binge eating scale in normal-weight (NW) and Obese participants. Values are presented as the mean and SD, n=3 in each group.

6.5.4.4 Power of food scale

Three domains were measured in this questionnaire: food available, food present, food tasted. The data for this questionnaire was available for analysis from 3 NW and 3 Obese participants. There was a trend for higher scores in the NW group compared with the Obese group in all domains and total score (Figure 6.20).

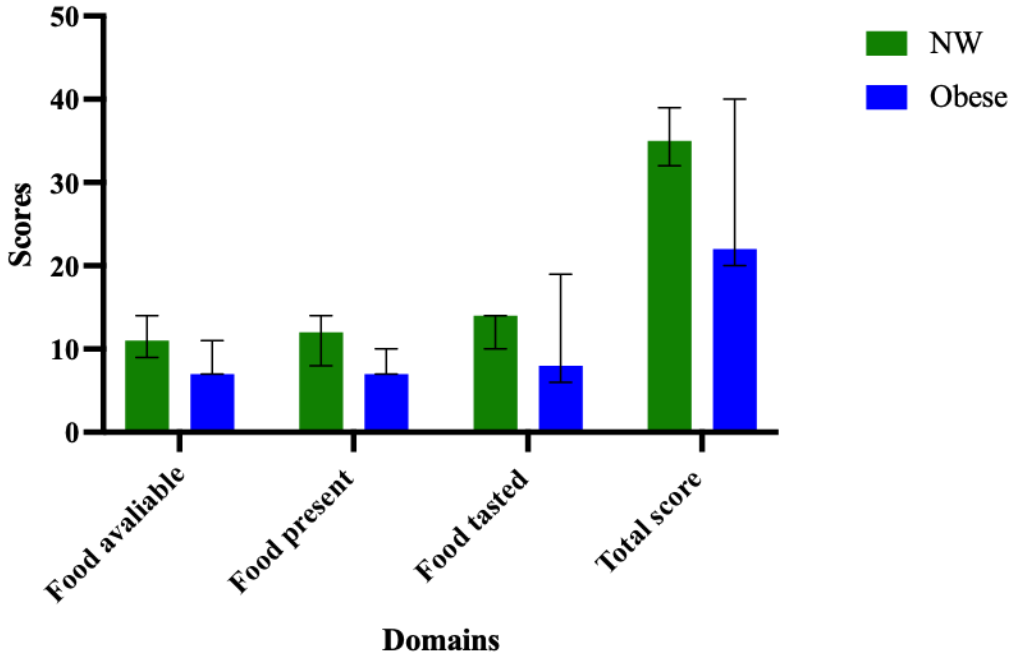


Figure 6.20. Scores for power of food scale in normal-weight (NW) and Obese participants. Values are presented as the median and range, n=3 in each group.

1.1.1.1 Food preference questionnaire

Four domains of food preference were measured: sweet liking, sweet and fat liking, salt liking, and salt and fat liking. Interestingly and unexpectedly, the NW group showed to have higher ratings for sweet liking, sweet and fat liking, and salt liking than the Obese group. No differences appear to show between groups in preference for the salt and fat liking (Figure 6.21).

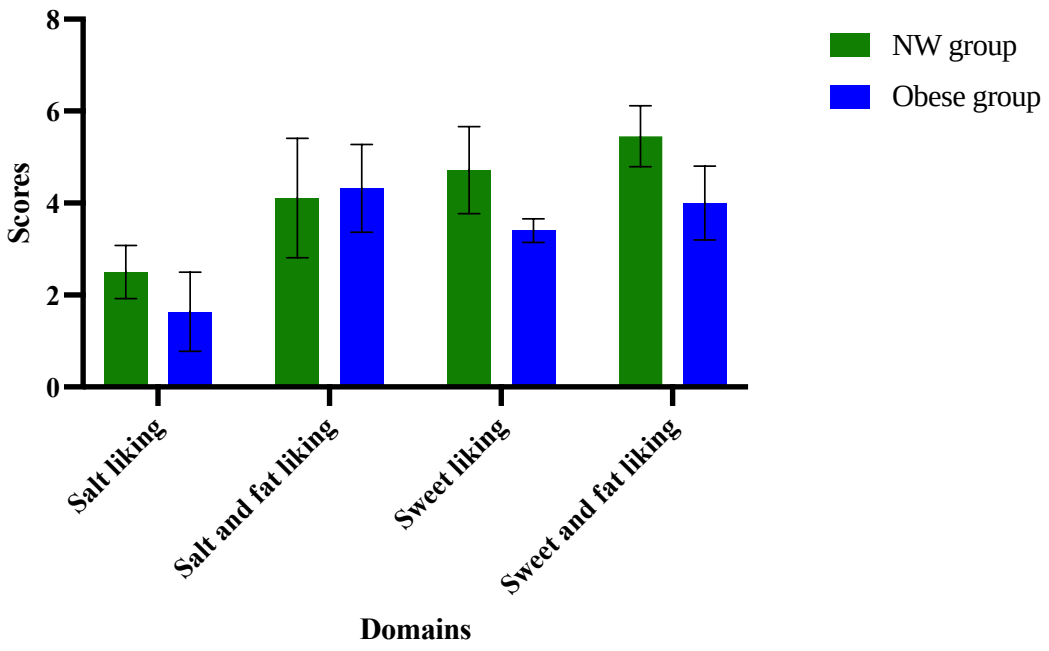


Figure 6.21. Food preference ratings for normal-weight (NW) and 3 Obese participants. Values are presented as the mean and SD, n=3 in each group.

6.6 Discussion

This study has developed HF and HC drinks that are isoenergic, isovolumic, and isoviscous, thus carefully controlling for key factors that influence appetite and satiety, facilitating the investigation of GI responses to fat and carbohydrate meals/drinks. Due to the low number of participants, the effects of these drinks on GCV, SBWC and SMA blood flow, as well as subjective satiety, were only compared statistically in combined data for NW and Obese participants.

Gastric volume measured immediately after consuming the two test drinks was almost identical. However, although not reaching statistically significance, the gastric volume following the HF drink was observed to be higher when compared with the HC drink. These differences may potentially become statistically significant with a larger sample size, as originally planned with a total of 22 participants. Since the drinks were carefully matched for energy content, the variation in GCV may be related to differing osmolality of the drinks. Previous MRI

study have reported differences in the GE curve for iso-caloric fat and glucose solutions, even when the calorie load was controlled (Goetze et al., 2007). In contrast to the findings of this study, Marciani et al. (2015) reported higher GCV and slower GE rate following the consumption of the HC rice pudding when compared to the HF rice pudding in NW participants. It's important to note that the design of that study was different from the current study, as the calories were not matched across the meals. Additionally, unlike the current study, which assessed liquid meals, the authors of that study evaluated GCV for solid meals.

SBWC revealed significant difference between HF and HC drink. This aligns with Hussein et al. (2015) findings, where the authors showed a significant increase in SBWC following the ingestion of a high fat emulsion (large droplet size with LBG). These observations could be explained by the secretions of pancreases and bile ducts, which are released following fat intake to aid fat digestion and absorption (Phan and Tso, 2001, Mu and Høy, 2004). In contrast to the HF drink, the results from the SBWC showed little changes following the HC drink in both NW and Obese groups. These findings are in agreement to Murray et al. (2014) who reported little changes in SBWC following a glucose drink (400 ml, 40 g glucose, 156 kcal).

SMA blood flow*:* Studies using ultrasound, have revealed distinct patterns in SMA blood flow response following the consumption of HF meals in comparison to HC meals, with a far more sustained response after fat meals (Sidery et al., 1994). In the current study, a similar difference in SMA blood flow pattern were observed for the HF and HC drinks. These results suggest that dietary fat may stimulates intestinal blood flow more effectively than carbohydrates. However, the results of Qamar and Read (1988) do not align with the findings mentioned above. In their study, isovolumic and isocaloric fat and carbohydrate liquid meals were compared and the SMA blood flow was found to be slower after the fat meal compared to the carbohydrate meal at 5-, 10- and 15-minutes post-ingestion. The current results indicate that the increase in SMA blood flow was more pronounced in the Obese group. However, a larger number of participants is needed to confirm this observation.

The results of VAS ratings between the meals and across the groups were inconclusive. However, previous studies showed conflicting results when comparing subjective satiety rating from fat and carbohydrate drinks. For example, Yang et al. (2009) compared the satiating effects between isoenergic HF (25% carbohydrate, 4% protein and 71% fat) and HC (88% carbohydrate, 4% fat and 8% protein) meals in 12 NW and 15 Obese participants. They found lower satiety VAS scores after HF meals compared to HC meals in both Obese and NW participants. In contrast, Gibbons et al. (2016) reported no differences in hunger and fullness VAS scores in Obese participants after isoenergic, iso-volume HF and HC meals. Further research is needed to determine the impact of carbohydrates and fat on satiety and weight management, considering factors like meal volume and energy density (as discussed in section 2.2.1.2), which will also affect GE rate.

Regarding results for the eating behaviour questionnaires, it was expected that the Obese group would have higher scores in the TEF questionnaires compared to the NW group. This was the finding of a previous study conducted by Jáuregui-Lobera et al. (2014) which showed that Obese participants have higher scores of cognitive restraints than NW participants. A study carried out by Vainik et al. (2019) demonstrated that uncontrolled eating and BMI are typically independent phenomena that interact with one another. Unexpectedly, the NW group had a trend for higher scores for BES, CoEQ (positive mood domain) and PFS than the Obese group. Additionally, NW group reported higher ratings for sweet liking, and sweet and fat liking than the Obese group. These results could be possibly linked to the under-reporting of the Obese group, as previous studies have found instances of misreporting dietary intake among individuals with obesity (Wehling and Lusher, 2019)

6.7 Conclusion

In this pilot study, a high fat emulsion drink was developed with an isoenergic, isovolumic and iso-viscous carbohydrate drink. No definitive conclusion could be made in the outcomes of this study because of a small number of participants

included. However, the present study suggests that a HF meal causes an increase in GCV, SBWC and SMA blood flow compared to HC meal, in both NW and Obese participants. This study is currently continuing, and a larger sample size will enable a more robust assessment of the effect fat and carbohydrate on gastrointestinal responses (GCV, SBWC and SMA blood flow) in NW and Obese participants. A further aim was to investigate the interactions between the brain and the GI tract in relation to food intake. Unfortunately, the brain data was not included in this work as there was not enough time to acquire the skills needed to analyse the data. However once this data is finalised, it will provide valuable understanding of the physiological mechanisms underlying appetite and satiety regulations in normal weight participants and alterations with obesity.

7 Assessing appetite and satiety responses to a high protein drink in adults with obesity and without obesity

This chapter studies the satiety responses to a high protein drink in individuals with and without obesity by using ad-libitum meal intake and subjective satiety rating measures. The study also explores satiety sensations in individuals who were formerly Obese and have lost weight (referred to as Ex-Obese in this chapter). The initial stage of this research involved the development of a high protein drink and a control carbohydrate drink with were matched in viscosity and of similar caloric content. Subsequently, an eating behaviour trial was conducted to evaluate satiety sensations in Obese, Ex-Obese and NW participants.

7.1 Introduction

Hight-protein (HP) meals are defined as 25-81% of a meal's energy from protein (Johnson and Vickers, 1993, Stubbs et al., 1996, Blom et al., 2006, Brennan et al., 2012), whereas high-carbohydrate (HC) meals are defined as 47-88% of a meal's energy from carbohydrate (Blom et al., 2006, Yang et al., 2009, Brennan et al., 2012). It has been suggested that an increase in protein intake may facilitate weight loss (Skov et al., 1999, Farnsworth et al., 2003, Weigle et al., 2005). However, while the intake of higher protein is suggested to reduce satiety and promote weight loss, it is often confounded by carbohydrate content. Several studies have attempted to untangle the effect of dietary protein from carbohydrate by assessing the satiety effects of HP intake and comparing the outcomes with high carbohydrate intake (Blom et al., 2006, Brennan et al., 2012, Ghazzawi and Mustafa, 2019). However, the satiety effects of HP meals versus HC meals remains an ongoing debate. For instance, a study conducted by Barkeling et al. (1990) involving 20 participants with NW found that participants consumed 12% less ($P<0.05$) of an ad libitum meal, 4-hour after a HP lunch (meat casserole, 43 % of total energy from protein) compared to a HC lunch (vegetarian casserole, 69% of total energy from carbohydrate). However, Ghazzawi and Mustafa (2019) reported no differences in any of the VAS

ratings at baseline, 30 minutes, and 60 minutes between the HP meal (400 kcal, 51% protein, 36% fat, and 13% carbohydrate) and the HC meal (403 kcal, 9.9% protein, 27% fat and 63% carbohydrate). However, they reported higher VAS ratings at 120 minutes in all VAS ratings following the HP meal when compared to the HC meal in a group of 30 NW participants. Similarly, Blom et al. (2006) showed no significance differences between a HP breakfast (394 kcal, 58.1% protein, and 14.1% carbohydrate) and HC breakfast (389 kcal, 19.3% protein, and 47.3% carbohydrate) meals on satiety VAS ratings and ad libitum energy intake in 15 NW participants.

