Development of Protocols to Identify High Hunger and Low Satiety Phenotypes

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Dedication

For the souls of my father and my mother

This PhD thesis is for you

Declaration

I declare that all the material presented in this thesis is my own work, unless stated otherwise. All contents reflect my own knowledge, ideas and interpretations and have not been submitted for a degree or award elsewhere.

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Hameida Elfarssi

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Abstract

Obesity has become a worldwide epidemic. Impaired appetite control is associated with weight gain. People who have difficulties in recognising their appetite sensations before and after a meal and who do not eat in response to their appetite sensations seem to be more susceptible to weight gain. These individuals may represent the 'low satiety phenotype.'

The objective of the present study was to extend and test the work identified in the literature regarding the identification of a satiety phenotype. To identify, at baseline, individuals who struggle to lose weight on weight management programmes, due to reduced satiety, to help clinical professionals to identify those people at baseline so that they can use personalised weight loss strategies to help them.

The work included studies in an acute laboratory setting and clinical studies. The first laboratory setting study was a pilot study to allow the researcher to gain expertise in the methods used to assess satiety phenotypes in people with obesity by analysing blood samples for gut hormone levels, subjective ratings of appetite response to a test meal, Three Factor Eating Questionnaire (TFEQ) subscales, food diaries and food craving. A second laboratory based study identified a satiety phenotype in individuals with normal weight by measuring energy intake in an *ad libitum* test meal, as well as energy intake from a 3 day food diary. Subjective ratings of appetite response, gut hormones, TFEQ and food cravings were also assessed. This study developed methods to subsequently use in a clinical community by designing a heat map, which is a visual presentation tool including independent variables, scores to help clinical professionals working in clinical settings to follow this scoring system to identify individuals who have low or high satiety prior to participation in weight management programmes.

Clinical studies were carried out on individuals with obesity by determining at baseline, variables prior to participation in a weight management programme to identify those who struggle to lose weight. In the study carried out in a Tier 3 setting, fasting samples of plasma gut hormones, subjective ratings of appetite response and TFEQ subscales were used to predict subsequent weight loss and reduced satiety. In a Tier 2 setting

study, food diaries, food craving and TFEQ subscales were used to identify those who may struggle to lose weight.

In the acute laboratory setting, subjective ratings of appetite response were found to be the best measure to identify satiety phenotypes and this was combined with other predictive measures to build the heat maps.

In the clinical studies, subjective ratings of appetite response were the best baseline measures to predict weight loss. In the Tier 3 study, subjective ratings of appetite response predicted weight loss and people with reduced satiety, as determined using the heat map tool, on average lost more body mass, BMI and waist circumference although the difference was not significant. In the Tier 2 study, carbohydrate food craving predicted subsequent weight loss.

In conclusion, the novel findings in this study are the further development of protocols to identify high hunger and low satiety phenotypes. These will inform researchers and staff in clinical community settings to identify people who have a low satiety phenotype and may inform personalised treatments. However, further studies are needed with larger sample sizes to fully elucidate and validate the above findings.

Abbreviations

AA	Amino acids
AGB	Adjustable gastric banding
AgRP	Agouti-related protein
Alpha-MSH	Alpha-melanocyte-stimulating hormone
AOD	Anti-obesity drug
AP	Area postrema
ARC	Arcuate nucleus
AUC	Area under curve
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CART	Cocaine and amphetamine-regulated transcript
CCK	Cholecystokinin
CHO	Carbohydrate
CNS	Central nervous system
CVD	Cardiovascular disease
DMN	Dorsomedial hypothalamic nucleus
DPPIV	Dipeptidyl- peptidase IV
DVC	Dorsal vagal complex
DVN	Dorsal motor nucleus of vagus
EDTA	Ethylenediaminetetra-acetic acid
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
FCI	Food Craving Inventory
GE	Gastric empting
GHS-R1	Growth hormone secretagogue receptor
GI	Gastrointestinal
GIP	Gastric inhibitory peptide
GLP-1	Glucagon-like peptide-1
HGL	High glycaemic load
HMHMAG34K	Human Metabolic Hormone Magnetic Bead Panel
HTN	Hypertension
ICV	Intracerebroventricular
kcal	Kilo calorie
LCD	Low-calorie diet
LEP	Leptin gene
LEPR	leptin receptor
LGL	low glycaemic load
LHA	Lateral hypothalamic area
LSG	Laparoscopic sleeve gastrectomy
MC3R	Melanocortin-3 receptor
MC4R	Melanocortin-4 receptor
NAFLD	Non-alcoholic fatty liver disease
NPY	Neuropeptide tyrosine
NTS	Nucleus of the tractus solitarius

PA	Physical activity
pg/ml	picogramme per milliliter
POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
PVN	Paraventricular nucleus
PYY	Peptide YY
RIA	Radioimmunoassay
SD	Standard deviation
SE	Satiety efficiency
SI	Satiety index
SNP	Single-nucleotide polymorphism
SPSS	Statistical Package for the Social Science
SQ	Satiety quotient
T2DM	Type 2 Diabetes mellitus
TEI	Total energy intake
TFEQ	Three-Factor Eating Questionnaire
VAS	Visual analogue scales
VLCD	Very low-calorie diet
VMN	Ventromedial hypothalamic nucleus
WC	Waist circumference
WHO	The World Health Organization
WHR	Waist-to-hip ratio
µL	Microliter

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General introduction

1 General introduction

1.1 Obesity overview

Despite recent improvements in our knowledge of the physiological mechanisms that regulate energy intake (EI), energy expenditure and body mass, obesity continues to be a substantial worldwide health issue with an array of metabolic, vascular and psychosocial co-morbidities (Suzuki, Jayasena and Bloom 2012). Obesity is the result of a long-term imbalance in energy, where EI exceeds energy expenditure (Crino *et al.,* 2015). Furthermore, obesity has been recognised as a worldwide epidemic and prevalence has increased rapidly in high-income countries since the 1970s; middle and low–income countries have an increased prevalence of obesity in both adults and children (Swinburn *et al.,* 2011).

1.2 Definition of obesity

Obesity is broadly defined as an abnormally high accumulation of fat in adipose tissue that associates with significant morbidity and mortality (Chan and Woo, 2010). The World Health Organization (WHO) has stated that overweight and obesity are the fifth leading cause of global deaths (Posovszky and Wabitsch, 2015). It is responsible for reducing life expectancy by 6 to 7 years; a body mass index (BMI) of 30–35 reduces life expectancy by 2–4 years while severe obesity (BMI > 40) reduces life expectancy by 10 years (Fock and Khoo, 2013).

Moreover, there is extensive evidence that obesity associates with increased risk of comorbidities such as cardiovascular disease (CVD), hypertension (HTN), type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) hyperlipidaemia, stroke, thromboembolic disease, certain cancers (e.g. colorectal and breast), asthma, gall bladder disease, sleep apnea, liver and gastroesophageal reflux disease, gynaecological and osteoarthritis problems (Finelli *et al.,* 2014).

1.3 Classification and anthropometric measurement of obesity

Measurement of body composition is required for accurate classification to describe a person as normal weight, overweight or obese. However, measuring body fat can be an expensive and difficult method, with no precise method that is easily accessible for clinical practice. Therefore, BMI remains the most widely and simple indicator for

determining obesity. It is expressed as an individual's weight in kilograms (kg) divided by their height in meters squared (m^2) (Buchholz and Bugaresti, 2005; James, 2004).

WHO has categorized obesity and overweight in an individual based on different BMI cut-offs. People who have a BMI ≤ 18.5 kg/m^2 are underweight. 18.5-24.9 kg/m^2 are normal weight. 25.0 kg/m^2 or greater are considered to be overweight and those with BMI of 30.0 kg/m^2 or greater are obese. A BMI of 40 kg/m^2 or above is classified as morbid obesity. The BMI cut-offs are set depending on the associated comorbidities risk (table 1.1) (WHO, 2012). However, BMI does not differentiate between body mass in relation to either fat or muscle, and the relationship between the content of body fat and BMI differs depending on body build and proportion (Ho-Pham *et al.*, 2015). Conversely, several studies indicate that central fat accumulation or intra-abdominal measurement is a better predictor than BMI for reflecting the risk factors of cardiovascular disease and other forms of chronic disease (Klein *et al.,* 2007; Wang *et al.,* 2009).

Central fat accumulation assessment can help in defining obesity (Chan and Woo, 2010). Waist circumference (WC), hip circumference and waist-to-hip ratio (WHR) are the anthropometric indices commonly used in assessing abdominal obesity and health risks related to obesity (Kitzinger and Karle, 2013). Visceral fat or excessive abdominal fat is defined as a waist circumference > 102 cm in men or > 88 cm in women, which can increase risks of morbidity and mortality (Table 1.2). Additionally, there are metabolic symptoms that are connected to excessive visceral fat such as dyslipidaemia, insulin resistance and hypertension (Chan and Woo, 2010).

Table 1-1: Classification of overweight and obesity in adults according to BMI (Adapted from Chan and Woo, 2010).

Classification	**BMI (kg/m^2)**	**Risk of co-morbidities**
Underweight	<18.5	Low (but increase of risk other clinical problems)
Normal range	18.5−24.9	Average
Overweight (pre-obese)	25.0−29.9	Increased

obese	≥ 30	
Obesity class I	30.0−34.9	Moderate
Obesity class II	35.0−39.9	Severe
Obesity class III	≥40	Morbid

Table 1-2: Disease risk associated with visceral or abdominal obesity (Adapted from Cheah and Kam, 2005).

Disease risk relative to waist circumference		
Category	**Men < 102 cm** **Women < 88 cm**	**Men ≥ 102 cm** **Women ≥ 88 cm**
Underweight	-	-
Normal weight	Minimal	Slightly increased
Overweight	Increased	High
Obesity I	High	Very high
Obesity II	Very high	Very high
Obesity III (Extreme obesity)	Extremely high	Extremely high

1.4 Prevalence of obesity

The rising prevalence of obesity poses a significant health threat to national and global public health in terms of prevalence, incidence and economic burden (Tremmel *et al.,* 2017). The WHO estimates that the global prevalence of obesity has nearly tripled between 1975 and 2016. In 2016, overweight adults who were aged 18 years and older accounted for more than 1.9 billion, of which over 650 million adults were deemed obese. Approximately 13% of the global adult population were classed as obese (11% of men and 15% of women). WHO has estimated that in 2016, about 41 million children who were aged under 5 years were overweight or had obesity and more than 340 million children and adolescents aged 5-19 years were considered to be overweight or obese (WHO, 2018).

Worldwide, it is projected that sixty percent of the world's population (3.3 billion people) will be overweight (2.2 billion) or obese (1.1 billion) by the year 2030, if recent trends of obesity and overweight rates continue (Kelly *et al.*, 2008).

In the UK, 25% of adults are obese and if overweight adults are included this increases to two-thirds of the adult population (Booth, Prevost and Gulliford, 2015). Furthermore, predictions from the UK Foresight report show that men will overtake women for obesity (47% and 36% respectively by 2025). Conversely, men with morbid obesity (BMI ≥ 40 kg/m^2) are likely to be less common than women (Boyers *et al.,* 2015).

In England, if the current trend continues it shows that by 2050, 60% of males and 50% of females could be obese. It has been estimated that on current trends around 8% of 1–2-year-old children with obesity are predicted to be obese adults, and 80% of 10–14-year olds are predicted to be adults with obesity. Moreover, it has been suggested that by 2050, among children aged 6–10 years, the number of obese boys will be more than girls with an estimate of 50% of boys will become obese by 2050 (Agha and Agha, 2017).

1.5 The aetiology of obesity

The aetiology of obesity is multifactorial and represents a complex interaction between genetics, hormones, environmental, physiological, psychological, economic, social and even political factors. These factors interact to enhance the risk of obesity development (Wright and Aronne, 2012; Aktar, Qureshi and Ferdous, 2017). Though several genetic markers have been implicated in the pathogenesis of obesity, these findings are inconsistent. These genes include the beta-3-adrenergic receptor gene, melanocortin-4 receptor gene, peroxisome-proliferator-activated receptor gamma 2 genes, chromosome 10p and other genetic polymorphisms (Mutch and Clement, 2006).

Genetic predisposition affects obesity risk and there is ample evidence that single gene mutations can cause rare forms of monogenic obesity e.g., in the genes coding for leptin (LEP), leptin receptor (LEPR), melanocortin-4 receptor (MC4R) and pro-opiomelanocortin (POMC). However, common single-nucleotide polymorphisms (SNP) or genetic variants might also play a significant role in the obesity epidemic. These

SNPs have different impacts on an individual's vulnerability to common forms of obesity (Nguyen and EL-Serag, 2010).

Several hormones are also included in the pathophysiology and regulation of obesity, involving gut-related hormones, adipokines and others. Ghrelin is an orexigenic hormone which is responsible for stimulating appetite, whereas anorectic gut hormones such as Peptide YY (PYY), Cholecystokinin (CCK), and Glucagon-like peptide-1 (GLP-1) are responsible for limiting food intake (Aktar, Qureshi and Ferdous, 2017).

These hormones are released, in response to ingested food, from several sites in the gut, including the stomach, proximal/distal small intestine, pancreas and colon (Schwartz, 2006; Konturek *et al.,* 2004). Peripheral signals from adipose tissue and the gut integrate in the hypothalamus to control short-term food intake and achieve long-term energy balance; centrally released hormones and neurotransmitters also contribute to appetite regulation (Chaudhri, Small and Bloom, 2006; Yu and Kim, 2012).

Homeostatic and hedonic systems in the brain also impact on appetite and satiety. The physiologic need to eat during negative energy balance is a primitive survival function, mediated through the hindbrain and hypothalamus (Lean and Malkova, 2016).

In contrast, a desire to consume attractive foods arises from mesolimbic reward circuits and regions in the orbitofrontal cortex that regulates hedonic food intake based on the sensations of taste, smell, texture and sight. The hedonic systems are in constant interaction with homeostatic mechanisms and sensory attractiveness of foods can become dominant to contribute to weight gain and obesity (Lean and Malkova, 2016).

Low physical activity, adoption of a sedentary lifestyle, high energy density diet, increased portion size, as well as eating disorders have been considered as important risk factors for obesity (McKenney and Short, 2011). Moreover, drugs (steroids and antipsychotic) and neuroendocrine diseases (hypothalamic, pituitary, thyroid and adrenal) can lead to obesity (McKenney and Short, 2011).

In addition, low socioeconomic groups tend to have BMIs that are higher than middle and high socio-economic groups; in migrants, obesity is common, perhaps because they live under new socio-economic and cultural conditions (Wright and Aronne, 2012; Ali and Crowther 2009). Reduced physical education in schools and increased time

undertaking sedentary behaviours such as computer use, watching television and playing video games have also been linked to the increasing prevalence of obesity (Wright and Aronne, 2012). Sleep debt also contributes to increasing body mass. Evidence of some studies has shown that hours of sleep per night are negatively associated with BMI and sleep restriction has been shown to contribute to increased hunger and appetite (Wright and Aronne, 2012).

Mental wellbeing can impact on eating habits; some individuals eat in response to negative emotions. For instance, stress not only increases food consumption but also biases consumption toward higher caloric foods that are normally avoided (Ali and Crowther 2009).

1.6 Obesity management strategies

The treatment of obesity is more than just weight loss alone and must also focus on health improvement and reduction of risk of comorbidities (Aktar, Qureshi and Ferdous, 2017). To achieve significant and enduring weight loss, numerous studies have focused on developing several interventions, including: 1) - life style modification using several diet restrictions, alone or in conjunction with physical activity, 2) - pharmacotherapy and 3) - bariatric surgery.

1.6.1.1 Life style modification

Lifestyle modification strategies (diet and exercise) are the initial intervention in mitigating obesity risk and encouraging weight loss. Diet is dependent on the principles of metabolism and work by decreasing the intake of calories (energy) to create a negative energy balance (i.e. more energy is used than is consumed). Diet programmes can produce meaningful weight loss over the short term (Zhang *et al.,* 2014). In general, there are four types of dietary regimens suggested for weight loss which are Low-Calorie Diet (LCD), low-fat diet, low-carbohydrate diet and Very Low-Calorie Diet (VLCD). All these diets provide 800–1500 kcal/day, while VLCD is < 800 kcal/day (Fock and Khoo, 2013).

According to a systematic review of 34 randomised trials, LCD (55–60% carbohydrate) consumed during a 3–12-month period resulted in 8% body weight loss (Strychar, 2006). Low-fat diet used over 2–12 months in 16 trials showed a 5% reduction in body mass and improved cardiovascular risk factors such as hypertension and high levels of

low- density lipoprotein (LDL)-cholesterol (Astrup *et al.*, 2000). Low-carbohydrate diets reduced 5% of body weight in 2–12 months (Fock and Khoo, 2013). Whereas, VLCD should only be used under supervision by trained medical personnel, because they are associated with electrolyte imbalance, low blood pressure and increased risk of gallstones (Fock and Khoo, 2013).

Obesity is associated with inactivity and increased sedentary behaviour. Therefore, physical activity (PA) is an essential part of a well-rounded treatment programme for obesity. PA leads to lipolysis and as a result, free fatty acids are released from triglycerides stored as fat for use as an energy source by muscle, thus there is an increase in energy expenditure (Fujioka *et al.*, 2000). Although some reports show that PA alone can produce a 2% to 3% decline in BMI, it is a more effective body mass loss strategy when combined with dietary modification. Most of the research advocates that exercise alone is ineffective in achieving initial weight loss or results in only modest weight loss of a few kilograms. However, it can help in long-term weight loss maintenance (Shaw *et al.,* 2010). The consensus UK guideline recommendation for weekly physical activity has been prescribed as 150 minutes of moderate-intensity activity, or more per week. On approach is to do 30 minutes on at least 5 days a week or 75 minutes of vigorous-intensity activity, or some combination of moderate and vigorous activity with resistance exercise on 2 days (Chief Medical Officer, 2011).

1.7 Appetite terminology: satiety and satiation

Blundell, Rogers and Hill (1987) described the overlapping processes occurring after food intake until the next period of eating in their satiety cascade over 25 years ago. But later Mela (2006) developed a modified framework of the satiety cascade and added long-term regulation to it. Since then, numerous other papers e.g. Halford and Harrold (2012) and Van Kleef *et al.,* (2012) have also described the satiety cascade. The satiety cascade combines the physiological events that control appetite with the simultaneous behaviours and psychological experiences that are integral to the eating process. Hunger is defined as a state that leads to the initiation of the eating process, particularly as it links to meals. The stomach is the most commonly perceived hunger signal where electrical (vagus nerve) signals relate the state of emptiness (or fullness), reinforced by the release of the hormone ghrelin and by metabolic signals such as blood glucose (hypoglycaemia) (Amin and Mercer, 2016).

The satiety cascade expresses two distinct processes: satiation and satiety. Satiation (intra-meal satiety) is defined as the processes that develop during eating and tends to bring the period of eating to an end; therefore, it inhibits hunger (Gibbons, 2014; Blundell *et al.,* 2010b; Gerstein *et al.,* 2004). Whereas, satiety (inter-meal satiety) refers to the post-ingestive processes that occur following a meal and inhibit further eating, and includes the suppression of hunger and a feeling of fullness during the inter-meal period, and is usually associated with measures of time until the next meal and/or later meal or snack intake. The satiety cascade describes how cognitive, sensory, post-ingestive and post-absorptive factors stimulate and suppress appetite (Blundell *et al.,* 2010b).

Sensory and cognitive processes also guide meal expectation and learned relations to anticipate reward and pleasure. Early pre-ingestive signals from sensory and cognitive signals play a vital role in food choice, meal initiation, satiation and the early stages of satiety (Blundell *et al,* 2010b). After food enters the gastrointestinal system post-ingestive information is provided by the stomach and intestines via the physical signals of stretch/distension, as well as osmotic load, providing feedback related to meal quantity. Medium-term satiety is controlled by gut peptide hormones including GLP-1, CCK and PYY. These hormones are considered to have meal-processing roles in addition to their inhibitory effects on food intake. Cognitive, sensory, post-ingestive and post-absorptive signals are integrated to determine the experience of satiety (Amin and Mercer, 2016; Blundell *et al.,* 2010b). The combination of these satiety signals occurs in the hypothalamus and cortico-limbic structures in the brain that are involved in the control of food intake (Chambers, 2016). The post-absorptive phase contributes to long-term satiety. It is controlled by insulin, glucose and amino acid concentrations in the blood and oxidation of nutrients in the liver (Amin and Mercer, 2016).

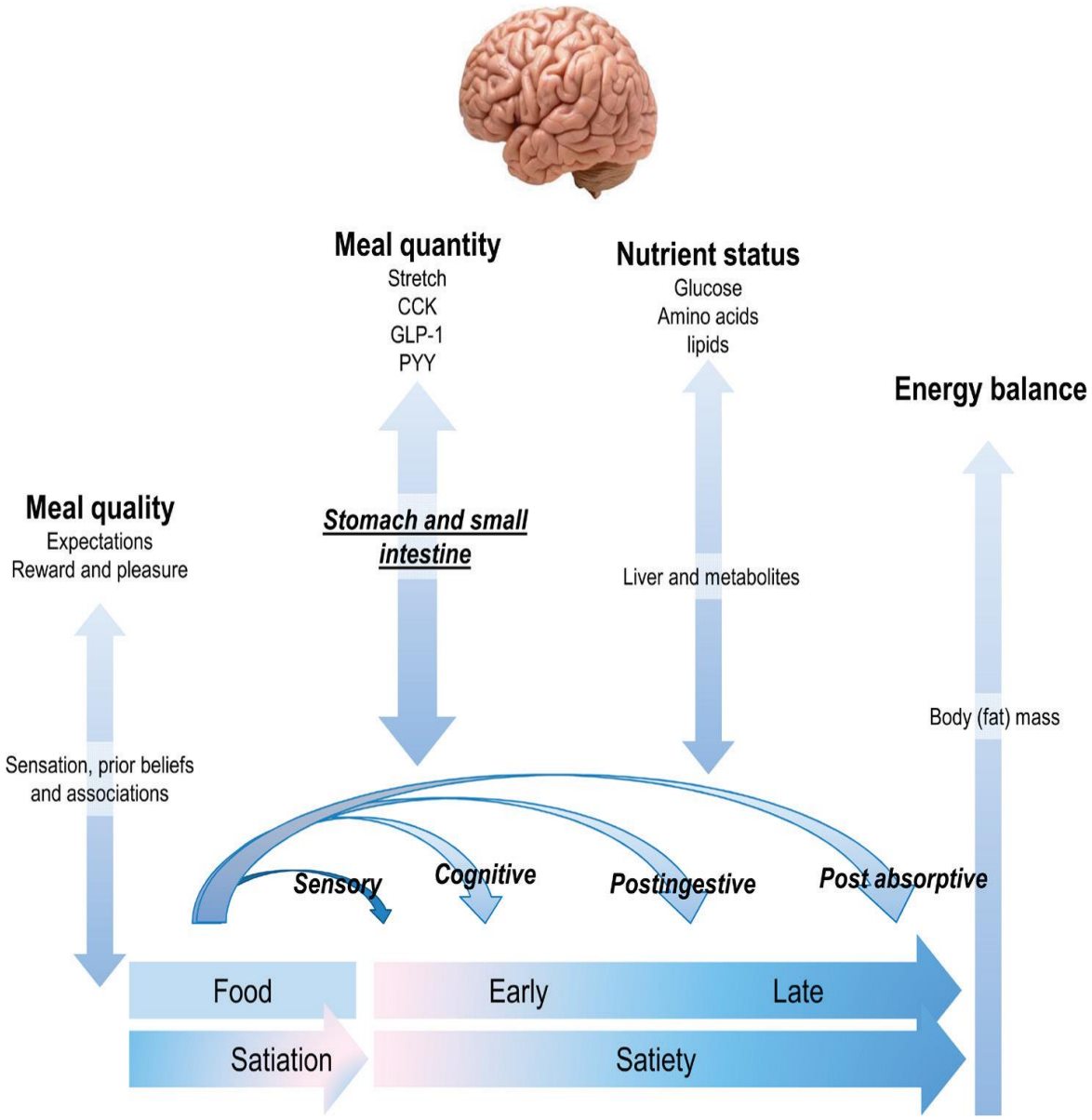

Figure 1-1: The "satiety cascade", first constructed by Blundell, Rogers and Hill (1987) (Taken from Blundell *et al.,* 2010a). CCK = Cholecystokinin, GLP-1= Glucagon-like peptide-1 and PYY = Peptide YY

1.8 Energy Homeostasis and appetite regulation

The role of the gut/brain axis and peripheral hormones in appetite regulation has received widespread attention in the literature owing to the increasing worldwide obesity crisis. Of particular interest has been the potential of these peripheral signals for providing novel targets for developing anti-obesity therapies (Bewick, 2012).

Energy balance and appetite are regulated by neural signals that are released from adipose tissue and endocrine, neurological and GI systems and are integrated by the

central nervous system (CNS). The CNS relays signals to multiple organs in the periphery in order to control EI and expenditure and maintain energy homeostasis over long periods of time (Chaptini and Peikin, 2016; MacLean *et al.,* 2017).

1.8.1 Role of the CNS in energy homeostasis and appetite regulation

1.8.1.1 Hypothalamus

The hypothalamus and brainstem are the two areas in the CNS which are responsible for the metabolic aspects of appetite control and energy homeostasis (Sam *et al.,* 2012). Based on early hypothalamus lesioning experiments in rats, it was believed that the lateral hypothalamic area (LHA) acted as the 'hunger centre' while the ventromedial hypothalamic nucleus (VMN) was a 'satiety centre'. However, it is now clear that several hypothalamic nuclei and neuronal circuits interact with the brainstem and higher cortical centres in appetite regulation. In addition, peripheral signals relay through the brainstem and vagus nerve, with some researchers suggesting the presence of an incomplete blood-brain barrier (BBB) at the median eminence of the hypothalamus and area postrema of the brainstem, permitting peripheral circulating factors direct access to the CNS (Suzuki *et al.,* 2010).

The arcuate nucleus (ARC) of the hypothalamus is a group of neurons which has a significant role in the integration of signals regulating appetite (Wójcik-Gładysz and Szlis, 2016). The ARC contains two populations of neurons with opposing effects on food intake. Orexigenic neurons have the overall effect of increasing appetite by expressing neuropeptide Y (NPY) and Agouti-related protein (AgRP).

These neuropeptides are inhibited by leptin and insulin and stimulated by ghrelin (Bewick, 2012; Sorrentino and Ragozzino, 2017). Whilst anorexigenic neurons inhibit appetite by expressing alpha-melanocyte-stimulating hormone (alpha-MSH) derived from pro-opiomelanocortin (POMC), and cocaine and amphetamine-regulated transcript (CART). Both populations project to the hypothalamic areas, which are involved in appetite control including dorsomedial hypothalamic nucleus (DMN), paraventricular nucleus (PVN) and LHA (Bewick, 2012).

Alpha-MSH produced within POMC neurons binds to melanocortin-4 receptor (MC4R) and melanocortin-3 receptors (MC3R) in the PVN to inhibit food intake (Schwartz *et al.,* 2000; Marić, 2014). Hyperphagia and obesity have also been noticed in MC4R knock-

out mice (Huszar *et al.,* 1997). It is similar in humans, MC4R mutations which associate with 6% of severe early-onset obesity and more than 70 different mutations have been related to obesity (Tao, 2005).

Neuronal expression of CART in the ARC co-localises with POMC and animal studies have proved that intracerebroventricular (ICV) administration of CART suppresses feeding, whereas ICV injection of antibodies to CART can increase food intake (Suzuki *et al.,* 2010).

NPY/AgRP neurons have extensive projections within the hypothalamus, including the PVN, DMN and LHA. ICV administration of NPY has been found to stimulate food intake in rats and repeated daily injections of NPY can result in chronic hyperphagia and increased weight gain (Suzuk*i et al.,* 2010). The orexigenic effect of NPY can be mediated by stimulation of hypothalamic NPY receptors (Y1R) and (Y5R) (Wójcik-Gładysz and Szlis, 2016) in addition to local inhibition of POMC neurons in the ARC (Suzuki *et al.,* 2010). Furthermore, AgRP serves as a selective antagonist at MC3R and MC4R in the PVN. The PVN also contains corticotrophin-releasing hormone and anorectic thyrotropin-releasing hormone. Destruction of the PVN leads to hyperphagia and obesity (Suzuki et al., 2010). Other nuclei within the hypothalamus are also involved in the control of food intake. The LHA has the orexigenic hormones, melanin-concentrating hormone and orexin, and the DMN receives NPY/AgRP projections from the ARC (Wynne *et al.,* 2006). In the VMN, brain-derived neurotrophic factor (BDNF) is highly expressed and suppresses food intake through MC4R signalling (Xu *et al.,* 2003).

1.8.1.2 The brainstem

The dorsal vagal complex (DVC) is located within the brainstem and consists of three major areas which are the dorsal motor nucleus of vagus (DVN), area postrema (AP), and the nucleus of the tractus solitarius (NTS). The DVC is thought to be a crucial communication link between peripheral signals from the gut to the hypothalamus (Schwartz, 2010).

Vagal afferent, within the brainstem express receptors for a variety of hormones that have a role in controlling food intake including cholecystokinin (CCK) 1R and CCK 2R at which both CCK and gastrin act (Moriarty *et al.,* 1997), insulin receptors, GLP-1 (Nakagawa *et al.,* 2004) and GLP-2R (Nelson *et al.,* 2007), growth hormone

secretagogue receptor (GHS)-R1 at which ghrelin acts (Date *et al.,* 2002), the orexin receptor, Orexin receptor-1 (OX-R1) (Burdyga *et al.,* 2003), and leptin (Burdyga *et al.,* 2002).

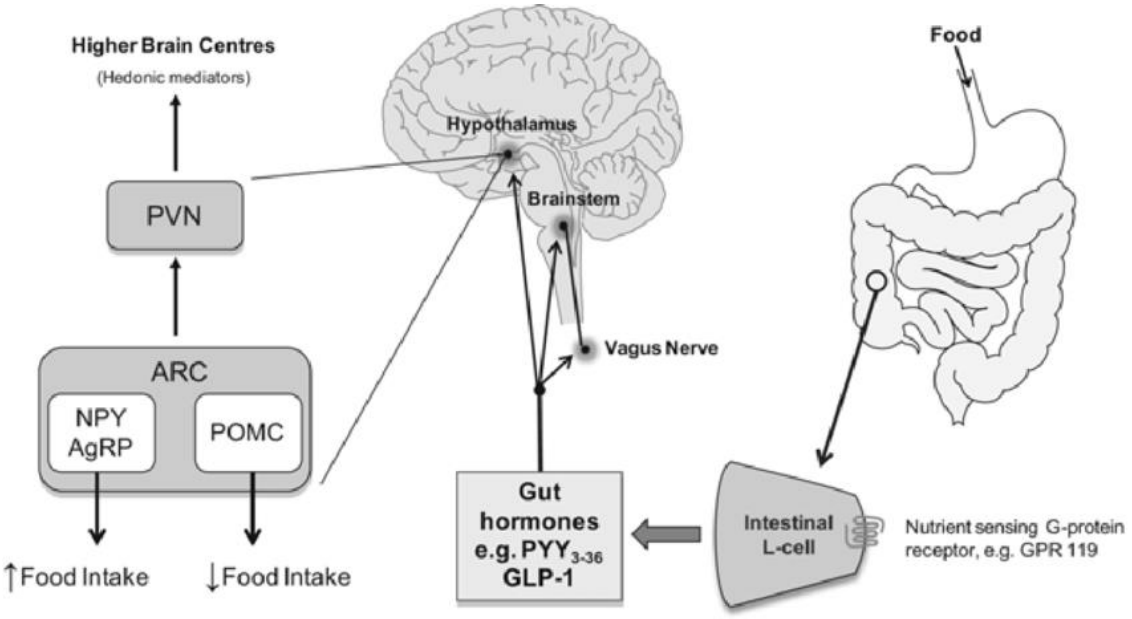

Figure 1-2 : Gut-brain axis: regulation of food intake. (Taken from (Sam *et al.,* 2012)
PVN = paraventricular nucleus, ARC= arcuate nucleus, NPY = neuropeptide Y, AgRP= Agouti-related protein and POMC = pro-opiomelanocortin, PYY = Peptide tyrosine tyrosine and GLP-1= Glucagon-like peptide 1.

1.9 Role of peripheral adiposity signals in energy homeostasis and appetite regulation (long-term signal or tonic)

Adiposity signals modulate energy balance via the regulation of food intake and energy expenditure. They are peripheral signals that circulate in proportion to body fat and inform the brain about the stored energy state (Roh, Song and Kim, 2016). It is widely accepted that insulin and leptin are adiposity signals (Schwartz *et al.,* 1992 and Zhang *et al.,* 1994). Adiposity signals are implicated in the long-term regulation of energy balance (Perry and Wang, 2012).

Insulin is produced by the β cells of the islets of Langerhans in the pancreas and is secreted rapidly following a meal to exert hypoglycaemic effects. As it penetrates the BBB, insulin also exerts an anorexigenic effect by inhibiting the NPY/AgRP pathways and stimulating the POMC pathways (Wynne *et al.,* 2006). The circulating concentrations of insulin are proportional to fat mass (Suzuki *et al.,* 2010). Central

administration of insulin can reduce food intake and body weight in rodents (Perry and Wang, 2012).

Leptin is exclusively secreted and expressed by white adipose tissue adipocytes. It circulates at levels proportional to fat mass (Perry and Wang, 2012). Increasing leptin concentration produces an anorexigenic action by inhibiting the NPY/AgRP neurons and stimulating the POMC neurons (Cowley *et al.,* 2001). Peripheral or central administration of leptin resulted in reduction of food intake and body mass and increased energy expenditure in rodents (Friedman and Halaas, 1998). Paz-Filho *et al.,* (2009) reported an inverse correlation between circulating leptin concentrations adjusted for body fat and body weight in men with obesity, suggestive of a leptin deficient state linked to with obesity.

1.10 Role of gut hormonal signals in energy homeostasis and appetite regulation (short-term signal or episodic)

The GI tract can be defined as the largest endocrine organ in the body and it plays an important role in appetite-regulation because it is the source of various regulatory peptide hormones (Perry and Wang, 2012). The GI tract secretes more than 20 different hormones. These hormones perform several roles, which include digestion and absorption along with the prime role of nutrient uptake (Mishra, Dubey and Ghosh, 2016).