Contradicting outcomes of the satiety effects of HP vs HC meals have also been reported in people living with obesity. For instance, Brennan et al. (2012) reported less VAS ratings for hunger and ad libitum energy intake after a HP lunch meal (212 kcal, 30% carbohydrate, 45% protein, and 25% fat) compared to a HC lunch meal (213 kcal, 60% carbohydrate, 30% fat, and 10% protein) in 16 Obese participants. However, Witjaksono et al. (2018) reported no differences among low protein/HC (12.4% of protein and 68.2% of carbohydrate), HP/moderate carbohydrate (40.6% protein & 40.2% carbohydrate) and moderate protein/moderate carbohydrate (23.5% protein and 56.9% carbohydrate) meals in a cohort of 22 Obese participants.

The discrepancies observed in previous findings could arise from various factors including the energy density, portion size and/or viscosity of the test meals, rather than solely the macronutrient compositions. The impact of energy density and portion size on satiety has been demonstrated in a study by Kral et al. (2004) involving 39 women with NW. In this study, lunch meals were provided at two energy density levels (5.23 or 7.32 kJ/g) and three portion sizes (500, 700, or 900 g). It was found that higher energy density meal reduced food consumed and increased energy intake at a subsequent meal ($P<0.0001$). Additionally, larger portion sizes increased food consumed ($P<0.03$) and energy intake ($P<0.0001$). The viscosity of the meal also plays an important factor in satiety. Mattes and Rothacker (2001) demonstrated that a low viscous drink led to a greater sensation of hunger ($P<0.016$) compared to a higher viscous drink with the same macronutrient

composition and energy density. Therefore, when using a HP diet to promote weight loss and maintain body weight, a thorough evaluation of these factors is essential.

Interestingly, no study to date has considered matching viscosity, portion size and energy content of HP and HC meals to assess their satiety effects in individuals with obesity compared to those with NW. In addition, no study has compared the appetite and satiety responses to HP and HC meals in individuals with obesity who have successfully lose weight (Ex-Obese) compared to those with NW adults, or individuals with obesity who have not achieved weight loss. Also, it remains unclear whether satiety responses to HP meal in Ex-Obese individuals would follow the same patterns as in people living with obesity or those with NW.

7.2 Aims and hypothesis

Primary aim

The purpose of this study was to compare the effects of HP vs. HC drinks, which have similar caloric content and are matched in viscosity and volume, on satiety feelings assessed by ad libitum meal intake in Obese, Ex-Obese and NW participants.

Secondary aims

- To compare the effects of HP vs. HC drinks on subjective satiety measured by VAS scale.
- To compare the effects of HP vs. HC drinks on energy intake for the rest of the study days between the study groups.
- To compare 3-day energy and macronutrient intake between the study groups.
- To assess eating behaviour between the study groups measured by TFE, BES and DEBQ.

Hypothesis

People with obesity will have lower satiety feelings compared to NW participants following the consumption of HP, as opposed to the HC drink. Similarly, Ex-Obese will have lower satiety feelings compared to Obese participants who haven't lost weight or NW participants following the consumption of HP, as opposed to the HC drink.

7.3 Methods

7.3.1 Development work on designing the study drinks.

To uncouple the satiety effects of protein from carbohydrate, the study was designed to measure the satiety effects of pure HP and HC drinks rather than mixed macronutrient drinks/meal. This approach provides a clearer insight into the impact of individual macronutrients on satiety sensations.

Designing the high-protein drink

Type of protein: The protein drink in this study was prepared from whey protein. We have chosen whey protein as it is considered a 'fast protein', and evidence suggests it is more satiating than 'slow protein', such as casein, in supressing hunger feelings (Veldhorst et al., 2009) and reducing subsequent food intake (Hall et al., 2003). The casein protein delays GE and slows the postprandial increase in amino acids by coagulating in the acidic environment of the stomach. Whey protein, in contrast, is categorised as a fast protein that causes a rapid and temporary rise in postprandial amino acid levels. Additionally, whey protein appears more efficacious in stimulating secretion of gut hormones, especially gastric inhibitory polypeptide (GIP), GLP-1 and CCK (Hall et al., 2003).

Amount/content of protein: Adults should consume 45% to 65% of their total calories from carbohydrates, 20% to 35% from fat, and 10% to 35% from protein according to the acceptable macronutrient distribution range (Trumbo et al., 2002). Consumption of a high-protein diet (defined as 30% or more from protein of total energy intake) has been shown to induce higher satiety compared with consumption of normal-protein diet (defined as 18% from protein of total energy intake) (Moran et al., 2005, Leidy et al., 2007). Within a single meal, previous studies showed a

significant increase in protein-induced satiety from a single meal containing 25% to 81% protein energy contents (Johnson and Vickers, 1993, Stubbs et al., 1996, Blom et al., 2006, Brennan et al., 2012). Moreover, evidence suggests having a dose of 50 grams per meal or more of protein is necessary to see a significant effect of protein on satiety (Poppitt et al., 1998, Anderson and Moore, 2004, Belza et al., 2013). In this study, the amount of the protein in the test drink was designed based on the upper safe limit of protein intake that can be tolerated by healthy adults without side effects. This was defined as 3.5 g/kg/d divided over 4 meals (Bilsborough and Mann, 2006). The protein amount in this study was calculated at 3.5 g/kg for a 70 kg man, which results in 245 g/day divided across 4 meals (61.2 g/meal), which accounts for 37% of the daily energy intake of 2625 kcal. Based on this, the protein amount was based on 61.6 from whey protein. Seventy grams of Diet Whey Protein Isolate 90 (Protein Works, Chocolate Silk, www.theproteinworks.com) were used to prepare the protein drink which included 255 kcal, 61.6 g of protein (96.6% of the meal's energy), 1.4 g of carbohydrate (2.1% of the meal's energy), and 1.12 g of fat (3.9% of the meal's energy). Two-hundred and thirty millilitres of water was mixed with the protein amount to make 300 ml of the HP drink.

Designing the high carbohydrate drink

To match the energy content of the protein drink, a carbohydrate solution was made of 63 g maltodextrin dissolved in 237 ml of water to make 300 ml of the HC drink. Maltodextrin DE of 18 was chosen as it has moderate sweetness (Saldivar and Perez-Carrillo, 2016, Muhamad et al., 2018). The nutrient composition of the drinks is shown below in Table 7.1.

Table 7.1. Nutrition information of 300 ml of the high-protein (HP) and high-carbohydrate (HC) drink.

Nutrition	**HP**	**HC**
Energy (kcal)	255	285
Carbohydrate (g)	1.4	62
Of which sugars (g)	0.56	4.4
Of which dietary fibre (g)	0	5.8

Protein (g)	61.6	1.2
Fat (g)	1.12	1.26
Of which saturated fat (g)	0.56	0.063

Viscosity measurement

As highlighted, one of the main aims of this study is to study the satiety effects of match viscosity HP and HC drink. Viscosity measurements were carried out for the two drinks, in collaboration with colleagues from the Food Science group, using Physica MCR 301 rheometer (Anoton Parr, Benelux) at 25°C per second. Samples of 50 ml were tested for stress viscosity measurement using a concentric cylinder geometry for 5 minutes. Viscosity measurements were acquired at 50 logarithmic ramp shear rates ranging from 0.1 to 100 s^{-1} and 100 to 0.1 s^{-1}. The initial viscosity/ rheology measurements of the drinks showed that the HP drink is significantly more viscous compared to the HC drink at shear rate of the mouth 50 s^{-1} (Figure 7.1). To match the viscosity of the two drinks, different concentrations of the HPMC thickening agent were added to the HC drink. As explained in section 6.3.1, HPMC was used as a thickening agent due its low taste and odour properties (Sothornvit, 2009). The concentrations of HPMC were 0.1%, 0.2 %, 0.3%, 0.4%, 0.5, 0.6%, 0.8%, 1.2%, 1.4%, 1.6%, 2.3, 2.7%, 3.2%, 3.6%, and 4%. The HP drink and the fifteen HC drinks with different concentrations of HPMC were tested for viscosity measurements. The composition of the drinks tested for viscosity is shown in Table 7.2. The results showed that the HC drink with a concentration of 1.2% HPMC was the most closely match viscosity for the HP drink at the shear rate of 50 s^{-1}, as shown in Figures 7.2.

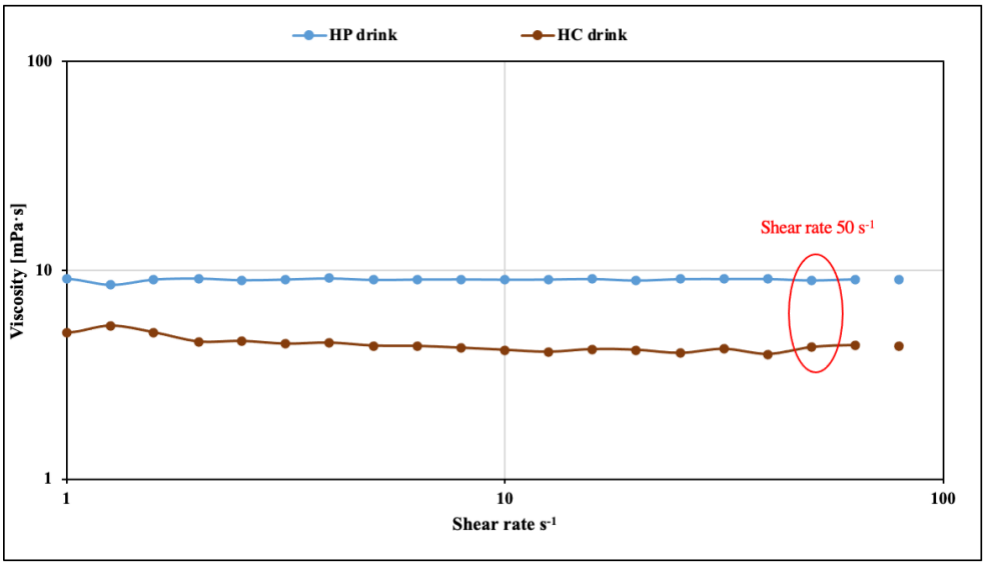


Figure 7.1. Shows rheology data for the high-protein (HP) and the carbohydrate drink (HC drink, maltodextrin).

Table 7.2. Composition of 100 g high carbohydrate (HC) drinks tested for viscosity.

Sample	**HPMC (g)**	**Water (g)**	**Maltodextrin (g)**
HC drink with 0.1 % HPMC	0.1	78.9	21
HC drink with 0.2% HPMC	0.2	78.8	21
HC drink with 0.3% HPMC	0.3	78.7	21
HC drink with 0.4% HPMC	0.4	78.6	21
HC drink with 0.5% HPMC	0.5	78.5	21
HC drink with 0.6 % HPMC	0.6	78.4	21
HC drink with 0.8% HPMC	0.8	78.2	21
HC drink with 1.2% HPMC	1.2	77.8	21
HC drink with 1.4% HPMC	1.4	77.6	21
HC drink with 1.6% HPMC	1.6	77.4	21
HC drink with 2.3 % HPMC	2.3	76.7	21
HC drink with 2.7% HPMC	2.7	76.3	21
HC drink with 3.2 % HPMC	3.2	75.8	21
HC drink with 3.6% HPMC	3.6	75.4	21
HC drink with 4% HPMC	4	75	21

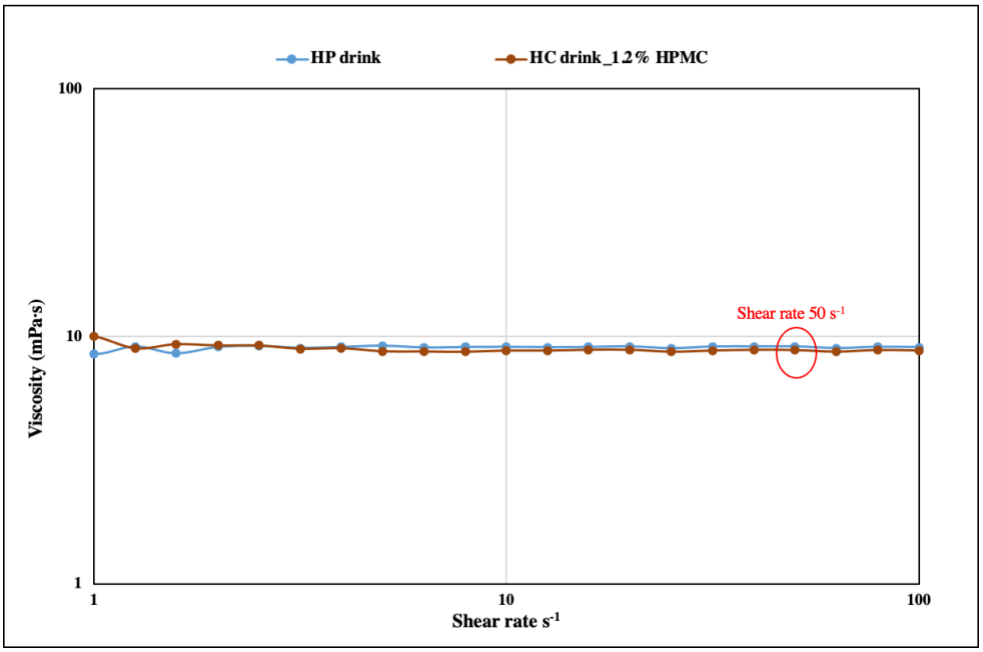


Figure 7.2. Shows rheology data for the high-protein (HP) and the carbohydrate drink (HC drink, maltodextrin). The plots show the matched viscosity profile of the HP drink with the addition 1.2% HPMC (hydroxypropyl methylcellulose) and the carbohydrate drink (HC drink, maltodextrin).

7.4 Intervention Study

7.4.1 Methods

7.4.1.1 Study design and ethics

This is a single-blinded, randomised crossover study, where the study participants were not aware of the nature of the test meals. Meals were randomised across participants using research randomizer software (https://www.randomizer.org/). Ethical approval for the study was obtained from the Medical School Research Ethics Committee at the University of Nottingham, reference FMHS 25-0520.

7.4.1.2 Recruitment

The study was advertised using poster. Interested volunteers were given participants' information to read. The local Slimming World Groups were used to advertise for the Ex-Obese group.

7.4.1.3 Eligibility criteria

Inclusion criteria

- Adults aged 18-65, male and female with body mass index (BMI) of:
    - Normal-body weight (BMI 18.5-24.9 kg/m^2).
    - Obese (BMI 30-40 kg/m^2) who have not lost weight over the past 6 months.
    - Ex-Obese (BMI 25-40 kg/m^2) who have lost 5% or more of their initial body weight and have been weight stable for at least the past three months. Successful weight loss is defined as a decrease in body weight of at least 5% of initial body weight (Ramage et al., 2014). This level of weight loss is thought to have beneficial clinical effects on comorbidities such as lipid profile, blood pressure, glycaemic control as well as osteoarthritis and gastroesophageal reflux disease (Lau et al., 2007).
- Able to understand the study requirements.
- Able to give voluntary written informed consent to participate in the study.
- Apparently healthy (judged by health questionnaire, and blood screening): no medical conditions which might affect study measurements.

Exclusion criteria

- Any reported history of metabolic, endocrine, renal disease or gastrointestinal disorders.
- Abnormal screening procedures (including depression and restrictive eating) and laboratory results that are clinically significant, including diabetes, dyslipidemia, impaired renal functions, liver diseases, pancreatitis, or untreated hypertension.
- Under medication (except aspirin/paracetamol), prescribed probiotic or antibiotic treatment in the past 12 weeks.
- Under medication that may have influenced appetite and sensory functioning.

- Following a self-prescribed - or medical diet during the two weeks before the pre-study examination and until the end of the study.
- Pregnancy or breastfeeding declared by candidate.
- Smoking.
- No understanding of the written and/or spoken English language.

7.4.1.4 Sample size calculation

The sample size calculation was based on previous data by Blom et al. (2006) on 15 NW men who consumed a high protein breakfast dairy product meal (393 kcal) with an average ad libitum energy intake of 4697 ± 1784 kJ (1118 ± 426 kcal) (mean ± SD) measured 3 hours after the meal. Employing this in the sample size calculation, 11 participants in each independent group would give 90% power (α= 0.05) to detect a 13% (627 kJ, 150 kcal) difference in the ad libitum energy intake in the Obese group. To allow for an estimated 10% dropout rate, the aim was to recruit 12 participants for each group.

7.4.1.5 Study protocol

Participants were invited to attend 2 study visits, in addition to the screening/consenting visit.

Screening visit

Interested participants were asked to sign an online consent form and to fill out online questionnaires. These questionnaires include a depression scale questionnaire, a health questionnaire, EAT, eating behaviour questionnaires (TEF, BES, and DEBQ) as well as a 3-day food intake diary, using the Intake24online dietary recall survey, designed and validated by Newcastle University (Intake24, 2023). Eligible participants were asked to come to the SPMIC for blood samples to measure full blood count, liver function tests, renal function tests, and HbA1c. Blood samples were collected by a research nurse from the SPMIC. Body weight, height, waist circumference and blood pressure were also measured. Before participants left the SPMIC, they were given a leaflet containing instructions to follow the day before the study visits. These instructions included limiting sporting activity, which may affect satiety.

Study visit 1

Eligible participants were invited to attend a study visit starting at 8:45 am, having fasted for at least 8 hours. They were required to eat their evening meal on the night before the study between 20:00 and 21:00 and have no further food or drink, other than water, after 10 pm. They were further instructed to consume nothing in the morning prior to the study visits. A further reminder of these instructions was provided immediately before the study day. Baseline measurements (T0) of VAS questionnaires were taken at 9:00 am. Following this, participants were instructed to have breakfast consisting of 300 ml of either the HP or HC drink within 15 minutes. These drinks were provided in random order and blinded to the participants. The VAS questionnaire was given to participants right after they had the test drink (T15). Thirty minutes after (T45), satiety and appetite feelings were measured using the VAS and every 30 minutes for the duration of the experiment (three hours until T195). Participants received an ad-libitum test meal at 12:00 pm (T200) and were asked to eat as much as they could in 30 minutes until they felt comfortably full. Participants were asked to note how much they had eaten for the rest of the day using the Intake24 online dietary recall survey (Intake24, 2023).

Study visit 2

At least one week after study visit 1, to allow for the washout period, participants conducted a second study visit that was identical to study visit 1, but in which the second test meal was given.

7.4.1.6 Test drinks

Each volunteer received a 300 ml serving of either a pure protein or pure carbohydrate drinks. The drink was served at room temperature in an opaque paper cup with a straw and a lid. The composition of each drink is detailed in Table 7.1. Cocoa powder was included in both drinks to ensure consistent flavour across the drinks. Appendix 10.2.6 and Appendix 10.2.7 gives a full description of the drinks' preparation. The palatability and flavour of the drinks were tested by the research team before commencing the study.

7.4.1.7 Ad Libitum lunch meal

The ad libitum lunch meal in this study was served 3 hours after the drinks intake to assess satiety.-The ad libitum meal was a standardised tomato-based pasta meal (Horner et al., 2014b, Alhussain et al., 2016). The composition of the meal was as the follow: 250 g of dried white pasta (Sainsbury's Supermarket, UK), 340 g of tomato sauce (Dolmio Bolognese Pasta Sauce, Freepost Mars Food UK, Dublin, UK), 30 g olive oil (Tesco Supermarket, UK), and 80 g of cheddar cheese (Sainsbury's Supermarket, UK). The preparation method of the meal is illustrated in Appendix 10.2.8. The macronutrient composition of the meal was 50% from the energy from carbohydrates, 12.8% from protein, and 36% from fat (Table 7.3).

The pasta was aliquot into ~1000 g portions and stored in the fridge overnight. On the study day, the pasta was heated in a microwave for 2 minutes, stirred hallway through, and then provided to participants straight away with 250 ml water . The pasta plate was always replenished when it was about three-quarters empty, ensuring that there was always enough hot food for participants and that they weren't prompted to stop eating by emptying their plate. Participants were asked to eat as much as they could within 30 minutes until they felt comfortably full. The energy intake was measured by the amount of food consumed.

Table 7.3. Nutrition information of 100 g of Ad Libitum lunch meal.

Nutrition	**Per 100 g of ad libitum meal**
Energy (kcal)	156.4
Carbohydrate (g)	19.5
Of which sugars (g)	10
Of which dietary fibre (g)	7.5
Protein (g)	5
Fat (g)	6.28
Of which saturated fat (g)	2.2

7.4.1.8 Subjective satiety

Satiety questionnaires were completed as previously described in section 2.6.1 using a 100 mm VAS scale at baseline, immediately after the drinks (T0), and then every 30 minutes (at 30, 60, 90, 120, 150, and 180 minutes) throughout the experiment (three hours) to assess feelings of DTE, hunger, fullness, and PFI.

7.4.1.9 Satiety quotient

SQ for each VAS domain was calculated to determine the satiety efficiency of the drinks relative to their energy content as discussed earlier in section 2.6.2 (Green et al., 1997).

7.4.1.10 Eating behaviour questionnaires

Eating behavior was assessed by TFEQ, DEBQ, and BES, with more details available in section 2.6.7.

7.4.1.11 Statistical analysis

Type of data presented, and the normality test was conducted as explained in section 5.3.8. A two-way ANOVA was used to test variations in baseline/fasting levels of VAS scales ratings and ad libitum meal intake across the different groups (NW vs. Obese vs. Ex-Obese) with the type of drink and BMI group as the main factors of interaction. A three-way analysis of variance (ANOVA) was used to identify differences in satiety VAS responses for repeated measurements with the type of drink, BMI group and time as the main effects of interactions. If there were significant differences between the groups detected by the two-way or three-way ANOVA analysis, further exploration was carried out using the Bonferroni post hoc test.

The total AUC for postprandial responses was calculated using the trapezoid rule. The postprandial AUC from 0-195 minutes was calculated by the GraphPad Prism for the area under the reading curve down to fasting baseline values. The peaks that were below the baseline value were also considered in the AUC calculation. A two-way ANOVA was used to test differences between groups (NW vs. Obese vs. Ex-Obese) in total AUC after the HP and HC drinks. Unpaired-t-test or Mann-Whitney

test were used to test differences between groups (NW vs. Obese vs. Ex-Obese) for eating behaviour questionnaires and dietary intake.

7.5 Results

7.5.1 Participant's descriptive results

One hundred and thirty participants contacted the research team and were assessed for eligibility. Forty-six participants were screened online for the study, 32 of whom were invited for a screening session. Six participants were excluded after screening (Figure 7.3). Twenty-three participants were included in the final analysis of the study, 12 NW and 11 Obese. Table 7.4 summarises the demographic characteristics of the included participants. Due to the consequences of COVID pandemic and difficulty of recruiting Ex-Obese participants, only two participants in this group were recruited, and were therefore not included in the statistical analysis of the study results. However, some of their individual data are presented in Figure 7.5 and Table 7.6.

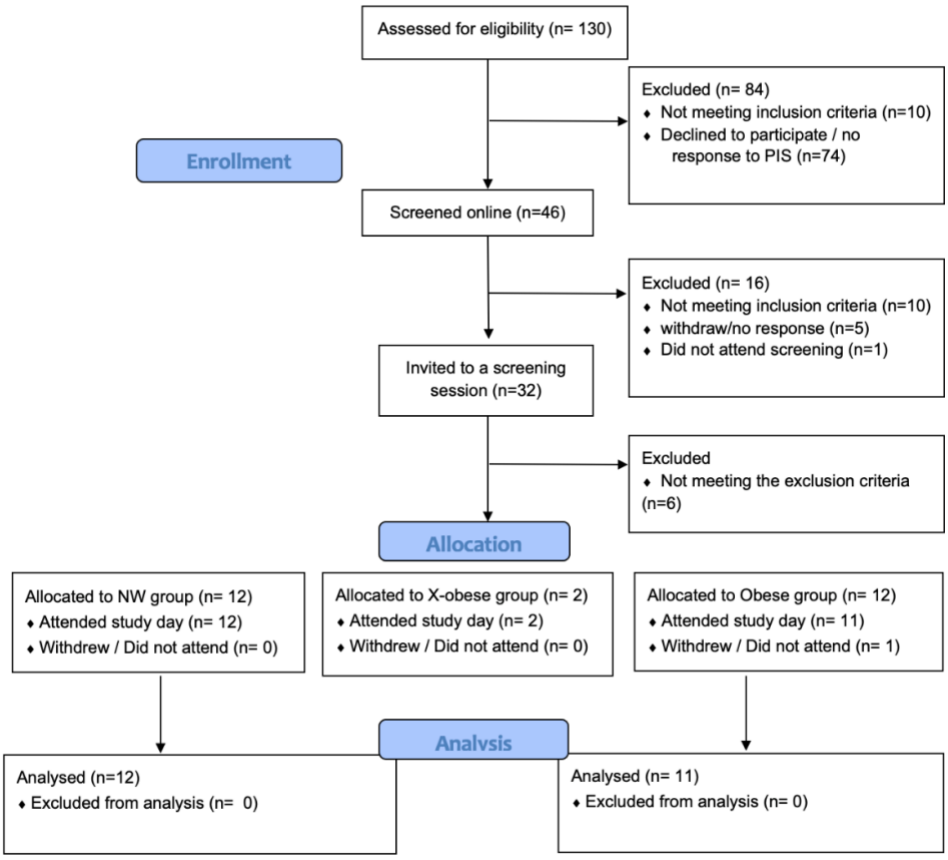


Figure 7.3. Recruitment flow cha

Table 7.4. Demographic characteristics of the included participants.