It is widely accepted that gut peptides are part of the energy homeostasis system that contributes to the regulation of body weight, and malfunctions in these peptides can lead to obesity (Blundell *et al.,* 2008). In this system these peptides are hypothesised to play a vital role in the translation of energy requirements into an orexigenic drive, or inhibit this drive via mediating in processes that produce satiety. Consequently gut peptides are envisaged to either stimulate or inhibit episodes of eating in accordance with the principles of energy homeostasis. This usually implies the modulation of the state of hunger, which is a marker of the orexigenic drive and a measure of the operation of satiety. Numerous peptides have been identified as biomarkers of satiation or satiety. Some of these peptides are considered as promoting obesity by weakening the operation of satiety (Blundell *et al.,* 2008). Figure 1.3 shows how central and peripheral signals communicate information about the current state of

energy balance to key brain regions (hypothalamus and hindbrain) and how hormones act on specific centres in the brain to affect the sensations of hunger and satiety (Mendieta-Zeron, Lopez and Diéguez, 2008).

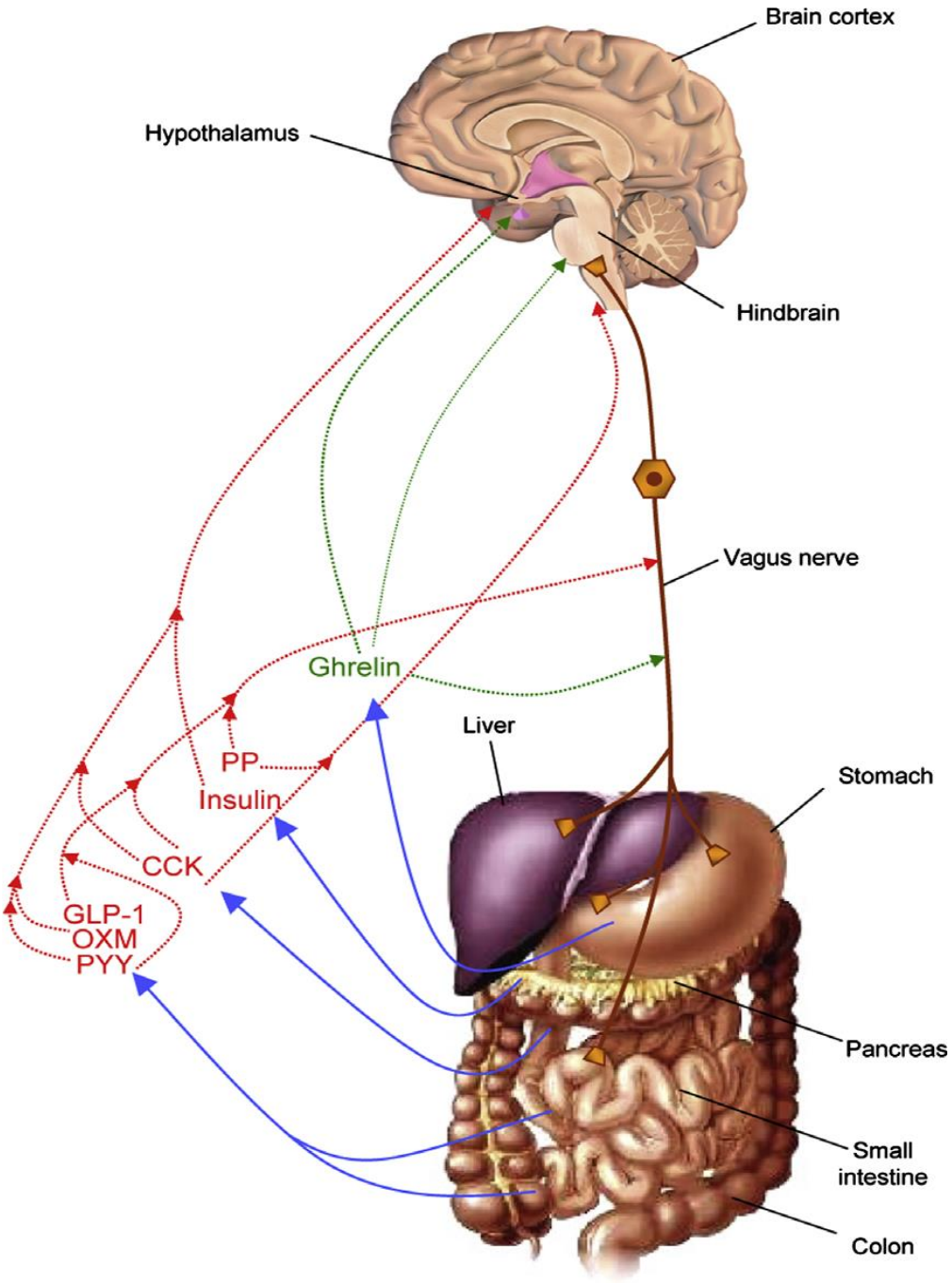

Figure 1-3 : Brain–gut mechanisms regulating food intake and the secretion of gut hormones (Taken from Mendieta-Zeron, Lopez and Diéguez, 2008). Red-labelled peptides are anorexigenic and ghrelin, the unique orexigenic hormone is green-labelled. PP= Pancreatic polypeptide CCK= Cholecystokinin GLP-1= Glucagon-like peptide 1 OXM= oxyntomodulin PYY= Peptide tyrosine tyrosine

Whilst the GI tract secretes multiple gut hormones, this chapter will now focus on the four hormones which were measured as part of the programme of research i.e. Ghrelin, PYY, PP and GLP-1.

1.10.1 Ghrelin

Ghrelin is an orexigenic gut hormone that consist of 28 amino acids derived from pre-proghrelin and is produced predominantly in the stomach by the X/A cells of the gastric oxyntic gland, but it is also secreted in the small intestine. Ghrelin circulates in two forms being acylated and deacylated. Acylated ghrelin binds to the G protein-coupled receptor 1a (GHS-R1a) to cross the BBB (Wójcik-Gładysz and Szlis, 2016).

The NPY/AgRP neurons situated in the ARC nucleus are the primary targets for ghrelin and this population of neurons plays an essential role in the mediation of the orexigenic effects of ghrelin. GHS-R1a is expressed at high levels in the whole hypothalamus area and studies conducted on rats have shown the presence of the GHS-R1a receptor in the ARC nucleus. Within the ARC, ghrelin stimulates NPY and AgRP transcription, as well as encourages secretion of both these orexigenic peptides in the PVN. Therefore, the central actions of ghrelin give rise to increased food intake and body weight gain (Wójcik-Gładysz and Szlis, 2016).

Ghrelin is called the 'hunger hormone' and since its discovery in 1999 it has been suggested to function as a meal initiator (Kojima *et al.,* 1999). It is the only known gut hormone to increase appetite and food intake. Ghrelin promotes gastric motility, acid secretion and pancreatic exocrine secretion in anticipation of meals, as a result preparing the GI tract to process food (Cummings, 2006).

It is worth emphasizing that ghrelin induces appetite in both lean people and people with obesity and is suppressed by feeding (Troke, Tan and Bloom, 2014). Circulating ghrelin levels rise before the start of a meal and fall again rapidly after the initiation of the meal in humans (Cummings *et al.,* 2004; Cummings *et al.,* 2001) and rodents (Tschop, Smiley and Heiman, 2000; Wren *et al.,* 2000).

Fasting ghrelin concentrations have been shown to be lower in people with obesity when compared with normal weight controls, which suggests ghrelin is not a cause of obesity; although there is a suggestion that obese people are actually more sensitive to

the hormone. However, more research is needed to confirm this (Cummings *et al.,* 2002).

The typically expected post-prandial fall in circulating ghrelin concentration is also attenuated, or even absent in people with obesity (English *et al.,* 2002) suggestive of a role of ghrelin in the pathophysiology of obesity (Perry and Wang (2012). Moreover, the circulating ghrelin concentration is reduced after gastric bypass surgery. This fall in plasma ghrelin levels may contribute to reduced appetite and weight loss following this procedure (Cummings *et al.,* 2002). In contrast, there is evidence demonstrating that plasma ghrelin levels are elevated in people with weight loss resulting from anorexia nervosa and bulimia nervosa or caloric restriction as well as from chronic exercise without hypophagia (Cummings, 2006).

Cummings *et al.,* (2004) have reported that there is a close overlap between ghrelin temporal profiles and subjective hunger scores, with ghrelin peaks preceding hunger. As a result, ghrelin has a critical role in meal initiation (Karra and Batterham, 2010). Many studies have commonly described ghrelin as a short-term signal, however, it has been argued that ghrelin can also be considered a long-term signal, since it relates inversely to adiposity and is important for body weight regulation (Karra and Batterham, 2010; Klok, Jakobsdottir and Drent, 2007).

Various studies have shown that intravenous administration of ghrelin in both lean and humans with obesity can increase feelings of hunger and EI from buffet meals (Druce and Bloom, 2006; Druce *et al.,* 2005; Wren *et al.*, 2001). In addition to this, chronic central ghrelin administration in rats elevates the release of enzymes that stimulate fat storage in adipose tissue (Theander-Carrillo *et al.,* 2006). Additionally, both central and peripheral infusions of ghrelin have been shown to enhance body weight gain (Nakazato, 2001). In rats, Wren *et al.,* (2000) have reported that central and peripheral administration of ghrelin resulted in stimulation of food intake. Daily administration of GHS-R 1a antagonists in diet-induced obese mice has been shown to decrease EI and result in ~ 15% weight loss compared with pair-fed controls. GHS-R antagonists reduced EI and decreased gastric emptying in lean mice and in *ob/ob* obese mice and decreased body weight gain and improved glycaemic control in *ob/ob* obese mice (Asakawa *et al.,* 2003).

How fast ghrelin levels decline after a meal, as well as the extent and the duration of the inhibition is determined by the caloric load, the size, the frequency and the composition of the meals (Klok, Jakobsdottir and Drent, 2007; Cummings, 2004). Dietary carbohydrates and protein suppress ghrelin release greater than dietary lipids (Cummings, 2006) and glucose-sweetened beverages are better at suppressing ghrelin concentrations compared to isocaloric fructose-sweetened beverages. Ghrelin levels are also influenced by age, gender, BMI, growth hormone, glucose and insulin (Cummings, 2006).

1.10.2 Peptide tyrosine tyrosine (PYY)

The PP-fold family includes NPY, PYY, and PP. PYY and PP are secreted from the GI tract, whereas NPY is distributed within the CNS (Suzuki, Jayasena and Bloom, 2011).

PYY is a 36 amino acid peptide synthesized and released from the L-cells in the GI tract. The first isolation of PYY was from porcine upper small intestine. There are two circulating forms of PYY being PYY (1-36) and PYY (3-36). PYY (1-36) is the major circulating form that is released by cleavage of the N-terminal Tyrosine-Proline residues via the enzyme dipeptidyl-peptidase IV (DPPIV) to give the active form, PYY (3-36). Recently, the DPP-IV inhibitors have been evaluated for their effects on obesity and metabolic traits (Abdalla, 2017).

PYY (3-36) reduces food intake through binding with highest affinity to the hypothalamic Y2 receptor which has highest affinity for PYY (Troke, Tan and Bloom, 2014). The secretion of PYY plays a pivotal role in satiety. In the fasted state, circulating PYY concentrations are low and they quickly rise following a meal, peaking at 1-2 hours and stay high for several hours (Suzuki, Jayasena and Bloom, 2012). The level of circulating PYY can rise through stress and exercise and it acts mainly as a satiety factor rather than a food terminator (Mishra, Dubey and Ghosh, 2016).

Many researchers highlight the effects of PYY on food intake in both lean and obese humans and rodents following peripheral administration. However, the findings of these studies are conflicting, peripheral administration of PYY (3-36) has been reported to reduce food intake. A previous study has reported an acute inhibition of feeding by peripheral administration of PYY (3–36) in rats or mice (Chelikani, Haver and Reidelberger, 2005).

Furthermore, peripheral PYY (3-36) administration to rodents resulted in reduction in food intake and body weight gain (Pittner *et al.,* 2004). A further study in the same year was conducted by Adams *et al.,* (2004) confirmed that intraperitoneal PYY (3–36) injection inhibited short-term food intake by up to 50% in overnight-fasted mice. Conversely, central administration of PYY has been reported to be associated with stimulating feeding in rats (Hagan, 2002).

IV administration of PYY (3-36) has also been demonstrated to lead to a reduction in appetite and an almost 30% restriction in caloric intake in lean and obese humans (Batterham *et al.,* 2003a). Moreover, peripheral infusion of PYY (3–36) leads to reduced appetite and 24 hour caloric intake in normal-weight humans. PYY 3-36 physiologically inhibits food intake (Batterham *et al.,* 2002). This is in contrast with Sloth *et al.,* (2007) who examined the effect of PYY (1-36) and PYY (3-36) on food intake reporting it had no inhibitory effect on food intake, although, PYY (3-36) showed increased perceptions of satiety and decreased perceptions of hunger.

Numerous reports have shown that PYY (3-36) may prompt weight loss in part by its effect on energy expenditure and fuel partitioning (defined as a steady supply of energy-yielding substrates to meet the needs of various tissues). However, there is a negative link between markers of obesity in adults, children and infants and fasting PYY (Mishra, Dubey and Ghosh, 2016).

A decrease in total fasting PYY, or no changes in fasting, or impaired postprandial PYY have been reported in overweight people (Moran *et al.,* 2007). Several studies have shown the retention of sensitivity to PYY (3-36) among people with obesity which is in contrast to leptin. As a result, it is a matter of debate as to whether PYY has a critical role in the pathogenesis of obesity (Mishra, Dubey and Ghosh, 2016).

In rodents, dietary protein results in a greater stimulus of PYY secretion (Batterham *et al.,* 2006), whereas, ingestion of a high carbohydrate, low fat diet appear to give the greatest rise in PYY levels in obese humans (Essah *et al.*, 2007).

1.10.3 Pancreatic polypeptide (PP)

PP is also a member of the PP-fold family. It is secreted as a 36 amino-acid peptide from the PP cells of the islets of Langerhans in the pancreas after eating (Troke, Tan and Bloom, 2014). PP is known to decrease food intake directly via the Y4 receptor in

the hypothalamus and brainstem (Suzuki Jayasena and Bloom, 2011). Circulating PP concentrations increase after meals and remain elevated for up to 6 hours post-prandially (Chaudhri, Small and Bloom, 2006). The vagus nerve has a significant role to play in stimulating PP release, along with some other stimulators like gastric distension, exercise and gut hormones such as gastrin, CCK, secretin and motilin, whereas somatostatin prevents its release. PP has the highest affinity for the Y4 receptor, which is widely expressed in the brain areas involved in the hypothalamus and brainstem, which have a key role in the central control of appetite (Chaudhri, Small and Bloom, 2006). Concentrations of PP are reduced in people with obesity and increase following a meal. The action of PP causes a reduction in food intake and gastric emptying (Troke, Tan and Bloom, 2014). There is growing evidence proposing that PP plays a vital role in the regulation of body weight (Karra and Batterham, 2010).

A number of studies have focused on the effect of PP on food intake in humans and rodents. According to a previous study in normal weight volunteers conducted by Batterham *et al.,* (2003b), there was a 22% decrease in hunger scores and food intake persisting for 24 hours following infusion of PP at 10 pmol/kg min. Further previous studies have observed that the infusion of PP at 5 pmol/kg min following a buffet meal, reduced EI by 11% compared with saline control, together with a decrease in hunger as analysed by visual analogue scales (VAS) (Jesudason *et al.*, 2007). Moreover, it has been reported that a reduction in PP release in obese patients with Prader-Willi syndrome (PWS) (Suzuki, Jayasena, and Bloom, 2012) and twice-daily infusion of PP in volunteers with PWS has been reported to reduce food intake by 12% (Berntson *et al.,* 1993).

Other studies were carried out in normal weight and genetically obese (*ob/ob*) rodents. These studies have confirmed that the peripheral administration of PP decreased food intake in rodents (Neary *et al., 2*008; Liu *et al.,* 2008; Asakawa *et al.,* 2006). Moreover, Asakawa *et al.,* (2003) reported that acute intraperitoneal injection of PP in fasted mice reduced food intake up to 24 hours after injection. Chronic infusion of PP to *ob/ob* mice over 6 days significantly decreased body weight gain. Ueno *et al.*, (1999) have reported that PP over-expressing mice have a decreased daily food intake and exhibit a lean phenotype.

1.10.4 Glucagon-like peptide 1 (GLP-1)

GLP-1 is cleaved from the product of the pre-proglucagon gene. It is continuously expressed in the α-cells of the pancreas, the L-cells of the small intestine and neurons located in the caudal brainstem and hypothalamus (Shah and Vella 2014). GLP-1 has several forms, but the major circulating form is GLP-1 (7–36) amide (Murphy and Bloom, 2006). GLP-1 receptors are found in the gut, pancreas and brain, specifically the hypothalamus and AP, all areas closely associated with appetite regulation (Suzuki *et al.,* 2010). GLP-1 is of relevance to appetite and weight maintenance because it has actions on the GI tract as well as the direct regulation of appetite. It is responsible for delaying gastric emptying, gut motility in humans and suppression of appetite (Troke, Tan and Bloom, 2014; Shah and Vella 2014).

Circulating concentrations of GLP-1 are low during the fasting state and increase rapidly post-prandially, especially after ingestion of carbohydrates and fat (Ronveaux, Tomé and Raybould, 2015). After meals, GLP-1 releases into the circulation and it acts as an incretin hormone that stimulates pancreatic insulin release and suppresses glucagon secretion (Holst, 2004; De Silva and Bloom, 2012).

GLP-1 receptor agonists have been used in chronic weight management programmes. The first GLP-1 receptor agonist was approved by the Food and Drug Administration (FDA) for this indication. GLP-1 agonists offer a novel treatment option that may be safer and better tolerated than the available alternatives. Other GLP-1 agonists have also been studied for their effects on weight loss in patients with or without diabetes (Isaacs, Prasad-Reddy and Srivastava, 2016). Numerous studies have confirmed that systemic administration of GLP-1 or GLP-1 receptor agonists decrease food intake, slows gastric emptying (a test that measures the time it takes for food to empty from the stomach and enter the small intestine) and reduces body weight (Marathe *et al.*, 2011; Van Bloemendaal *et al.*, 2014). It has consistently been found that peripheral and central administration of GLP-1 to rodents acutely decreases food intake and chronic administration of GLP-1 reduces weight gain in rats (Meeran *et al.,* 1999; Turton *et al.,* 1996).

However, GLP-1 receptor knockout mice showed no alteration in food intake or body weight. This may be because GLP-1 has a more significant physiological role in

controlling blood glucose than in regulating food intake or it may be that developmental changes compensate for the lack of GLP-1 signalling (Drucker, 2006).

In humans, the IV administration of GLP-1 to normoglycemic and people with diabetes prompts satiety and decreases food intake in short term studies. Chronic continuous infusion of GLP-1 to human with diabetes showed no association with modest weight loss (Verdich *et al.,* 2001). Moreover, it is well established that the IV administration of GLP-1 at doses up to 1.2 pmol /kg/min inhibits food intake in humans (Näslund *et al.,* 1999; Gutzwiller *et al.*, 1999). GLP-1 has been proposed for use in the treatment of obesity. However, it has a short circulating half-life and is rapidly broken down in the circulation by the enzyme DPP-IV to an inactive form, rendering native GLP-1 being excluded from therapeutic use (Bewick, 2012).

Dietary protein is more of a stimulator of GLP-1 release than carbohdrates (CHO) (Lejeune *et al.,* 2006). When comparing meals rich in protein, fat, carbohydrate or alcohol, GLP-1 secretion was the highest after a protein rich meal (Karhunen *et al.,* 2008).

1.11 Food macronutrients and satiety

Blundell and Macdiarmid (1997) suggested a hierarchy of satiating effects of macronutrients in the order of protein > carbohydrate > fat.

1.11.1 Protein (PRO)

Diets high in protein lead to greater satiety and reduced hunger scores (Lobley *et al.,* 2015). Acute studies have shown protein as the most satiating of the macronutrients in both people with obesity and those who are normal weight, rather than other macronutrients (Batterham *et al.,* 2006). The mechanism for the satiating action of protein remains unresolved, but a range of hypotheses include increased postprandial thermogenesis, decreased rate of gastric emptying, reduced digestion and absorption rates, supply of specific amino acids (AA) that serve as a precursor for brain metabolites and release of gut peptides involved in appetite regulation such as ghrelin and GLP-1 (Lobley *et al*., 2015).

1.11.2 Carbohydrate (CHO)

The satiating effect of CHO results from changes in blood glucose, liver metabolism, end products of CHO metabolism and secretion of satiety hormones including insulin, GLP-1 and amylin (Feinle, O'Donovan and Horowitz, 2002). It has been established that low glycaemic index foods induce satiety and suppress voluntary food intake (Ludwig, 2000). Conversely, high glycaemic index CHO tends to suppress short-term (1 hour) intake more effectively than low glycaemic index CHO, but the reverse occurs in long-term intake (Anderson and Woodend, 2003).

1.11.3 Fat

Fat can affect satiety by slowing gastric emptying, inhibiting the release of ghrelin and promoting the release of satiating gut hormones (Little *et al.,* 2007). However, it has been suggested that fat has a lesser effect on satiety than either protein or CHO (Blundell and MacDiarmid, 1997; Robinson, Yeomans and French, 2005). When people are provided with a range of foods with higher fat in free feeding experiments they consumed more energy than when they are provided with high CHO foods (Blundell, Green and Burley, 1994). Thus, offering high fat foods stimulated passive over-consumption (Blundell and Tremblay, 1995). Importantly, it seems to be that fat-related increased EI does not cause increased sensations of satiety (Blundell and Macdiarmid, 1997).

1.12 Methods to measure appetite

1.12.1 Measuring satiation

1.12.1.1 Food intake

Satiation can be measured by serving an *ad libitum* test meal to participants under standardised conditions at predetermined time points. Participants are requested to eat until comfortably full and satisfied and then the amount of food consumed is recorded. It has also been used to quantify the effect of dietary manipulations (Blundell *et al.,* 2010a). It has been shown that there is no difference in the frequency of eating between normal-weight and individuals who are obese, yet people with obesity tend to consume more calories, therefore indicating the importance of meal

size as a contributor to over-consumption and obesity development (Gibbons *et al.,* 2014).

Reproducibility of *ad libitum* energy intake during single meals has been shown to be high in both people with obesity and normal weight. A study was carried out on overweight and obese men and women, which demonstrated that *ad libitum* EI was highly reproducible (Lara, Taylor and Macdonald, 2010). Moreover, Horner, Byrne and King (2014) have assessed the reproducibility of subjective appetite ratings and *ad libitum* test meal EI after a standardised pre-load in men who were overweight or had obesity. Their findings showed the reproducibility of subjective appetite ratings and *ad libitum* energy intake is similar to that reported in lean adults (Gregersen *et al.,* 2008).

1.12.2 Measuring satiety

There are numerous approaches to measure satiety as follows.

1.12.2.1 Self-report scales

Subjective appetite ratings (as a measure of the motivation to eat) can be measured through VAS, which are the standard tools used to assess subjective appetite sensations (hunger, fullness, satiety and desire to eat). They have also been used to monitor depression and pain in clinical and research settings (Gibbons *et al.*, 2014).

Initial questionnaires have considered measurement of hunger through asking the question "how hungry are you right now" which ignored the fact that appetite is multidimensional (Hill and Blundell, 1982). This weakness was recognised and consequently, an additional seven questions were proposed covering various facets of appetitive sensations, involving fullness, satisfaction and prospective food consumption. These questions are 'How hungry are you right now? How strong is your desire to eat right now? How much could you eat right now? How full are you right now? How strong is your desire to consume something sweet right now? How strong is your desire to consume something savoury right now? How thirsty are you right now? (Drapeau *et al*., 2007).

VAS comprise of a 100–150 mm line with extreme subjective feelings anchored at either end, for example 'How hungry are you?', 'Not at all hungry' at one end versus 'As hungry as I have ever felt' at the other end (Stubbs *et al.,* 2000). Participants are requested to place a vertical mark on the scale at a point between the two extreme

ratings to rate their feeling in response to the questions that include appetite sensations (Chaput *et al.,* 2010).

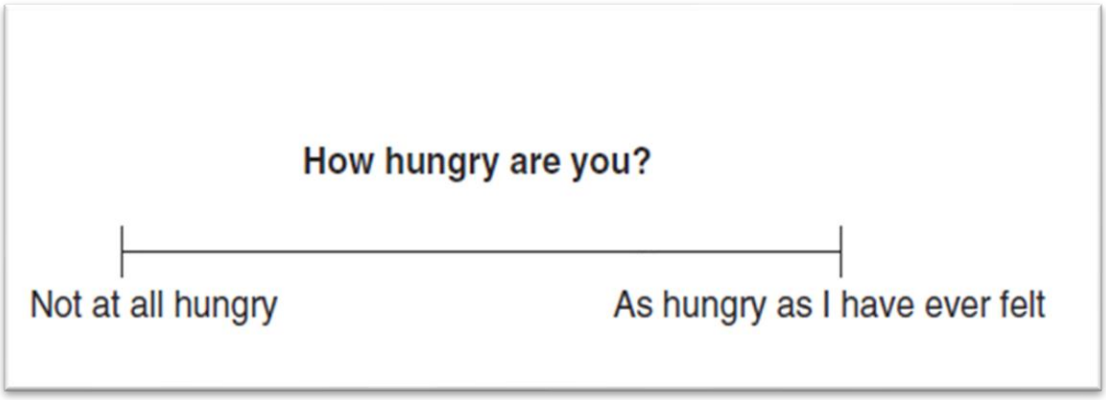

Figure 1-4: Example for visual analogy scale for hunger (Stubbs *et al.,* 2000). Calculation of VAS measurement can be done by measuring the distance from the left end of the line to the mark indicated by the participant (Chaput *et al.,* 2010).

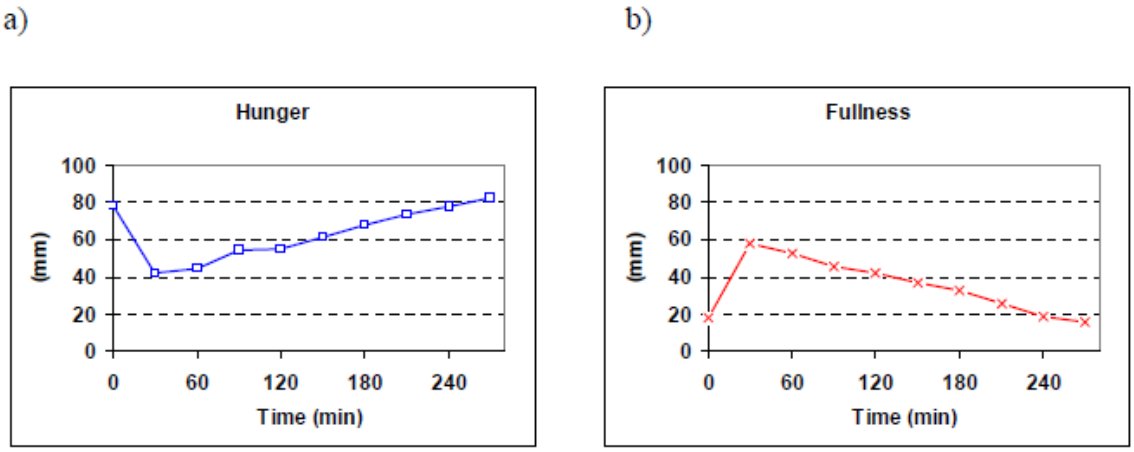

Figure 1-5: Feeling of a) hunger and b) fullness measured on a 100 mm VAS (Flint *et al.,* 2000). 0 min = fullness/hunger before eating the test food, other times after eating the test food.

VAS are applied using pen and paper, which are convenient and quick to complete (Rumbold, Dodd-Reynolds and Stevenson, 2013). However, data collection using this method is time consuming and susceptible to human error, because each line needs manual measurements and needs to be individually inputted into a spreadsheet. Electronic Appetite Ratings System (EARS) have been developed to reduce the measurement error. The benefits of using EARS are inexpensive cost and its practical benefits. Moreover, EARS include the use of an audio alarm as a reminder of when the

VAS needs to be completed, leading to compliance rates of 90% or better (Whybrow, Stephen and Stubbs, 2006; Hufford and Shields, 2002).

Numerous studies have used VAS in appetite research. Flin*t et al.,* (2000) has reported the validity and reliability of VAS. Additionally, previous studies that have used VAS for measuring appetite reported that these scales have a high degree of reproducibility (Delargy *et al.,* 1996, Stratton *et al.,* 1998, Stubbs *et al.,* 2001, Whybrow *et al.,* 2006, Stubbs *et al.,* 2000). VAS are predictive of EI in experimental conditions (laboratory and free-living) (Flint *et al.,* 2000; Stubbs *et al.,* 2000).

1.12.2.2 Biomarkers

The power of satiety can be inferred by levels of circulating appetite-related peptides, such as ghrelin, CCK, GLP1, PP and PYY, which have a vital role in the short-term control of appetite. These peptides are often measured as biomarkers of satiety (Gibbons *et al.,* 2014) and have been discussed in detail earlier in this chapter.

The degradation of the peptides and sample storage are also of importance and precautions must be taken to avoid this. As a result, it is important to treat blood samples with protease inhibitors immediately after blood collection. However, biomarkers cannot be used on their own to quantify hunger or satiety, but when combined with other measurements they are indicators of appetite and food intake (Delzenne *et al.,* 2010).

There are a number of analysis techniques that are used for measuring peptides – for example, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). ELISA technology is one of the methods most applied for measurement of specific peptides and proteins. The conceptual idea of an ELISA evolved to include multiplexing methods that permit simultaneous detection of multiple analytes (Albrechtsen, 2017). These are more cost effective, therefore these are useful in large-scale studies also multiplex allows for measuring many analytes with only a small amount of sample (Gibbons *et al.,* 2014)

1.13 Quantification of satiety

1.13.1 Satiety quotient (SQ)

The SQ was first introduced by Green *et al.,* (1997) to evaluate the satiating effect of an eating episode. SQ is calculated from the difference in hunger ratings pre- and post-

consumption of a food, divided by the weight or energy content of the food or drink being tested (Tremblay and Bellisle, 2015). When the motivation to eat is assessed on VAS and scored in mm, the result is expressed in mm/g or in mm/kJ. A higher SQ means a higher satiety level because there is a higher suppressive effect on the motivation to eat. The SQ has been applied in a wide range of research, a low SQ in response to a fixed-energy meal has been shown to be related to higher subsequent *ad libitum* energy intake measured under both laboratory and free-living conditions (Drapeau *et al*., 2005; Drapeau *et al*., 2007). This quotient uses a mean value for the post-meal assessed by hunger, fullness, desire to eat and prospective consumption scales rated every 10 min during a 60 min period, and multiplying the result by 100 (Drapeau *et al.*, 2005).

1.14 Factors affecting satiety

1.14.1 Body mass

People with obesity and lean people have different responses to tests of appetite and EI, as their energy requirements are different. People with obesity have higher energy requirements than lean participants. There are differences in some aspects of physiological appetite control between people with obesity and those with normal weight (Benelam, 2009). Gastric mechanisms of satiation, nerves communicate an increase in gastric volume to the brain when food or drink reaches the stomach. It is clear that gastric distension stimulates satiation, independently of nutrient content (Benelam, 2009).

When a food or drink is consumed and enters the GI tract it is digested and absorbed. Signals as a result of the ingestion of energy feed into specific areas of the brain that are involved in the regulation of EI, in response to the sensory and cognitive perceptions of the food or drink consumed and the distension of the stomach. These signals are integrated by the brain and satiation is stimulated. When nutrients reach the intestine and are absorbed, a number of hormonal signals that are again integrated in the brain to induce satiety are released. In addition to these episodic signals, satiety is also affected by fluctuations in hormones, such as leptin and insulin, which indicate the level of fat storage in the body (Benelam, 2009).

Furthermore, there is evidence that when food is removed from the stomach after ingestion, satiation does not occur. In experiments where cannulas are fitted that permit food to drain from the stomach, animals eat continuously, but quickly reach satiation when the cannula closes allowing food to fill the stomach normally (Davis and Smith, 1990). There is the possibility that gastric distension could be used as a biomarker of satiation. Kim *et al.,* (2001) have reported that people with obesity have greater gastric capacities, which could result in a greater EI before gastric distension-stimulated satiation occurs. However, this may be associated with binge eating and not strictly linked to obesity itself, and, although the likelihood of binge eating can increase with the degree of obesity, it has been reported that the gastric capacity of normal weight bulimic patients is larger than that of some people with obesity (Geliebter and Hashim 2001).

The gastric capacity of people with obesity is reduced after weight loss and it is possible that this could help restore a stronger satiation response (Geliebter *et al.,* 1996). Differences in satiety signalling in people with obesity are another physiological aspect that can affect satiety. Ghrelin hormone concentrations are found to be lower in people with obesity compared with lean people (English *et al.,* 2002). The inhibition of ghrelin in response to a meal may indicate some form of dysregulation of appetite hormones in obese individuals. In addition, ghrelin levels increase with subsequent weight loss, which has a role long-term role in weight regulation. It has also been suggested that a lowering in fasting ghrelin could be a consequence of downregulation caused by overeating. Two anorexigenic hormones, Peptide YY and GLP-1, have been reported to be lower in individuals with obesity, suggesting differences in these hormones could be contributing to a higher EI in the population with obesity (Brown *et al.,* 2014).

1.14.2 Gender

Another factor that has an effect on appetite and EI is gender. Men are likely to consume more food than women due to their higher resting metabolic rate and higher body mass (Rolls, Fedoroff and Guthrie 1991b). It has been reported that appetite ratings differed according to gender (Gregersen *et al.,* 2011). Food intake of women has been observed to fluctuate during the menstrual cycle. Food intake decreases in

the periovulatory phase and elevates in the luteal phase, compared to the follicular phase (Buffenstein *et al.,* 1995).