	NW	**Obese**	**Ex-Obese**	**P-value (NW *vs.* Obese)**
Age (mean ± SD)	24.1 ± 4.3	29.1 ± 6.78	30.5 ± 2.1	0.05
Gender	10 female & 2 males	8 female & 3 males	All female	
Height [cm, median (IQR)]	166 (13.75)	165 (20.1)	160.5 (15)	0.47
Weight [kg, median (IQR)]	64 (14.8)	87.3 (17)	69.2 (7.1)	<0.0001
BMI (mean ± SD) (kg/m^2)	22.2 ± 1.8	33.5 ± 2.6	26.8 ± 1.5	<0.0001

Waist circumference (mean ± SD) (cm)	Not measured	101.5 ± 11.48	76 ± 3.2	Not applicable
Waist-height ratio (mean ± SD)	Not measured	0.6 ± 0.06	0.74 ± 0.05	Not applicable

7.5.2 Ad libitum meal intake

The amount of food consumed and energy intake from the ad libitum meal did not differ between the NW and Obese groups for the HP and HC as shown in Table 7.5. No difference was found for the group × treatment interactions. Individual data for ad libitum meal intake following the HP and HC drinks for the Ex-Obese group are shown in Table 7.6.

Table 7.5. Amount consumed and energy intake of the ad libitum lunch meal. Data are presented as the mean ± SD.

Outcomes	**Group**	**HP drink**	**HC drink**	**Group**	**Treatment**	**Group x Treatment**
Amount consumed (g)	NW	399.5 ± 178	484.1 ± 246.9	0.25	0.59	0.35
	Obese	519.3 ± 144.6	496.9 ± 183.2			
Energy intake (kcal)	NW	648.1 ± 276.7	757.1 ± 386.2	0.31	0.68	0.42
	Obese	812.1± 226.1	777.2 ± 286.5			

Table 7.6. Individual data representation for amount consumed and energy intake of the ad libitum lunch meal of the Ex-Obese group.

Outcomes	**Participant ID**	**HP drink**	**HC drink**
Amount consumed (g)	XOB01	97	485
	XOB02	446	457

Energy intake (kcal)	XOB01	151.7	758.5
	XOB02	697.5	714.7

7.5.3 Subjective Satiety

Figure 7.4 shows the subjective satiety sensation measured by the VAS questionnaire. There were no differences in fasting levels in the DTE, PFI, hunger domains and CSS between the NW and Obese groups for the HP and HC drinks (Table 7.7). However, there was a trend for higher baseline fullness for the HC drink condition in the Obese group (P=0.07).

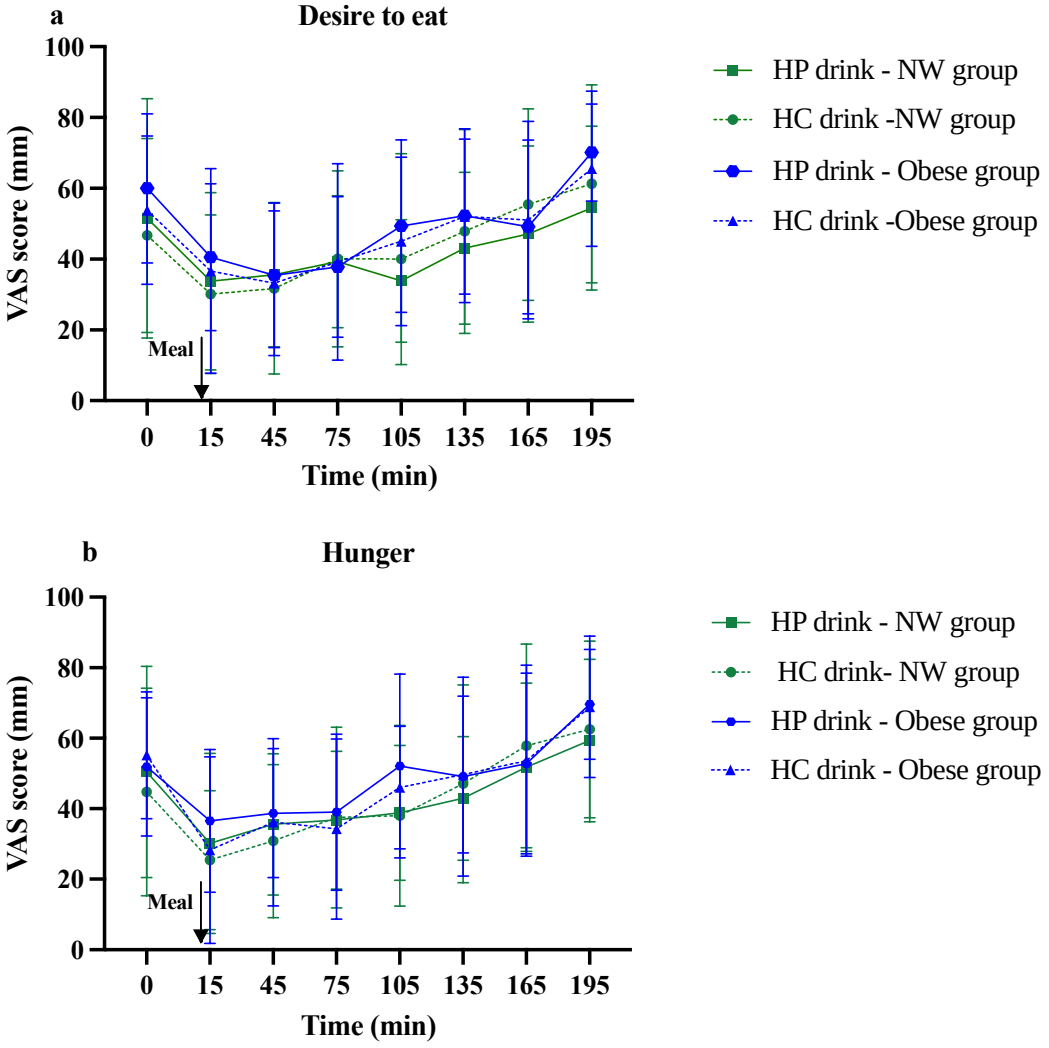
No significant differences were found in DTE, hunger, and PFI domains and CSS between the HP and HC drinks in the NW and Obese groups (Figure 7.4a, 7.4b, 7.4d, and 7.4e). There was a trend for group effect for fullness (P=0.09, Figure 7.4c) which could be explained for higher baseline for the HC drink in the Obese group. No differences were shown for the following interactions: treatment × group, time × treatment, group × time, and group × time × treatment for all VAS domains. Total AUC did not differ between the drinks in the NW and Obese groups for all VAS domains and CSS (Table 7.8). Individual data for VAS ratings following the HP and HC drinks for the Ex-Obese group are shown below in Figure 7.6.

Table 7.7. Bassline (fasting) levels of the visual analogue scale (VAS) domains and composite satiety scores (CSS). Data are presented as the mean ± SD.

	Group	**Treatment**		**P-value**		
		HP drink	**HC drink**	**Group**	**Treatment**	**Group x Treatment**
DTE (mm)	NW	51.5 ± 33.7	46.6 ± 27.4	0.32	0.49	0.93
	Obese	60 ± 21.1	53.8 ± 20.9			
Hunger (mm)	NW	50.4 ± 29.9	44.8 ± 29.5	0.43	0.87	0.55
	Obese	51.9 ± 19.6	55.2 ± 17.9			
Fullness (mm)	NW	21.3 ± 14.9	19.1 ± 12.7	0.07	0.35	0.16
	Obese	23.5 ± 21.4	34.6 ± 13.7			

PFI (mm)	NW	52.5 ± 17.3	51.5 ± 24.6	0.32	0.79	0.93
	Obese	59 ± 20.6	59.9 ± 15.7			
CSS (mm)	NW	42 ± 18.6	44.2± 19.1	0.60	0.54	0.84
	Obese	38.3 ± 17.2	42.5 ± 14.7			

DTE, desire to eat; PFI, prospective food intake.



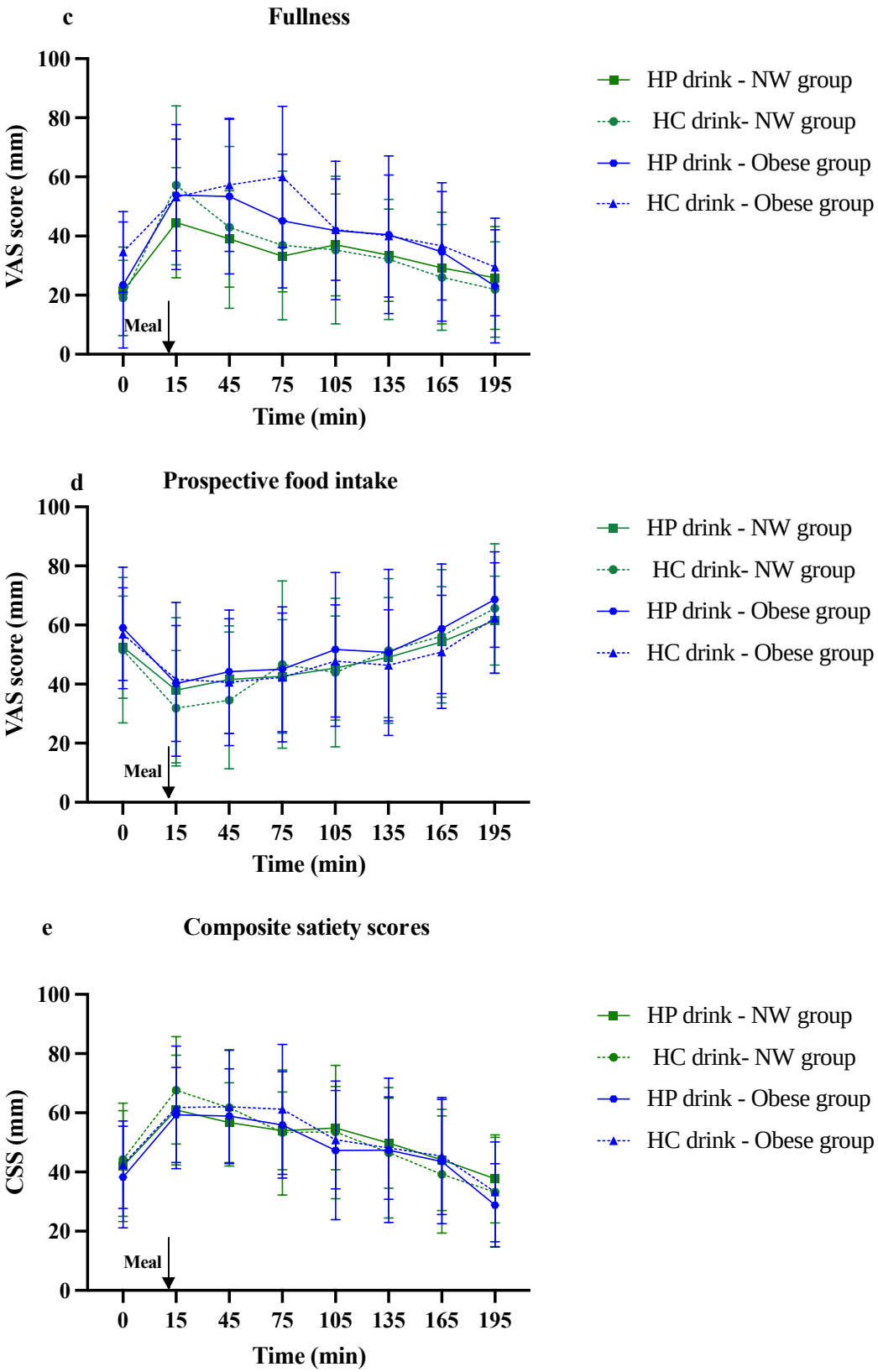
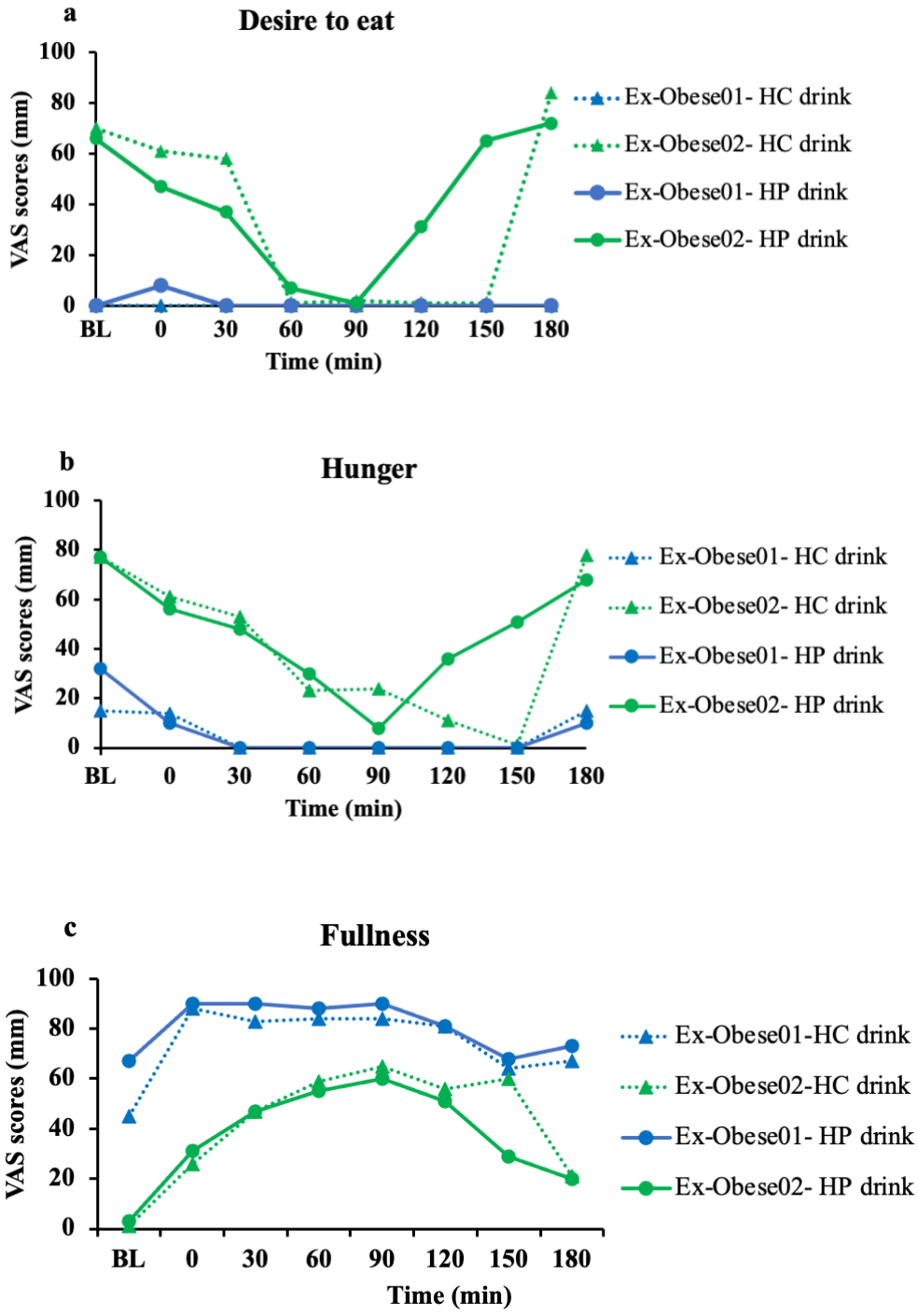

c
Fullness
VAS score (mm)
100
80
60
40
20
0
Meal
0
15
45
75
105
135
165
195
Time (min)
HP drink - NW group
HC drink- NW group
HP drink - Obese group
HC drink - Obese group
d
Prospective food intake
VAS score (mm)
Meal
Time (min)
HP drink - NW group
HC drink- NW group
HP drink - Obese group
HC drink - Obese group
e
Composite satiety scores
CSS (mm)
Meal
Time (min)
HP drink - NW group
HC drink- NW group
HP drink - Obese group
HC drink - Obese group

Figure 7.4. Subjective satiety ratings over time using Visual Analogue Scale (VAS) scores of (a) desire to eat, (b) hunger, (c) fullness, (d) prospective food intake, and (e) composite satiety scores at fasted and following the consumption of the high-protein (HP) and high-carbohydrate (HC) drinks in 12 normal-weight (NW) and 11 Obese participants. Values are presented as the mean and SD.

Table 7.8. Total area under curve (AUC) from 0-195 minutes of the visual analogue scale (VAS) domains and composite satiety scores (CSS). Data are presented as the mean ± SD.

Outcomes	**Group**	**Treatment**		**P-value**		
		HP drink	**HC drink**	**Group**	**Treatment**	**Group x Treatment**
DTE (mm)	NW	76.12 ± 138.2	61.05 ± 162.2	0.90	0.63	0.74
	Obese	95.26 ± 132.3	69.5 ± 145.5			
Hunger (mm)	NW	72.49 ± 135.8	69.81 ± 155.5	0.99	0.68	0.64
	Obese	53.19 ± 116.5	88.47 ± 136.9			
Fullness (mm)	NW	90.58 ± 107.2	117.20 ± 148.4	0.99	0.79	0.34
	Obese	128.4 ± 140	80.8 ± 121.3			
PFI (mm)	NW	51.2 ± 119.2	60.9 ± 148.4	0.72	0.86	0.94
	Obese	68.01 ± 134.9	71.69 ± 122.3			
CSS (mm)	NW	68.93 ± 95.26	70.66 ± 124.4	0.74	0.93	0.89
	Obese	83.98 ± 115.2	77.02 ± 111.2			

DTE, desire to eat; PFI, prospective food intake.



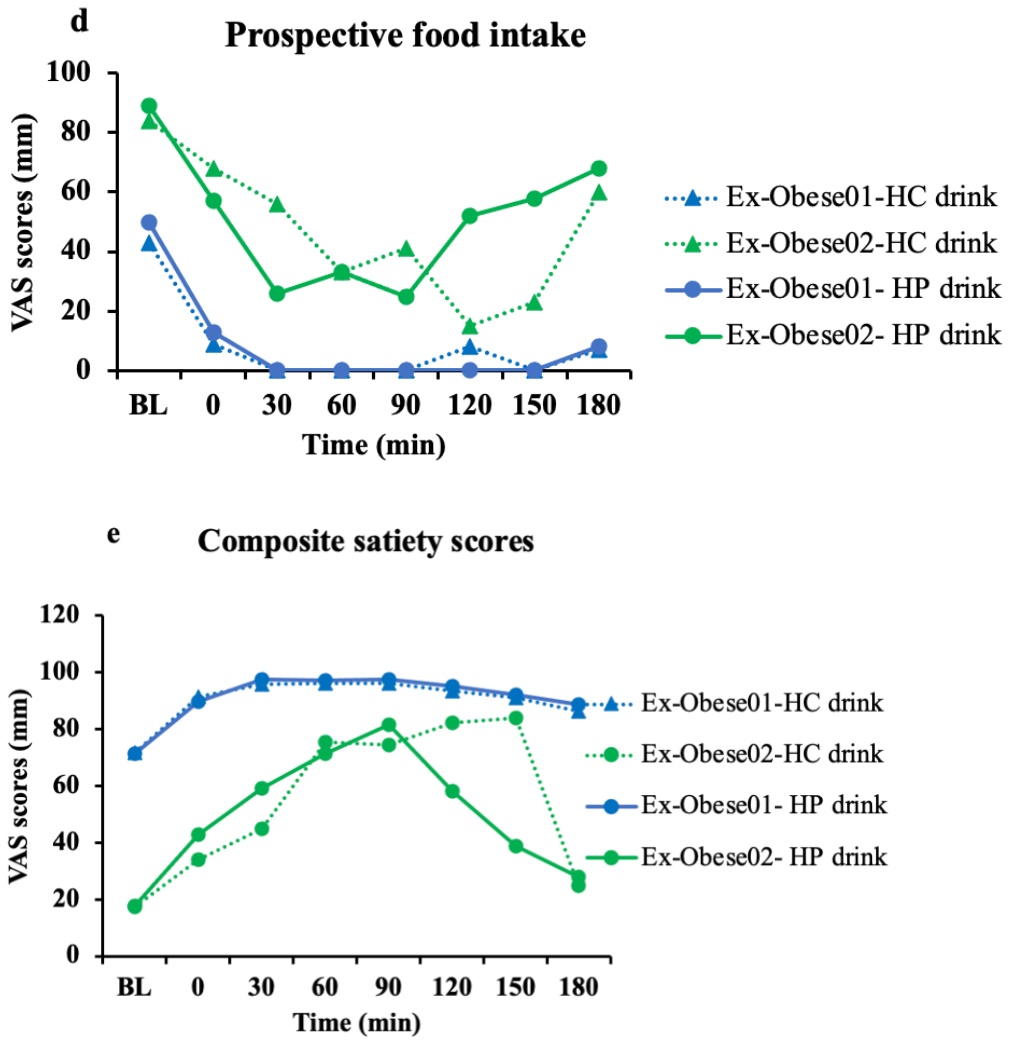
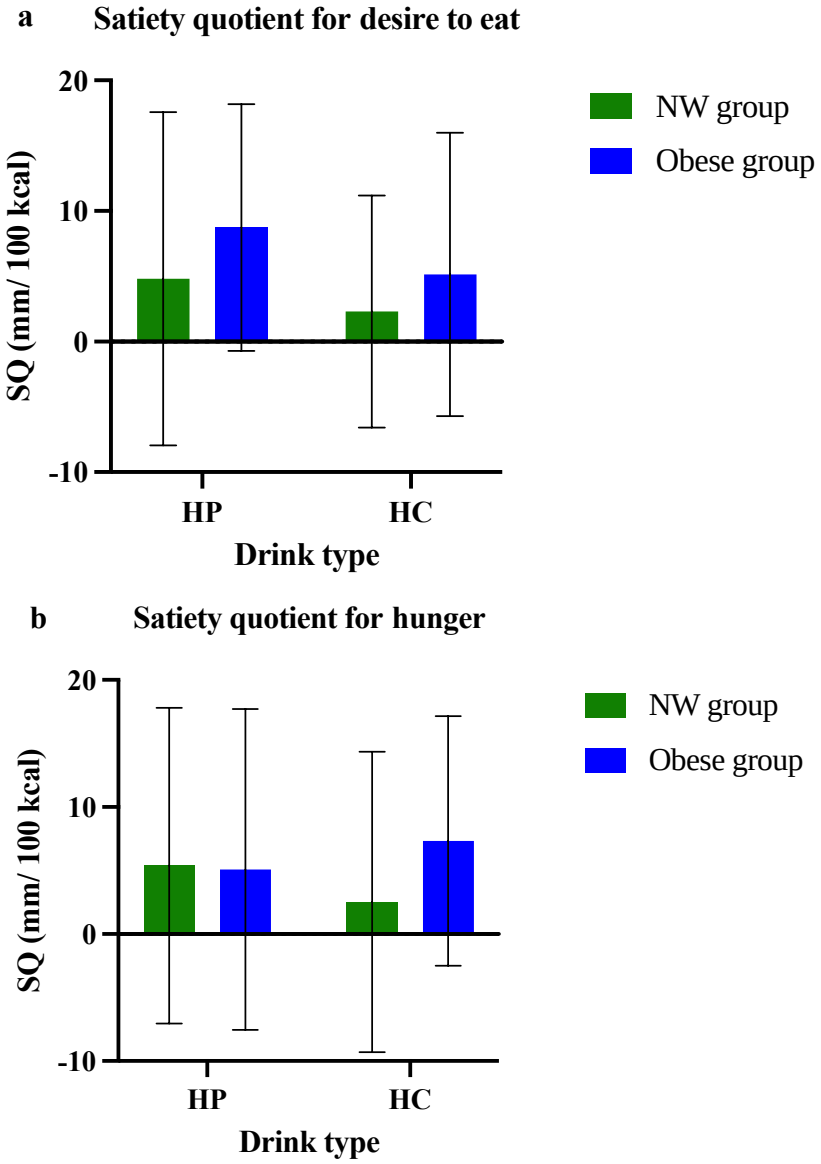


Figure 7.5. Individual values for subjective satiety ratings over time using Visual Analogue Scale (VAS) scores of (a) desire to eat, (b) hunger, (c) fullness, (d) prospective food intake, and (e) composite satiety scores at fasted and following the consumption of the high-protein (HP) and high-carbohydrate (HC) drinks in 2 participants who were obese and lost weight (Ex-Obese group).

7.5.4 Satiety quotient

The SQ for each VAS domain as well as the mean SQ following the HP and HC drinks were analysed. The mean SQ and SQ for each VAS domain did not differ between the NW and Obese groups for the HP and HC drinks (Figure 7.6). The mean SQ for the NW and Obese groups following the two drinks was considered to

have low satiety phenotypes as their mean SQ was lower than 8 mm/100 kcal, as shown in Figure 7.6e.