1.14.3 Age

Age can affect satiety responsiveness, in particular sensory specific satiety and changes in taste receptors. Sensitivity to sensory specific satiety seems to decrease with age and this is important if the test meal presented in research studies is in the form of a buffet, where subjects are provided with a variety of foods (Rolls and McDermott, 1991a). Many people experience a decrease in appetite with ageing. This condition is called anorexia of aging and is defined as age-related reduction in appetite and food intake (Wysokinski *et al.,* 2015). It has been estimated that between 15 and 30% of older people have anorexia of ageing, with higher rates in women, hospitalised people and nursing home residents (Malafarina *et al.,* 2013).

1.14.4 Sleep

It has been suggested that reduced sleep duration is associated with an increased risk of obesity and weight gain (Broussard *et al*., 2016). The main mechanism is that short sleep duration may predispose individuals to weight gain through changes in appetite-regulating hormones, leading to increased food intake. Indeed, experimental studies have consistently presented that sleep restriction increases caloric intake when free access to food is allowed (St-Onge *et al.,* 2011; Bosy-Westphal *et al.,* 2008). However, findings regarding the direct effects of sleep restriction on appetite-regulating hormones have been inconsistent; some studies have reported that increased levels of the appetite-stimulating hormone ghrelin and decreased levels of the satiety hormone leptin during controlled caloric intake (Spiegel *et al.,* 2004).

1.14.5 Social situations

It has been shown from decades of research that other people can influence our choices and food intake in a variety of ways (Higgs and Thomas, 2016). When eating in groups, people tend to consume significantly more energy than when alone. De Castro *et al.,* (1997) reported that people ate more than 50 per cent more food when they ate in groups than when eating alone. This may be due to a number of factors, such as a relaxation of dietary restraint and longer duration of eating (Benelam, 2009). However, this was not consistent for all people and all types of groups, Brindal *et al.,* (2015)

found that in a fast-food restaurants, women ate more when they were with other women when compared to mixed gender groups, similarly, men ate more when they were with other men compared to mixed gender pairs.

1.15 Identification of a satiety phenotype

Appetite is controlled by psychological, behavioural and physiological aspects (Blundell and Gillett, 2001). Some of these aspects promote food intake (excitatory processes) and are reflected by the subjective sensation of hunger, whereas others (inhibitory processes) are responsible for satiation and satiety signals and are reflected by the subjective sensation of fullness (suppression of the feeling of hunger) (Blundell and Gillett, 2001). The balance between hunger, satiation and satiety is complex but can have important implications for body weight gain and the development of obesity. It has been reported that a weaker satiety response to a test meal in certain individuals who were obese, who claimed their eating was unrelated to their appetite sensations (comparison of obese or normal weight controls) (Barkeling *et al.,* 2007). The people with obesity who expressed a weaker satiety response showed similar diurnal oscillations in hunger (and fullness) to the other two groups (obese or normal weight controls), indicating a weakened satiety response can occur despite normal daily satiety signalling mechanisms.

Moreover, higher disinhibition and hunger scores have been observed in people with obesity claiming problems with appetite sensations compared to lean or people with obesity (Barkeling *et al.,* 2007), which are eating behaviour traits that are related to overconsumption and higher body weights (Bryant *et al.,* 2008). Based on these experimental observations, it can be hypothesised that impaired satiety signals could favour overconsumption and increase the risk of weight gain in certain individuals, but not limited to people with obesity.

Numerous studies have attempted to predict satiety. Drapeau *et al.*, (2007) used appetite sensations and the satiety quotient of VAS to predict EI of people with obesity. They found that higher scores of baseline SQ for fullness were associated with low *ad libitum* test lunch energy intake in women. These findings indicate that individuals with low SQ have weaker appetite sensation responses after a meal. Drapeau *et al.,* (2005) also found a negative correlation between SQ and lunch energy intake. A further

previous study investigated the response of appetite sensations to fasting in obese and non-obese people. The study reported that during fasting, desire to eat, hunger and prospective consumption was reduced for the non-obese, whereas these appetite sensations were increased slightly for people with obesity (Oh, Kim and Choue, 2002). Lindroos *et al.,* (1997) and Provencher *et al.*, (2003) used the three factor eating questionnaire (TFEQ) to predict satiety phenotype. They found TFEQ-H (hunger) was positively associated with EI.

1.16 Predicting weight loss

Drapeau *et al.,* (2007) found that higher baseline fasting desire to eat, hunger and prospective food consumption VAS scores were associated with reduced weight loss. Other researchers measured gut hormones to predict weight loss. Hainer *et al.,* (2008) and Koska *et al.,* (2004) used fasting PP to predict weight loss, they reported that baseline PP values were inversely associated with body weight. Moreover, Williams *et al.,* (2016) found that a higher baseline level of ghrelin predicted greater weight loss at six months in males and females. Macronutrients in a food diary have been studied to predict weight loss. Buscemi *et al.,* (2017) using 3 day food records found that low initial levels of caloric intake were positively associated with changes in BMI. Moreover, Linde *et al.,* (2006) showed eating high fibre cereal, fruit and overall fibre intake at baseline were related to lower BMI in females.

Eating behaviour traits have been measured and are thought to be useful in predicting weight loss in clinical patients, to monitor progress during weight loss treatment and is considered to be one of the most widely used indicators to study eating behaviour in individuals with obesity (Beechy *et al.,* 2012). Food craving has also been used in predicting weight loss. Buscemi *et al.,* (2017) showed that there was a relationship between higher initial food cravings and more gradual and less steep reductions in BMI. Both findings warrant further consideration.

1.17 Eating behaviour

Eating behaviour can be described as a complex phenomenon encompassing the size and frequency of eating episodes and everyday food choices, which together can determine total energy and macronutrient intake (Martins, Robertson and Morgan, 2008). It is the result of constant physiological and environmental inputs. The latter are

especially important and it has been accepted that the physiological mechanisms that control food intake can be easily overridden by strong social and environmental factors. Appetite can be explained as the control of food intake and defined as a range of variables related to food consumption that predict normal eating behaviour (Martins, Robertson and Morgan, 2008). Moreover, eating behaviour is one of the most important factors contributing to body weight status (Dietrich *et al.,* 2014).

Many questionnaires have been developed to measure eating behaviours, especially dietary restraint. TFEQ was developed by Stunkard and Messick (1985) and consists of 51 items. It is designed to assess 3 aspects of eating behaviour—cognitive restraint (TFEQ-R) (21items), disinhibition (TFEQ-D) (16 items) and hunger (TFEQ-H) (14 items) (Keskitalo *et al.,* 2008). The TFEQ has been validated and shows good test-retest reliability (reliability coefficients for internal consistency (Cronbach's alpha) were 0.80 for the restraint factor of the TFEQ-R (Laessle *et al.,* 1989)). It is commonly used in appetite research to describe study populations (Harden *et al.*, 2009).

Cognitive restraint is generally accepted to be a tendency to consciously restrict one's food intake instead of using physiological cues (i.e. hunger and satiety) as regulators of intake (Cornelis *et al.,* 2014). Restrained eaters attempt to inhibit impulses to eat in order to pursue long-term weight goals. They avoid fattening foods and eat small portions (Hays *et al.,* 2006).

Disinhibition is a term that is defined as a tendency to overeat in the presence of palatable foods or other stimuli, such as boredom or sadness and hunger could be defined as the susceptibility to perceive body symptoms that signal the need for food (Hays *et al.*, 2006). Disinhibited eaters consume food because of external environmental cues, such as palatable food. They find it difficult to resist food stimulation and/or eat under emotional distress (Dietrich *et al.,* 2014).

Hunger is defined as the susceptibility to perceived body symptoms that signal the need for food (Hays *et al.*, 2006). TFEQ-H has been studied to predict the satiety phenotype of individuals. Provencher *et al.*, (2003) observed positive associations with EI and hunger. In theory, those who report chronically high levels of hunger are likely to be more susceptible to overeating compared with those who do not report being often hungry. Furthermore, Lindroos *et al.,* (1997) reported that hunger was positively associated with daily EI in individuals with obesity. Moreover, high scores on TFEQ-H

have been associated with higher body weight (Dykes *et al.,* 2004; French, *et al.,* 2014), which runs counter to the original idea that high scores on TFEQ-H might relate to greater interoceptive awareness (homeostatic information to consciousness) and consequently lower susceptibility to overeating. TFEQ-H has been used to predict weight change. Previous studies have shown that baseline hunger was negatively correlated with weight change (Batra *et al.,* 2013; Bas and Donmez, 2009).

1.18 Food Craving

Food craving is referred to as frequent, intense desires to consume a specific type of food (Kahathuduwa *et al.,* 2018). It is believed to be a multidimensional experience as it involves cognitive e.g. thinking about food, emotional e.g. desire to eat or changes in mood, behavioural e.g. seeking and consuming food and physiological e.g. salivation aspects (Rodríguez-Martín and Meule, 2015).

Psychometric self-report scales for the experience of food cravings have been developed by a number of researchers. The Food Craving Inventory (FCI) consists of 28 items measuring the frequency of cravings for specific foods comprising of 4 factors or subscales measuring cravings for high fats, CHO/starches, sweets and fast food and generates a total score (White and Grilo, 2005). FCI places the question about craving in the test rubric, ‘‘Over the past month, how often have you experienced a craving for the food?’’ and then assesses the participant on a number of specific foods. It has been consistently demonstrated that there are gender differences in food cravings: women tend to experience higher food cravings than men (Weingarten and Elston, 1991). These differences may be linked to hormonal differences between women and men, particularly as many women experience rises in food cravings perimenstrually and prenatally (Hormes, 2014). Additionally, White *et al.,* (2002), using the FCI found that people with obesity report more frequent cravings for high fats than their normal-weight counterparts. It has been reported that the FCI is a reliable and valid measure of general and specific food cravings (White *et al.,* 2002). It can be used in research related to overeating and binge eating. Moreover, FCI may be useful and valuable in treatment studies that target obesity and/or food cravings (White *et al.,* 2002).

1.18.1 Food craving and obesity

Food craving has been found to associate with calorie overconsumption (Hill, Weaver and Blundell, 1991; Lafay *et al.,* 2001; White and Grilo, 2005). A cross-sectional study of 646 adults, conducted by Chao *et al.,* (2014) showed that there was a significant positive relation between food craving and BMI. They also reported significant positive associations between cravings for specific calorie dense foods e.g. sweets, high fat foods, CHO and fast foods and actual consumption of those respective foods. It has also been reported from longitudinal evidence that there are relationships between food cravings, EI and BMI, with suggestions from experimental evidence that caloric restriction is associated with decreased food cravings and weight loss decreased food cravings (Anton *et al.,* 2012; Martin *et al.,* 2011).

Recently a study used the FCI and a three day dietary recall to explore relationships between food craving, caloric intake and BMI changes over the course of an 18 month weight loss trial. The finding of the study showed that from baseline to 6 months, there was a relationship between higher initial food cravings and more gradual and less steep reductions in BMI. Additionally, the relationship between changes in food craving and BMI changes were noted to vary by levels of change in caloric intake, such that there was a positive correlation between BMI change and change in food cravings and change in caloric intake at low levels, but at average and high levels the changes in caloric intake were unrelated. Similarly, from baseline to 6 months and from 6 to 18 months, the relationship between changes in food craving and BMI changes were also found to vary by initial levels of caloric intake (Buscemi *et al.,* 2017).

1.19 Food diaries and dietary assessment

Dietary records (also known as food diaries) are typically obtained for 3 to 7 days. They are considered a prospective, open-ended survey method where the subjects are given a recording form to collect data about their foods and beverages consumed over a previously specified period of time. Food diaries are often requested to record detailed information about food preparation methods, ingredients of mixed dishes and recipes, and even the brand name of commercial products, depending on the aim/hypothesis of the study. Food diaries can be applied to estimate the current diet of individuals and population groups, as well as to identify groups at risk of diet inadequacy. They have been used in epidemiological and clinical studies (Ortega, Pérez-Rodrigo and López-

Sobaler, 2015). It has been reported that 3 days of food diaries have high validity and precision (Yang *et al.,* 2010; Schlundt, 1998).

Food diaries with weighed portion size are known as one of the best instruments among dietary assessment methods. Nevertheless, when using food diaries concerns have been raised about the possibility that food behaviour may be influenced or changed by the recording process (Cantwell *et al.,* 2006). Biro´ *et al.,* (2002) highlighted the main concerns related to food diaries. For example, participants may forget to record items immediately following eating, the likelihood of omitting foods may increase when they later record their intake. Participants may experience difficulties in writing down the foods and beverages consumed or in describing the portion sizes. Increasing the number of days observed reduces the quality of completed diet records (Biro´ *et al.,* 2002). It should also be considered the high cost of coding and processing information collected in diet records (Ortega, Pérez-Rodrigo and López-Sobaler, 2015). Finally, the reliability of food diaries can be decreased over time due to respondent fatigue because they have a high degree of participation burden (Biro´ *et al.*, 2002).

Food diaries can also be used to measure the contribution of macronutrients to total energy intake (TEI) and BMI. In a study, subjects were classified into three BMI groups (underweight, normal and overweight). There was no difference in the TEI in all three BMI groups. However, when stratified by age, a positive correlation was observed between polyunsaturated fatty acid intake in younger women and animal protein intake in older women and BMI (Shin *et al.,* 2007).

1.20 Food preferences

Food preferences are influenced by a wide range of behavioural, social and economic variables, including food availability. However, the desire to select one food over another is more closely associated with taste and other sensory properties of foods (Mela, 2001). Food preferences can be measured by preference checklists or actual taste tests and are often considered to predict food consumption in everyday life (Drewnowski, 1997). Their use allows investigators to dispense with the less reliable food diaries. Again, attitudinal responses collected in the laboratory often fail to predict real-life behaviour, because many people do not overeat the foods they like. Potential reasons for dissociation between taste preferences and actual intake may include weight-related attitudes and behaviours and general concern with nutrition and health (Drewnowski, 1997). Previous studies have examined the relationship

between food preferences and obesity. Davis *et al*., (2007) found that preference for sweet and fatty foods were positively correlated with BMI. Moreover, it has been reported that there was a positive correlation between BMI and consumption of a diet characterized by higher intakes of meat, eggs, fats and oils (Maskarinec, Novotny and Tasaki, 2000). Whereas, preference for vegetables, beans, fruit and grains had a negative association with BMI (Maskarinec, Novotny and Tasaki, 2000). Drewnowski *et al.,* (1992) reported that women preferred high-fat, high-sugar foods, men appeared to prefer high-fat, high-protein foods.

1.21 Types of diet used to reduce weight

There are four types of diet that are recommended for weight loss which are Low-Calorie Diet (LCD), Low-fat diet, Low-carbohydrate diet and Very Low-Calorie Diet (VLCD). All these diets provide 800–1500 kcal/day while VLCD is< 800 kcal/day (Fock and Khoo, 2013).

One of the common diets that is used for weight loss is a high protein (HP) diet. Increased protein intake during calorie deficit is beneficial for appetite control and it is defined as a diet with around 30% protein, 30% fat and 40% CHO from energy (Johnstone, 2012). Thus, designing a diet with HP can help people with low satiety and those who struggle to lose weight. The guidelines from the Institute of Medicine permit the inclusion of higher amounts of protein than previously recommended in a healthy diet and it reported that there is no clear evidence that HP intake increases the risk of renal stones, osteoporosis, cancer or CVD (Johnstone, 2012). It has been shown that diets containing 30% protein are well-tolerated and may have a health benefit, providing that the increase in protein is not made up of red meat and provided CHO is not excluded (Halton and Hu 2004).

1.21.1 High protein and gut hormones

It has been hypothesised that there are relationships between protein -induced satiety and a larger increase in concentrations of anorexigenic hormones (GLP-1, cholecystokinin, PYY) or large reduction in 'orexigenic' (ghrelin) hormones (Veldhorst *et al.,* 2008). Previous studies have shown that HP increases GLP- 1 and decreases the concentration of ghrelin, which are satiety effects (Blom *et al.,* 2006; Veldhorst *et al.,* 2009).

Veldhorst *et al.,* (2009) reported that HP (25%) increased GLP-1 and decreased the concentration of ghrelin in twenty five subjects fed breakfast containing whey or whey without glycomacropeptide (GMP) as a type of protein. Several amino acids were increased more after the breakfast with whey than after the breakfast with whey without GMP, regardless of the protein concentration, namely serine, threonine, alanine, alpha-aminobutyric acid and isoleucine. It may be hypothesized that these amino acids play a role in the reduction of food intake

A further study carried out by Blom *et al.,* (2006) assessed blood samples and subjective measures of satiety in fifteen healthy men. The study aimed to investigate whether a HP breakfast is more satiating than a high-CHO breakfast (HC) due to suppression of postprandial ghrelin concentrations. At the end of the study the HP breakfast decreased postprandial ghrelin secretion more than the HC breakfast. The HP breakfast also decreased gastric emptying, probably through increased secretion of cholecystokinin and GLP-1.

1.21.2 High protein diet and weight loss

1.21.2.1 Short-term effects of a high-protein weight-loss diet

A study included twenty people with obesity with a mean BMI 34.8 kg/m^2 conducted by Neacsu *et al.,* (2014) in a crossover design with either a meat-based HP weight-loss (Meat- HPWL) or a vegetarian HPWL (Soy-HPWL) diet for 2 weeks. Both diets comprised 30% protein, 30% fat and 40% CHO. Over 2 weeks there was a significant decrease in participant's weight on both diets, approximately 2.41 kg for (Soy-HPWL) and 2.27 kg for Meat- HPWL. Gut hormone profiles indicated diet effects for ghrelin and PYY but not for GLP-1 plasma concentrations. The diet effect on plasma concentrations of amino acids was not related to a change in appetite. Since appetite control and weight loss were similar in both the Soy-HPWL and Meat-HPWL diet groups, a vegetarian HP diet could be a healthier alternative to a meat-based HPWL diet, achieving desired results without any negative health effects (e.g. risk of colon disease).

A short term study was conducted by Johnstone *et al.,* (2011) on 16 people with obesity. They did a randomised, cross-over design for 4 weeks with two HP diets (30%) with Low CHO (LC, 4 % CHO) and a moderate CHO (MC, 35 % CHO). The result of study

showed that participants lost about 6.75 kg of their weight on HP and LC and 4.32 kg on HP and MC diet. A study was carried out by Johnstone *et al.,* (2008) on seventeen obese men. They were offered two types of HP for 4 weeks with LC and MC. The finding of this study reported that there was significant reduction in subject's weight with LC diet compared with the MC diet.

1.21.2.2 Long-term effects of a high-protein weight-loss diet

Due *et al.,* (2004) reported that at 12 months there was greater abdominal fat loss in subjects that followed a HP diet of about 25% protein from total energy rather than those following a medium protein diet. Two further clinical intervention studies were carried out by Brinkworth *et al.,* (2004) on different groups of individuals with obesity; one group had T2DM and the other had hyperinsulinemia. Participants were randomly assigned to either a HP diet (30%) or a standard protein (SP) diet (15%). The duration of the study carried out on T2DM subjects was 15 months and the duration of the study on the hyperinsulinemia group was 17 months. The results of both studies reported that a HP diet reduced body weight of T2DM and hyperinsulinemia participants by 3.7 kg and 4.1 kg respectively, whereas weight loss for SP diet subjects was 2.2 kg and 2.9 kg respectively.

1.22 Aims of thesis

The aim for this thesis is to extend and test the work identified in the literature regarding the identification of a satiety phenotype. To identify at baseline individuals who struggle to lose weight on a weight management programme due to reduced satiety, in order to help clinical professionals to identify those people to develop strategies to help them lose weight. This was achieved by the following objectives.

In a pilot study

1- To investigate the relationship between VAS appetite/hunger and total energy and macronutrient intake.

2- To investigate the relationship between TFEQ subscales and total energy and macronutrient intake.

3- To investigate the relationship between plasma gut hormone levels and total energy and macronutrient intake.

4- To investigate the relationship between food craving and total energy and macronutrient intake.

5- Design a diet for people with low satiety.

6- To investigate whether a high-protein diet is an effective weight-loss strategy for individuals with reduced satiety compared to other diet strategies.

In a laboratory setting, study individuals with normal weight

1- To investigate the relationship between VAS, *ad libitum* lunch and food diary energy intake.

2- To investigate the relationship between plasma gut hormone concentrations and *ad libitum* lunch and food diary energy intake.

3- To investigate the relationship between the TFEQ-H subscale and *ad libitum* lunch and food diary energy intake.

4- To investigate the relationship between food craving and *ad libitum* lunch and food diary energy intake.

5- Use this data to develop a method to identify an individual's satiety phenotype.

In a clinical Tier 3 setting

1- To investigate the relationship between VAS, appetite rating scores and subsequent weight loss.

2- To investigate the relationship between fasting concentrations of plasma gut hormone levels and subsequent weight loss.

3- To investigate the relationship between the TFEQ subscales and subsequent weight loss.

In a clinical Tier 2 setting

1- To investigate the relationship between a 3 day food diary and weight loss.

2- To investigate the relationship between the food craving scores and weight loss.

3- To investigate the relationship between the TFEQ subscales and subsequent weight loss.

Chapter 2

General methods

2 Materials and methods

2.1 General Methods

The purpose of this chapter is to give an overview and description of all the general methods used as part of the programme of research in this thesis. The laboratory work of this thesis was carried out at the Biomolecular Sciences Research Centre and Sheffield Business School Specialist Facilities (Food and Nutrition laboratories) at the City Campus of Sheffield Hallam University (SHU).

2.2 Participants, recruitment and timings

2.2.1 Pilot study (study 1)

Healthy, overweight and people with obesity aged between 18-60 years with BMI ≥ 25 kg/m^2 participated voluntarily. They were recruited from staff and students at SHU through advertisement posters asking for people who would be interested in taking part in the studies. The participant's characteristics are described in chapter 3. Prior to the main trials, each eligible participant was interviewed at the first visit for preliminary screening using a Pre-Test Medical Questionnaire (Appendix 1). Volunteers were excluded from participation in the study if they were over 60 years, pregnant, those had a BMI 25 kg/m^2 or they had a history of chronic disease such as diabetes, hypertension or heart disease. The ethical approval for the study was granted by the Faculty of Health and Wellbeing Research Ethics Committee, SHU (Appendix 2).

All volunteers were provided with a participant information sheet 1 (Appendix 3) explaining the purpose and giving a brief description of the procedures of the study. They completed and signed an informed consent form (Appendix 4). They were given an explanation of the experimental protocol and any questions were answered by the investigator (HE). After enrolment, participants completed a number of questionnaires. These included questionnaires about dieting and weight history (Appendix 5), food and beverage preferences (Appendix 6), a food diary (Appendix 7), food craving inventory (FCI) (Appendix 8) and the three factor eating questionnaire (TFEQ-51) (Appendix 9) (Stunkard and Messick, 1985).

Anthropometric measurements were taken by the investigator for the participants at the first visit including height, weight, waist and hip measurements.

Participants were asked to fast for 12 hours overnight prior to a second visit for the main trial, which included blood sample collection and VAS questionnaires (Appendix 10 Appetite scale 1). This visit lasted approximately 3 hours 30 minutes. Finger prick blood samples were taken for hormone analysis five times from participants during this period and the VAS questionnaires were used at the same time points to evaluate sensations of appetite (hunger, satiety, desire to eat and fullness). Volunteers were instructed to fill out the first VAS questionnaire before the breakfast and another VAS questionnaire was filled out again directly after they consumed their breakfast, at 30 minutes after breakfast and then at 1, 2 and 3 hours post breakfast. A test breakfast was served to volunteers after taking the first blood sample for monitoring metabolic and hormonal responses. It consisted of standard breakfast products, which were all commercially available. It comprised of 200 ml of Tesco Smooth orange juice (Tesco's own brand, Tesco, UK), 100 ml of Tesco British Semi Skimmed Long life UHT Milk (Tesco's own brand, Tesco, UK) and 50 g of Tesco Corn Flakes Cereal (Tesco's own brand, Tesco, UK). Total calories provided by the breakfast test meal were 327 kcal (83.3% CHO, 10.7% protein, 6% fats). Participants were allowed to read or work during the trial and were not exposed to food cues.

2.2.1.1 Personalised diets

Three types of diet were tested to investigate which diets would be the most effective in terms of weight loss in the current study. The diets were personalised by giving participants suggested food plans based on their stated food preferences. These were 1) an increased or high protein diet (HP), 2) NHS Choices 12 week guide and 3) the intermittent fasting diet (5 days normal food consumption and 2 days fasting).

2.2.1.2 Diet allocation of participants

Volunteers were allocated to a diet, taking into account which types of diet they had tried before. After the second visit, participants were allocated to meal plans based on the diet and information to help them follow the diet day to day. In the HP diet, the number of calories per day was restricted, for most men, this meant consuming no more than 1,900 kcal a day, and for most women, 1,400 kcal. Men were instructed to consume 142.5 g of protein per day, this amount comes from dividing 1,900 kcal by 30% of total protein and after that divide this number by 4, which is calories of protein

per gram. Women were instructed to consume 105 g of protein per day using the same calculation based on 1,400 kcal.

Participants on the intermittent fasting 5:2 diet were required to limit calorie intake for two days, consecutively or otherwise each week. The calorie limit for fasting days was approximately 600 kcal for men and 500 kcal for women. On non-fasting days subjects were able to eat a normal calorie level (approximately 2500 kcal for men and 2000 kcal for women).

The NHS Choices guide helps individuals to work towards losing weight using an online programme. For most men, this meant consuming no more than 1,900 kcal a day, and for most women, 1,400 kcal.

2.2.1.3 Follow up diet of participants

Participants were met after 6 weeks for repetition of the anthropometric measurements and to discuss how their diet was going and to give them advice, then another meeting after 12 weeks for repeating hormone analysis, questionnaires (Dieting and weight history, food and beverages preferences, a food diary, FCI and the TFEQ-51 and taking measurements.

2.2.2 Identification of a satiety phenotype in people with normal weight (Study 2)

Students were recruited from Sheffield Business School Specialist Facilities at SHU, UK. They were aged between 18-45 years. Participant characteristics included in this study are described in chapter 4. This study was approved by the Faculty of Health and Wellbeing Research Ethics Committee, SHU.

Before the main trial, student classes were visited to describe the study protocol and procedures and the students were given a pack with participant information sheet and informed consent form to consider prior to participation in the study, the pack also included questionnaires. Volunteers were instructed to attend one practical session in the laboratory over a one week period. Participants arrived at the laboratory first thing in the morning after 12 hours fasting.

The finger prick blood samples were collected five times from participants, details are given in section 2.6. The first samples were collected in the morning when participants first arrived, then a further 4 samples were taken over the next 3 hours. Anthropometry measurements were also taken. Participants were instructed to fill out

the first VAS before breakfast and another VAS was completed directly after they consumed their breakfast and at 30, 90 and 150 minutes after the end of breakfast the VAS was completed. The exact time they finished their breakfast was noted. After the participants gave the first blood sample and completed the VAS questionnaire they were asked to not look at earlier pages during the time they completed the remaining pages. For the standardised breakfast test meal, cornflakes were weighed and recorded then put in bowls. This breakfast test meal was served to them. An *Ad libitum* test lunch of fusilli Tesco pasta (Tesco's own brand, Tesco, UK) and tomato sauce (Sacla, Tesco, UK) were weighed out for participants.

The protocol for cooking pasta was undertaken in the following steps: first of all, a large mixing bowl was weighed and the exact weight recorded. Then 400g fusilli pasta was weighed out. After that, unsalted water was boiled in a kettle and 2 litres of boiling water added to the pasta in the bowl. The bowl was put in to a 900W microwave and heated for 7 minutes. Pasta was stirred gently and a further 400ml of boiling water was added and then the bowl then was placed in the microwave at 900W for 7 minutes. Pasta was stirred gently again and then the drained in a colander for 2 min (total). After that the bowl was transferred from the colander three times with shaking each time, to remove the water. The pasta was left in the colander for the remaining time. It was dried and returned to the bowl and 500g in the pasta bowl was placed on a balance and 400g of sauce was added to the bowl and the exact weight of sauce was recorded on the sheet, the sauce was then mixed into the pasta until the pasta was evenly coated. Finally, the bowl was covered with cling film and placed in the fridge overnight. 32 g of extra-virgin olive oil was added to the pre-prepared pasta and mixed until homogenous and then it warmed up and served to participants.

Participants were instructed to consume the pasta lunch after the 150 minute VAS and blood sample were taken. Pasta was consumed in the sensory suite where participants ate in isolation and were devoid of other food cues.

The plate with 500g mixed pasta and sauce was served with 1 glass of water and participants were instructed to eat until they felt comfortably full and satisfied. Participants were not allowed to read or to access their phones during this part of the trial. The plates were monitored at intervals, as soon as the bottom of the plate was visible the plate was topped up with additional pasta and sauce. The amount of mixed

pasta and sauce participants ate was recorded. The EI provided by the *ad libitum* lunch was calculated using the information provided by the food manufacturer.

2.2.3 Tier 3 study (Study 3)

People with obesity and overweight people aged between 25-80 years were recruited from Rotherham Institute for Obesity (RIO, Rotherham, UK) prior to starting a weight loss programme by letter of invitation. Participants' characteristics included in this study are described in chapter 5. The inclusion criteria for this study were the same as the inclusion criteria for acceptance of participants on to the RIO weight management programme. This is: BMI>40 kg/m^2, BMI>30 kg/m^2 with comorbidities such as diabetes, hypertension, heart disease and arthritis, waist circumference over 102 cm (males) or 88 cm (females). Exclusion criteria for the study was anyone unwilling or unable to provide informed consent to undertake the study. After patients agreed to take part in the study two appointments were arranged. The first 30min appointment was immediately after the participant's initial consultation with the clinicians and dieticians. During this appointment, volunteers were interviewed and were provided with a participant information sheet 2 (Appendix 11). They were asked to sign the consent form and then questionnaires were provided for them to complete. These included TFEQ-51.

A second appointment was arranged during this meeting and participants chose the best day to come back to RIO a few days after meeting 1 or during the following week. The second appointment was for 2 hours. In this appointment one finger prick blood sample was collected in the morning between 8:00 -10:00 AM at the RIO meeting room, after the participant had fasted for 12 hours overnight. Then, they were asked to complete a VAS questionnaire (Appendix 12 Appetite Scale 2) after which a breakfast test meal was served to them. Participants completed multiple VAS questionnaires for the next 1 hour and 45 minutes. During this time participants needed to stay at RIO but were free to read or use their phones.

2.2.4 Tier 2 study (Study 4)

People with obesity and overweight people were recruited from Rotherham Leisure Complex (Rotherham, UK) prior to starting a community group-based weight loss programme. Participants were aged between 25-80 years and their characteristics are described in chapter 6.

Volunteers were recruited via two visits by the researcher, during two weeks. At the first visit, a presentation was made about the study for participants during their first group session. They were provided with information and a description of the study protocol. They were given the opportunity to ask questions regarding the study. Researchers (Hameida Elfarssi and Caroline Dalton) gave out packs with questionnaires including the FCI and the TFEQ-51. They took the information sheet including information on food diaries and the questionnaires home for a week so that they could consider about taking part. After the session in week 1, questionnaires were collected the following week. Participants completed and signed an informed consent form. Data from food diaries from for the participants were collected including breakfast, lunch, dinner and snacks for seven days. Three consecutive following days from the diary were analysed from these forms.

2.3 Anthropometry

2.3.1 Height

Height was measured to the nearest 0.1 cm. Participants were asked to remove their shoes and stand with their back to the height rule. They were told that the back of the head, back, buttocks, calves and heels should be against the wall, with feet and knees together. Participants were asked to look straight ahead, with their head in the Frankfort horizontal plane (It is referred to the as lower orbits of the eyes and the external auditory meatus).

In study 1 (chapter 3), the height of participants was taken by using a wall-mounted stadiometer (Seca, Hamburg, Germany). In the nutrition study (study 2, chapter 4), a 213 Height Measure (Leicester) (Seca, Germany) was using for measuring participants height.

In studies 3 and 4 (chapters 5 and 6 respectively), the height of participants was taken by trained RIO and Rotherham Leisure Complex staff and the data was then provided to the researcher.

2.3.2 Weight

Body weight was measured to an accuracy of 0.1 kg. Participants were asked to remove their heavy outer garments (jacket and coat) and shoes. They were told to remove personal belongings in their pockets prior to measurement. They were also

asked to stand in the centre of the platform with head erect and eyes looking straight ahead.

In study 1 (chapter 3), the weight of participants was measured by using a balance beam scale (Seca, Williams Medical Supplies, Germany). In the nutrition study (study 2, chapter 4), 899 portable electronic scale (Seca, Germany) was used for measuring participant's weight.

In studies 3 and 4 (chapters 5 and 6 respectively), weight of participants was measured using 899 portable electronic scale (Seca, Germany) by trained RIO and Rotherham Leisure Complex staff and then staff provided the measurements on a spreadsheet containing participant data. In all studies, BMI was then calculated by dividing the participant's weight in kilograms by the square of their height in metres.

2.3.3 Circumference Measurements

Waist and hip circumferences were measured using a tape measure (Seca 201 Fitness Body Waist to Hip Ratio Ergonomic Body Fat Measuring, Germany). Waist circumference was measured at the narrowest diameter between the xiphoid process and iliac crest. Hip circumference was measured as the maximal circumference over the buttocks.

In study 1 (chapter 3), waist and hip circumference of participants was measured using a tape measure. In studies 3 and 4 (chapters 5 and 6 respectively) the circumferences of participants were measured by trained RIO and Rotherham Leisure Complex staff and then staff provided the data. These staff were trained for reliability in undertaking these measurements.

2.4 Questionnaires

A number of questionnaires were given to participants to complete after enrolment, including the following.

2.4.1 Three factor eating questionnaire (TFEQ)

The TFEQ-51 developed by Stunkard and Messick, (1985) was used to measure eating behaviour subscales of volunteers, including hunger. The 51-item questionnaire assesses eating attitudes and behaviour across three scales: dietary restraint (21 items), disinhibition (16 items) and hunger (14 items). The 51 items are measured on a 4-point

response scale (definitely true for me: 1, quite true for me: 2, occasionally true for me: 3 definitely not true for me: 4 and scores were summated into subscale scores for: cognitive restraint, disinhibited eating and hunger.