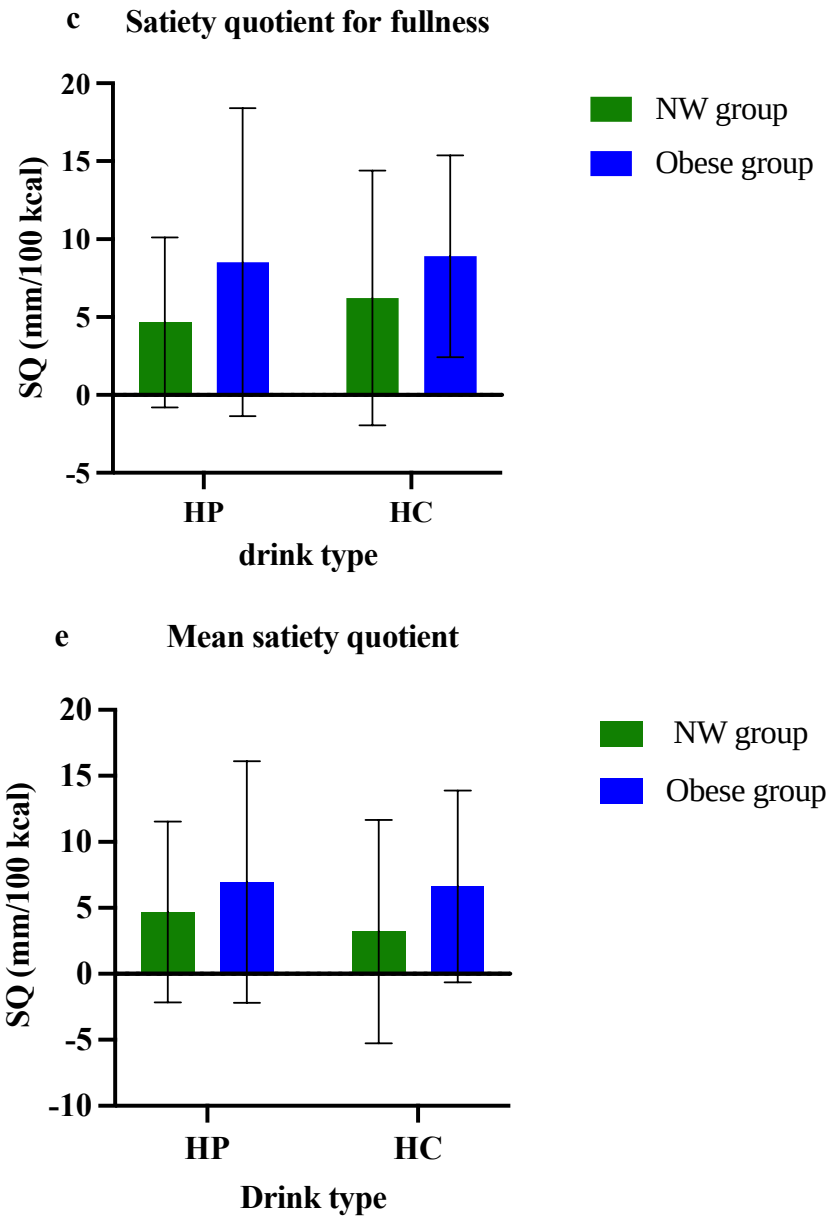


Figure 7.6. Satiety quotient for (a) desire to eat, (b) hunger, (c) fullness, (d) prospective food intake and (e) mean satiety quotient of the high-protein (HP) and high-carbohydrate (HC) in 12 normal weight participants (NW group) and 11 participants with obesity (Obese group). Values are presented as the mean and SD.

7.5.5 Energy intake for the rest of the study day

This outcome was available for 11 NW and 10 Obese participants. One NW and one Obese participant did not fill out the survey for one study visit, and their data for the other study visit were not included in the analysis. There were no differences

between the NW and Obese groups in energy intake for the rest of the study days following the HP and HC drinks (Table 7.9). Individual data in energy intake for the rest of the study days following the HP and HC drinks for Ex-Obese participants are shown below in Table 7.10.

Table 7.9. Energy intake of the rest of study days following the high-protein (HP) and high-carbohydrate (HC) drinks of the NW and Obese groups. Data are presented as the median and IQR.

Outcomes	**Group type**	**HP drink**	**HC drink**	**P-value**		
				Group	**Treatment**	**Group x Treatment**
Energy intake (kcal)	NW	940 (1144)	1209 (833.1)	0.44	0.99	0.97
	Obese	893 (976)	1070 (648.5)			

Table 7.10. Individual data for energy intake of the rest of study days following the high-protein (HP) and high-carbohydrate (HC) drinks of the Ex-Obese group.

Outcomes	**Participant ID**	**HP drink**	**HC drink**
Energy intake (kcal)	XOB01	1071	1435
	XOB02	862	570

7.5.6 Three days average intake of energy and macronutrients

No differences between the NW and Obese groups in the average intake of energy and macronutrients was found, as shown below in Figures 7.7a and 7.7b.

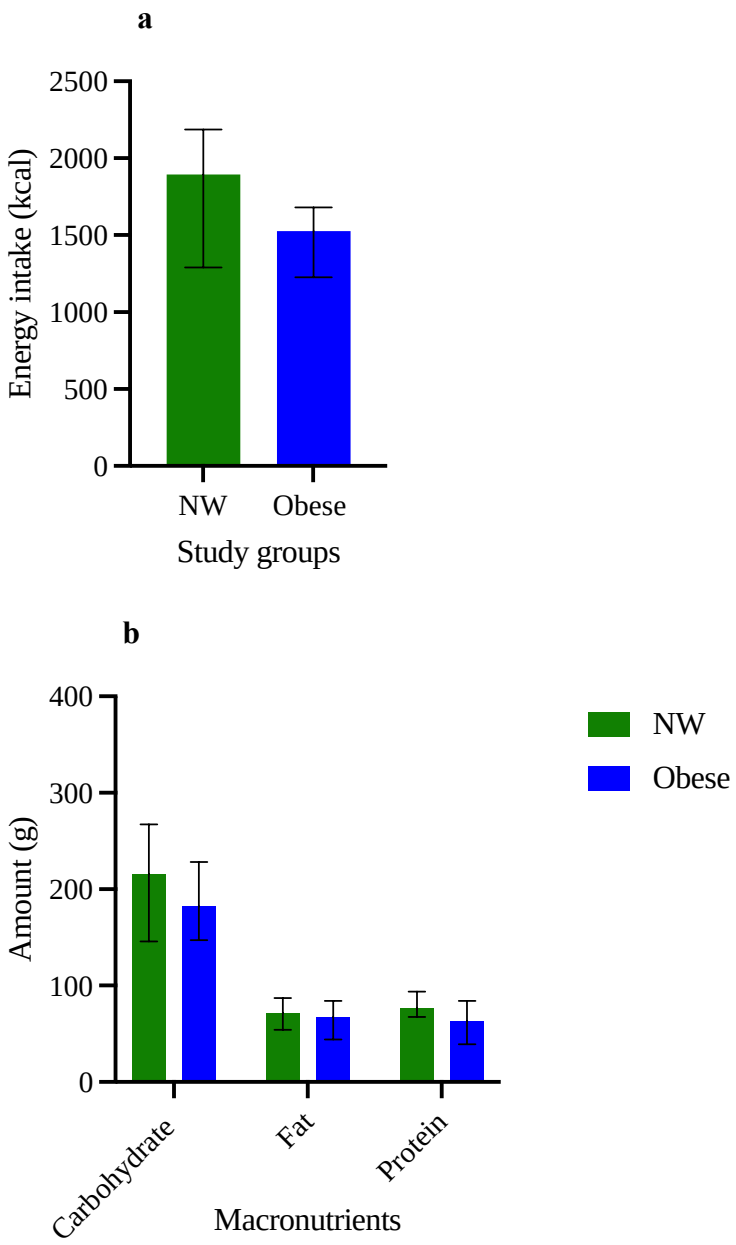


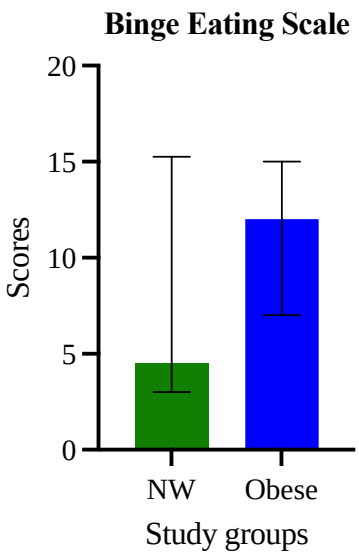
Figure 7.7. Three days average of energy and macronutrient intake of 12 normal-weight (NW) participants and 11 participants with obesity (Obese). Values are presented as the median and IQR.

7.5.7 Eating behaviour scores

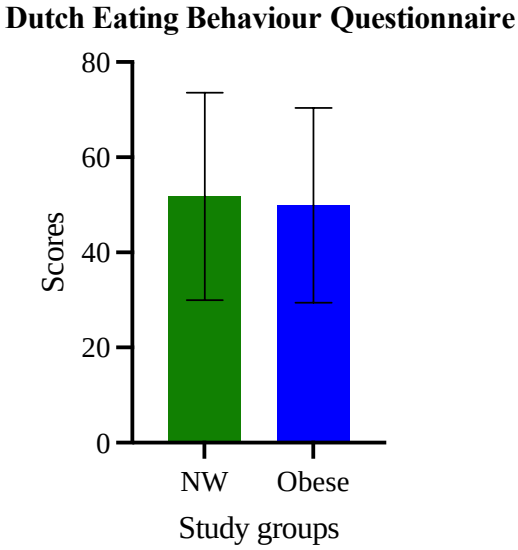
No differences between the NW and Obese groups in scores of BES (Figure 7.8a) and DEBQ (Figure 7.8b). Similarly, no differences were observed between the groups in TFEQ-Restraint and TFEQ-hunger and TFEQ-Total scores, however,

there was a trend for higher scores in the TFEQ-Disinhibition factor for the Obese group compared with the NW group (P=0.06, Figure 7.8c).

a



b



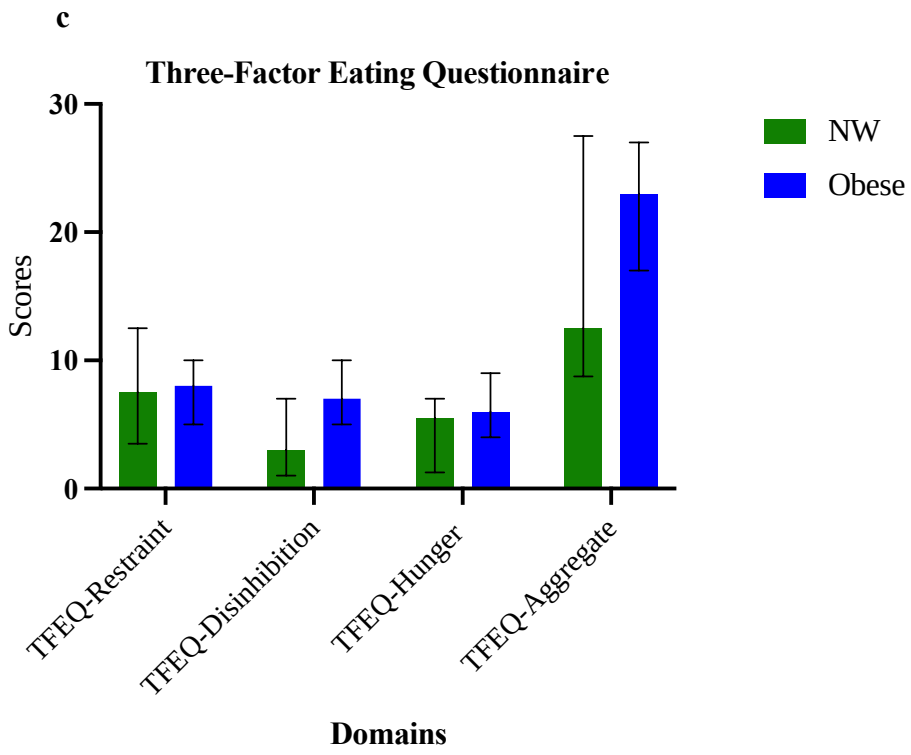


Figure 7.8. Scores for binge eating scale (BES, a), Dutch eating behaviour questionnaire (DEBQ, b) and Three-factor eating (TFE, c) in 12 normal-weight (NW) participants and 11 participants with obesity (Obese, DEBQ only 10). Values are presented as the median and IQR except for DEBQ where values presented as the mean and SD.

7.6 Discussion

This is the first study to compare satiety responses between HP and HC drinks in people with and without obesity, using drinks that were matched for volume and viscosity, and similar in energy content. Protein plays an important role in satiety sensation and could potentially have a significant impact on weight loss and managements. A significant proportion, ranging from 30% to 40% of individuals with obesity who have undergone weight loss treatment have experienced weight regain within one year (Foster et al., 2010), and approximately 51% have returned to their initial weight by the fifth year of their weight-loss treatment (Weintraub et al., 2023). Therefore, understanding satiety patterns in these individuals is crucial for maintaining body weight. While one of the objectives of this chapter was to

evaluate satiety responses to high protein intake in individuals who were Obese and have successfully lost weight (Ex-obese), and to assess whether their satiety responses follow NW or Obese responses, this was unfeasible within the constraints of the current study due to the limited number of participants recruited (only 2 participants). Consequently, the subsequent sections of the discussion primarily focus on the outcomes from the NW and Obese groups.