2.4.2 Food craving inventory questionnaire (FCI)

The FCI was used to assess frequency of desire to eat specific foods. It includes 28 items classified into four subscales: 1) high fat foods (fried chicken, sausages, gravy, fried fish, bacon, steak, sausage rolls), (2) sweets (brownies, cookies, chocolate, doughnuts, cakes ice-cream, sweets, sponge cake), (3) CHO (breads, pancakes, biscuits, sandwich bread, rice, baked potato, pasta, cereal, Danish pastry, crisps) and (4) fast food (hamburgers, French fries, pizza). Each subscale had multiple choices and the summation score of these multiple choices represented the frequency of desire for each food. The questionnaire measured the frequency of cravings for a specific food item on a scale of 0 -never, to 4- almost every day.

2.4.3 Food diaries

Food diaries were used to measure nutrient intake and participants were asked to record their food. They were provided with instructions for recording their food in food diary sheets and were instructed to fill out their food for 3 days, noting down what they had eaten as close as possible to the time when they ate it (to help them to remember accurately). They were told that they should be as specific as possible about the amount of each item, what time they ate it and any brand names. For example: Breakfast 8am - 1 piece of Warburton's white bread with Olivio spread, 2 scrambled eggs, I large cup of coffee with 1 level teaspoon sugar.

Lunch 12.30pm - 1 small plate of lettuce and tomatoes, half a large tin (160g) of Princes tuna in brine, 2 small new potatoes, boiled, small portion of full fat salad dressing, 1 can Coke.

Evening meal 7pm - Beef chilli con carne with white rice (average portion). Cup of black coffee with 1 level teaspoon sugar, 1 medium slice Tesco own brand blackcurrant cheesecake. They were asked to include all drinks and any between meal snacks and were told that they did not need to change their diet in any way, all information was anonymised.

Nutritics software (Libro, Dublin, UK) (version 1.8) was used to calculate TEI and macronutrient intake. This provides users with a report that contained all micro and macro nutrient information from the food diaries.

2.5 Assessment of appetite

2.5.1 Visual Analogue Scale

VASs were used in chapters 3, 4 and 5 to assess subjective appetite sensations (hunger, fullness, satiety and desire to eat). It is the most commonly used method to assess appetite sensations. VAS are comprised of a 100-mm horizontal line with words anchored at each end expressing the extreme of each rating, e.g. 'I am not hungry at all' (0 mm) / 'I have never been more hungry' (100 mm). Pen and paper were used to administer the VAS, which were easy and quick to use. Participants were instructed to mark a vertical line on the scale at a point between the two extreme ratings, which they considered to reflect their current feeling. The VAS scores were quantified by measuring the distance in millimetres from the 0 mm point to the position of the mark.

The questionnaire that was used consisted of four scales adapted from Flint *et al.,* (2000). The VAS booklet included four questions and they were presented in the format ("How hungry do you feel", "How satisfied do you feel" "How full do you feel" and "How much more do you think you can eat".

The area under curve (AUC) was calculated by using the trapezoidal method which draws four triangles and 4 squares on the graphs (Yeh, 2002). The summation of the area of the 4 squares and 4 triangles for each graph determined the AUC for the participants for those questions over the time of the experiment. AUC composite of appetite score were summated using the AUC for hunger, full, desire to eat and can eat more, by using this equation hunger score+ desire score + prospective food intake score + (100-fullness score) (Hill and Blundell, 1982). The figure below shows an example of the calculation of an AUC from a participant's hunger scores.

AUC= Square 1 = score 30 x 0.5, Square 2= score 30, Square3= score 1hr, Square 4= score 2hr, triangle 1=score 0- score 30/2 x 0.5, triangle 2=score 1hr-30/2x0.5, triangle 3=score 2hr-score 1hr/2x1 and triangle 4=score 3hr-score 2hr/2x1.

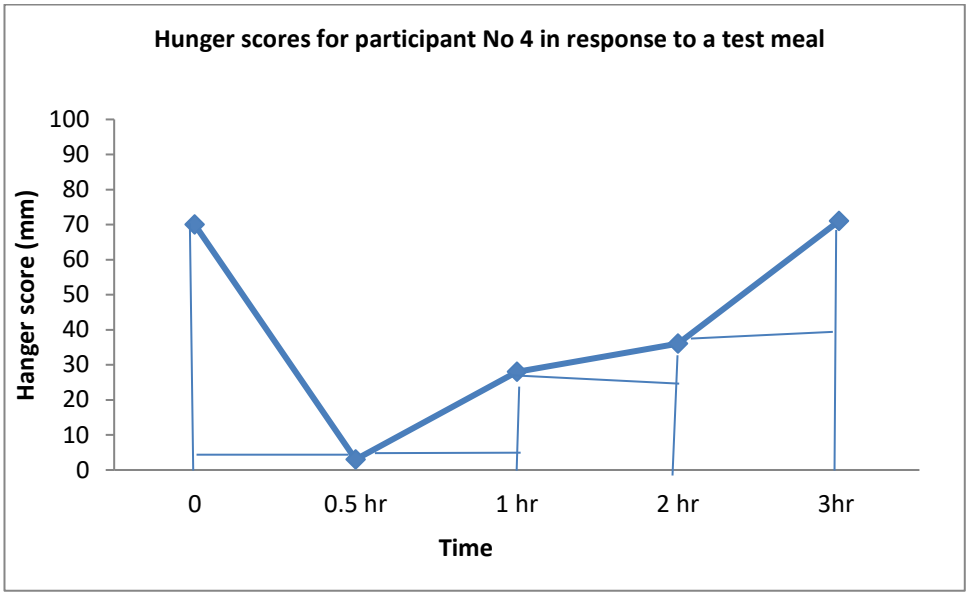

Figure 2-1: An example of an AUC calculation of hunger scores for participant No 4 in response to a test meal.

2.5.2 Satiety Quotient (SQ)

The SQ was used in chapters 3, 4 and 5. It is a formula to measure the satiating effect of foods relative to energy content. VAS scores were used to calculate SQ, in response to a fixed-calorie meal. Hunger VAS ratings were used to calculate the average SQ. SQ composite appetite scores were the summation of SQ hunger, fullness, desire to eat and can eat more, by using this equation hunger score+ desire score + prospective food intake score + (100-fullness score) (Hill and Blundell, 1982).

A higher SQ is representative of a stronger appetite response to the ingested food, whereas a lower SQ represented a weaker response (Drapeau *et al.,* 2005; Dalton *et al.,* 2015).

In chapters 3 and 4 this equation below was used to calculate the SQ.

$$SQ = \frac{(\text{Fasting AS} - \text{mean 60 min post meal AS})}{\text{Energy content of the test meal (kCAL)}} \; X\ 100$$

In chapter 5 this equation below was used to calculate the SQ following that reported in Dalton *et al.,* 2015).

$$SQ = \frac{(\text{Fasting AS} - \text{mean 75min post meal AS})}{\text{Energy content of the test meal (kCAL)}} \; X\ 100$$

AS = appetite sensation

2.6 Blood sample collection

Blood samples were taken by using mechanical finger-prick devices, Accuchek Safe-T-Pro Plus Single Use Lancets (Mannheim, Germany). These samples were collected into ice-chilled microvette tubes containing 500 µl potassium ethylenediaminetetra-acetic acid (EDTA)-coated as an anti-coagulant (Sarstedt, Nümbrecht, Germany). Blood samples were dispensed into EDTA tubes for the determination of plasma gut hormone concentrations.

Each microvette tube contained EDTA plus 2.5µL each of the 3 protease inhibitors (3.p-hydroxymercuribenzoic acid (PHMB), DPPIV inhibitor and Pefabloc) to prevent the degradation of gut hormones.

To prepare the PHMB solution, 30 µL of NaOH was added to 2.5 mL of phosphate buffered saline (PBS). 500 µL of PHMB was added to 500 µL of the NaOH/PBS solution before being frozen in aliquots. On the morning of the study, 5 microvette blood tubes were prepared per participant. Each tube contained 2.5 µL of the PHMB/NaOH/PBS solution, 2.5 µL of Pefabloc and 2.5 µL of DPPIV inhibitor.

All blood samples were kept in an ice bath until centrifugation. The microvettes were spun at 1000xg for 10 min at 4°C and plasma supernatants were then aliquoted into plastic Eppendorf tubes for storage. 100 µL of 1 M HCl was added per mL of plasma to neutralise proteases and allow for acylated ghrelin and GLP-1 determination.

After HCl was added to each plasma sample the sample was spun at 1000xg for 5 minutes in a refrigerated centrifuge (Eppendorf® Minispin® and MiniSpin Plus personal microcentrifuge, Germany) at 4˚C. All prepared plasma samples were labelled with a numerical code and stored at – 80°C in BMRC laboratory until the time of the assay.

During blood collection, the subjects were allowed to sit in a room (at university for the pilot and nutrition studies and in a meeting room at RIO centre for the RIO study). They could bring their computer or iPad to work or listen to the radio, toilet visits were permitted and they were monitored to make sure they did not access anything related to food.

2.7 Laboratory methods

Prior to commencing experimental trial days, all reagents were prepared in advance.

2.7.1 Preparation of reagents for the multiplex assay

Preparation of reagents, controls and working standards were carried out using the reagents and assay protocol provided by the manufacturer (EMD Millipore, Darmstadt, Germany). Hormone quantification used the human metabolic hormone magnetic bead panel, 96 well plate assays.

2.7.1.1 Preparation of Antibody-Immobilized Beads

Each antibody-bead vial was vortexed for 1 minute and 150 µL from each vial was added to the mixing bottle and the final volume made up to 3.0 mL with Bead Diluent. The mixed beads were vortexed well again.

Example 2: When using 3 antibody-immobilized beads, 150 µL from each of the 3 bead vials was added to the Mixing Bottle. Then 2.55 mL Bead Diluent was added.

2.7.1.2 Preparation of Quality Controls

Before use, Quality Control 1 and Quality Control 2, supplied with the kit, were reconstituted with 250 µL deionized water. The vial was inverted several times to mix and vortexed.

2.7.1.3 Preparation of Wash Buffer

60 mL of 10X Wash Buffer was diluted with 540 mL deionized water.

2.7.1.4 Preparation of Serum Matrix

1 mL of deionized water was added to the bottle containing lyophilized Serum Matrix and it was mixed well and then allowed to stand for at least 10 minutes for complete reconstitution.

Prior to use, the Human Metabolic Hormone Standard was reconstituted with 250 µL deionized water and then the vial was inverted several times to mix and then vortexed for 10 seconds. The vial was left to stand for 5-10 minutes. This was used as standard number 7.

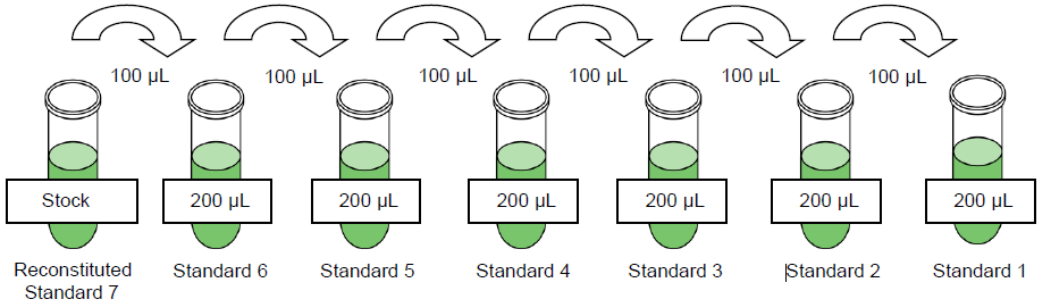

Figure 2-2: Preparation of standards for the multiplex assay.

2.7.2 Plate washing procedure

The plate was rested on a handheld magnet (EMD Millipore, Darmstadt, Germany) for 60 seconds to allow the complete attraction of the antibody coated magnetic beads. The well contents were removed by gently decanting the plate into an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid, after that the plate was washed with Wash buffer by removing the plate from the magnet and then adding Wash buffer (200 µL) to each well again and the plate was shaken for 30 seconds and then reattached to the magnet and beads were left to settle for 60 seconds and then well contents were removed as described above.

2.7.3 Immunoassay procedure

Controls 1 and 2 and test samples were recorded on a 'Well Map Worksheet' in a vertical configuration to ensure accurate placement in the wells. First of all 200 µL of assay buffer was added to each well of the plate and then it was sealed and mixed and after that it was shaken for 10 minutes on a plate shaker at room temperature (20-25°C). Assay Buffer was decanted and the residual amount was removed from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. After that, 25 µL of each standard or control were added into the appropriate wells in duplicate and Assay Buffer was used as the 0 pg/mL standard (background) then 25 µL of Assay Buffer was added to the sample wells and 25 µL of appropriate matrix solution was added to the background, standards and control wells, then 25 µL of each sample was placed into the appropriate wells and the bottle of Mixed Beads were vortexed and mixed. Thereafter, 25 µL of Mixed Beads was taken and added to each well and the plate was sealed with a plate sealer, wrapped in foil and incubated with agitation on a plate shaker overnight (16-18 hours) at 4°C. After overnight incubation

well contents were gently removed and the plate washed 3 times. After that 50 µL of Detection Antibody was added to each well and the plate sealed and covered with foil and incubated with agitation on a plate shaker for 1 hour at room temperature (20-25°C). 50 µL Streptavidin-Phycoerythrin was added to each well and the plate was sealed and covered as before and incubated with agitation on a plate shaker for 30 minutes at room temperature (20-25°C). After that, well contents were gently removed and the plate washed 3 times. Then 100 µL of Drive Fluid was added to all wells and the beads were resuspended on a plate shaker for 5 minutes. Finally the plate was run on a Luminex® 200™, HTS, FLEXMAP 3D® or MAGPIX® with xPONENT® software and data was saved on a USB.

2.8 Gut hormone analysis

All hormone assays were performed at the laboratory in the Biomolecular Sciences Research Centre at SHU. Samples for each participant were run in duplicate.

Plasma acylated ghrelin, PYY, PP and GLP-1 concentrations were measured by enzyme-linked immunosorbent assay using a commercially available kit (Human Metabolic Hormone Magnetic Bead Panel (HMHMAG-34K) following the manufacturers' instructions and it was assayed by Luminex MAGPIX instrument with xPONENT software version 4.2 (Austin, Texas, USA).

2.9 Statistical analysis

All data were analysed using the Statistical Package for the Social Science (SPSS) software version 24.0 (IBM) for Windows (SPSS Inc., Chicago, IL, U.S.A.) and Prism version 7.03 (GraphPad Software Inc., La Jolla, CA, U.S.A.). All data was normally distributed within the software and tests used were non parametric. All AUC values for appetite perception and appetite hormone levels in chapters 3, 4, 5 were calculated using the trapezoidal rule. T-tests were used to assess differences in gender, age, height, body mass, BMI and other dependant variables between groups. T-tests were also used to assess differences in mean values of BMI, body weight, waist change, TFEQ subscales, macronutrient and food cravings in high satiety participants (chapter 6).

Correlation coefficients were used to assess relationships between variables. Linear regression analysis was used to establish a relationship between dependent variables

(*Ad libitum* pasta and TEI measured by food diary) and independent variables (the predictors such as VAS (fasting time, hunger, fullness, desire to eat and prospective food intake), 30 min fullness, post breakfast desire to eat, AUC of 4 questions and SQ of 4 questions, gut hormones and TFEQ-H of the heat map. One way ANOVA was used to determine whether there were any statistically significant differences between the average BMI and TFEQ subscales of low, medium and high hunger groups (chapter4). One way ANOVA was used in chapter 5 to determine whether there were any statistically significant differences between the average BMI, weight, waist and TFEQ subscales in low, medium and high hunger participants.

Data are represented as mean ± SD, unless otherwise indicated. A p value < 0.05 was considered to be statistically significant. Cohen d=0.2 was considered a 'small' effect size, 0.5 represents a 'medium' effect size and 0.8 a 'large' effect size.

Chapter 3

Pilot study for developing methods to identify individuals with blunted satiety measured by hormone, appetite and eating behaviour

3 Introduction

The use of pilot and feasibility studies to develop interventions is an important step in scientific research (Lancaster, 2015). Until recently, much of this preliminary work, including decision-making processes, methods optimisation and formal testing prior to a full-scale study, has gone unreported (Hoddinott, 2015). However, data generated from such studies is of interest to the scientific community, most notably for those individuals undertaking similar research. Pilot studies supply data to encourage scientific rigour and ensure that future study protocols are optimised to best investigate their aims and objectives (Van Teijlingen and Hundley, 2002).

3.1 Low satiety phenotype and high protein diet

Impaired appetite control is one factor implicated in weight gain. Individuals who have difficulties in recognising their appetite sensations, before and after a meal, and who do not eat in response to their appetite sensations seem to be more susceptible to weight gain. These individuals represent the 'low satiety phenotype'. This phenotype is associated with specific behavioural and metabolic profiles that could explain their susceptibility to overeating. Drapeau and colleagues (2005) reported individual variability in satiety responsiveness among both people with obesity and normal weight and have identified a 'low satiety phenotype' by using the satiety quotient (SQ) (Dalton *et al.,* 2015). The SQ is calculated from the difference in hunger ratings before and post-consumption of a food, then divided by the weight or energy content of the food or drink being tested (Tremblay and Bellisle, 2015).

With an increasing prevalence rate of obesity in western countries, authorities emphasise the importance of averting weight gain, improving weight loss and sustaining weight-loss status. However, this requires a great effort from people who live in the modern obesogenic environment that encourages food intake and limits energy expenditure to maintain their healthy body weight. Several dietary strategies have been suggested to reduce body weight and prevent weight gain (Gilbert *et al.,* 2011).

Previous studies have demonstrated that prescribing a diet with 30% of the calories obtained from protein reduces feelings of hunger, as protein has a greater effect on satiety than fats or CHO (Astrup, Raben and Geiker 2015). Therefore this study

hypothesised that people with low levels of satiety hormones may find that a high protein diet is easier to adhere to than other types of diet. Studies have shown that diets containing 30% protein are well-tolerated and may have some health benefits, providing that the increase in protein is not made up of red meat, and that CHOs are not excluded. Higher protein diets also enhance weight loss due to increased dietary thermogenesis (Halton and Hu, 2004) and protein-induced alterations in gluconeogenesis to improve glucose homeostasis (Pesta and Samuel, 2014).

Over the past two decades, high protein diets have proven to be a successful strategy that can prevent or treat obesity through improvements in the management of body weight. This is thought to be due, in part, to modulations in appetite, energy metabolism and EI (Leidy *et al.,* 2015). Diets with high protein have a long history (Denke, 2001). Many people have also been encouraged to try higher protein diets due to their poor experiences in failing to achieve and maintain clinically relevant weight loss, which is typically stated as 5% (NICE, 2006).

The rationale for the use of this type of diet is that it offers a more accentuated satiety response for a longer period of time, when compared with other macronutrients such as CHO and fat (Lepe, Bacardi and Jimenez, 2011). Moreover, it affects both sides of the energy balance, i.e. EI and energy expenditure, thereby providing a strategy for weight loss and weight maintenance after weight loss (Veldhorst, Westerterp and Westerterp-Plantenga, 2012).

Identifying food preferences at the beginning of a weight loss programme would help to design diets that stick to the required protein content but reflect individual preferences.

3.1.1 Rationale for the methods used to measure satiety and hunger in laboratory settings

3.1.1.1 Visual analogue scales

VAS have been described previously and the reproducibility, validity and power of this tool has been well established (Parker *et al.*, 2004). Appetite sensations reflect objective and subjective components of appetite control (Stubbs *et al.,* 2000). The Satiety Quotient in response to a standardised meal gives a numerical measure of

satiety, and is expressed per unit of intake (e.g. kcal) when calculated (Dalton *et al.,* 2015).

3.1.1.2 Test meal

The single-course test meal is used to measure both satiation (energy consumed to fullness) and later EI following a period of satiety. It is the most widely utilised approach to quantifying the food consumed to fullness, in response to the variable being tested. This approach has advantages and limitations. The benefit of the single-course meal is that it is easy to implement and interpret and enables a clear measure of quantitative intake to fullness. The limitation of this approach is that when restricting eating to a single meal item, the focus is only on quantity consumed and not on food choice, therefore poorly reflecting a real life meal, where several food items are available and can be freely consumed (Chapelot, Blundell and Bellisle, 2013; Hill, Rogers and Blundell, 1995). Participants can also become bored of the homogenous food and therefore are not eating until comfortably full and satisfied.

3.1.1.3 Three factor eating questionnaire (TFEQ)

Completing the TFEQ is an empirical approach to identifying eating behaviours (Stunkard and Messick, 1985). The TFEQ was originally developed to investigate the theory that people with obesity were thought to have different eating behaviours and different behavioural and emotional responses to situations involving food choices, compared to people who are normal weight. The TFEQ measures three distinct constructs of eating behavior: 1) Restraint, 2) Disinhibition and 3) Hunger. Restraint is described as the degree to which a person exerts behavioral control over their own eating behavior. Disinhibition refers to a person's stable underlying readiness to eat in response to environmental triggers, such as the sight and smell of palatable food, social or emotional eating. Hunger represents a person's stable underlying sensitivity to hunger feelings and predisposition to eat (French *et al.,* 2014). The TFEQ has been validated and shown good test-retest reliability (Laessle *et al.,* 1989).

There are fewer studies that have shown associations between TFEQ hunger scores and outcome, compared to studies considering restraint and disinhibition. Provencher *et al.,* (2003) have observed positive associations with EI. In theory, those who report chronically high levels of hunger should be more susceptible to overeating, compared with those who do not report being hungry. French *et al.,* (2014) reported that there

was a significant correlation between each of the eating behavior measures and EI, and they found a strong correlation between TFEQ Hunger and food intake. Fundamental for the studies in this thesis it is vital that restrained eaters are excluded, otherwise they would not eat to fullness.

3.1.1.4 Gut hormones

Appetite-related peptides are known to play a physiological role in the regulation of hunger and satiety (Delzenne *et al.,* 2010). This study measured four gut hormones: ghrelin, GLP-1, PP and PYY. These four hormones were selected as they represent the key group of hormones that have been shown to influence hunger and satiety. Ghrelin is the only known orexigenic hormone and fasting ghrelin concentrations have been shown to be lower in people with obesity when compared with normal weight controls (Cummings *et al.,* 2002). Moreover, GLP-1, PP and PYY have been shown to lead to reduced food intake and body weight (Suzuki *et al.,* 2010).

Ghrelin increases with subsequent weight loss and so may have a role in long-term weight regulation. It has also been suggested that decreases in fasting ghrelin could be a consequence of downregulation caused by overeating (Geliebter, Hashim and Gluck, 2008). GLP-1 acts as a satiety stimulator, which decreases hunger and promotes meal cessation (Figlewicz, 2003, Woods *et al.,* 1998). It has been reported to be lower in people with obesity, suggesting differences in GLP-1 could contribute to higher EI in people with obesity (Brown *et al.,* 2014).

PP increases following a meal, and acts as a satiety stimulator. The action of PP involves a reduction in food intake and it is reduced in people with obesity (Troke, Tan and Bloom, 2014). It has a role in the central control of appetite because it has high affinity for the Y4 receptor, which is widely expressed in brain areas (the area postrema) (Chaudhri, Small and Bloom, 2006).

PYY also acts as a satiety stimulator. It inhibits food intake through binding with, the highest affinity to the hypothalamic Y2 receptor (Troke, Tan and Bloom, 2014). People with obesity have low plasma concentrations of total fasting PYY, or no changes in fasting, or impaired postprandial PYY, as a result it has a role in the pathogenesis of obesity (Moran *et al.,* 2007).

3.1.1.5 Food diaries

EI can be determined by meal frequency, portion size and specific macronutrient contents of food consumed. Food diaries request participants to record all foods and beverages that have been consumed over a specific period of time, usually 3 to 7 days with at least one weekend day. However, week-day food records need highly motivated participants, and they are expensive to administer in large samples, thus 3-day food records are commonly used in free living studies (Yang *et al.,* 2010). The validity and reliability of 3-day food diaries for recording food intake has been reported in several studies (Schlundt, 1988; Yang *et al.,* 2010).

Food diaries can provide details about the macronutrient content of each participant's diet and their overall daily calorific intake. Using a 3-day food diary can identify a relationship between average daily EI and *ab libitum* food intake on the study date. For example, it could be predicted that participants who record a high carbohydrate diet across the 3-day food diary may consume more of a high carbohydrate meal in *ad libitum* conditions and participants who record a sustained high caloric intake would also consume a larger quantity of food in *ad libitum* conditions (Yang *et al.,* 2010). The main limitation is the potential under reporting that has been described previously in chapter 1.

3.1.1.6 Food cravings

Food cravings have been suggested as a conditioned expression of hunger, where food cravings develop from pairing consumption of certain foods with hunger (Gibson and Desmond, 1999). It has been suggested that food cravings play a role in maintaining the excessive eating patterns observed in binge eating, bulimia and obesity. Numerous studies have examined the role of cravings for specific food classes (e.g. CHOs, sweets, and fats) (White *et al.,* 2002) and have confirmed differences in the types of foods craved according to age, gender, time of day, hunger state and phase of the menstrual cycle (White *et al.,* 2002). CHO craving has been most frequently studied (White *et al.,* 2002). Martin *et al.,* (2008) tested the association between food cravings and consumption of specific foods in a laboratory taste test. The results indicated that food cravings are significantly related to food intake, with specific food cravings correlating with the types of foods consumed.

3.1.2 Aims

The main aim of the present study was to pilot methods to assess satiety phenotypes.

Aims of the present study are:

1- To investigate the relationship between the VAS rating and total energy and macronutrient intake.

2- To investigate the relationship between TFEQ subscales and total energy and macronutrient intake.

3- To investigate the relationship between gut hormones and total energy and macronutrient intake.

4- To investigate the relationship between food craving and total energy and macronutrient intake.

5- Design a diet for people with low satiety.

6- To investigate whether a HP diet is an effective weight-loss strategy for individuals with reduced satiety, compared to other diet strategies.

3.2 Materials and methods

3.2.1 Participant recruitment

Twenty-seven healthy overweight participants and people with obesity were recruited from SHU. Of those, data is presented for twenty-two participants (n= 20 females and n= 2 males) as 5 people either did not give blood samples (n=2) or did not return the questionnaires (n=3). Participants were aged between 18-55 years (Mean ± SD 42.09 ± 10.98 years). Participants were interviewed at the first visit for preliminary screening using a Pre-Test Medical Questionnaire (Appendix 1). Exclusion criteria were people aged over 60 years, pregnant, breast feeding, those with BMI≤ 25 kg/m^2 or had a history of chronic diseases such as diabetes, hypertension or heart disease. Participants were excluded if they disliked or were allergic to the foods used in the study, or if they took medication that may affect their appetite and / or metabolism (more details are provided in Chapter 2).

Intra assay % CV (The Average Coefficient of Variation) for ghrelin, PP, PYY and GLP-1 was < 10 and inter assay % CV for those hormones was < 15.

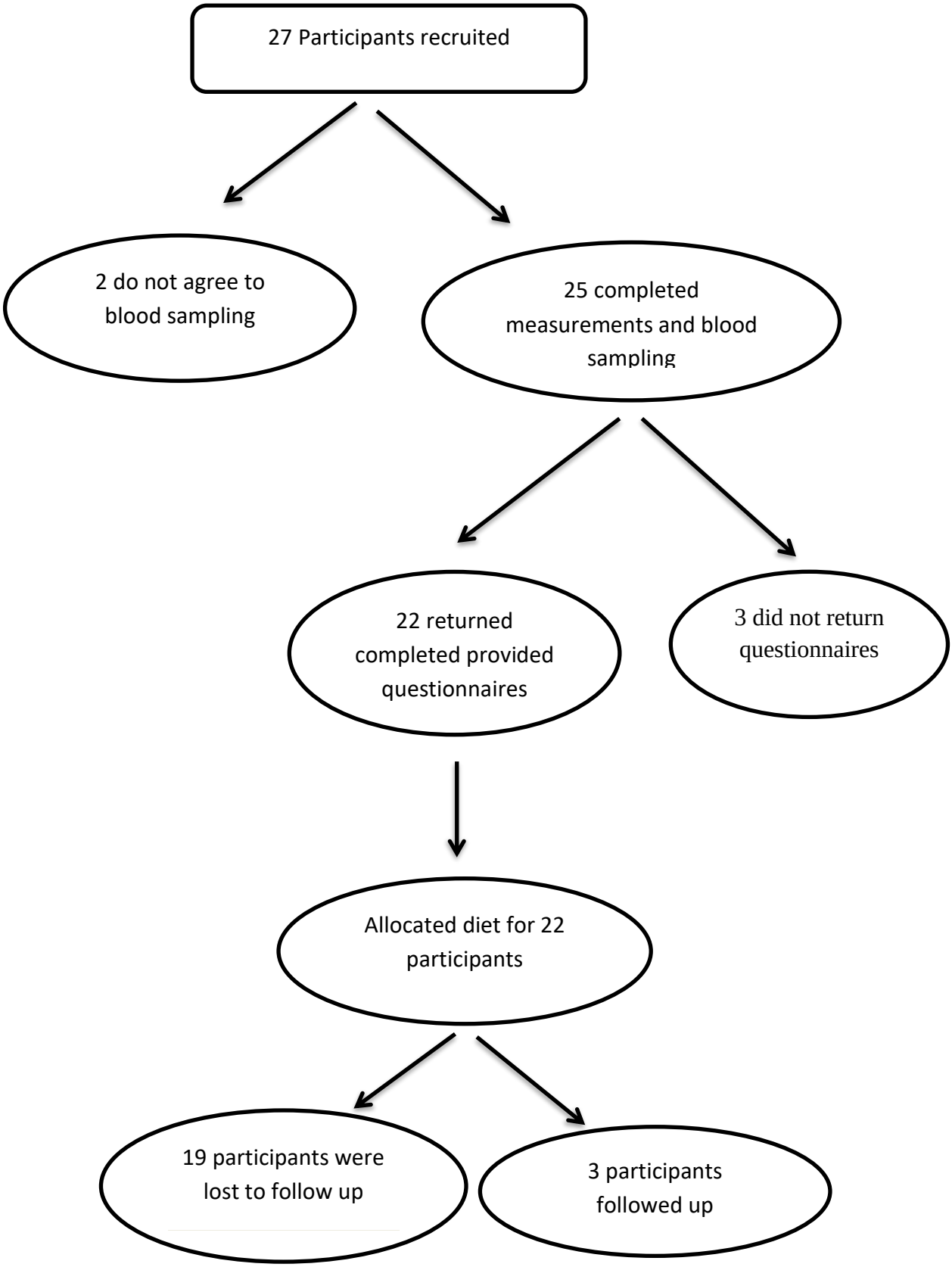

Figure 3-1: Flow chart of participants in the study

3.2.2 Statistical analysis

All data were analysed using (SPSS) and Prism version 7.03 (GraphPad Soft-ware Inc., La Jolla, CA, U.S.A.). Nutritics software (Libro, Dublin, UK) was used to calculate the percentage of macronutrients as well as EI from the completed food diaries.

Normal correlation was used to assess relationships between variables and total energy and macronutrients. Independent t-tests were used to assess for any differences in gender, age, height, body mass, BMI, TFEQ scores, macronutrient intake and food craving scores. A p value < 0.05 was considered to be statistically significant. Cohen d=0.2 be considered a 'small' effect size, 0.5 represents a 'medium' effect size and 0.8 a 'large' effect size.

3.3 Results

3.3.1 Baseline characteristics of the study participants

The baseline characteristics of all participants is shown in table 3.1, from where it is observed there are no statistical differences in measures between males and females apart from height and body mass, which were significantly different.

Table 3-1: Baseline characteristics of the study participants

Variables	**All** (n=22) Mean ± SD	**Female** (n=20) Mean ± SD	**Male** (n=2) Mean ± SD	t	p	d
Age (y)	42.1±11	41.8±11.0	44.5±14.8	-0.318	0.754	0.202
Height (cm)	164±7.7	163 ±5.8	177.6±13.6	-3. 038	**0.007**	1.397
Body mass (kg)	93.3±30	82.3±10.2	132.5±38.9	-3.400	**0.003**	1.766
BMI (kg/m^2)	32 ±5.2	30.2±4	41.5±5.9	-1.819	0.317	2.239
Waist (cm)	117.9±21.9	111.6±9.1	129.5±26.2	-.0.963	0. 509	0.915
TFEQ Hunger	6.9±3.8	6.9±3.8	9.5±2.1	-0.940	0.359	0.850
TFEQ Restraint	8 ±3.6	8.7±3.5	5.00±2.8	1.412	0.174	1.149
TFEQ Disinhibition	8.8±3.2	8.7±3.4	9.5±0.7	-0.313	0.758	0.317

Values are mean ± standard deviation, n= number of participants, t = t-test and Cohen's d. BMI: body mass index. SD: standard deviation. $p < 0.05$ significant differences between males and females.

3.3.2 Scores of TEI, macronutrient intake, based on food diaries and food craving questionnaires of participants

Table 3.2 presents the data of TEI and macronutrient intake using food diaries and food craving questionnaires for all participants. As can be seen in the table there was no significant difference in TEI and macronutrient intake based on the food diaries between genders. There was a significant difference in fat cravings between women and men with men having stronger fat cravings. No significant difference was found between females and males for other food cravings.

Table 3-2 : The mean ± standard deviation of TEI, macronutrient intake based on food diaries and food craving questionnaires of participants

Variables	All (n=22) Mean ±SD	Females (n= 20) Mean ± SD	Males (n= 2) Mean ± SD	t	p	d
Food diary-TEI (kcal)	1747.7±499.4	1728.5±517.4	1939.5±262.3	-0.560	0.581	0.514
Food diary-CHO (% of TEI)	45.7± 7.9	45.6±±8.0	46.5±8.8	-0.161	0.874	0.114
Food diary-Protein (% TEI)	19.3± 5.5	19.6±5.6	16.3±0.8	0.816	0.424	0.827
Food diary -Fat (% of TEI)	32.5± 7	32.4±7.2	34.3±5.5	-0.372	0.714	0.306
Food diary-Fibre (g)	14.8±5.0	15.1±5.1	11.6±1.9	0.929	0.364	0.893
Fast food craving	13.4± 4.3	13.3±4.5	14.5±2.1	-.0370	0.715	0.344
Sweet craving	27.4±7.1	26.8±6.6	33.5±12.0	-1.297	0.209	0.691
Fat craving	9.4±2.7	9.1±2.4	13.0±2.82	-2.184	**0.041**	1.506
CHO craving	15.4±5.1	15.2±5.0	17.0±7.1	-1.907	0.643	0.293

Values are mean± standard deviation, n=number of participants, t= t-test and Cohen's d. BMI: body mass index. SD: standard deviation. $p < 0.05$ significant the differences between males and females.