The results of this study did not reveal significant differences in ad libitum energy intake and VAS scores among the drinks/treatments or across the NW and Obese groups. These findings are consistent with previous study by Blom et al. (2006) where no differences in satiety VAS ratings and ad libitum energy intake were observed between HP and HC isovolumic (400 g), isoviscous, and isoenergic dairy breakfast products: HP (394 kcal, 14.1% carbohydrate, 58.1% protein, 27.8% fat) and HC [389 kcal, 47.3% carbohydrate, 19.3% protein (57.2 g), 33.3% fat] in a group of 15 males with NW. Similarly, another study by Fischer (2004) did not identify differences in energy intake during the ad libitum lunch meal among isoenergic (398 kcal) and isovolumic (400 ml) preloads, including protein (10.5 g dried chicken egg white powder and 94.7 g milk protein), carbohydrate (84.2 g maltodextrin + 10.5 g glucose+ 10.5 g starch rice) and lipid (31.5 g double cream+ 15 g palm oil+ 15 g soyabean oil) in 17 males with NW. Additionally, a study by Potier et al. (2010) did not find differences in satiety VAS ratings between isoenergic and isovolumic (250 ml) preloads consisting of pure macronutrients: protein (243 kcal, 45 g whey and 5 g casein), carbohydrate (245 kcal, 50 g maltodextrin), and lipid (242 kcal, 11.2 g palm oil and 11.2 g soya bean oil). It is important to note that, while the above studies did control for energy and volume contents between the liquid test meals, the viscosity of the meals/drinks was matched only in the study of Blom et al. (2006).

The energy content/density of a meal, as well as the portion size/volume have been demonstrated to independently reduce the amount of food consumed and the subsequent energy intake at a later meal (Kral et al., 2004). In addition to the energy content and volume, meal viscosity is also known to influence satiety. Previous

studies have found that increasing the viscosity of a meal can lead to delay GE (Camps et al., 2016) and slow nutrient digestion and absorption, ultimately promoting satiety (Zavoral et al., 1983, Blackburn et al., 1984, Haskell et al., 1992). Therefore, the fact that the viscosity, energy content, and volume were all matched between the test meals may explain the lack of significant differences in satiety sensation between the HP and HC drinks between and across the NW and Obese groups in the present study.

Here, there was an observable trend towards increased fullness over time in the Obese group for both drinks compared to the NW group. This trend might be attributed to the higher baseline fullness observed in the Obese group during the HC drink condition.

The results of the VAS ratings for our study contradict findings from a previous study by Ghazzawi and Mustafa (2019). In their study, they reported higher ratings for desire to eat and prospective food intake and lower fullness after participants consumed a HC breakfast meal (403 kcal, 63% carbohydrate, 9.9% (10 g) protein & 27% fat) when compared to a HP breakfast meal (400 kcal, 13% carbohydrate, 51% (51 g) protein & 36% fat). Another study by Brennan et al. (2012) also found lower VAS ratings for hunger and lower ad libitum energy intake following a HP lunch meal (212 kcal, 30% carbohydrate, 45% (23.3 g) protein and 25% fat) in comparison to a HC lunch meal (213 kcal, 60% carbohydrate, 30% fat and 10% (6.2 g) protein) in the NW and Obese participants. The discrepancies between these and the current study, could be attributed to differences in meal viscosity and the use of solid meal. These distinctions in meal texture might account for the variation in satiety responses, as evidenced by a previous systematic review involving 48 experiments, which revealed that physical texture of a food can significantly impact satiety, with as solid and semi-solid food being more satiating than liquid forms (Almiron-Roig et al., 2013).

Data from the eating behaviour questionnaires including the DEBQ, BES, and TFEQ did not find significant different between the NW and Obese groups. However, there was a noticeable trend towards higher scores in the disinhibition

domain for the Obese group, as expected. Disinhibition is the overconsumption of food in response to various stimuli, such as emotional stress, which is accompanied by a lack of control over food intake (Stunkard and Messick, 1985). The increased disinhibition trend in the current study is consistent with a previous review by Vainik et al. (2019), which demonstrated that uncontrolled eating and BMI are typically independent phenomena that can interact with one another.

7.7 Strength and limitations of the study

This is the first study to compare satiety responses between HP and HC drinks in NW and Obese participants while matching, volume, energy content, and viscosity. Unlike other studies, the drinks used in this study were pure macronutrients, allowing better understanding of individual macronutrient effects on satiety.

However, it is important to acknowledge that this study has some limitations which should be considered when interpreting the results. One of the limitations is the absence of data for the Ex-Obese group in the main analysis due to a small number of individuals recruited (only 2 participants). Instead, their individual data for the ad libitum meal intake, VAS ratings and energy intake were presented for these participants. Several factors impacted the recruitment of the Ex-Obese group. Firstly, the study experienced interruptions due to the COVID pandemic and related lockdown measures, with recruitment activities halted from March 2020 until June 2021. Secondly, recruiting participants for this group proved challenging as, although, advertisements were sent to local Slimming World Groups, these groups had been adversely affected by the pandemic, resulting in no participants contacting the research team through this route. Only two participants were recruited through the study advertisement posters placed around the three University's Campuses.

The other limitation of this study is the inclusion of fibre in the HC drink, with methylcellulose added to achieve the required viscosity (1.9 g of fiber/100 ml). Fibre can potentially impact satiety by increasing viscosity, forming gels in the stomach, and fermenting in the GI tract (Slavin and Green, 2007). However, a study conducted by Berthold et al. (2008) measured the effects of six highly cross-linked

cellulose capsules compared to six placebo capsules, administrated 30 minutes before a standardised meal in 19 Obese participants. Their findings demonstrated that supplementation with cross-linked cellulose did not influence GE and satiety sensation.

The study included both male and female participants, which may have introduced variability into the results. Female sex hormones, particularly oestrogens, can influence peripheral and central signals from several hormones involved in the regulation of food intake , including CCK, ghrelin, insulin, and leptin (Asarian and Geary, 2006). These hormones may also play a role in the inhibitory effect of oestrogenic on food consumption during meals. It has been shown that higher energy intake was seen in the luteal phase when compared to the follicular period (Davidsen et al., 2007). It is worth noting that female participants in this study were not recruited during the early phase (follicular period) of menstrual cycle due to logistical challenges in coordinating study visits and availability of the participants. Also, we were unable to conduct separate analysis to assess the impact of gender on satiety responses due to the relatively small sample size in each gender group.

While the use of pure macronutrient drinks in this study provides a significant advantage in understanding the specific effects of individual macronutrients on satiety, it's important to acknowledge that these drinks may not entirely represent the complexity of human diets. In reality, foods people consume are typically combinations of various macronutrients in different proportions.

7.8 Conclusion

This study did not reveal significant differences in ad libitum energy intake, or satiety VAS ratings between the HC and HP drinks among adults with a normal-weight and obesity. Similarly, no differences were found in eating behaviour traits between the two groups. However, participants with obesity tended to have higher TFEQ-Disinhibition scores. While subjective satiety rating provides a validated method of measure satiety response, further studies should consider including more objective measures of satiety, such as gut hormones and GE rate, allowing

comprehensive understanding of the satiety effect between macronutrients. In addition, other factors that might affect satiety, such age and gender should be considered. Furthermore, investigating satiety responses in the Ex-Obese group holds significant promise, as it can provide valuable insights into effective dietary strategies for weight maintenance, thereby contributing to the prevention of weight regain and the management of the obesity epidemic.

8 General discussion

The work in this PhD thesis aimed to use MRI measurements combined with behavioural and biochemical analysis to investigate and understand the potential mechanisms of the regulation of food intake in normal-weight adults and alterations in people living with obesity. Specific hypotheses to be tested in this thesis were that people with obesity when compared with normal weight participants will show A) an alteration in neurohormonal gut-brain signalling, B) an increase in gastric emptying rate, SBWC, and SMA with associated appetite scores, and C) a change in eating behaviour. These hypotheses were addressed through a systematic review and functional MRI meta-analysis and three eating behaviour studies, two of which used MRI techniques.

Recent advances in MRI methods allowed measuring the physiological responses to food intake in the GI system non-invasively (Carbone et al., 2010, Fruehauf et al., 2011). MRI can capture dynamic changes during the process of GE, making it suitable for studying the process under various conditions. As described in Chapter 2, MRI has been validated against the gold standard methods (scintigraphy) of measuring GE in healthy participants after both liquid and solid meals (Kunz et al., 1999, Teramoto et al., 2012, Khalaf et al., 2020a) and also in patients with gastroparesis (Hayakawa et al., 2017), diabetes (Parkman et al., 2010), and dysphagia (Menys et al., 2017). In this thesis, MRI was employed extensively to investigate and understand the GI responses to food consumption in people with obesity. This is demonstrated in Chapter 5 and Chapter 6. Chapter 5 assessed the GI responses to a standard pasta meal, while Chapter 6 used advances in MRI to assess food intake to different macronutrients (fat vs carbohydrate) with the same energy content, volume, and viscosity. To date, no previous studies have used MRI techniques to compare GI responses following food intake between normal-weight participants and those with obesity.

In terms of GI responses to a pasta meal, no significant differences were found in GCV, GE rate, SBWC and SMA blood flow, appetite, and satiety hormones when

people with obesity were compared with normal-weight adults. This suggests that the immediate GI responses to the meal were similar between Obese and normal-weight individuals. Despite the similarities in GI responses, the studies revealed that the Obese group had lower satiety feelings compared to the NW group. This difference in satiety feelings implies that individuals with obesity may experience less satisfaction or fullness after eating, which could potentially lead to overeating and weight gain. In addition to lower satiety feelings, the Obese group displayed higher insulin and triglyceride concentrations. These hormonal differences could be important factors contributing to weight gain in individuals with obesity.

In chapter 6, no definitive conclusion could be made from the outcomes of this study because of the relatively small number of participants included. However, the study does suggest that a HF meal causes an increase in GCV, SBWC, and SMA blood flow compared to a HC meal in both NW and Obese participants. The completion of this work with a larger sample size will enable a more robust assessment of the effect of fat and carbohydrate on GI responses (GCV, SBWC, and SMA blood flow) in NW and Obese participants. Additionally, this study aimed to investigate the interactions between the brain and the GI tract in relation to food intake. Unfortunately, the brain data was not included in this work; however, once this data is finalised, it will provide a valuable understanding of the physiological mechanisms underlying appetite and satiety regulations in normal weight participants and alterations with obesity. Understanding the physiological mechanisms that regulate appetite and satiety could lead to the development of food products that enhance satiety feelings, prevent weight gain, and/or enhance weight loss. It is important to note that despite the great benefits of MRI to assess brain and GI responses, the technique is expensive and may not be readily available in all clinical or research settings. Also, MRI might not be suitable for individuals who are claustrophobic, or have MRI contraindications (i.e., pacemaker, metallic implants).

The work in this thesis also conducted eating behaviour trial to evaluate satiety sensations to acute high-protein (HP) consumption compared to high-carbohydrate

(HC) in Obese, Ex-Obese and NW participants. The results did not reveal significant differences in ad libitum energy intake or subjective satiety ratings between the acute consumption of HC and HP drinks among NW and Obese participants. The study challenges, at least in the short term, the widely held belief that protein is significantly more satiating than carbohydrate. It suggests that the specific form in which macronutrients are presented within food may have a major impact on their satiating effect. In other words, factors beyond macronutrient composition alone, such as the texture and overall composition of the food, can influence satiety. It's important to note that long-term and real-world dietary patterns may yield different results, and further research is needed to explore the complexities of macronutrient satiety in various contexts. Unfortunately, the initial aim of comparing these satiety effects in individuals who had lost weight following obesity was not possible to achieve or complete in this study due to the impact of COVID pandemic on recruitment within this group. Further research is needed for this group.

8.1 Advantages and limitations

The advantages and limitations of each study was discussed in detail in relevant chapters. This section highlights the general advantages and limitations of this PhD work.

However, the main strength on this thesis is the use of a multidisciplinary approach, through utilising and integrating different approaches, to measuring the regulation of food intake, including behavioural assessments (subjective satiety ratings) and physiological measurements obtained through MRI to potentially unravel the mechanisms of alerted eating behaviour in individuals with obesity. This holistic approach facilitates a comprehensive understanding of the impact of macronutrient composition on satiety, hormonal influences, gastrointestinal responses, neural signalling, and eating behaviours in the regulation of food intake.

One of the limitations of this work is the absence of brain MRI measurements in response to meal intake (Chapter 5). The brain plays a major role in regulating food intake, and future studies should include brain measurements in response to food

intake to allow a better understanding of the interactions between the gut and brain to tackle obesity. Understanding these dynamics should contribute to a better comprehension of the metabolic and neural alterations that often accompany obesity.

Participants in Chapter 7 were mostly females, which could have affected satiety responses. Female sex hormones, particularly oestrogens, can influence peripheral and central signals from several hormones involved in the regulation of food intake, including CCK, ghrelin, insulin, and leptin (Asarian and Geary, 2006). These hormones may also play a role in the inhibitory effect of oestrogen on food consumption during meals. On the other hand, the work conducted in Chapter 5 was collected from male-only participants, which cannot be generalised on female participants.

Another limitation in this thesis are small sample sizes which could not allow for more comprehensive comparisons between groups. However, it has been challenging to recruit people with obesity for this thesis for the following reasons. Initially, posters advertising the study were placed around the three University's Campuses, and the majority of participants were typically normal-weight individuals. Second, people with obesity frequently have other medical conditions like diabetes or hypertension, making it difficult to include them in the study. Third, the COVID pandemic had a negative impact on recruiting people with obesity who lost weight since it prevented any participants from contacting the research team via advertisements sent to local Slimming World Groups. Hence, future studies should be designed to recruit larger sample sizes, to allow for more comprehensive comparisons between different groups with altered eating behaviour. This will strengthen the findings and enhance the generalisability of the research.