3.3.3 Fasting and AUC appetite, TEI and macronutrient intake

Table 3.3 displays the relationship between fasting and AUC appetite scores, measured using VAS and TEI and macronutrient intake from the food diary. As can be shown in the table, there was a positive correlation between fasting prospective food intake (r=0.441*) (p=0.040) and TEI. AUC prospective food intake (the amount of food that is expected to be eaten) was also positively correlated (r=0.497*) (p=0.019) with TEI. However, for the rest of the AUC and fasting scores for the 4 questions of the VAS there was no significant correlation with TEI. Fasting prospective food intake was significantly negatively correlated with percentage of CHO intake (r=-0.485*, p=0.019). However, for the rest of the fasting and AUC VAS measures there was no significant correlation with percentage CHO intake, AUC satisfaction was negatively correlated with the percentage of CHO intake but this did not reach significance.

Table 3-3 : Correlation coefficients between fasting and AUC appetite, TEI and macronutrient intake

Correlation	TEI	% CHO	% Fat	% Protein	Fibre
Fasting hunger	0.230	-0.180	0.265	-0.165	-0.003
AUC hunger	0.045	0.048	0.358	- 0.246	-0.118
Fasting satisfied	-0.036	-0.032	-0.030	- 0.038	-0.198
AUC satisfied	0.239	- 0.255	-0.184	0.326	0.394
Fasting fullness VAS	-0.022	0.086	0.100	0.068	-0.032
AUC fullness	0.155	0.155	-0.064	-0.088	0.318
Fasting prospective food intake	**0.441***	**-0.485***	0.339	0.119	0.132
AUC Prospective food intake	**0.497***	0.218	0.090	-0.024	0.261
AUC composite appetite score	0.269	0.023	0.331	-0.376	0.017

All significance tests were two-tailed (*$p < 0.05$) and values in table are r values.

3.3.4 SQ, SQ composite appetite score, TEI and macronutrient intake

Table 3.4 presents the relationships between SQ of VASs for 4 questions and the SQ composite appetite score from the VAS and TEI and macronutrient intake taken from the food diary. There was a positive correlation between SQ composite appetite score and percentage CHO of food diary (r=0.504*, p=0.017). SQ fullness was negatively correlated with percentage protein intake (r=-0.431*, p=0.045). Remaining SQs were not significantly correlated with TEI and macronutrient intake.

Table 3-4 : Correlation coefficients between TEI and macronutrient intake of food with SQ.

Correlation	TEI	% CHO	% Fat	% Protein	Fibre
SQ hunger	-0.055	-0.195	0.182	-0.195	0.182
SQ satisfied	-0.049	0.025	-0.245	-0.050	-0.111
SQ fullness	-0.041	0.088	0.011	**-0.431***	0.093
SQ prospective food intake	0.352	-0.289	0.134	0.037	0.323
SQ composite appetite score	-0.069	**0.504***	-0.366	-0.111	0.154

All significance tests were two-tailed (*p <0 .05) and values in table are r values.

3.3.5 TFEQ subscales, TEI and macronutrient intake

Table 3.5 shows the relationships between TFEQ subscales, TEI and macronutrient intake based on the food diary. No correlation was found between the TFEQ and to TEI. TFEQ Hunger and TFEQ Disinhibition were negatively correlated with percentage protein consumed, but this finding did not reach significance.

Table 3-5 : Correlation coefficients between TFEQ subscales, TEI and macronutrient intake

Correlation	TFEQ Hunger	TFEQ Restraint	TFEQ Disinhibition
TEI	0.122	0.041	0.068
% CHO	0.052	-0.128	0.020
% Fat	0.182	0.091	0.208
% Protein	-0.313	-0.111	-0.310
Fibre	-0.170	0.013	-0.025

Values in table are r value

3.3.6 Fasting sample and AUC of gut hormone, TEI and macronutrient intake based on food diaries

Table 3.6 shows the relationships between fasting and AUC of gut hormone levels and TEI and macronutrient intake based on food diaries. There was a positive correlation between fasting PYY and protein (r= 0.812**, p= 0.008). There was a negative correlation between the AUC for PYY, PP and GLP-1 and TEI. AUC for ghrelin was positively correlated with the TEI but this did not reach significance. Correlation between fasting samples of all hormones and TEI also did not reach significance. It was observed that there was some sample degradation on storage; repeat testing of samples, after being frozen for 2-3 weeks, gave much lower or undetectable values, indicating that samples had degraded. This affected analysis of 14 ghrelin samples, 10 GLP-1 samples, 6 PP samples and 12 PYY samples. Data from these samples was excluded from the analysis.

Table 3-6 : Correlation coefficients between fasting and AUC for gut hormones, TEI and macronutrient intake

Correlation	TEI	% CHO	% Fat	% Protein	Fibre
Fasting ghrelin	-0.178	-0.403	0.316	0.225	0.092
AUC ghrelin	0.423	-0.626	0.590	0.002	0.597
Fasting GLP-1	-0.101	-0.085	0.494	-0.104	-0.306
AUC GLP-1	-0.205	-0.202	0.508	0.408	-0.275
Fasting PP	-0.081	-0.262	0.288	0.023	-0.068
AUC PP	-0.248	0.336	0.040	-0.302	0.064
Fasting PYY	0.119	-0.432	0.088	**0.812****	0.344
AUC PYY	-0.661	0.034	0.282	-0.208	-0.531

All significance tests were two-tailed (* p<0.01) and values in table are r value

3.3.7 Food cravings, TEI and macronutrient intake

Table 3.7 represents the relationships between food cravings and TEI and macronutrient intake from the recorded food diaries. As can be observed in the table there was a correlation between food cravings and TEI and macronutrient intake. CHO craving was inversely correlated with TEI, percentage of fat and protein intake but these did not reach significance. CHO craving was positively correlated with percentage CHO intake, but this did not reach significance. Sweet craving was positively correlated with percentage of fat intake but this did not reach significance. All food cravings were negatively correlated with percentage of protein intake, but these did not reach significance.

Table 3-7 : Correlation coefficients between food cravings, TEI and macronutrient intake.

Correlation	**TEI**	**% CHO**	**% Fat**	**% Protein**	**Fibre**
Fast food craving	0.113	0.132	-0.056	-0.101	0.022
Sweet craving	0.081	0.060	0.266	-0.334	-0.100
Fat craving	-0.188	0.138	0.034	-0.241	-0.412
Carbohydrate craving	-0.286	-0.138	-0.223	-0.205	-0.091

Values in table are r values

3.3.8 Food cravings, fasting and AUC appetite

Table 3.8 shows the relationships between food cravings and appetite scores from the VAS. There was an inverse correlation between AUC for satisfied (r=-0.541**, p= 0.009) and fat craving. There was a negative correlation between fasting prospective food intake (r=-0.494*, p=0.019) and CHO craving. The AUC satisfied values and fasting prospective food intakes were negatively correlated with fasting food craving but these did not reach significance. Fasting fullness rating was positively correlated with fat craving but this did not reach significance. There was an inverse correlation between fasting satisfied and AUC fullness and sweet and CHO craving but these did not reach significance. Fasting hunger, fasting satisfied, AUC fullness were inversely correlated with CHO intake but these did not reach significance.

Table 3-8 : Correlation coefficients between food cravings, fasting and AUC appetite

Correlation	**Fast food craving**	**Sweet craving**	**Fat craving**	**Carbohydrate craving**
Fasting hunger	-0.300	0.021	-0.138	-0.268
AUC hunger	-0.155	0.039	-0.033	0.078
Fasting satisfied	-0.024	-0.341	-0.077	-0.398
AUC satisfied	-0.283	-0.213	**-0.541****	-0.204
Fasting fullness	0.217	0.077	0.235	0.147
AUC fullness	-0.212	-0.274	-0.117	-0.268
Fasting prospective food intake	-0.303	-0.187	-0.251	**-0.494***
AUC prospective food intake	-0.131	0.086	0.093	-0.049
AUC composite appetite score	-0.077	0.066	0.199	0.183

All significance tests were two-tailed (*$p < 0.05$; ** $p<0.01$) and values in table are r value

3.3.9 Food cravings and satiety quotient (SQ)

Table 3.9 presents the relationships between food cravings and SQ from the VAS and SQ composite score. As can be seen in the table no significant correlations were found between food cravings and SQ scores.

Table 3-9 : Food cravings and satiety quotient and SQ composite score.

Correlation	**Fast food craving**	**Sweet craving**	**Fat craving**	**CHO craving**
SQ hunger	-0.129	-0.004	0.040	-0.148
SQ satisfied	0.309	0.351	-0.008	0.245
SQ fullness	-0.066	0.068	-0.113	0.105
SQ prospective food intake	-0.122	-0.260	-0.305	-0.198
SQ composite appetite score	0.071	0.189	0.112	-0.085

Values in table are r value

3.3.10 Food cravings and three factor eating questionnaire subscales

Table 3.10 represents the correlation between food cravings and TFEQ. There was a positive correlation between TFEQ Hunger (r= 0.547*, p=0.010) and fast food cravings. TFEQ Disinhibition and TFEQ Restraint were also positively correlated with fast food cravings, but these did not reach significance. Moreover, TFEQ Disinhibition and TFEQ

Hunger were positively correlated (r=0.470*, p=0.032) and (r=0.628**, p=0.002) respectively with sweet cravings. However, TFEQ Restraint did not correlate with sweet cravings. TFEQ Hunger was positively correlated with fat cravings but this did not reach significance. TFEQ Restraint and TFEQ Disinhibition did not correlate with fat craving. TFEQ subscales were positively correlated with CHO craving but these did not reach significance.

Table 3-10: Correlation between food cravings and three factor eating questionnaire subscales

Correlation	TFEQ Hunger	TFEQ Restraint	TFEQ Disinhibition
Fast food craving	**0.547***	0.375	0.358
sweet craving	**0.628****	0.083	**0.470***
Fat craving	0.267	-0.070	0.182
CHO craving	0.328	0.429	0.242

All significance tests were two-tailed (*$p < 0.05$; **$p < 0.01$) and values in table are r values

3.3.11 Food cravings and gut hormones

Table 3.11 shows the correlation between food cravings and gut hormone concentrations. Fasting ghrelin and PYY levels were negatively correlated with all food cravings but these did not reach significance. AUC PYY was negatively correlated with fat, fast and carbohydrate food craving and positively with sweet cravings but these did not reach significance. There was a positive correlation between AUC for GLP-1 and fast food, sweet and fat food cravings but these did not reach significance. AUC GLP-1 did not correlate with CHO craving. AUC for ghrelin was negatively correlated with fat and fasting PP was positively correlated with fat craving but these did not correlate with fast food, sweet food and CHO food cravings. AUC for PP was positively correlated with fast food and negatively with fat food cravings and did not correlate with sweet and CHO craving.

Table 3-11 : Correlation coefficient values between food cravings and gut hormone levels.

Correlation	**Fast food craving**	**Sweet craving**	**Fat craving**	**CHO craving**
Fasting ghrelin	-0.529	-0.665	-0.707	-0.417
AUC ghrelin	0.178	0.092	-0.203	-0.195
Fasting GLP-1	0.278	0.256	0.267	-0.014
AUC GLP-1	0.326	0.276	0.242	0.077
Fasting PYY	-0.361	-0.367	-0.508	-0.441
AUC PYY	-0.260	0.256	-0.272	-0.398
Fasting PP	-0.048	0.119	0.346	-0.010
AUC PP	0.205	0.006	-0.309	0.160

3.3.12 Food preferences of participants

3.3.12.1 Dairy, meat, fish and bread preferences of participants

Table 3.12 displays the food preferences of participants. As can be seen in the table the percentage of participants that dislike dairy was 9% for milk, 4.5% cheese and 23% for yoghurt. Moreover, percentages of participants that dislike meat were 14% for beef, 18% for lamb, 9% for chicken and 28% for bacon. The percentages of participants who disliked fish was 14% for tuna fish, 9% for salmon and 28% for cod. Furthermore, the percentages of participants who disliked bread was 9% for white bread, 14% for wholemeal bread, 18% for rye bread and 23% for bread with nuts and seeds.

Table 3-12 : Food preferences of participants

Participant No	Dairy preferences			Meat preferences				Fish preferences			Bread preferences			
	Milk	Cheese	Yoghurt	Beef	Lamb	Chicken	Bacon	Tuna fish	Salmon	Cod	Whole meal	Rye bread	Bread with nuts and seeds	White bread
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✓	✓	✓	✕	✓	✓	✓	✓	✓	✓
6	✓	✓	✓	✓	✕	✓	✓	✓	✓	✓	✓	✓	✕	✓
7	✕	✕	✕	✓	✓	✓	✓	✓		✕	✓	✕	✕	✓
8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	✓	✓	✓	✓	✓	✓	✕	✕	✓	✓	✓	✓	✓	✕
10	✓	✓	✓	✓	✓	✓	✕	✓	✓	✕	✓	✓	✓	✕
12	✓	✓	✓	✕	✕	✕	✕	✓	✓		✓	✓	✓	✓
13	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
15	✓	✓	✓	✕	✕	✕	✕	✕	✕	✕	✓	✓	✓	✓
18	✓	✓	✕	✓	✓	✓	✕	✓	✓	✕	✓	✕	✕	✓
20	✓	✓	✕	✓	✓	✓	✓	✓	✓	✓	✕			✓
21	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✕	✕	✓
25	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✕	✓
26	✓	✓	✓	✓	✓	✓	✓	✕	✕	✕	✓	✓	✓	✓
27	✓	✓	✕	✓	✕	✓	✕	✓	✓	✓	✕	✕	✓	✓
28	✓	✓	✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
29	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
31	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✕	✓	✓	✓
33	✕	✓	✓	✕	✓	✓	✓	✓	✓	✕	✓	✓	✓	✓
36	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

3.3.13 Examples of food recipes in the diets provided

(See appendix 13).

3.3.14 Diet allocation

Table 3.14 presents the allocation of diets for the twenty-two participants based on diets they had attempted before.

Table 3-13 : Allocation of diet based on diets tried previously which were unsuccessful.

PN	Type of diet tried before	Diet Allocation
3	low CHO, Low fat	5:2
4	High protein diet, Atkins, caloric counting	5:2
5	Caloric counting ,5:2, slimming word	increased protein
6	None	increased protein
7	5:2	NHS
8	None	NHS
9	None	NHS
10	None	5:2
12	Caloric counting, weight watch, slimming world	increased protein
13	None	increased protein
15	high protein diet	5:2
18	None	NHS
21	All types except high protein	Increased protein
20	None	5:2
25	None	5:2
26	Caloric counting, weight watch, Slimming World	NHS
27	None	5:2
28	Slimming World, other	NHS
29	Slimming World., weight watchers, 5,2	increased protein
31	Slimming World, caloric counting	increased protein
33	Slimming World, Caloric counting	NHS
36	None	5:2

3.3.15 Follow up

Only three participants out of twenty-two adhered to the diet and completed sent food diaries after 6 weeks of starting the diet. Of these participants, one followed the 5:2 diet and one followed the NHS diet. Due to the low sample size, statistical analysis was not undertaken as the study was under-powered.

3.4 Discussion

The main aim of this study was to familiarise the researcher with the methods and test the suitability of the methods to assess satiety phenotypes and design diets for people with low satiety for future studies. The aim was also to investigate the relationship between subjective appetite sensations, TFEQ subscales, plasma gut hormone levels and food cravings and total energy and macronutrient intake. An additional aim was to investigate whether a HP diet is an effective weight-loss strategy for individuals with reduced satiety compared to other diet strategies. However, too many participants were lost to follow up and therefore the sample size was inadequately powered to undertake statistical analysis for the diet part of the study.

3.4.1 Baseline characteristics and scores of TEI and macronutrient intake based on food diaries and food craving questionnaires of participants.

Recruitment of males was difficult within this study. This is typical of weight loss studies; there is previous evidence to show that females predominantly participate in weight loss trials. Ahern *et al.,* (2017) recruited 1,267 participants, about 90% of whom were females and another study carried out in the UK by Crane, Jeffery and Sherwood (2017) included only 18.4% males. This compares reasonably well with the present study in which the proportion of males was only 9.09%.

3.4.2 Fasting sample and AUC appetite, TEI and macronutrient intake

3.4.2.1 Fasting sample and AUC of VAS and TEI

Two previous studies by Douce*t et al.,* (2003) and Drapeau *et al.,* (2007) agree with the findings of the present study. Doucet *et a*l., (2003) showed that AUC prospective food consumption was significantly correlated with reported 24 hour EI in males. Drapeau *et al*., (2007) reported a strong correlation between AUC desire to eat, and AUC for prospective food and TEI, as measured using the food diary.

3.4.2.2 Fasting and AUC of VAS and macronutrient intake of food diary

Johnstone *et al.,* (2008) reported that people who had a lower percentage of CHO in their diet had higher scores for hunger and prospective food intake using the VAS. This finding agrees with the present study that found that people who had higher VAS

scores for fasting prospective food intake consumed a lower percentage of CHO and a higher percentage of fat in their diet. Furthermore, people who had higher satisfaction scores and fullness scores consumed a higher percentage of protein and fibre in their diet.

3.4.3 SQ, SQ composite score, TEI and macronutrient intake

3.4.3.1 SQ and TEI

The previous finding by Drapeau *et al.,* (2005) carried out with a cohort of 53 individuals who were overweight and/or had obesity found that SQ fullness scores predicted daily EI. Although SQ fullness in the present study did not reach significance and the r value of SQ fullness in both studies showed an inverse relationship, indicating similar findings.

3.4.3.2 SQ, composite appetite score and macronutrient intake

Only one other study in the literature has focused on SQ and macronutrient intake. In agreement with the present study, McNeil *et al.,* (2014) studied 102 women and found that the SQ predicted the percentage of CHO intake, when measured using food diaries.

3.4.4 TFEQ subscales, TEI and macronutrient intake

3.4.4.1 TFEQ subscales and TEI

In contrast to the results of the current study, where no correlation was found, French *et al.,* (2014) reported a strong and significant correlation between TFEQ Hunger, TFEQ Disinhibition and TFEQ Restraint and TEI. The potential explanation for the contrasting findings could be the larger sample size in the French *et al.,* (2014) study, with 233 participants. Another explanation could be the statistical tests used to find relationships between TEI and TFEQ scales in French *et al.,* (2014). They used multiple regression analysis and the present study used Spearman correlation. The Spearmen correlation analyses two variables but multiple regression analyses multiple variables, which could explain the differences in findings between the studies. Moreover, another previous study has observed positive associations between hunger and EI (Provencher *et al.,* 2003) in a large study of 244 men and 352 women. They report people with chronically high levels of hunger are more susceptible to overeating compared with those who do not report being hungry.

3.4.4.2 TFEQ subscales and macronutrient intake

In contrast with the findings of the current study, De Lauzon *et al.,* (2004) observed that females with a high cognitive restraint score had a higher proportion of EI derived from protein. The possible interpretation of this different finding could be linked to age. Lauzon *et al.,* (2004) included teenagers and older adults. Teenagers have different eating behaviours and people eat less and make different food choices as they get older (Drewnowski and Shultz, 2001) which could explain the differences in findings with the current study, where age range was 18-60 years only with two teenagers.

The findings of the present study are also in contrast with Lindroos *et al.,* (1997) who showed no correlation between TFEQ Hunger and daily EI recorded using a food diary and the percentage of macronutrients (protein, CHO and fat) in a non-obese group. The possible explanation is that Lindroos *et al.,* (1997) included people of all weight statuses. It has been suggested that high BMI associates with lower restraint and higher disinhibition scores (Stunkard and Messick, 1985). With regards to gender, Lindroos *et al.,* (1997) included women and men. Restraint and disinhibition scores were higher in women compared to men (Provencher *et al.,* 2003).

3.4.5 Fasting and AUC of gut hormone, TEI and macronutrient intake

3.4.5.1 Fasting and AUC of gut hormone and TEI

The present study indicated that the fasting sample and AUC of gut hormones were not significantly correlated with TEI. This was also observed by Ratliff *et al.,* (2010) in a study which included only 21 men with an age range from 20 to 70 years. They also found that there was no correlation between macronutrient intake and PYY and GLP-1 levels in serum.

3.4.5.2 Fasting and AUC of gut hormone and macronutrient intake based on food diaries

In a study by Kong *et al.,* (2009), plasma ghrelin levels did not correlate with habitual macronutrient intake in postmenopausal women. However, they found higher habitual intake of dietary fat associated with ghrelin levels. They found a statistically significant positive association between percentage intake of saturated fat and ghrelin concentrations. The interpretation for this may be the method for measurement of macronutrient intake assessment. Kong *et al.*, (2009) used food frequency

questionnaires (FFQs) and the present study used 3 day food diary. A possible interpretation may be food diary records have revealed relationships not observed in the FFQ (Freedman *et al.,* 2006). However, most likely the difference in age of participants may account for the difference in findings with Kong *et al.* having post-menopausal women in their study, compared to the current study where most women participants were premenopausal.

People who had high scores of AUC PP consumed a higher percentage of CHO and lower percentage of protein in their diet and the remainder of fasting sample and AUC satiety hormones showed different findings. These samples had low values as a result of deterioration on storage and were excluded from the analysis. The researcher analysed these samples twice and found the sample had lower values after storage. So a higher level of protease inhibitors was added to samples for the remainder of the studies in this thesis to mitigate this issue. One of the purposes of the pilot study was to ensure robust procedures were in place for the larger studies.

3.4.6 Food cravings and TEI and macronutrient intake based on food diary records

3.4.6.1 Food cravings and TEI

The present study showed that food craving was not significantly correlated with TEI.

3.4.6.2 Food cravings and macronutrient intake of food diary

In agreement with the present study findings Chao *et al.,* (2014) found significant positive relationships between specific categories of food cravings and habitual intake of those foods. Fast food craving was associated with increased CHO in the diet. The present study reported that people who crave all food types had a lower percentage of protein in their diet. Sweet cravings were significantly associated with intake of fat measured by food diary records in the findings of Chao *et al*., (2014). These findings partially agree with the present study where people who crave sweet food had a higher percentage of fat in their diet reported by their food diary. The potential explanation for the current study may be age, as participants in Chao *et al.,* (2014) were aged between 18-50 years where, average age of the current study was 18-60 years with only two teenagers. It has confirmed differences in the types of foods craved according to age (White *et al.,* 2002).

This finding agrees with Martin *et al.,* (2008) who reported that specific food cravings were significantly correlated with consumption of corresponding types of foods.

3.4.7 Food cravings, fasting and AUC of appetite

In the present study, people who had higher scores of fasting hunger and prospective to eat, crave less CHO and fast food based on food craving questionnaires. People who had higher scores of VAS 'satisfied' had less fat craving. This finding agrees with Hopkins *et al.,* (2016) who found alterations in the physiological signals arising from the fat and CHO content of the meals may underlie the differences in satiety.

3.4.8 Food cravings and fasting and AUC hormone

Only one previous study examined the effect of gut hormones on food cravings. Chao *et al.,* (2017) reported that individuals with higher baseline total ghrelin had significantly higher food cravings at 6 months. This study, in contrast to the current study, indicates that people who had higher scores of fasting ghrelin had less food cravings for all subscales. A possible explanation for this difference may be to measure change in food craving during 6 months and their correlation with ghrelin. In Chao *et al.,* (2017) and the present study only baseline of food craving was assessed. This would give different results because appetite at baseline could change with weight loss over a long duration.

3.4.9 Food cravings, satiety quotient and SQ composite score

In the current study no significant correlation was found between food cravings and SQ and SQ composite appetite score.

3.4.10 Design and development of increased protein diet

In the current study the 30% HP diet was designed for females and males based on their food preferences. The design of a HP diet was difficult. One reason for this was that it was designed for both sexes with different grams of protein intake. Another reason high protein diet from animal sources influences health, so there is a need to vary the diet by adding protein from plants. High protein diet was designed based on food preference and it would be easier to consider food preferences before the diet is designed in future studies.

3.4.11 Follow up diet

The current study did not follow up the diet with participants. In the present study, just three people continued with their diet and completed the food diary sheets. Two allocated with the 5:2 diet and one with the NHS diet. There were no participants who followed up the HP diet contacted after 6 weeks from starting the diet. Participants had less contact for the follow up diet, for this reason, the protocol developed for the following study focused on a food diary at baseline in the community to identify individuals prior to their participation in a weight management programme, who may struggle to lose weight due to impaired satiety. In the current study, some participants did not return questionnaires when they came for the trial day. In the present study participants did not communicate with the researchers in the follow up diet. Participants were contacted by email several times to arrange a meeting to discuss their diet but they did not reply. Low follow up made it impossible to undertake statistical analysis. To mitigate this effect, the protocol was changed for the remainder of the studies to a formal setting (Tier 2 and 3) to avoid this issue with regards to lack of follow up.

3.4.12 Summary

The key findings of this chapter are that people who had higher scores on the VAS (fasting and AUC prospective food intake) ate more, according to their food diary TEI. They had a lower percentage of CHO and had a higher percentage of fat in their diet. People who had higher scores for satisfied and fullness had higher percentages of protein and fibre in their diet. In the current study, SQ did not correlate with TEI or food cravings. However, SQ composite appetite score was positively correlated with percentage CHO in food diaries and SQ full was negatively correlated with percentage protein intake. TFEQ subscale scores did not correlate with TEI in the present study but the results of macronutrient intake indicated that people who had high scores for eating behaviour, based on TFEQ subscales, had a lower percentage of protein in their diet and had more food cravings.

Plasma gut hormone results showed that fasting samples and AUC of gut hormones were not significantly correlated with TEI and people who had higher scores on the fasting ghrelin sample and AUC ghrelin had a lower percentage of CHO and higher percentage of fat in their diet. Moreover, people who had higher scores of fasting

ghrelin had less food cravings for all subscales. The findings of the present study also indicated that food craving was not significantly correlated with TEI and people who had more food cravings, had a lower percentage of protein in their diet. In the present study, people who had higher scores of fasting hunger and prospective to eat, craved less CHO and fast food.

Based on this preliminary pilot study the protocols were changed for future studies. This included:

- Changing the VAS sheets to a more relevant version
- Adding a higher concentration of protease inhibitors (Pefabloc) (DPP IV inhibitor) to prevent sample degradation
- Stopping the HP diet.
- Using only baseline assessments, due to lack of follow up in the pilot study.

3.4.13 Study strengths and limitations

This study had both strengths and limitations. The strength of the study was that it has developed a method to study the satiety phenotype of individuals to avoid any issue that may be faced in the remainder of the studies in this thesis. The limitations of the study were: the small number of participants, lack of follow up on completion and the study sample consisted of only two male participants.

Chapter 4

Measurement of VAS, gut peptide hormones and eating behaviour traits to predict individual satiety phenotypes in normal healthy students

4 Introduction

Research surrounding appetite control and satiety in relation to energy balance has recently increased substantially, in an attempt to elucidate and aid the battle against obesity. Both hunger and satiety play major roles in excessive eating (Beaulieu *et al.,* 2016). Appetite refers to the desire to fulfil a bodily need and is divided into three components: hunger, satiation and satiety (Mattes *et al., 2*005). The definition of satiety was discussed earlier in Chapter 1. Impairment in appetite control has been recognised as an essential contributor to over consumption and weight gain (Blundell and Finlayson, 2004; Dalton *et al.*, 2013) with one potential marker of susceptibility to over eating and obesity being a weakened satiety response to food (Blundell and Gillett, 2001).

Research examining individual differences in satiety responsiveness has shown that people with obesity who report no relationship between their eating behaviour and appetite sensations, exhibited a weaker satiety response during a test meal compared to people with obesity who reported that their eating behaviour was associated with their appetite sensations (Barkeling *et al.,* 2007). Interestingly, people with obesity with weak satiety responsiveness had higher scores on the TFEQ subscales of disinhibition (TFEQ-D) and hunger (TFEQ-H) compared to controls (Barkeling *et al.,* 2007), which are eating behaviour traits linked to over consumption and a higher BMI (Bryant, King and Blundell, 2008).

A weaker satiety response is not limited to people with obesity, indeed, Drapeau and colleagues identified a "low satiety phenotype" highlighting individual variability in satiety responsiveness among both individuals with obesity and normal weight individuals (Drapeau *et al.,* 2005; Drapeau *et al.,* 2013). These studies calculated the SQ to identify individuals as high or low in satiety responsiveness. A low SQ in response to a fixed-energy meal has been shown to be related to greater subsequent *ad libitum* EI, measured under both laboratory and free-living conditions (Dalton *et al.,* 2015).

When investigating appetite regulation, the most common study design employed is the single day preload study, where short-term postprandial effects of an intervention are assessed. These studies measure the effects of a fixed nutrient, food or meal (termed the 'preload') on postprandial appetite-related ratings, such as hunger and fullness, and also measure *ad libitum* food intake from one or more subsequent meals (termed the outcome 'test meal') from which the participant eats freely (Bertenshaw,

Lluch and Yeomans, 2008; Blundell *et al.,* 2010). Several studies used appetite sensations and *ad libitum* test meals to measure EI (Horner, Byrne and King, 2014). There are several methods to assess satiety and hunger in laboratory settings such as VAS, gut hormones, eating behaviour, test meal, food diary and food craving. All these methods have been described in detail in earlier chapters.

4.1 Factors affecting the food intake in laboratory settings

Measuring food intake within laboratory settings is challenging, it is important to take into account a range of factors that may significantly influence or bias the amount of energy consumed until fullness. These include environmental cues within the test room such as plate size, choice of utensils, food label information, visual cues that could stimulate appetite, including the smell or sight of food. Every effort should be made to keep these factors controlled and constant from one session to the next to ensure comparable experimental conditions across trials. Furthermore, it has been suggested that consumption within a laboratory environment may not reflect normal eating behaviour (Meiselman, 1992). One concern about laboratory based assessment of eating is that if participants understand that their intake is being observed this may affect how much food is consumed. It is suggested that when participants are directly monitored by an experimenter present in the same room, they eat less food than when a researcher is not present (Roth *et al.,* 2001). This inhibitory effect of monitoring on eating can extend to situations when the experimenter is not in the same room, but participants feel as though the experimenter will know how much food they have eaten (Polivy e*t al.,* 1986).

4.1.1 The 'low satiety phenotype'

The low satiety phenotype reflects weaker appetite sensation responses after a caloric load (Dalton *et al.,* 2015). Previous studies have studied the low satiety phenotype; Drapeau *et al.,* (2005) demonstrated that appetite sensations measured in response to a standardised breakfast test meal represented markers of overall intake. They used 1h post meal AUC and the SQ as predictors of EI.

Drapeau *et al.,* (2007) also used VAS (fasting, fullness, desire and prospective) to identify low satiety phenotype. In this case, the negative relationship between SQ for fullness and TEI was stronger for women. These results indicate that individuals

characterised with a low SQ have weaker appetite sensation responses after a meal and could be more vulnerable to overeating. These individuals represent the 'low satiety' phenotype. Dalton *et al.,* (2015) also examined SQ to identify low satiety phenotype. They found that a low SQ was associated with a greater implicit wanting for high fat foods and higher scores on the TFEQ Disinhibition subscale.

4.1.2 Aims

The present study was designed to identify a satiety phenotype in individuals**.** The hypothesis was that by measuring VAS (fasting, fullness, desire and prospective), gut hormone levels in blood, TFEQ and food craving it is possible to identify people with low and high satiety phenotype who would eat low or high EI at an *ad libitum* test meal, as well as EI from a 3 day food diary.

The aims of the present study were:

1- To investigate the relationship between VAS, *ad libitum* lunch and food diary EI.

2- To investigate the relationship between gut hormone concentrations and *ad libitum* lunch and food diary EI.

3- To investigate the relationship between the TFEQ-H subscale and *ad libitum* lunch and food diary EI.

4- To investigate the relationship between food craving and *ad libitum lunch* and food diary EI.

5- Use this data to develop a method to identify an individual's satiety phenotype.

4.2 Materials and methods

4.2.1 Participants and recruitment

Participants were aged between 18-45 years (mean ± SD 22.55 ± 5.93 years). All participants were recruited using posters and word of mouth at SHU, Sheffield, UK. Exclusion criteria were any chronic disease such as heart disease, diabetes, hypertension or conditions known to affect metabolism. Participants were excluded if they disliked or were allergic to the foods used in the study and they were also excluded if they took medication that may affect their appetite or if they were pregnant or breast feeding. The ethical approval for the study was granted by the Faculty of Health and Wellbeing Research Ethics Committee, SHU.

Study design, study setting, experimental design of the study day, anthropometry, questionnaires, test meal, food intake and laboratory methods were performed as indicated in the relevant sections in chapter 2.

Forty seven healthy students initially volunteered for the study. Of those, data is presented for forty-five participants (n=19 males, n= 26 females). Two participants were excluded from the study due to not providing blood samples (n= 1) and not consuming all the breakfast (n= 1).

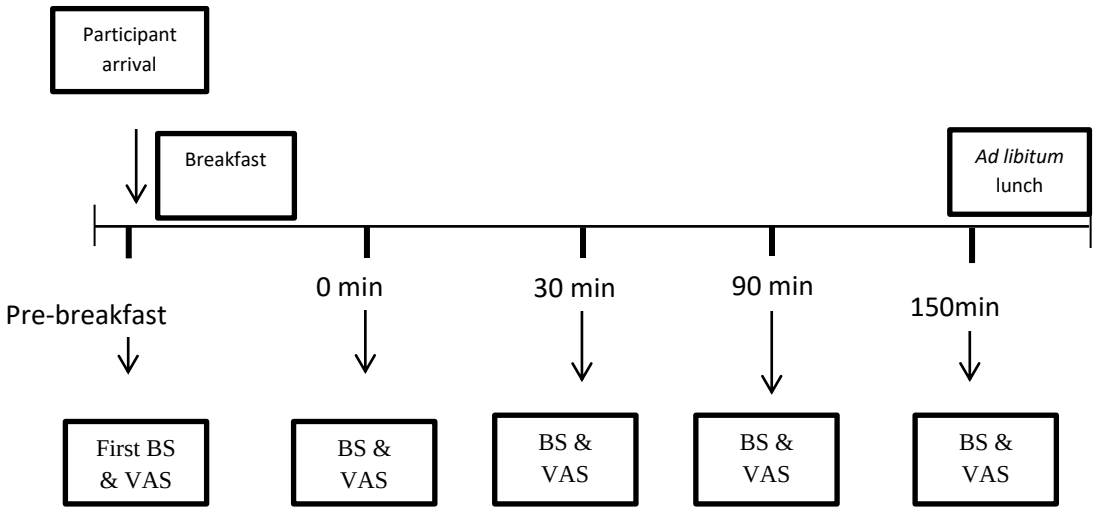

Figure 4-1 : Representation of the laboratory period of the trial days. **VAS=** visual analogue scale for all questions, **BS**=blood sample

4.2.2 Questionnaires

Three questionnaires were given to participants to complete on a separate day from the day of the laboratory experiment. These were the TFEQ, the FCI and food diaries, over 3 days as described in Chapter 2.

4.2.3 Statistical analysis

4.2.3.1 Software

All data were analysed using SPSS software version 24.0 (IBM) for Windows (SPSS Inc., Chicago, IL, U.S.A.) and Prism version 7.03 (GraphPad Soft-ware Inc.). Nutritics software (Libro, Dublin, UK) (version 1.8) was used to calculate percentage of macronutrients as well as EI from the completed food diaries.

4.2.3.2 Data analysis

Normal correlation was used to assess relationships between independent variables and *ad libitum* pasta and TEI. Independent t-tests were used to assess for any differences in sex, age, height, body mass, BMI, TFEQ scores, macronutrient intake and food craving scores. Linear regression analysis was used to establish a relationship between dependent variables, *ad libitum* pasta and total energy, measured by food diary and independent variables, the predictors such as VAS (fasting hunger, fullness, desire to eat and prospective food intake), 30 mins fullness, post breakfast desire to eat, AUC of 4 questions and SQ of 4 questions), gut hormone levels and TFEQ Hunger. AUC was calculated by using the trapezoidal method (chapter 2). One way ANOVA was used to determine whether there were any statistically significant differences between the average BMI and TFEQ subscales of low, medium and high hunger groups. Data are represented as mean ± SD, unless otherwise indicated. A p value < 0.05 was considered to be statistically significant. Cohen d=0.2 was considered a 'small' effect size, 0.5 represents a 'medium' effect size and 0.8 a 'large' effect size.

4.2.3.3 AUC and SQ calculation

A composite appetite score was calculated by summing AUC scores at individual time points. This equation was used to calculate composite appetite score (hunger score+ desire score + prospective food intake score + (100-fullness score) (Hill and Blundell, 1982).

The SQ was calculated for each appetite sensation at 60 min as described in chapter 2.

SQ composite appetite score were a summation of SQ scores at individual time points. This equation was also used to calculate SQ composite appetite score (hunger score+ desire score + prospective food intake score + (100-fullness score). AUC fasting composite appetite score, which was the summation of fasting time individual time points, (hunger score+ desire score + prospective food intake score + (100-fullness score) (Hill and Blundell, 1982) was used to calculate SQ composite appetite score.

4.2.3.4 Heat map design

A heat map was developed to model the satiety phenotype of participants. All independent variables (fasting appetite VAS, VAS 30 mins fullness, post breakfast prospective food intake, AUC (for 4 questions), AUC composite appetite score, AUC fasting appetite composite score, SQ composite score, fasting ghrelin, PP and GLP-1 levels, TFEQ-H and BMI. All these independent variables were analysed using linear regression and those that were significant and showed the most important predictors for *ad libitum* lunch and total energy of food diary were used to generate the heat maps.

The heat maps were generated by two methods to assess the optimal method.

Method one

In this method participants were spilt by gender and analysed separately. For each variable, participants were ranked in order from low to high values of the variable. The participants were then split into tertiles according to the value of the variable. Each participant was allocated a colour for that variable: i.e. green cells for low scores, orange for medium and red for high scores for variables, where a low score was associated with satiety e.g. ghrelin, or hunger and a high score associated with hunger or low satiety. For other measures e.g. fullness or PP hormones, high values were green and small values red. This process was repeated with each variable so that each participant was allocated a colour for each variable and this was used to build a heat map for each person. Participants were then determined as low, medium or high satiety phenotypes by using the equation = (R*2+O*1), R= red cells and O=orange cells. After that the heat map was reordered by *ad libitum* lunch intake and the same process was used to rank by food diary TEI.

Method two

In this method, participants were again spilt by gender. In this method participants were spilt into three groups based on values of variables taken from the literature (Carlson *et al.,* 2009; Chungchunlam *et al.,* 2015; Cummings *et al.,* 2004 Gibbons *et al.,* 2013; Lemmens *et al.,* 2011). For hormones, ranges were taken from published work, split into tertiles and used to determine the 3 groups. For VAS scores 0-33 mm were green, 34-66 mm - orange) and 67-100 mm - red for e.g. hunger. High scores for ghrelin (8.06-252 pg/ml) GLP-1 (2.36-141 pg/ml) and PP (5.46-477 pg/ml) cut off levels and VAS fullness were green and low scores red. Participants were again allocated a colour for each variable and a heat map was built. After these steps the process was the same as in method 1.

4.3 Results

4.3.1 Baseline characteristics of the study participants

The baseline characteristics of all the subjects are shown in table 4.1.

Table 4-1: Baseline characteristics of the study participants

Variables	All (n=45) Mean ± SD	Female (n=26) Mean ± SD	Male (n=19) Mean ± SD	t	p	d
Age (y)	22.5 ± 5.9	23.9 ±7.5	21± 1.1	2.190	**0.037***	0.597
Height (m)	171.1 ± 9.3	165.1 ± 6.1	179.1 ± 6.4	-7.692	**0.000***	2.262
Body mass (Kg)	70 ± 13.4	65 ± 11.7	77± 12.7	-3.347	**0.002***	0.982
BMI (Kg/m²)	23.9 ± 4.1	23.8± 4.3	24± 3.8	-0.157	0.876	0.049
TFEQ Hunger	6 ± 3.4	6.3 ± 3.1	6.3 ± 3.9	-0.104	0.918	0.030
TFEQ Restraint	7.11±5.0	8.5 ±4.9	5.30±4.7	2.225	**0.031***	0.663
TFEQ Disinhibition	6.2 ±3.1	7.0±3.2	5.1±2.6	2.204	**0.033***	0.664

Values are mean± standard deviation, n=45, t= t-test and d= Cohen's d. BMI: body mass index.
$p < 0.05$ for significant differences between males and females.

4.3.2 Food diary and food craving questionnaires

Table 4.2, presents the data from the food diary and the food craving inventory for all participants, grouped into females and males. There was a significant difference in *ad libitum* lunch intake between males and females. There was a significant difference between males and females in total energy, protein (g) and fat (g) intake, measured by the food diary and also a difference in CHO craving. Females craved more CHO than males. However, no differences were found between the percentage of total energy of protein, CHO and fat for females and males. Moreover, there was no significant difference in fibre intake and there was no significant relationship between sweet, fast food and high fat food cravings in females and males.

Table 4-2: Food diary scores, *ad libitum* lunch intake and food craving inventory for participants

Variables	All (n=45) Mean ±SD	Females (n= 26) Mean ± SD	Males (n= 19) Mean ± SD	t	p	d
TEI (kcal)	1738.8 ± 672	1532.3 ± 470.9	2007.3±802.2	-2.513	**0.016***	0.722
Food diary-CHO (g)	204.8 ± 89.6	192.5 ± 80.9	220.9 ± 99.7	-1.066	0.292	0.3127
(% of TEI)	46±7.7	47.1±7.4	44.0 ±7.9	1.282	0.208	0.398
Food diary-Protein (g)	71.1 ± 30.6	59.6 ± 16.6	86.2 ± 37.8	-3.206	**0.003***	0.908
(% of TEI)	17.7±4.4	16.7±3.4	18.8±5.3	-1.319	0.195	0.473
Food diary-Fat (g)	62.5 ± 26.5	52.5 ± 17.3	75.6± 30.9	-3.217	**0.002***	0.922
(% of TEI)	31.9±8.4	31±10.3	33.4±6.2	-1.086	0.285	0.281
Food diary-Fibre (g)	16.5± 8.8	14.91 ± 5.3	18.5 ± 11.8	-1.389	0.172	0.395
***Ad libitum* lunch intake (kcal)**	620.7 ± 245.1	551.6 ± 194.4	714.0±278.9	-2.355	**0.023***	0.676
Fast food craving	8.3 ± 3.7	8.2 ± 3	8.31 ± 4.2	-0.102	0.920	0.042
Sweet craving	10.1 ± 4.6	11.6 ± 4.9	9.1 ± 4.2	1.468	0.154	0.554
Fat craving	5.6 ± 4.2	5.2 ± 3.8	5.9 ± 4.5	-0.440	0.663	0.171
Carbohydrate craving	7.4 ± 4.8	9.5 ± 4.2	5.9 ± 4.8	2.085	**0.047***	0.806

Values are mean ± standard deviation, n=45, t= t-test and d= Cohen's d. BMI: body mass index. $p < 0.05$ for the differences between males and females. TEI= Total energy intake and nutrients estimated from 3-day food records. g= gram, kcal= kilocalories

4.3.3 VAS measurements versus time during the test morning

Figure 4.2 shows VAS measurements versus time for female and male participants. There were significant differences in VAS hunger (p=0.047), fullness (p=0.017), and prospective food intake (p=0.006) scores between females and males. However, no significant difference was found between females and males in VAS desire to eat (p=0.251) during the test morning.

4.3.4 Plasma gut hormone concentrations versus time during the test morning

Figure 4.3 shows there was no significant difference in ghrelin (p=0.608), GLP-1 (p=0.385) PP (p=0.536) and PYY (p=0.407) hormone concentrations between females and males.

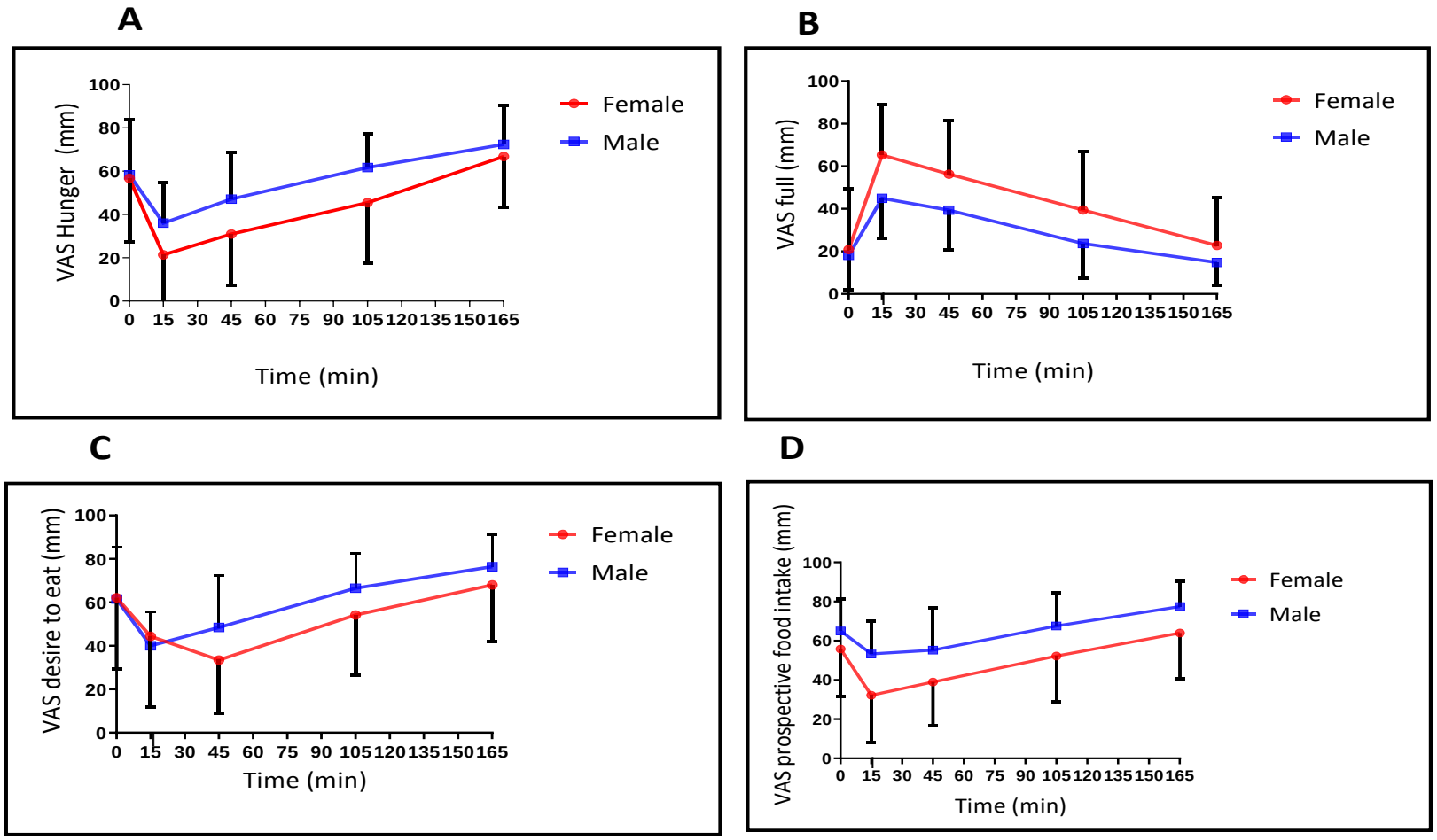

Figure 4-2 VAS scores: hunger (A), fullness (B), desire to eat (C) and prospective food intake (D) for participants versus time, during the test morning.

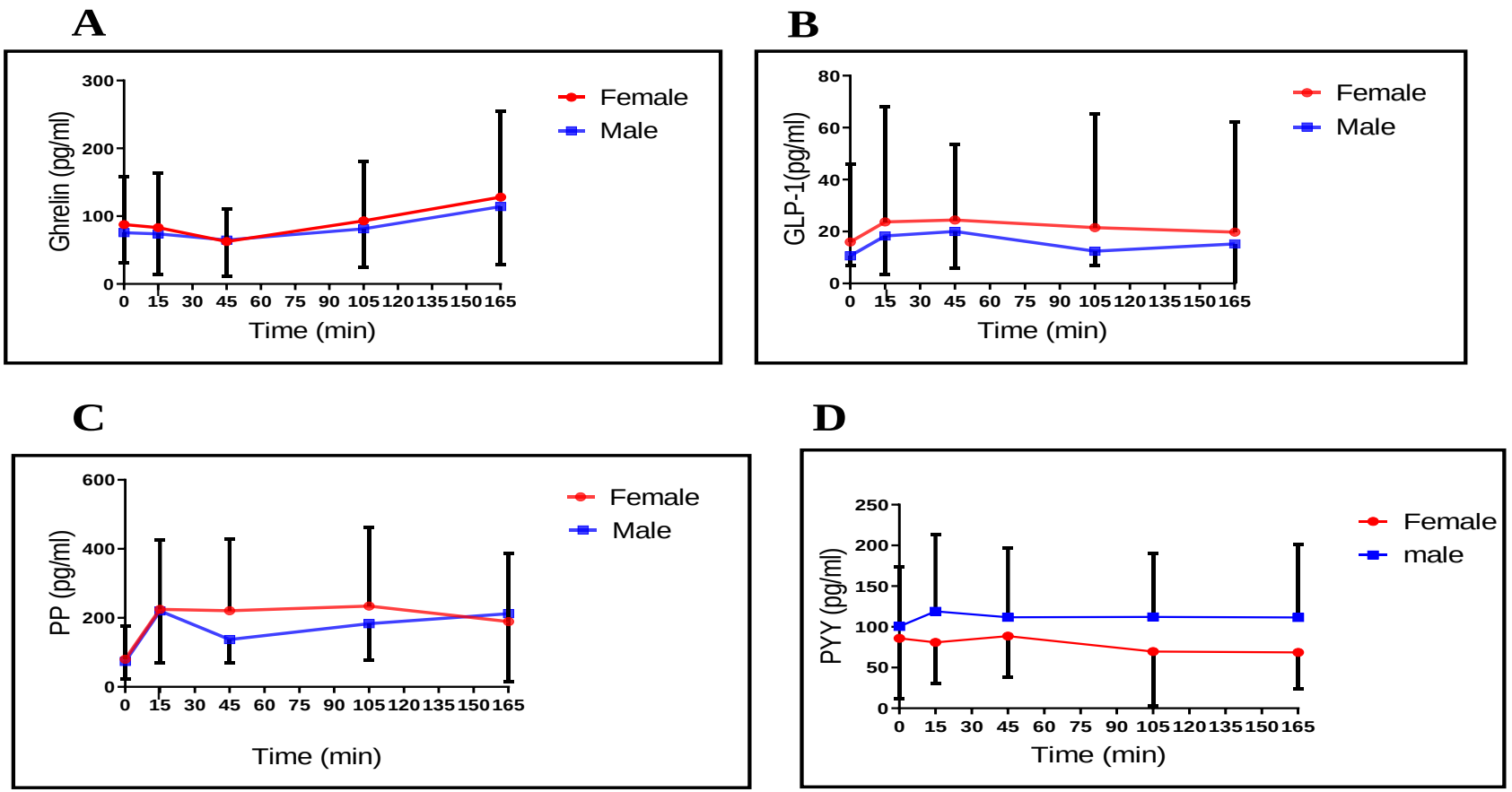

Figure 4-3: Concentration of plasma hormones (ghrelin (A), GLP-1(B), PP (C) and PYY (D) for participants versus time during the test morning.

4.3.5 VAS, TFEQs, gut hormones, food cravings and TEI and macronutrient intake.

4.3.5.1 Relationships between VAS (fasting scores and AUC fasting composite appetite score) and TEI and macronutrient intake

Tables 4.3 and 4.4 present fasting scores for VAS and TEI and macronutrient intake as measured using the food diaries. The fasting prospective food intake score was positively correlated (r=0.505**, p=0.009) with TEI of females. AUC fasting composite was positively correlated (r=0.396*, p=0.045) with TEI of females. In males, fasting hunger (r=-0.570*, p=0.014), fasting desire to eat (r=-0.478*, p=0.045) and fasting prospective food intake (r=-0.551*, p=0.018) were negatively correlated with percentage fat intake, as measured by the food diary. AUC fasting composite was negatively correlated with percentage of fat but not significantly.

The data in table 4.3 also shows that in women, all VAS measures correlate with EI reported in the food diary, but this effect was not as strong in men. Also strong relationships between fat intake and all VAS scores in both men and women, and men who eat more CHO are hungrier. There was a negative correlation between VAS fasting hunger (r= -0.586*, p=0.011) and fat measured by the food diary.

After adjusting for BMI the correlation remained between VAS fasting, prospective food intake and TEI in females (r= 0.505*, p=0.010), AUC fasting composite (r=-0.606*) (p=0.010) and TEI of females and, after adjusting for age, the correlation also remained between VAS fasting prospective food intake and TEI (r=0.499*, p=0.011) in females and between AUC fasting composite (r=-0.528*, p=0.029) and TEI. For males after adjusting for age the correlation remained between fasting hunger and percentage fat intake (r=-0.534*), p=0.027).

Table 4-3: The correlations of fasting and AUC fasting composite appetite score of VAS and TEI and macronutrient intake.

Female	**Fasting hunger**	**Fasting fullness**	**Fasting desire to eat**	**Fasting prospective food intake**	**AUC fasting composite appetite score**
TEI (kcal)	0.295	-0.272	0.373	**0.505****	**0.396***
Male	**Fasting hunger** **Fasting fullness** **Fasting desire to eat** **Fasting prospective food intake** **AUC fasting composite appetite score**	**Fasting fullness** **Fasting desire to eat** **Fasting prospective food intake** **AUC fasting composite appetite score**	**Fasting desire to eat**	**Fasting prospective food intake**	**AUC fasting composite appetite score**
TEI (kcal)	0.156	-0.047	0.208	0.082	-0.177

Correlations of primary variables for females and males. All significance tests were two-tailed (*p <0 .05; **p < 0.01).

Table 4-4: The correlations of fasting and AUC fasting composite appetite score of VAS and macronutrient intake

Female	**Fasting hunger**	**Fasting fullness**	**Fasting desire to eat**	**Fasting prospective food intake**	**AUC fasting composite appetite score**
% CHO	-0.039	-0.122	-0.072	-0.174	-0.035
% Protein	0.041	0.225	-0.039	-0.035	-0.075
% Fat	**-0.586***	-0.402	**-0.402**	**0-402**	0-.402
Fibre (g)	0.218	-0.121	0.269	0.313	0.005
Male	**Fasting hunger** **Fasting fullness** **Fasting desire to eat** **Fasting prospective food intake**	**Fasting Fullness** **Fasting desire to eat** **Fasting prospective food intake**	**Fasting desire to eat**	**Fasting prospective food intake**	**AUC fasting composite appetite score**
% CHO	0.206	0.097	0.213	0.335	0.197
% Protein	-0.266	-0.034	-0.181	-0.040	0.013
% Fat	**-0.570***	0.166	**-0.478***	**-.0551***	**-0.525***
Fibre (g)	.086	0.191	0.034	0.259	0.222

Correlations of primary variables for females and males. All significance tests were two-tailed (*p <0 .05; **p < 0.01).

4.3.5.2 Post-breakfast VAS scores and TEI and macronutrient intake

Tables 4.5 and 4.6 show post breakfast VAS scores and TEI and macronutrient intake. There was a positive correlation between VAS post-breakfast hunger (r= 0.412*, p= 0.037) and TEI in females. Whereas, in males there were a negative correlation between VAS post-breakfast desire to eat scores (r=-.499*, p=0.035) and VAS post breakfast prospective food intake (r=-0.500*) (p=0.035) percentage of fat intake. Moreover, there was a negative correlation between prospective food intake (r=-0.556*, p=0.17) and percentage CHO in food diary. The data in this table also shows that in women and men VAS measures correlate with EI reported in the food diary but these did not reach significance. Also, there were strong relationships between fat intake and all VAS scores in women and men who eat more CHO are hungrier.

After adjusting for BMI, the correlation remained between VAS post-breakfast hunger (r= 0.417*, p= 0.038) and TEI in females, after adjusting for age the correlation remained between VAS post-breakfast hunger and TEI in females (r= 0.407*, p= 0.043). After adjusting for BMI, the correlation remained between VAS post-breakfast desire to eat and VAS post-breakfast prospective food intake (r=-0.516*, p=0.034), percentage of fat intake (r=0.498*, p=0.042) in males after adjusting for age, the correlation also remained (r=-0.505*, p=0.039), (r=-0.496*, p=0.043). After adjusting for BMI, the correlation remained between VAS post breakfast and post-breakfast prospective food intake (r=-0.575*, p=0.20), after adjusting for age the correlation also remained between VAS post-breakfast post breakfast prospective food intake (r=-0.570*, p=0.17).

Table 4-5 : The correlations of VAS post-breakfast and TEI.

Female	**Post-breakfast hunger**	**Post-breakfast fullness**	**Post- breakfast desire to eat**	**Post- breakfast prospective food intake**
TEI (kcal)	**0.412***	-0.306	0.186	0.337
Male	**Post-breakfast hunger**	**Post-breakfast fullness**	**Post- breakfast desire to eat**	**Post- breakfast prospective food intake**
TEI (kcal)	.0124	0.037	-0.034	-0.328

Correlations (r values) of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p < 0.01).

Table 4-6: The correlations of VAS post-breakfast and TEI and macronutrient intake

Female	Post-breakfast hunger	Post-breakfast fullness	Post-breakfast desire to eat	Post-breakfast prospective food intake
% CHO	0.118	-0.007	0.168	0.143
% Protein	0.279	-0.232	0.146	0.211
% Fat	-0.248	-0.080	-0.038	-0.032
Fibre (g)	0.122	-0.223	0.261	0.279
Male	**Post-breakfast hunger**	**Post-breakfast fullness**	**Post-breakfast desire to eat**	**Post-breakfast prospective food intake**
% CHO	0.152	0.014	0.461	**0.556***
% Protein	0.413	0.111	-0. 103	0. 176
% Fat	-0.303	0.168	**-0.499***	**-0.500***
Fibre (g)	**-0.491***	0.159	0.251	**0.537***

Correlations of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p < 0.01).

4.3.5.3 Thirty minute VAS scores and TEI and macronutrient intake

Tables 4.7 and 4.8 represent the relationship between thirty minute VAS scores and TEI and macronutrient intake. As can be observed, there was a positive correlation between VAS at 30 min hunger (r=0 .390*, p= 0.049) and VAS 30 min desire to eat (r= 0.536**, p= 0.005) and TEI in females. In addition, VAS 30 min fullness was negatively correlated (r=-0.480*, p=0.013) with TEI in females. Whereas, for males the VAS at 30 min fullness rating was negatively correlated with TEI and VAS 30 min prospective food intake was positively correlated with percentage of CHO intake but these did not reach significance.

After adjusting for BMI, the correlation remained between desire to eat (r= 0.535*, p= 0.006) and VAS 30 min fullness (r=-0.481*, p=0.015) TEI in females. Moreover, after adjusting for age the correlation remained between VAS 30 min desire to eat (r= 0.541*, p= 0.005) and VAS 30 min fullness (r=-0.483*, p=0.014) TEI in females.

Table 4-7: The correlations of VAS 30 min and TEI.

Female	30 min hunger	30 min fullness	30 min desire to eat	30 min prospective food intake
TEI (kcal)	**0.390***	**-0.480***	**0.536****	0.345
Male	Fasting hunger Fasting fullness, Fasting desire to eat Fasting prospective food intake AUC fasting composite appetite score	Fasting fullness Fasting desire to eat Fasting prospective food intake	Fasting desire to eat	Fasting prospective food intake
TEI (kcal)	-0.229	-0.291	-0.020	-0.147

Correlations of primary variables for females and males. All significance tests were two-tailed (*$p < 0.05$; **p <0 .01).

Table 4-8: The correlations of VAS 30 min visual analogue scale and TEI and macronutrient intake.

Female	30 min hunger	30 min fullness	30 min desire to eat	30 min prospective food intake
% CHO	-0.065	-0.005	0.005	0.143
% Protein	0.053	0.135	0.016	0.010
% Fat	-0.155	0.177	-0.210	-0.143
Fibre (g)	0.029	-0.173	0.241	0.090
Male	**30 min hunger**	**30 min fullness**	**30 min desire to eat**	**30 min prospective food intake**
% CHO	0.330	0.045	0.198	0.401
% Protein	0.265	0.246	0. 255	0.264
% Fat	-0.392	-0.149	-0.284	-0.391
Fibre (g)	0.141	0.322	-0.032	0.122

Correlations of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p <0 .01).

4.3.5.4 AUC hunger, fullness, desire to eat, prospective food intake, composite appetite score and TEI and macronutrient intake

Tables 4.9 and 4.10 show AUC hunger, fullness, desire to eat, AUC prospective food intake, composite appetite score and TEI and macronutrient intake. There was a positive correlation between AUC desire to eat (r=0.601**, p=0.001) and AUC prospective food intake (r= 0.417*, p=0.034) and TEI. Moreover, AUC composite appetite score (r= 0.510**, p=0.008) was positively correlated with TEI of females. In males, the only significant relationship was between AUC composite of appetite score, which was inversely correlated (r=-0.525*, p=0.025) with percentage fat intake.

After adjusting for BMI, the correlation remained significant between AUC composite of appetite score (r=0.513*, p=0.009) and TEI, AUC desire and TEI (r=0.607, p=0.001) and AUC prospective food intake (r= 0.417*, p=0.038) and TEI in females, after adjusting for age, the correlation also remained between AUC prospective food intake (r= 0.430*, p=0.030) and TEI, AUC composite of appetite score (r=0.540**, p=0.005) and TEI in females. After adjusting for BMI, the correlation also remained between AUC composite of appetite score (r=0.513**, p=0.009) and percentage fat intake.

Table 4-9: Correlations between AUC hunger, AUC fullness, desire to eat, AUC prospective food intake, AUC composite of appetite score and TEI.

Female	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite appetite score**
TEI kcal)	-0.265	-0.388	**0.601****	**0.417***	**0.510****
Male	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite appetite score**
TEI (kcal)	0.002	-0.053	-0.028	-0.201	-0.240

Correlations of primary variables for females and males. All significance tests were two-tailed (*$p < 0.05$; **$p < 0.01$).

Table 4-10: Correlations between AUC hunger, AUC fullness, desire to eat, AUC prospective food intake, AUC composite of appetite score and macronutrient intake.

Female	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite appetite score**
% CHO	0.090	0.006	0.010	0.038	0.030
% Protein	-0.006	-0.005	-0.071	-0.056	-0.137
% Fat	-0.324	0.217	-0.306	-0.444	-0.273
Fibre (g)	-0.315	-0.192	0.334	0.161	0.255
Male	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite appetite score**
% CHO	0.195	0.124	0.063	0.366	0.356
% Protein	0.276	0.046	0. 297	0.266	-0.040
% Fat	-0.267	-0.076	-0.274	-0.348	**-0.525***
Fibre (g)	0.360	0.174	-0.051	0.168	0.307

Correlations of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p < 0.01).

4.3.5.5 SQ of VAS (4 questions), SQ composite score, TEI and macronutrient intake

Tables 4.11 and 4.12 show SQ of VAS for hunger, fullness, desire to eat and prospective food intake and TEI and macronutrient intake. No significant correlation was observed between SQ of VAS and TEI and macronutrient intake.

Table 4-11: Correlation between SQ of VAS and TEI.

Female	SQ hunger	SQ fullness	SQ desire to eat	SQ prospective food intake	SQ composite appetite score
TEI (kcal)	-0.045	0.192	-0.046	0.195	-0.033
Male	**SQ hunger**	**SQ fullness**	**SQ desire to eat**	**SQ prospective food intake**	**SQ composite appetite score**
TEI (kcal)	0.302	0.146	0.258	0.322	-0.014

Correlations of primary variables for females and males. All significance tests were two-tailed (*$p < 0.05$; **p <0 .01).

Table 4-12: Correlation between SQ of VAS and macronutrient intake.

Female	SQ hunger	SQ fullness	SQ desire to eat	SQ prospective food intake	SQ composite appetite score
% CHO	-0.024	-0.120	-0.080	-0.294	-0.053
% Protein	.019	.099	-0.055	-0.042	-0.056
% Fat	-0.256	0.267	-0.222	-0.060	-0.243
Fibre (g)	0.331	0.047	0.090	0.664	0.136
Male	**SQ hunger**	**SQ fullness**	**SQ desire to eat**	**SQ prospective food intake**	**SQ composite appetite score**
% CHO	-0.100	0.031	0.012	-0.230	-0.100
% Protein	-.459	-0.112	-0.446	-0.126	-0.201
% Fat	-0.317	0.234	-0.232	-0.016	-0.275
Fibre (g)	0.032	-0.210	0.059	-0.423	-0.184

Correlations of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p <0 .01).

4.3.5.6 Subscales of TFEQ (Hunger, Restraint and Disinhibition) and TEI and macronutrient intake

Table 4.13 and 4.14 present data of TFEQ subscales and TEI and macronutrient intake. There was no significant correlation between TFEQ Hunger and TFEQ-Disinhibition scores and TEI, or macronutrient intake recorded in the food diaries of both females and males. However, there was a significant negative correlation between TFEQ Restraint (r=-0.664**, p=0.003) and percentage fat intake in females. Moreover, TFEQ Restraint was negatively correlated (r=0-.445*, p= 0.049) with TEI in males.

After adjusting for BMI, the correlation remained between TFEQ Restraint (r=-0.665**, p=0.004) and percentage fat intake in females. After adjusting for age, the correlation remained between TFEQ Restraint (r=-0.673**, p=0.003) and percentage fat intake in females. After adjusting for BMI, the correlation remained between TFEQ-Restraint (r=-0.461*, p=0.047) and TEI in males.

Table 4-13: Relationship between subscales of TFEQ and TEI.

Female	TFEQ Hunger	TFEQ Restraint	TFEQ Disinhibition
TEI (kcal)	0.256	-0.140	0.066
Male	TFEQ Hunger	TFEQ Restraint	TFEQ Disinhibition
TEI (kcal)	-0.220	**-0.445***	-0.163

Correlations of primary variables for females and males. All significance tests were two-tailed (*p <0 .05; **p < 0.01).

Table 4-14: Relationship between subscales of TFEQ and macronutrient intake of food diary.

Female	**TFEQ Hunger**	**TFEQ Restraint**	**TFEQ Disinhibition**
% CHO	0.193	-0.131	0.069
% Protein	-0.217	0.276	-0.248
% Fat	-0.022	**-0.664****	-0.175
Fibre (g)	-0.042	-0.086	0.024
Male	**TFEQ Hunger**	**TFEQ Restraint**	**TFEQ Disinhibition**
% CHO	0.088	0.083	-0.084
% Protein	-0.210	0.366	-0.119
% Fat	0.011	-0.175	0.239
Fibre (g)	-0.408	-0.199	-0.277

Correlations of primary variables for females and males. All significance tests were two-tailed (*p <0 .05; **p < 0.01).

4.3.5.7 Fasting gut hormones and AUC hormones and TEI and macronutrient intake

Tables 4.15 and 4.16 display fasting gut hormone concentrations, AUC of hormones and TEI and macronutrient intake. There was a negative correlation between fasting levels of PYY (r= -0.854**, p=0.007) and AUC PYY (r=-0.933**, p=0.007) and TEI. Moreover, AUC ghrelin was inversely correlated (r=-0.440*, p=0.024) with fibre intake in females. However, all fasting gut hormones and AUC PP and AUC GLP-1 showed no significant correlation with macronutrient intake in females. There was a negative correlation between fasting PP hormone and TEI (r=-0.467*, p=0.038) and fasting ghrelin was negatively correlated (r=-0.651**, p=0.003) with percentage protein intake in males. AUC GLP-1 showed a positive correlation with percentage fat intake in males (r=0.579*, p=0.012). Some of the samples for PPY measurement were missed and for this reason it was not included in the regression model.