8.2 Recommendations for future work

The need of more a holistic approach to unravel the mechanisms of altered eating behaviour in people with obesity: The functional neuroimaging meta-analysis in this thesis identified the caudate nucleus and hypothalamus as key areas associated with satiety regulators in NW participants. These findings provide valuable information about the neural mechanisms that underlie appetite control in individuals with a normal weight and can be used as a valuable reference point for future studies exploring alterations in gut-brain interactions associated with obesity. Although the work aimed to generate ALE maps for people with obesity, the limited number of studies conducted in this area hindered the ability to draw conclusive findings about the brain regions associated with appetite and satiety regulation in individuals with obesity. This gap in research emphasises the need for further investigation to better understand the neural correlates of appetite control in this population.

Individuals with obesity who have lost weight: A possible extension of this thesis is to investigate satiety responses in individuals with obesity who have lost weight. This area holds significant promise. It can provide valuable insights into effective dietary strategies for weight maintenance, contributing to the prevention of weight regain and the management of obesity. Another aspect to consider is to compare satiety responses between people who have lost weight but are still obese and people who have lost weight and are no longer obese. Previous research revealed that higher basal and postprandial ghrelin concentrations and hunger scores in individuals who lost weight but are still obese compared to prior weight loss (Coutinho et al., 2018, Nymo et al., 2018). However, another study carried out by Hernández Morante et al. (2020) showed no effect of weight loss on ghrelin concentrations in people who remain obese after weight loss. DeBenedictis et al. (2020) assessed satiety responses in individuals who had lost weight but were no longer obese. They found that after losing weight, there was an increase in basal and postprandial ghrelin concentrations as well as hunger scores. These areas need

further exploration to understanding whether alterations in satiety scores that might arise during obesity are reversable following weight loss.

Comparing protein types: In this thesis, behaviour scores to satiety responses were measured following whey protein intake, as evidence suggests it is more satiating than casein in supressing hunger feelings (Veldhorst et al., 2009) and reducing subsequent food intake (Hall et al., 2003). However, it is still inconclusive if one protein is more satiating than others (Bendtsen et al., 2013). Hence, it will be valuable for future studies to compare appetite and satiety responses between whey and casein protein in people living with obesity. Measuring the satiety responses to meals that combine casein and whey with carbohydrates is also another extension.

Larger sample sizes: future studies should be designed to recruit larger sample sizes, to allow for more comprehensive comparisons between different groups with altered eating behaviour. This will strengthen the findings and enhance the generalisability of the research.

A longitudinal study: a future longitudinal MRI study is recommended to assess factors of impaired appetite and satiety mechanisms that contributes to obesity. This study should follow participants from birth to adulthood to evaluate the factors that regulate food intake regulation which include the following: gastric emptying rate, concentrations of appetite hormone (ghrelin) and satiety hormones (PYY, GLP-1, CCK, insulin, leptin) during hunger and fed states, brain responses in homeostatic and hedonic areas, and ratings of subjective satiety feelings. This study should follow body weight status of participants to know if they develop obesity during adulthood. To determine if the factors mentioned above are risk factors or consequences of obesity, they should be assessed both before and after weight gain in participants who will have obesity during adulthood. It is important to take into account additional risk factors for obesity, such as level of physical activity, alcohol consumption, emotional eating, social network, genetics, and medical problems and medications that may increase weight gain.

An aging population: Examining satiety responses in older individuals is another potential extension for this thesis. The "anorexia of ageing" is a common issue, and

understanding how macronutrient intake affects satiety in this population can have implications for nutrition and health in older people (Morley and Silver, 1988). Around 15% to 30% of the elderly have been affected by anorexia of ageing, with rates higher in women, residents of nursing homes, and hospitalised patients (Malafarina et al., 2013). A decrease in appetite can result in a decrease in the amount of food and nutrients consumed (Payette et al., 1995), which raises the possibility of malnutrition and weight loss (Wilson et al., 2005, Brownie, 2006). Elderly people have a higher need for protein intake (1.1 to 1.2 g/kg) to support recovery from illness and improve muscle function and health (Bauer et al., 2013), but they may not usually to get their protein requirements due to the satiating effects of protein. Most studies compare satiety effects of macronutrients focused on young adults, hence, it is unclear if older people have different satiety responses to intake (Dericioglu et al., 2023). Future work should study satiety feelings, GE rate, hormonal, and neural responses following macronutrient intake in older individuals.

8.3 Conclusion

Different studies were conducted in this PhD thesis, including a systematic review and functional MRI meta-analysis and three eating behaviours studies, two of which used MRI techniques. The utilisation and integration of different approaches to measuring the regulation of food intake offer a holistic understanding of food intake regulation. Although the work performed in this thesis did not find significant difference in most of the behavioural and physiological measurements related to regulation of food intake between people with obesity compared to those without obesity, it paved the way for further research opportunities to address the identified limitations from the current work and expand our knowledge in this critical area of nutrition and health.

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10 Appendices

10.1 Visual analogue scale (VAS)

Participant ID number:_____ Date:_____ Time-point:_____

Please rate the degree of desire to eat/hunger/fullness you would typically feel? Please put a slash (/) mark somewhere on the lines below

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

   Very weak ——————————— Very strong

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

   Not at all ——————————— Extremely

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

   Not at all ——————————— Extremely

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

   Not at all ——————————— Very full

5. **How much food do you think you could eat?**

   Nothing at all ——————————— A large amount

10.2 Preparation methods of drinks and meals used in the thesis

10.2.1 Materials and equipment used in preparation of drinks and meals in the thesis

- Mineral water (Evian, French Alps, France)
- Tween 20 (SIGMA, Aldrich, Gillingham, U.K)

- Hydroxypropyl methylcellulose (HPMC, Benecel™, K15M, Ashland, IMCD UK Ltd)
- Coffee flavour (Nielsen Massey Vanillas, Inc, USA)
- Artificial sweeteners (Hermesetas, Switzerland)
- Rapeseed oil (Sainsbury's Supermarket, UK)
- Dolmio Bolognese Original Pasta sauce (Freepost Mars Food UK, Dublin, UK)
- Dried white Pasta (Sainsbury's Supermarket, UK)
- Olive oil (Sainsbury's Supermarket, UK)
- Cheddar cheese (Sainsbury's Supermarket, UK)
- Whey protein (Protein Works, Chocolate Silk, www.theproteinworks.com)
- Maltodextrin DE 18 (Maltosweet 180, Azelis UK)
- Cocoa powder (Tesco Supermarket, UK)
- Kitchen scales
- Measuring cups
- Heidolph stirrer (model RZR 2021, shown in Figure 10.1)
- Silverson (model L5R, shown in Figure 10.2)
- Metallic spoon
- Food thermometer
- Food blender
- Large saucepan
- Kitchen scales
- Colander
- Mixing bowl
- Measuring bow
- Glassware (beakers, cylinders, and bottles)

10.2.2 Area and equipment cleaning

- Hands were thoroughly washed with soap and water (warm or cold) and dried before handling food.

- Utensils were washed before and after preparing food.
- Worktops were cleaned and sanitised before and after preparing food.

10.2.3 Preparation method of the standard pasta meal in Chapter 5

Ninety-five grams of the pasta was cooked in boiling water for eleven minutes. The pasta was drained well and rinsed in cold water until it was no longer warm. One hundred and ninety-five grams of Dolmio Bolognese Original Pasta sauce, 40 g of cheddar cheese, and 4.2 g of olive oil were added to the pasta and stirred until it became well combined. The pasta was aliquoted into ~500 g portions and stored in the refrigerator overnight. On the study day, the pasta was heated in a microwave for 4 minutes, stirred hallway through, and then provided to participants straight away.

10.2.4 Preparation of high-fat emulsion (High-fat drink) in Chapter 6

To prepare 500 ml of the fat emulsion, 148 g of water was added in a beaker with a 5 g Tween 20 emulsifier. The water and emulsifier were mixed in the Silverson (Figure 10.1) at full speed for two to three minutes. Then, 110 g of rapeseed oil was added to the mixture and mixing continued for 10 minutes. A Silverson homogenizer was used to achieve a highly uniform particle size distribution. This process involves three stages of mixing and homogenising, employing a high-speed rotor, centrifugal force, and hydraulic shear. A fat emulsion droplet size of approximately 6 m was produced. The fat emulsion was transferred to a 500-ml bottle, cooled to a temperature below 5°C, and then kept in the fridge until used.

Figure 10.1. Silverson homogenizer (model L5R). The picture taken from/source: the manufacture's website (https://www.silverson.co.uk).

To prepare the HPMC mixture, a Heidolph magnetic stirrer (Figure 10.2) was used to prepare the HPMC solution. Water was divided into two equal quantities (150 g) and placed in two beakers. One beaker was covered with cling film and kept in the fridge, while the other beaker was placed on the heater, which was adjusted to a temperature of 100–150 °C. The paddle on the stirrer was set to a speed of 2, with the power level at 1, and the beaker was also covered with cling film to reserve heat. HPMC (4.5 g) was gradually introduced into the stirring water when the water reached a temperature of 70 °C. HPMC was added while stirring when the water reached 70 °C. Subsequently, the cold water was added while continuing to stir. The resulting mixture was covered with cling film and transferred to a 500-ml bottle, cooled to a temperature below 5°C, and kept in a fridge until used. Before being served to participants on the study days, drinks were removed from the fridge and allowed to warm up to room temperature for thirty to forty minutes. The drink was served in an opaque paper cup with a straw and a lid.

Figure 10.2. Heidolph magnetic stirrer (model RZR 2021).

To prepare the drink for a study visit day, 210 g of the fat emulsion was mixed with 90 g of the HPMC mixture. Next, 5 artificial sweeteners and 18 ml of the coffer flavour were added to the drink. Following the mixing of these components, the drink—roughly 280 ml—was put into a cylinder. Water was subsequently added to make 300 millilitres.

10.2.5 Preparation of high-carbohydrate drink in Chapter 6

To prepare 500 ml of the high-carbohydrate drink, two hundred and sixty grams of maltodextrin were mixed with 240 g of water by a metallic spoon until the mixture became smooth and no lumps were visible.

To prepare the drink for a study visit day, 300 g of the mixture was weighted, and then 5 artificial sweeteners and 18 ml of the coffer flavour were added to the mixture. Following the mixing of these components, the drink was put into a cylinder. Water was subsequently added to make 300 millilitres. To match the components of the HF and HC drinks, 3.3 g of Tween 20 was added to 300 ml of carbohydrate drink. Before being served to participants on the study days, drinks were removed from the fridge and allowed to warm up to room temperature for thirty to forty minutes. The drink was served in an opaque paper cup with a straw and a lid.

10.2.6 Preparation method of high-protein drink in Chapter 7

To prepare 500 ml of the high-protein drink, 116.6 g of whey protein was mixed with 383 g of water in the food blender at full speed for two minutes. The drink was transferred to a 500-ml bottle and kept in the fridge overnight at a temperature below 5 °C. To prepare the drink for a study visit day, 300 g of the mixture was weighted, and then the drink was put into a cylinder. Water was subsequently added to make 300 milliliters. Before being served to participants on the study days, drinks were removed from the fridge and allowed to warm up to room temperature for thirty to forty minutes. The drink was served in an opaque paper cup with a straw and a lid.

10.2.7 Preparation method of high-carbohydrate drink in Chapter 7

The HPMC mixture for this drink was prepared by mixing 7.2 g of HPMC with 300 g of water. The preparation method was explained previously in section 10.2.4.

To prepare 300 ml of the drink, using a food blender, mix 150 g of the maltodextrin mixture with 150 g of the HPMC mixture, 12 g of cocoa powder, and 3 artificial sweeteners. Following the mixing of these components, the drink was put into a cylinder. To prepare the drink for a study visit day, 300 g of the mixture was weighted, and then the drink was put into a cylinder. Water was subsequently added to make 300 milliliters. The drink was transferred to a 500-ml bottle and kept in the fridge overnight at a temperature below 5 °C. Before being served to participants on the study days, drinks were removed from the fridge and allowed to warm up to room temperature for thirty to forty minutes. The drink was served in an opaque paper cup with a straw and a lid.

10.2.8 Preparation of ad libitum pasta meal in Chapter 7

Two hundred and fifty grams of the pasta was cooked in boiling water for eleven minutes. The pasta was drained well and rinsed in a cold water until it was no longer warm. Three-hundred and forty grams of Dolmio Bolognese Original Pasta sauce, 80 grams of cheddar cheese, and 30 g of olive oil were added to the pasta and stirred until it became well combined. The pasta was aliquot into ~1000 g portions and stored in the fridge overnight. On the study day, the pasta was heated in a microwave for 2 minutes, stirred hallway through, and then provided to participants straight

away with 250 ml water . The pasta plate was always replenished when it was about three-quarters empty, ensuring that there was always enough hot food for participants and that they weren't prompted to stop eating by emptying their plate.