After adjusting for BMI, the correlation remained between AUC ghrelin (r=-0.422*, p=.036) and fibre intake in females and fasting PP hormone (r=-0.490*) (p=0.033) and TEI. After adjusting for BMI, correlation between fasting ghrelin and protein (r=-0.641*, p=0.006), and AUC GLP-1 and % fat intake (r=0.561*, p=0.019) remained. After adjusting for age, the correlation remained between AUC ghrelin and fibre (r=-0.576*, p=0.003) fasting ghrelin and percentage protein intake (r=-0.652*, p=0.005) in males. After adjusting for age, the correlation remained between fasting PP hormone and TEI (r=-0.471*, p=0.042). AUC GLP-1 (r=0.593*, p= 0.012) and percentage fat intake in males.

Table 4-15: Correlation between fasting hormones and AUC hormones and TEI

Female	**Fasting PP**	**Fasting GLP-1**	**Fasting PPY**	**Fasting ghrelin**	**AUC PP**	**AUC GLP-1**	**AUC PYY**	**AUC ghrelin**
TEI (kcal)	-0.291	-0.275	**-0.854****	-0.041	-0.060	-0.142	**-0.933****	-0.192
Male	**Fasting PP**	**Fasting GLP-1**	**Fasting PPY**	**Fasting ghrelin**	**AUC PP**	**AUC GLP-1**	**AUC PYY**	**AUC ghrelin**
TEI (kcal)	**-0.467***	0.115	-0.228	0.338	-0.225	-0.039	-0.319	0.379

Correlations of primary variables among females and males. All significance tests were two-tailed (*p <0 .05; **p < 0.01).

Table 4-16: Correlation between fasting hormones and AUC hormones and total energy intake and macronutrient intake.

Female	Fasting PP	Fasting GLP-1	Fasting PPY	Fasting ghrelin	AUC PP	AUC GLP-1	AUC PYY	AUC ghrelin
% CHO	-0.269	0.067	0.036	-0.435	-0.236	-0.256	-0.004	-0.342
% Protein	0.325	0.024	0.233	0.039	0.331	-0.164	0.343	0.097
% Fat	-0.039	-0.225	-0.161	-0.041	0.014	-0.105	-0.107	0.089
Fibre (g)	-0.273	-0.272	0.139	-0.385	-0.107	-0.233	0.090	**-0.440***
Male	**Fasting PP**	**Fasting GLP-1**	**Fasting PPY**	**Fasting ghrelin**	**AUC PP**	**AUC GLP-1**	**AUC PYY**	**AUC ghrelin**
% CHO	-0.041	-0.257	0.524	-0.035	0.190	-0.328	0.507	-.099
% Protein	0.375	0.331	-0.466	**-0.651****	-0.062	-0.072	-0.467	-0.455
% Fat	0.209	0.481	0.162	-0.014	-0.252	**0.579***	-0.243	-0.058
Fibre (g)	-0.371	0.166	-0.331	0.312	-0.057	-0.092	0.023	0.189

Correlations of primary variables among females and males. All significance tests were two-tailed (*p <0 .05; **p < 0.01).

4.3.5.8 Food cravings and TEI and macronutrient intake

Tables 4.17 and 4.18 show data for food cravings and total energy and macronutrient intake. There was a correlation between food craving scores and TEI and macronutrient intake but these did not reach to significance in females. Whereas, there was a strong negative correlation between fast food cravings (r=-0.802**, p=0.005) and TEI in males. Moreover, sweet food craving was positively correlated (r=0.651**, p=0.006) with protein intake.

After adjusting for BMI, the correlation remained between fast food cravings and TEI (r= -0.785*) (p=0.001), sweet food cravings (r=0.630*, p=0.012) and protein intake and fast food cravings (r=-0.803**, p=0.000) and fibre intake. In males moreover, after adjusting for age, the correlation remained strong between fast food cravings and TEI (r=-0.833**, p=0.000), sweet food cravings (r=0.641*, p=0.010) and protein intake in males.

Table 4-17: Correlation between food craving and TEI.

Female	Fast food craving	Sweet craving	Fat food craving	CHO craving
TEI (kcal)	0.407	0.410	-0.256	0.146
Male	**Fast food craving**	**Sweet craving**	**Fat food craving**	**CHO craving**
TEI (kcal)	**-0.802****	-0.468	-0.248	-0.475

Correlations of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p <0 .01).

Table 4-18: Correlation between food craving and macronutrient intake.

Female	Fast food craving	Sweet craving	Fat food craving	CHO craving
% CHO	-0.060	-0.137	-0.008	0.149
% Protein	-0.349	-0.231	0.139	-0.076
% Fat	-0.302	-0.143	-0.337	-0.086
Fibre (g)	.384	-.241	-.171	.543
Male	**Fast food craving**	**Sweet craving**	**Fat food craving**	**CHO craving**
% CHO	-0.405	-0.005	-0.202	-0.072
% Protein	-0.274	**0.651****	0.047	0.075
% Fat	0.352	0.028	0.221	0.178
Fibre (g)	-0.293	0.027	-0.096	-0.065

Correlations of primary variables for females and males. All significance tests were two-tailed (*p < 0.05; **p <0 .01).

4.3.6 VAS, TFEQ, gut hormones and *ad libitum* lunch intake

4.3.6.1 Relationships between fasting and AUC fasting and *ad libitum* lunch intake

Figure 4.4 presents fasting and AUC fasting VAS scores and *ad libitum* lunch intake. There was a positive correlation between VAS fasting hunger (r=0.400*, p=0.042) and *ad libitum* lunch intake in females. However, there were no significant correlations between VAS fasting fullness, desire to eat and prospective food intake and ad *libitum* lunch intake in males there were no significant correlations between fasting VAS scores and *ad libitum* lunch intake.

After adjusting for BMI, the correlation remained between VAS fasting hunger (r=0.440*, p=0.013) and *ad libitum* lunch intake in females. However, after adjusting for age there was no correlation between VAS fasting hunger (r=.385*, p=0.058) and *ad libitum* lunch intake in females.

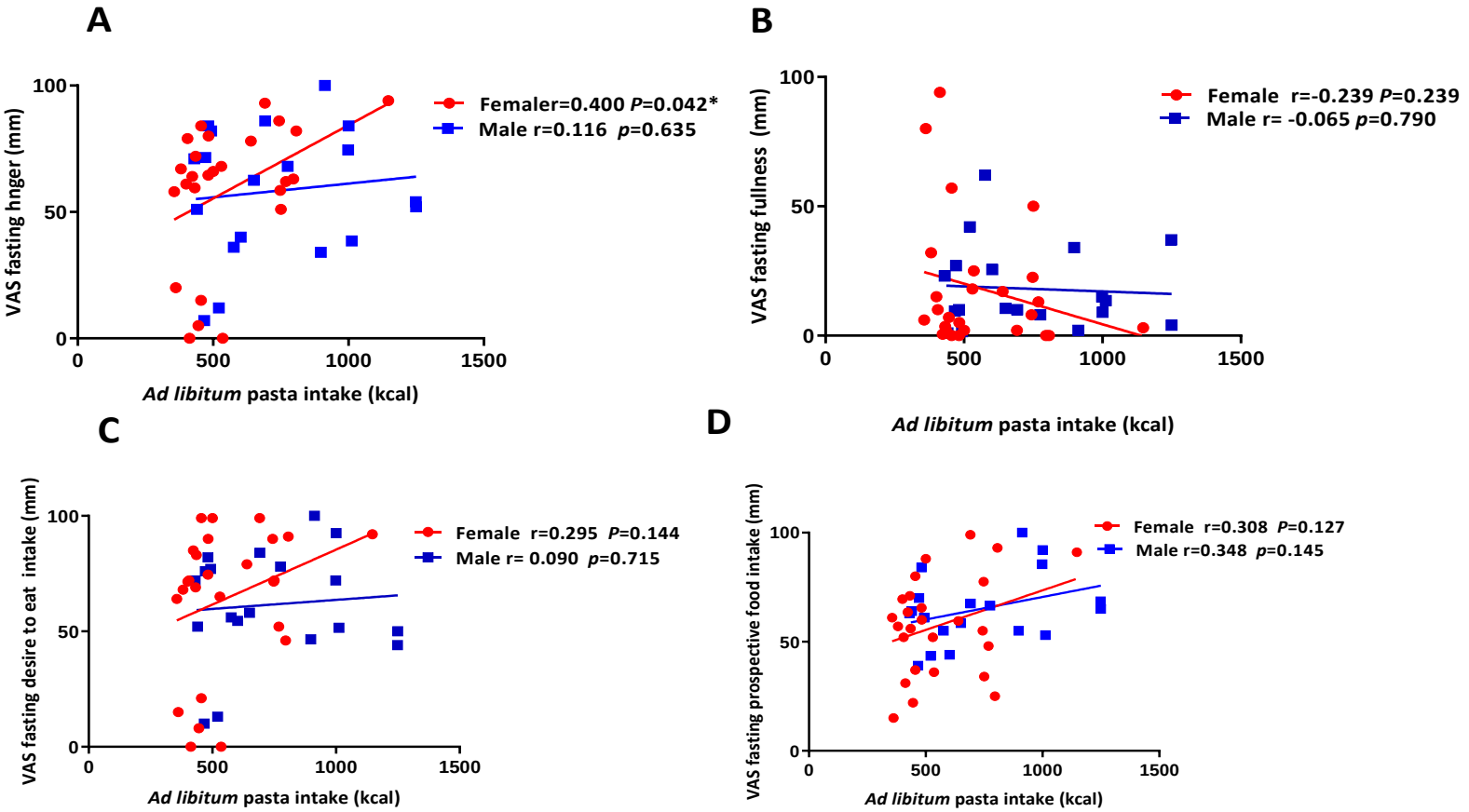

Figure 4-4 : VAS fasting and AUC fasting scores and *ad libitum* lunch intake (kcal) scores for females and males (A= VAS fasting hunger, B= VAS fasting fullness, C= VAS fasting desire to eat and D=VAS fasting prospective food intake)

4.3.6.2 Post-breakfast results of VAS appetite scale and *ad libitum* lunch intake

Figure 4.5 displays the post-breakfast VAS measurement and *ad libitum* lunch intake. As can be seen VAS post-breakfast hunger (r=0.536**, p=0.005) and VAS post-breakfast prospective food intake (r=0.494*, p=0.010) were positively correlated with *ad libitum* lunch intake in females. Whereas, VAS post-breakfast fullness was negatively correlated (r=-0.446*, p=0.022) with *ad libitum* pasta intake in females. However, post-breakfast desire to eat was correlated with *ad libitum* pasta intake in females but did not reach significance. There was a positive correlation between post-breakfast hunger, fullness, desire to eat and prospective food intake and *ad libitum* lunch intake in males, but these did not reach significance.

After adjusting for BMI, the correlation remained between VAS post-breakfast hunger (r=0.536*, p=0.006), VAS post-breakfast prospective food intake (r=0.496*, p= 0.012) and VAS post breakfast fullness (r=0-.445*, p= 0.026) and *ad libitum* lunch intake in females. After adjusting for age the correlation remained between VAS post-breakfast hunger (r=0.532*, p=0.006), VAS post-breakfast prospective food intake (r=-.510**, p= 0.009) and VAS post-breakfast fullness (r=0.436*, p= 0.030) and *ad libitum* lunch intake in females.

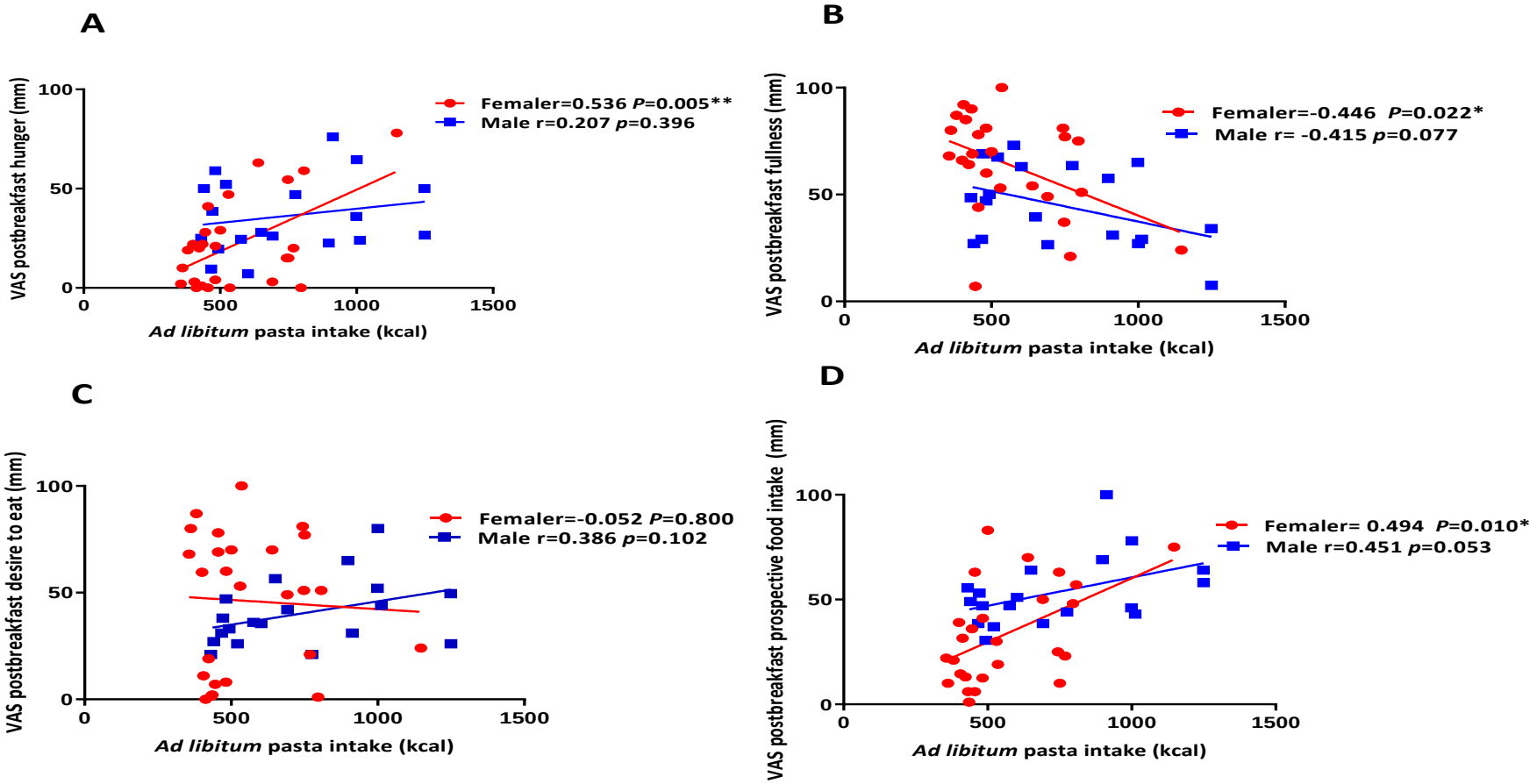

Figure 4-5: The relationship of VAS post-breakfast scores and *Ad libitum* lunch intake (kcal) scores for females and males (A= VAS post breakfast hunger, B=VAS post breakfast fullness, C= VAS post breakfast desire to eat and D=VAS post breakfast prospective food intake)

4.3.6.3 Thirty-minute VAS and *ad libitum* lunch intake

Figure 4.6 represents thirty-minute VAS scores and *ad libitum* lunch intake. As can be seen there was a positive correlation between VAS 30 min prospective food intake (r=0.443**, p=0.023) and *ad libitum* lunch intake in females. However, VAS 30 min hunger, fullness and desire to eat were not significantly correlated with *ad libitum* lunch intake in females. In males, VAS 30 min prospective food intake was positively correlated with *ad libitum* lunch intake (r=0.493*, p=0.032). However, there was no significant correlation between VAS 30 min hunger, fullness and desire and *ad libitum* lunch intake.

After adjusting for BMI, the correlation remained between VAS 30 min prospective food intake (r=0.452*, p=0.023) and *ad libitum* lunch intake in females. Moreover, after adjusting for age the correlation remained between VAS 30 mins prospective food intake (r=0.452*, p=0.023) and *ad libitum* lunch intake in females. After adjusting for BMI, the correlation remained between VAS 30 mins prospective food intake (r=0.588*, p=0.010) and *ad libitum* lunch intake in males. Moreover, after adjusting for age the correlation remained between VAS 30 min prospective food intake (r=0.494*, p=0.037) and *ad libitum* lunch intake in males.

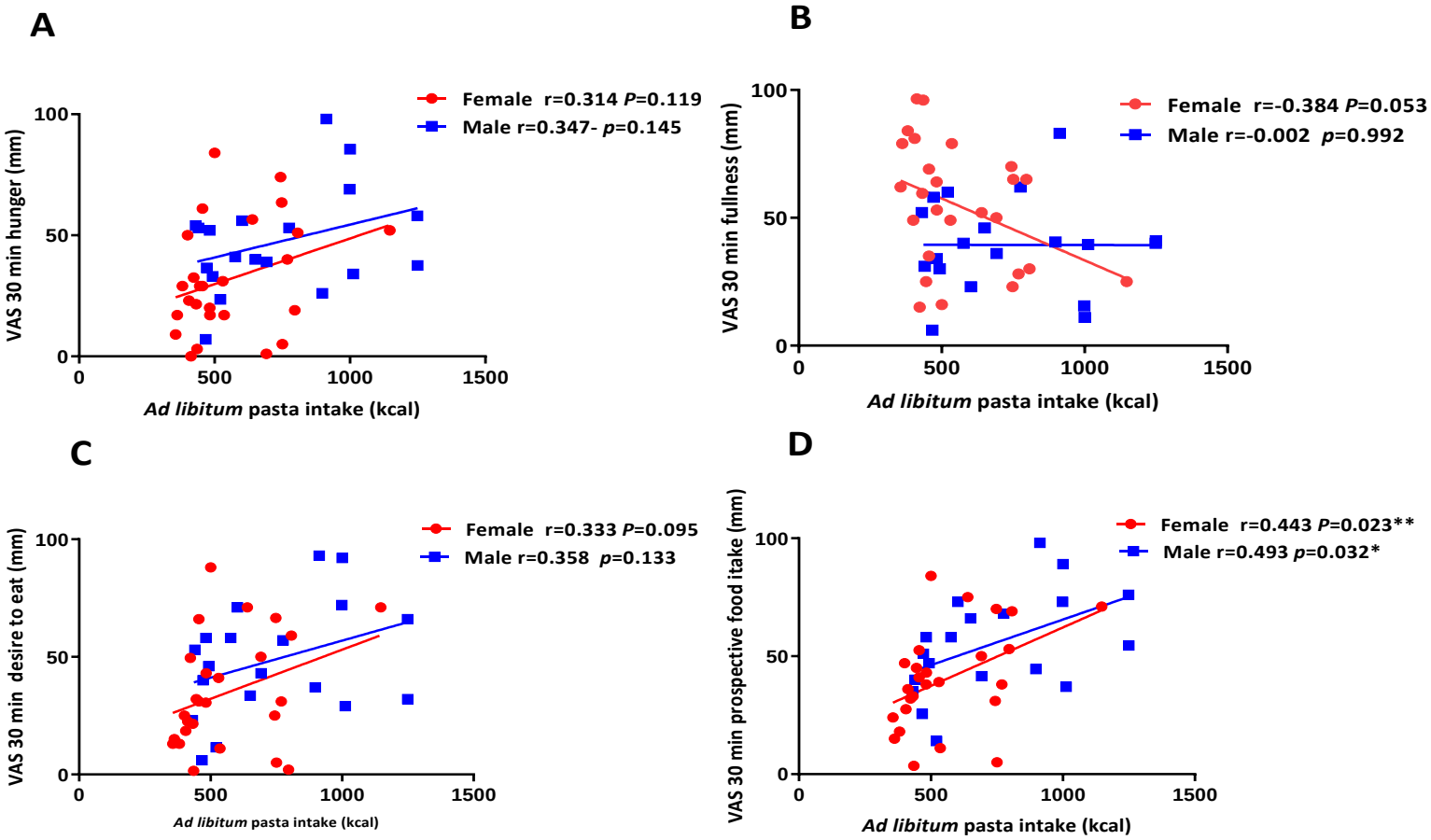

Figure 4-6 : The relationship of 30 min scores of four questions of VAS and *Ad libitum* lunch intake (kcal) scores for females and males (A= VAS 30 min hunger, B=VAS 30 min fullness, C=VAS 30 min desire to eat and D= VAS 30 min prospective food intake)

4.3.6.4 AUC hunger, fullness, desire to eat, prospective food intake, composite appetite score and fasting composite appetite score and *ad libitum* lunch intake

Figure 4.7 presents scores of AUC from VAS appetite ratings and *ad libitum* lunch intake. There was a positive correlation between AUC hunger (r= 0.460*, p= 0.017) and *ad libitum* lunch intake in females. Moreover, AUC prospective food intake (r= 0.0415*, p=0.035) were positively correlated with *ad libitum* lunch intake in females. However, AUC desire to eat, composite appetite score and fasting composite of appetite score were correlated with *ad libitum* lunch intake in females but these did not reach significance. AUC fullness was negatively correlated (r= -0.495*, p= 0.010) with and *ad libitum* pasta intake in females. Whereas, in males there was a correlation between AUC of all VAS appetite scores and *ad libitum* lunch intake, but these did not reach significance.

After adjusting for BMI, the correlation remained between AUC hunger (r= 0.477*, p= 0.016), AUC prospective food intake (r=0.429*, p=.032) and AUC fullness (r= -0.594*, p= 0.012) and *ad libitum* lunch intake in females. Furthermore, after adjusting for age, the correlation remained between AUC hunger (r= 0.562*, p= 0.020), AUC fullness (r= -0.599*, *p*= 0.011), AUC prospective food intake (r=0.432*, p=0.031) and *ad libitum* lunch intake in females.

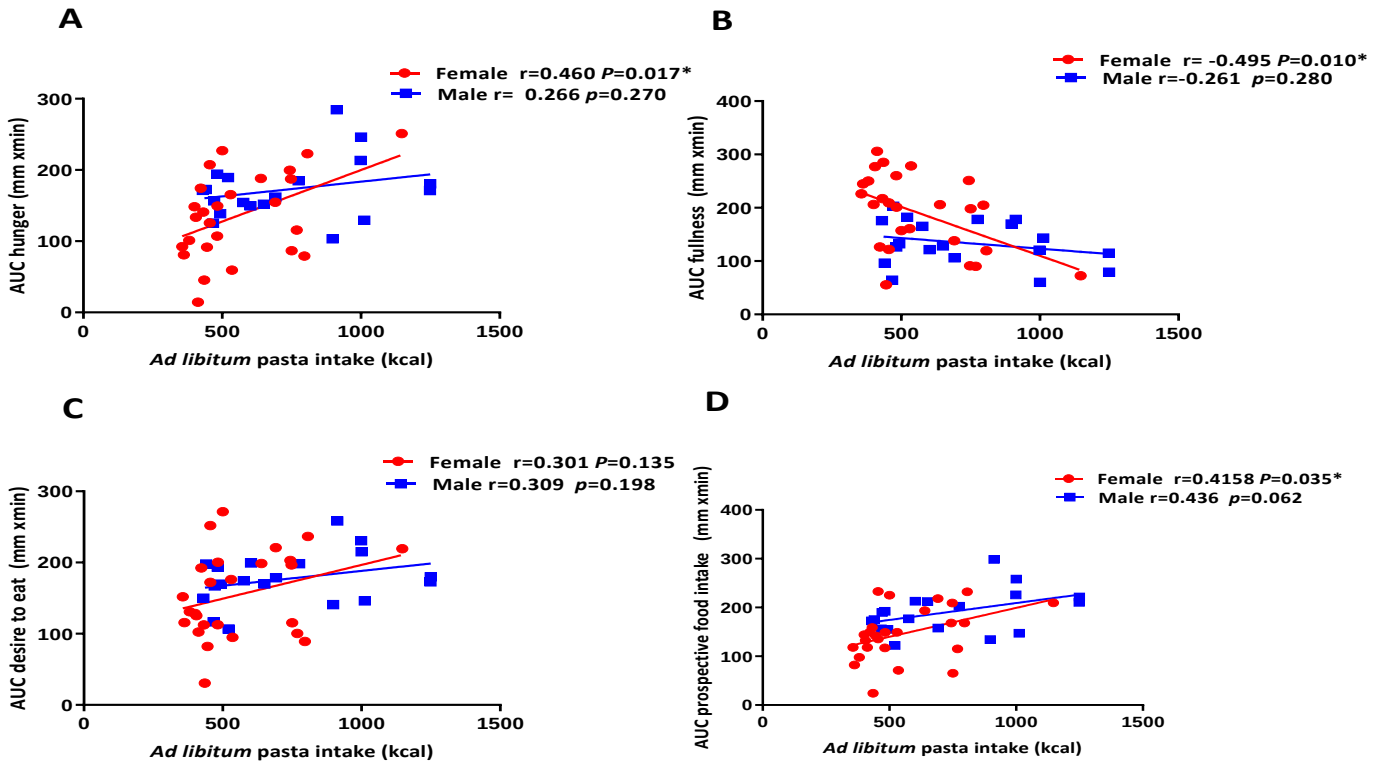

Figure 4-7: The relationship of AUC hunger, fullness, desire to eat and prospective food intake and *ad libitum* lunch intake (kcal) scores for females and males. (A= AUC hunger, B= AUC fullness, C= AUC desire to eat and D= AUC prospective food intake).

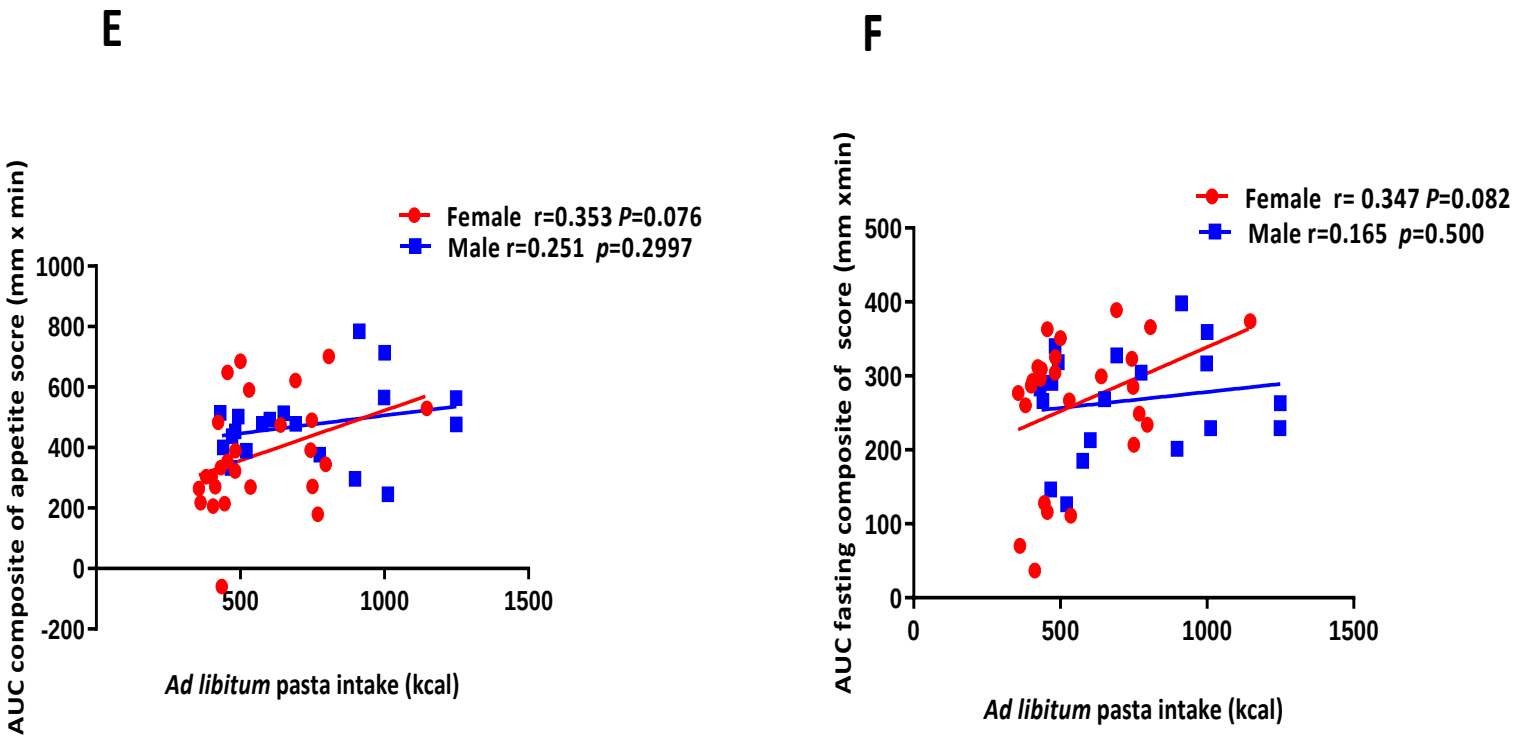

Figure 4-7: The relationship of AUC composite of appetite score and fasting composite of appetite score and *ad libitum* lunch intake (kcal)scores for females and males (E=AUC composite appetite score and F=AUC fating composite score) (continued)

4.3.6.5 SQ for all questions (hunger, fullness, desire to eat and prospective food intake) and SQ composite and *ad libitum* lunch intake

There were no significant correlations between SQ and (hunger, fullness, desire to eat and prospective food intake) and SQ composite and *ad libitum* lunch intake.

4.3.6.6 Subscales of TFEQ (hunger, cognitive restraint and disinhibition) and *ad libitum* lunch intake

Figure 4.8 represents TFEQ subscales and *ad libitum* lunch intake. As can be observed in the figure there was no significant correlation between TFEQ Hunger, TFEQ Restraint and TFEQ Disinhibition and *ad libitum* lunch intake.

4.3.6.7 The fasting gut hormones and AUC hormones and *ad libitum* lunch intake

Figure 4.9 shows the fasting gut hormone levels and AUC of gut hormones and *ad libitum* lunch intake**.** There was no significant correlation between fasting and AUC of gut hormones and *ad libitum* lunch intake in females and males.

4.3.6.8 Food cravings and *ad libitum* lunch intake

Figure 4.10 represents food craving (fast, sweet, fat and CHO) and *ad libitum* lunch intake. No significant correlation was found between fast food cravings and *ad libitum* lunch intake in both females and males

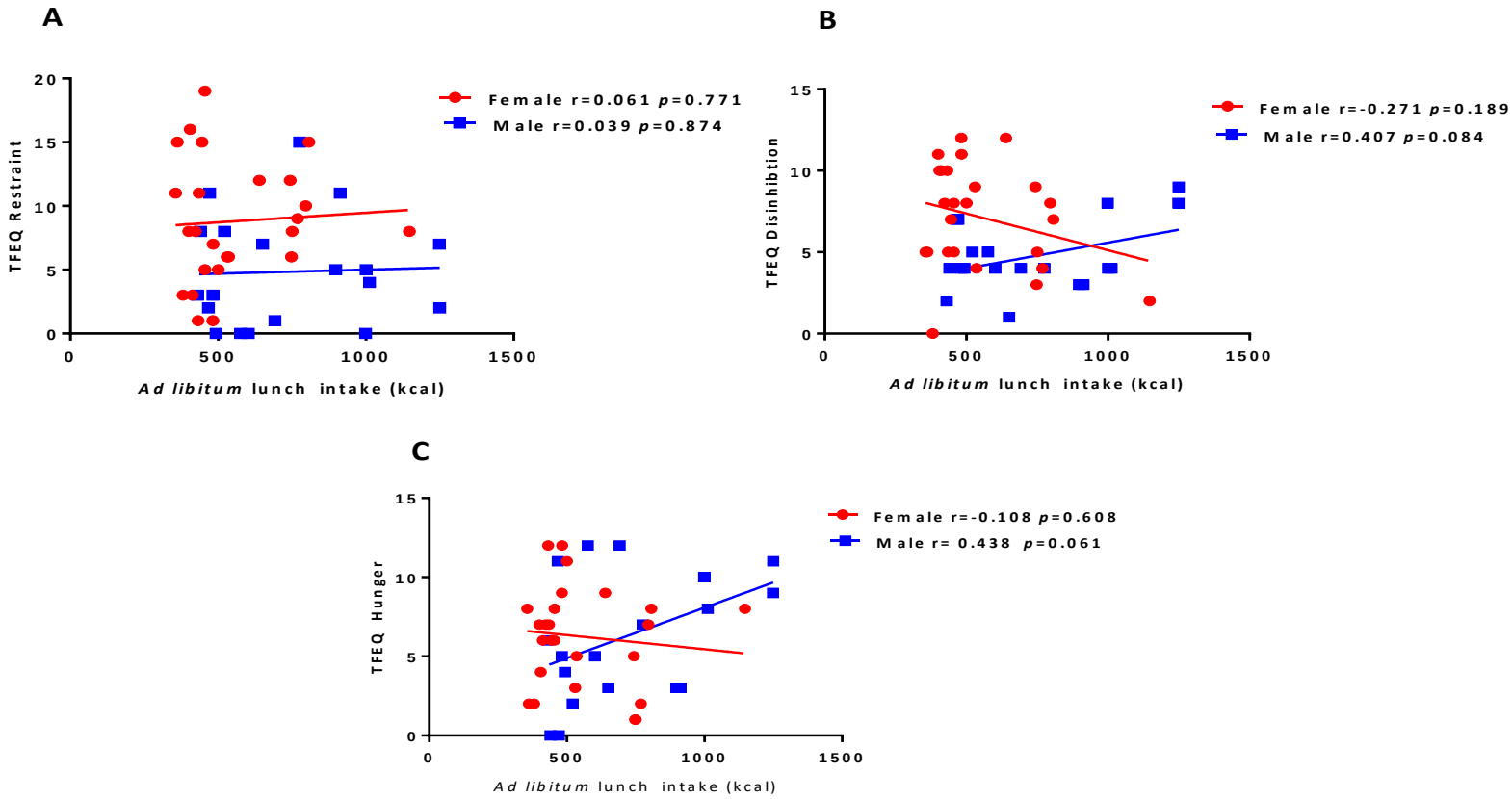

Figure 4-8: The relationship between TFEQ subscales and *ad libitum* lunch intake (A= TFEQ restraint, B=TFEQ Disinhibition and C= TFEQ hunger).

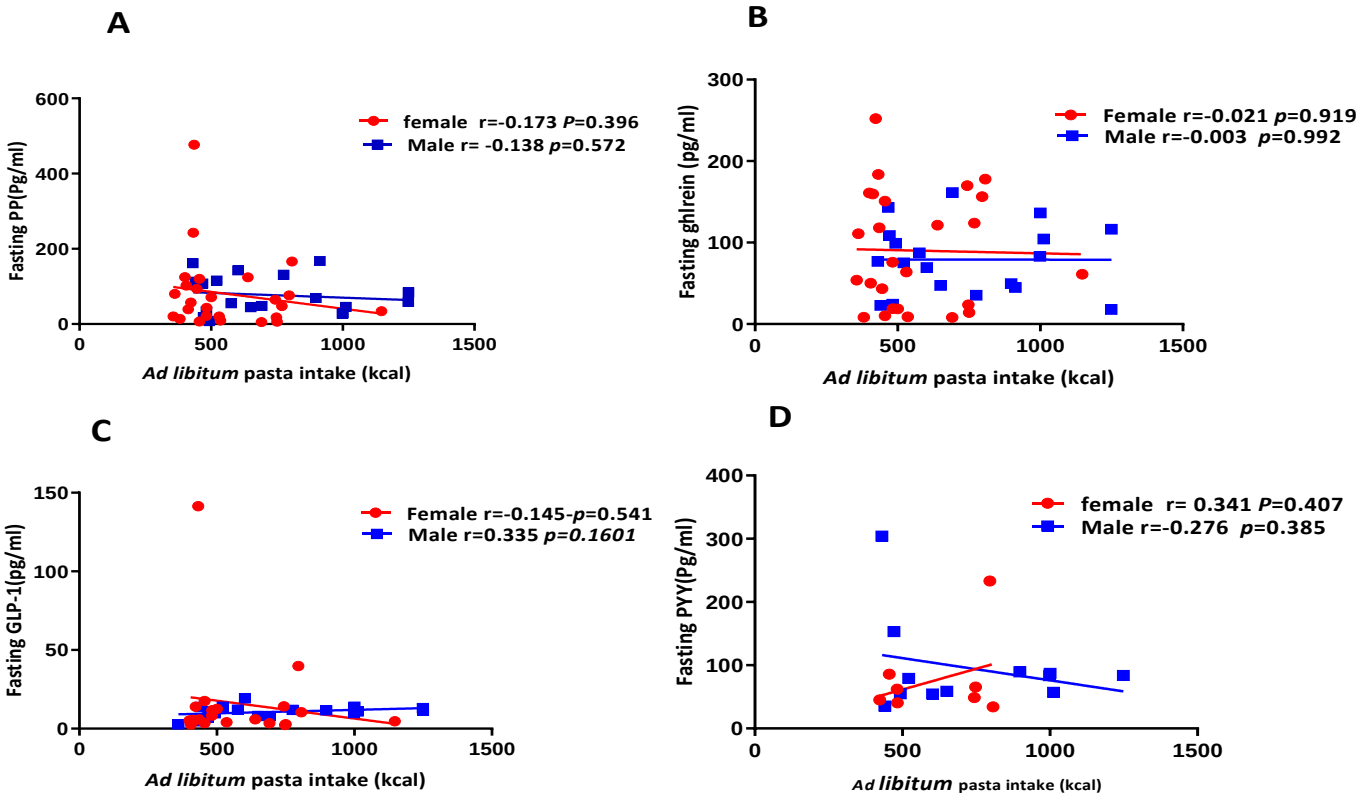

Figure 4-9: The relationship between fasting gut hormones and *ad libitum* lunch intake scores for females and males (A= fasting PP, B= fasting ghrelin, C= fasting GLP-1 and D= fasting PYY)

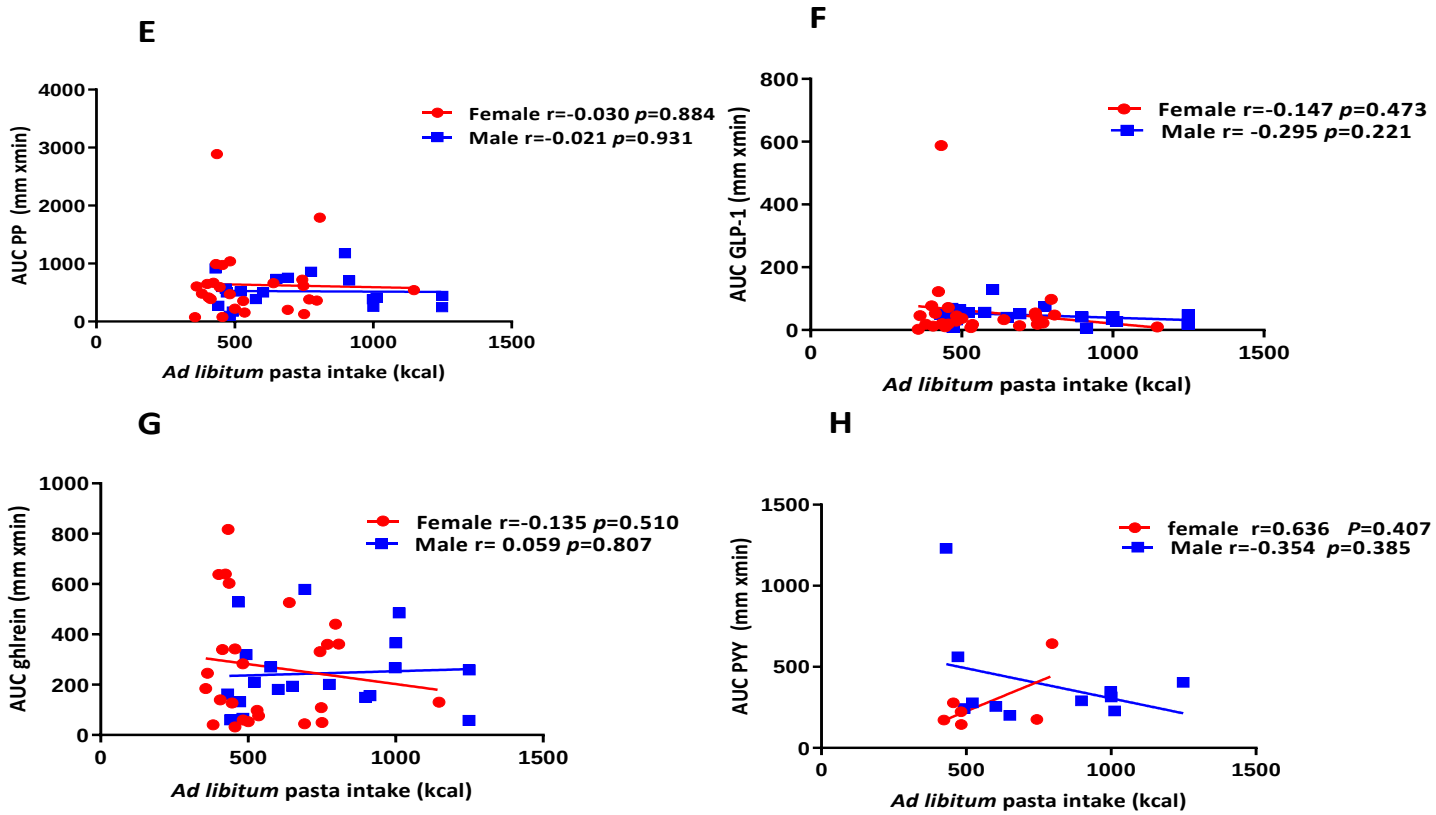

Figure 4-9: The relationship between AUC gut hormones and *ad libitum* lunch intake scores for females and males (E = AUC PP, F=AUC ghrelin, G= fasting GLP-1 and H= fasting PYY) (continued)

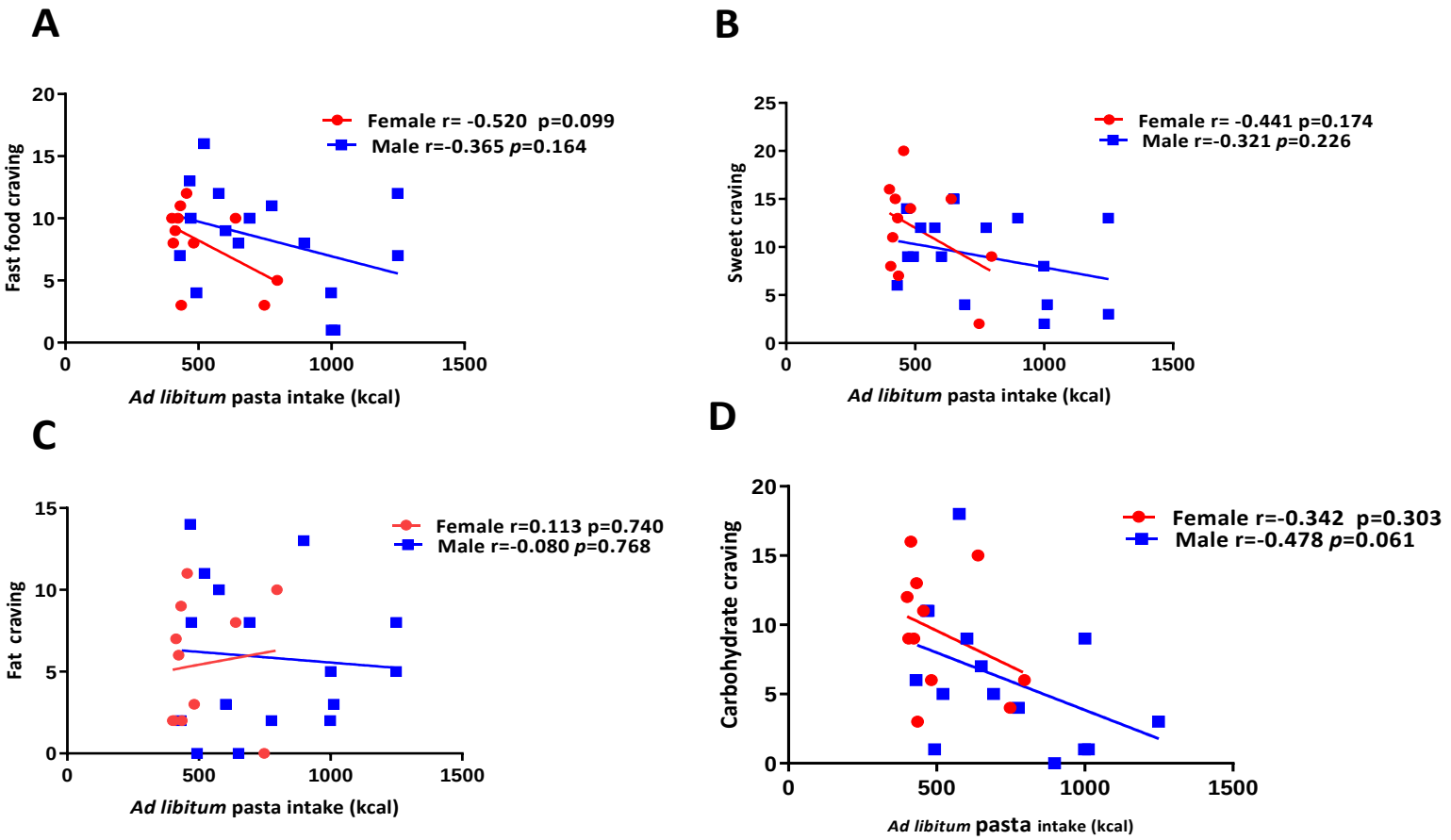

Figure 4-10: The relationship between food cravings and *ad libitum* lunch intake (A= fast food craving, B= Sweet craving, C= fat craving and D= CHO craving).

4.3.7 Models for baseline of variables, as predictors to identify individual satiety phenotypes.

4.3.7.1 TEI model

Table 4.19 presents the regression model of all independent variables that have the largest correlations for females based on TEI from the food diary. Table 4.20 presents the regression model of all independent variables that have the largest correlations for males based on energy food diary intake. Independent variables in both tables were chosen based on significant and non-significant correlation with TEI from the food diary.

Table 4-19: Regression model identifying predictors (VAS appetite scores at different time points, gut hormones) of energy food diary intake in females

Coefficients			
Independent variable	**Beta ± SE**	**t**	**p**
AUC composite appetite score	-2.20±0.44	-13.52	0.04
VAS fasting prospective food intake	2.29±4.19	12.93	0.05
AUC desire to eat	2.32±1.65	10.74	0.06
Fasting PP	-0.70±0.48	-6.85	0.09
VAS post breakfast desire to eat	0.40±0.93	6.58	0.10
AUC prospective food intake	-1.68±2.21	-6.359	0.10
VAS fasting desire to eat	-2.53±7.85	-5.15	0.12
VAS fasting fullness	0.94±4.16	4.46	0.14
VAS fasting hunger	1.67±9.16	3.23	0.19
Fasting GLP-1	0.89±15.61	3.22	0.19
Fasting ghrelin	0.33±0.69	3.13	0.20
VAS30 min fullness	-0.99±8.23	-2.32	0.26
TFEQ-H	0.15±11.65	2.29	0.26
AUC Fasting composite appetite score	2.55± 3.95	1.230	0.29
SQ composite score	0.36± 3.74	1.89	0.31
AUC fullness	0.14±1.61	0.61	0.65
AUC huger	0.12±0.36	0.485	0.712
BMI	0.11±14.29	0.34	0.75

Table 4-20: Regression model identifying predictors (VAS appetite scores at different time points, gut hormones) of energy food diary intake in males

Coefficients			
Independent variable	**Beta ± SE**	**t**	**p**
AUC fullness	1.59±31.55	0.96	0.51
TFEQ-H	0.83±204.34	0.79	0.57
VAS fasting fullness	-1.392±87.412	-0.77	0.58
BMI	0.18±39.19	0.47	0.67
VAS fasting hunger	-2.32±140.19	-0.54	0.68
Fasting PP	-0.42±13.47	-0.53	0.692
AUC composite appetite score	0.95±16.59	0.41	0.75
Fasting GLP-1	0.52±304.49	0.41	0.75
AUC prospective food intake	-0.61±33.05	-0.41	0.75
VAS 30 min fullness	-0.67±83.45	-0.39	0.76
VAS post breakfast desire to eat	0.32±46.23	0.35	0.79
VAS fasting prospective food intake	0.70±119.83	0.32	0.80
VAS fasting desire to eat	0.73±87.17	0.29	0.82
Fasting ghrelin	-0.27±18.47	-0.27	0.83
SQ composite score	0.69±142.83	0.21	0.87
AUC huger	-0.21±35.92	-0.14	0.91
AUC desire to eat	0.12±39.47	0.08	0.95
AUC Fasting composite appetite score	0.04±10.70	0.02	0.99

4.3.7.2 *Ad libitum* lunch intake model

Table 4.21 presents the regression model of independent variables that have the largest correlations for females, based on *ad libitum* lunch intake. Table 4.22 presents the regression model of independent variables that have the largest correlations for males, based on *ad libitum* lunch intake. Independent variables in both tables were chosen based on significant and non-significant correlation with *ad libitum* lunch intake.

Table 4-21: Regression model identifying predictors (VAS appetite scores at different time points, gut hormones) of *ad libitum* lunch intake in females

Coefficients			
Independent variable	**Beta ± SE**	**t**	**p**
AUC prospective food intake	-4.37±3.09	-4.73	0.01
VAS fasting hunger	4.15±6.63	4.28	0.02
VAS fasting prospective food intake	2.37±5.79	3.61	0.03
Fasting GLP-1	0.77±1.44	3.55	0.04
AUC hunger	1.19±0.23	3.11	0.05
SQ composite score	-1.74±5.43	-2.55	0.08
TFEQ hunger	-0.49±13.24	-2.52	0.09
VAS fasting desire to eat	-2.45±6.38	-2.36	0.10
AUC composite score	1.29±.59	2.20	0.12
AUC desire to eat	1.25±2.19	1.72	0.18
AUC Fasting composite appetite score	2.55±3.95	1.23	0.29
VAS30 mins fullness	1.11±7.95	1.08	0.36
VAS fasting fullness	0.61±4.20	1.01	0.39
Fasting PP	-0.28±0.53	-0.99	0.39
AUC fullness	-0.34±1.85	-0.52	0.64
Fasting ghrelin	0.07±0.51	0.37	0.74
BMI	0.11±14.29	0.34	0.75
VAS post breakfast desire to eat	0.03±1.26	0.16	0.88

Table 4-22: Regression model identifying predictors (VAS appetite at different time points, gut hormones) of *ad libitum* lunch intake in males

Coefficients			
Independent variable	**Beta ± SE**	**t**	**P**
AUC hunger	-2.02±6.15	-2.71	0.22
VAS fasting prospective food intake	2.52±17.92	2.73	0.22
TFEQ-H	1.54±40.58	2.61	0.23
Fasting GLP-1	1.59±64.91	2.08	0.28
VAS fasting fullness	-1.43±12.33	-1.98	0.30
VAS fasting desire to eat	-1.84±12.63	-1.79	0.33
AUC composite of appetite score	1.68±2.76	1.55	0.36
AUC fullness	1.04±4.72	1.48	0.38
VAS 30 min fullness	0.96±11.86	1.41	0.39
VAS post breakfast desire to eat	0.4±6.64	1.15	0.45
AUC desire to eat	0.81±6.19	1.13	0.46
VAS fasting hunger	-2.14±23.65	-1.04	0.49
SQ composite of appetite score	1.37±27.26	0.76	0.59
BMI	0.57±62.08	0.64	0.59
Fasting ghrelin	0.25±2.68	0.60	0.66
AUC Fasting composite of appetite score	-0.94±9.86	-0.40	0.73
Fasting PP	-0.09±1.95	-0.27	0.83
AUC prospective to food intake	0.14±4.89	0.22	0.86

4.3.8 TEI, *ad libitum* lunch intake based on food diaries and independent variables that identify individual satiety phenotypes.

4.3.8.1 *Ad libitum* lunch intake

Table 4.23 presents the heat map of *ad libitum* lunch intake and independent variables that have the largest correlations from the regression model for females. Participants were determined as low, medium or high satiety phenotype based on the colour coding as previously described. Satiety scores were calculated from this equation = (R*2+O*1). Participants were divided into 3 groups being low, medium and high satiety by using method one. The lowest score of satiety from the equation was 2 and the highest was 13. The first group had high satiety scores from 2-9, 10-11 scores were medium satiety and 12-13 scores was low satiety.

Table 4.24 presents the heat map of *ad libitum* lunch intake and independent variables that have the largest correlations from the regression model for males. Participants were determined as low, medium or high satiety phenotype with the colour coding as previously described. Satiety scores were calculated as in Table 4.15.

Table 4.25 displays the heat map of *ad libitum* lunch intake and all independent variables that that have the largest correlations from the regression model. Females were determined as low, medium or high satiety phenotype the colour coding as previously described. Satiety scores were calculated from this equation = (R*2+O*1). Participants were divided into 3 groups being low, medium and high satiety by using method two. Satiety scores were from 2-15, with the first group with high satiety scores from 2-6, 7-11 scores medium satiety and 12-15 was low satiety.

Table 4.26 presents the heat map of *ad libitum* lunch intake and all independent variables for males according to satiety scores. Males were determined as low, medium or high satiety phenotype and the colour coding was as previously described. Satiety scores were calculated as in Table 4.25.

Table 4-23: Heat map of satiety phenotype of females based on *ad libitum* pasta intake in female using method one

Participants number	Ad libitum lunch intake	TFEQ Hunger	fasting pp	VAS fasting prospective food intaket0mins	SQ composite	VAS post breakfast desire	VASfastingfull0mins	VAS fasting desire to	VASfastinghunger0mins	Phenotype	Satiety score
N22	356									2	High satiety
N30	362									4	High satiety
N17	381									5	High satiety
nut22	400									6	High satiety
nut12	405									6	High satiety
nut30	413									6	High satiety
nut33	423									5	High satiety
nut24	432									10	Medium satiety
nut14	435									9	Medium satiety
N8	445									7	Medium satiety
N10	455									12	Low satiety
nut10	455									12	Low satiety
nut15	482									11	Low satiety
N9	483									9	Medium satiety
N12	500									11	Low satiety
N35	530									4	High satiety
N5	535									4	High satiety
nut21	639									6	High satiety
N13	691									11	Low satiety
N4	744									10	Medium satiety
nut17	748									4	High satiety
N24	750									7	Medium satiety
N3	769									8	Medium satiny
nut7	796									10	Medium satiny
N2	807									11	Low satiety
N14	1147									9	Medium satiety

N and nut =participants divided in two groups and nut groups did not have food craving data

Table 4-24: Heat map of satiety phenotype of males based on *ad libitum* pasta intake in by using method one

Participants number	Ad libitum lunch intake	TFEQ Hunger	fasting pp	VAS fasting prospective food intaket0mins	SQ composite appetite	VAS post breakfast desire to eat	VASfastingfull0mins	VAS fasting desire to eat0mins	VASfastinghunger0mins	Phenotype	Satiety score
nut19	431									10	Low satiety
N26	440									6	High satiety
nut31	467									5	High satiety
nut18	471									11	Low satiety
N21	482									11	Low satiety
nut34	493									9	Medium satiety
nut3	521									5	High satiety
nut32	576									7	Medium satiety
nut1	602									8	Medium satiety
nut5	650									7	Medium satiety
nut36	692									13	Low satiety
nut27	776									9	Medium satiety
nut2	898									6	High satiety
N11	913									11	Low satiety
nut6	999									10	Low satiety
nut35	1000									10	Low satiety
nut11	1012									6	High satiety
nut16	1249									9	Medium satiety
nut20	1250									6	High satiety

Table 4-25: Heat map of satiety phenotype of females based on *ad libitum* pasta intake in females by using method two

Participants number	*Ad libitum lunch* intake	VAS fasting hunger	VAS fasting prospective food intake	VAS fasting desire to eat	SQ composite appetite	TFEQ Hunger	post break desire to eat	fasting PP	VAS fasting fullness	Satiety score	Phenotype
N22	356									13	Low satiety
N30	362									3	High satiety
N17	381									13	Low satiety
nut22	400									10	Medium hunger
nut12	405									9	Medium hunger
nut30	413									2	High satiety
nut33	423									9	Medium satiety
nut24	432									11	Medium satiety
nut14	435									10	Medium satiety
N8	445									3	High satiety
N10	455									6	High satiety
nut10	455									12	Low satiety
nut15	482									13	Low satiety
N9	483									13	Low satiety
N12	500									12	Low satiety
N35	530									10	Medium satiety
N5	535									11	Medium satiety
nut21	639									11	Medium satiety
N13	691									13	Low satiety
N4	744									12	Low satiety
nut17	748									10	Medium satiety
N24	750									11	Medium satiety
N3	769									7	Medium satiety
nut7	796									8	Medium satiety
N2	807									11	Medium satiety
N14	1147									13	Low satiety

Table 4-26: Heat map of satiety phenotype of males based on *ad libitum* pasta intake in males by using method two

Participants number	*Ad libitum lunch* intake	VAS fasting hunger	TFEQ Hunger	VAS fasting prospective food intake	VAS fasting desire to eat	VAS post breakfast t desire to eat	VAS fasting fullness	fasting PP	SQ composite appetite	Satiety score	Phenotype
nut19	431									10	Medium satiety
N26	440									6	High satiety
nut31	467									5	High satiety
nut18	471									12	Low satiety
N21	482									12	Low satiety
nut34	493									11	Medium satiety
nut3	521									2	High satiety
nut32	576									8	Medium satiety
nut1	602									6	High satiety
nut5	650									8	Medium satiety
nut36	692									14	Low satiety
nut27	776									8	Medium satiety
nut2	898									6	High satiety
N11	913									9	Medium satiety
nut6	999									13	Low satiety
nut35	1000									15	Low satiety
nut11	1012									9	Medium satiety
nut16	1249									9	Medium satiety
nut20	1250									9	Medium satiety

4.3.8.2 Energy intake food diary

Table 4.27 displays the heat map of all scores of TEI and all independent variables that have the largest correlations from the regression model. Females were determined as low, medium or high satiety phenotype, with the colour coding as previously described. Satiety scores were calculated from this equation = (R*2+O*1). Participants were divided into 3 groups, being low, medium and high satiety by using method one. The lowest score of satiety from the equation was 2 and the highest was 13. The first group with high satiety scores from 2-9, 10-11 scores were medium and 12-13 scores who low satiety.

Table 4.28 shows all scores of TEI and all independent variables that have the largest correlations from the regression model. Males were determined as low, medium or high satiety phenotype with the colour coding as previously described. Satiety scores were calculated as in Table 4.27.

Table 4.29 displays all scores of TEI and all independent variables that have the largest correlations from the regression model. Females were determined as low, medium or high satiety phenotype with the colour coding as previously described. Satiety scores were calculated from this equation = (R*2+O*1). Participants were divided into 3 groups being low, medium and high satiety by using method two, with satiety scores from 2-14. The first group with high satiety scores from 2-5, 6-9 scores medium and 10-14 low satiety.

Tables 4.30 presents all scores of TEI and all independent variables that have the largest correlations from the regression model. Males were determined as low, medium or high satiety phenotype, with the colour coding as previously described. Satiety scores were calculated as in Table 4.29.

Table 4-27: Heat map of satiety phenotype of females based on TEI and independent variables by using method one

Participants number	TEI	Fasting PP	VAS fasting prospective food intaket0mins	VASfastinghunger0mins	VAS fasting desire to eat0mins	VAS post breakfast desire to eat	TFEQ hunger	SQ composite appetite	VASfastingfull0mins	Phenotype	Satiety score
nut 24	432									10	Medium satiety
nut7	651									4	High satiety
N30	836									4	High satiety
nut14	1021									7	High satiety
nut21	1134									8	Medium satiety
nut12	1136									9	Medium satiety
N24	1155									6	High satiety
N17	1196									9	Medium satiety
N3	1248									3	High satiety
N22	1271									7	High satiety
nut30	1345									5	High satiety
N8	1416									4	High satiety
nut15	1453									10	Medium satiety
nut17	1585									8	Medium satiety
N5	1625									4	High satiety
N2	1631									11	Low satiety
nut10	1650									12	Low satiety
N10	1703									5	High satiety
N12	1717									10	Medium satiety
N13	1737									10	Medium satiety
N35	2001									7	High satiety
nut22	2006									9	Medium satiety
N4	2074									11	Low satiety
nut33	2146									7	High satiety
N9	2331									12	Low satiety
N14	2629									9	Medium satiety

Table 4-28: Heat map of satiety phenotype of males based on TEI and independent variables by using method one

Participants number	TEI	Fasting PP	VAS fasting prospective food intaket0mins	VASfastinghunger0mins	VAS fasting desire to eat0mins	VAS post breakfast desire to eat	TFEQ hunger	SQ composite appetite	VASfastingfull0mins	Phenotype	Satiety score
nut16	1052									9	medium satiety
N11	1142									9	medium satiety
nut31	1480									3	high satiety
nut1	1511									5	high satiety
nut5	1561									8	medium satiety
N26	1584									4	high satiety
nut19	1613									9	medium satiety
nut18	1658									12	low satiety
nut27	1689									8	medium satiety
nut3	1706									4	high satiety
nut32	1754									7	high satiety
nut20	1890									7	high satiety
nut36	1907									14	low satiety
N21	2532.3									12	low satiety
nut2	2646									7	high satiety
nut35	2886									12	low satiety
nut34	3022									11	medium satiety
nut6	3458									12	low satiety
nut11	3937									7	high satiety

Table 4-29: Heat map of satiety phenotype of females based on TEI and independent variables by using method two

Participants number	TEI	VAS post breakfast desire to eat	TFEQ hunger	VAS fasting desire to eat	VAS fasting prospective food intake	VAS fasting hunger	SQ composite appetite	VAS fasting fullness	Fasting PP	Satiety score	Phenotype
nut24	432									9	Medium satiety
nut7	651									8	Medium satiety
N30	836									3	High satiety
nut14	1021									9	Medium satiety
nut21	1134									11	Low satiety
nut12	1136									9	Medium satiety
N24	1155									11	Low satiety
N17	1196									13	Low satiety
N3	1248									6	Medium satiety
N22	1271									13	Low satiety
nut30	1345									2	High satiety
N8	1416									2	High satiety
nut15	1453									11	Low satiety
nut17	1585									10	Low satiety
N5	1625									9	Medium satiety
N2	1631									12	Low satiety
nut10	1650									12	Low satiety
N10	1703									7	Medium satiety
N12	1717									12	Low satiety
N13	1737									13	Low satiety
N35	2001									11	Low satiety
nut22	2006									10	Low satiety
N4	2074									13	Low satiety
nut33	2146									9	Medium satiety
N9	2331									13	Low satiety
N14	2629									12	Low satiety

Table 4-30: Heat map of satiety phenotype of males based on TEI and independent variables by using method two

Participants number	TEI	VAS fasting prospective food intake	VAS fasting desire to eat	VAS fasting hunger	TFEQ Hunger	VAS pot breakfast desire to eat	SQ composite appetite	Fasting PP	VAS fasting fullness	Satiety score	Phenotype
nut16	1052									8	Medium satiety
N11	1142									10	Low satiety
nut31	1480									5	High satiety
nut1	1511									7	Medium satiety
nut5	1561									8	Medium satiety
N26	1584									6	Medium satiety
nut19	1613									10	Low satiety
nut18	1658									13	Low satiety
nut27	1689									10	Low satiety
nut3	1706									2	High satiety
nut32	1754									7	Medium satiety
nut20	1890									10	Low satiety
nut36	1907									14	Low satiety
N21	2532									13	Low satiety
nut2	2646									5	High satiety
nut35	2886									14	Low satiety
nut34	3022									11	Low satiety
nut6	3458									13	Low satiety
nut11	3937									8	Medium satiety

4.3.9 Participants averages of BMI of low, medium and high satiety participants based on TEI and *ad libitum lunch* intake.

4.3.9.1 Participants average for BMI in low, medium and high satiety phenotype based on *ad libitum* lunch intake

Figure 4.11 (A) presents average BMI of females based on *ad libitum* lunch intake for females by using method one. One-way ANOVA analysis shows no significant differences between groups (F=2.267) (p= 0.126). (B) presents average BMI of females based on *ad libitum* lunch intake by using method two. One-way ANOVA again showed no significant differences between groups (F=2.441) (p=0.1103). Figure 4.12 (A) presents average BMI of males based on *ad libitum* lunch intake according using method one. One-way ANOVA showed no significance differences between low, medium and high satiety males in average BMI (F=1.972) (p=0.1716). (B) presents average BMI of males based on *ad libitum* lunch intake using method two. One-way ANOVA showed no significant differences between the groups (F= 0.071) (p=0.931).

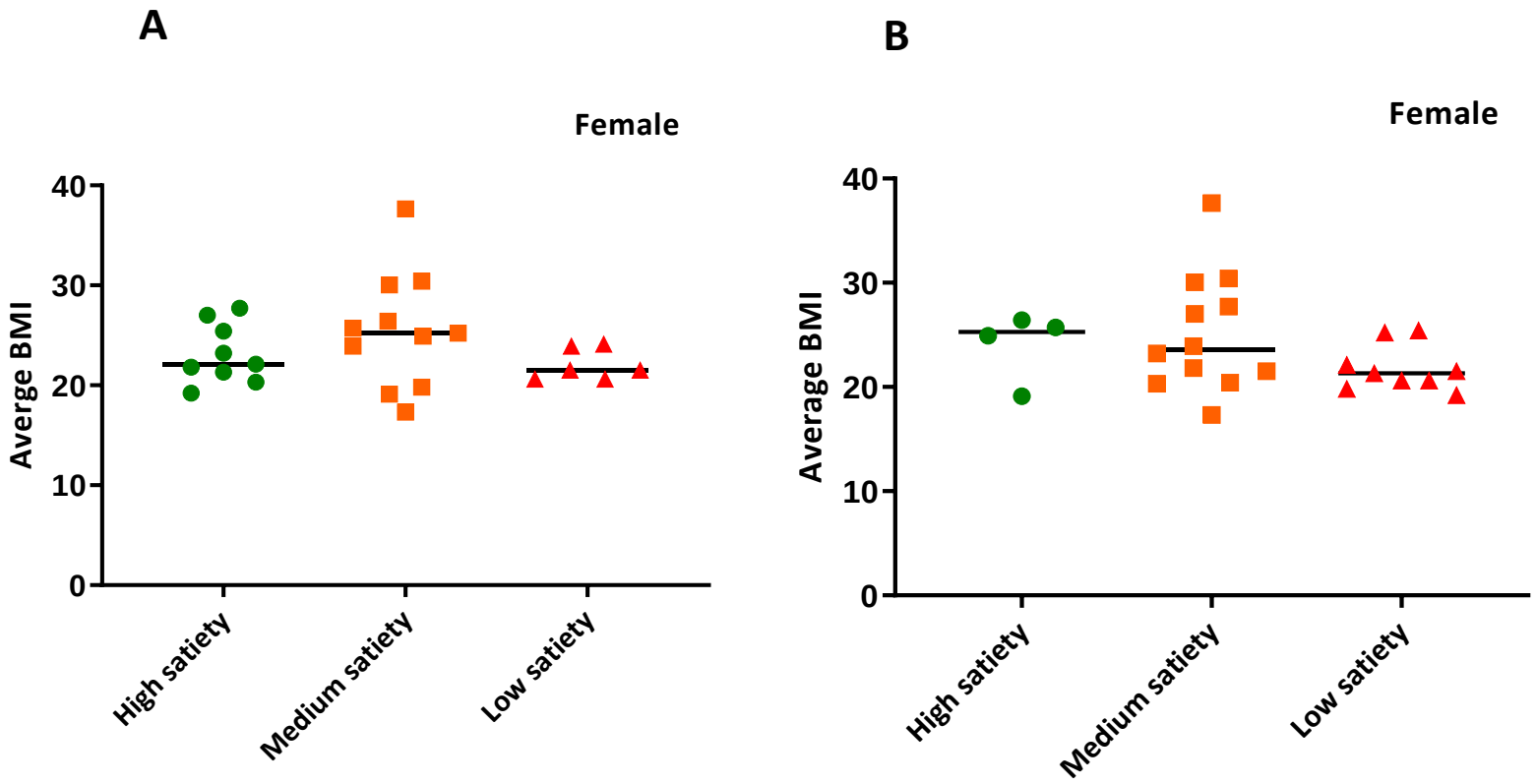

Figure 4-11 : Average BMI female based on *ad libitum* lunch intake by using (A) method one and (B) method two.

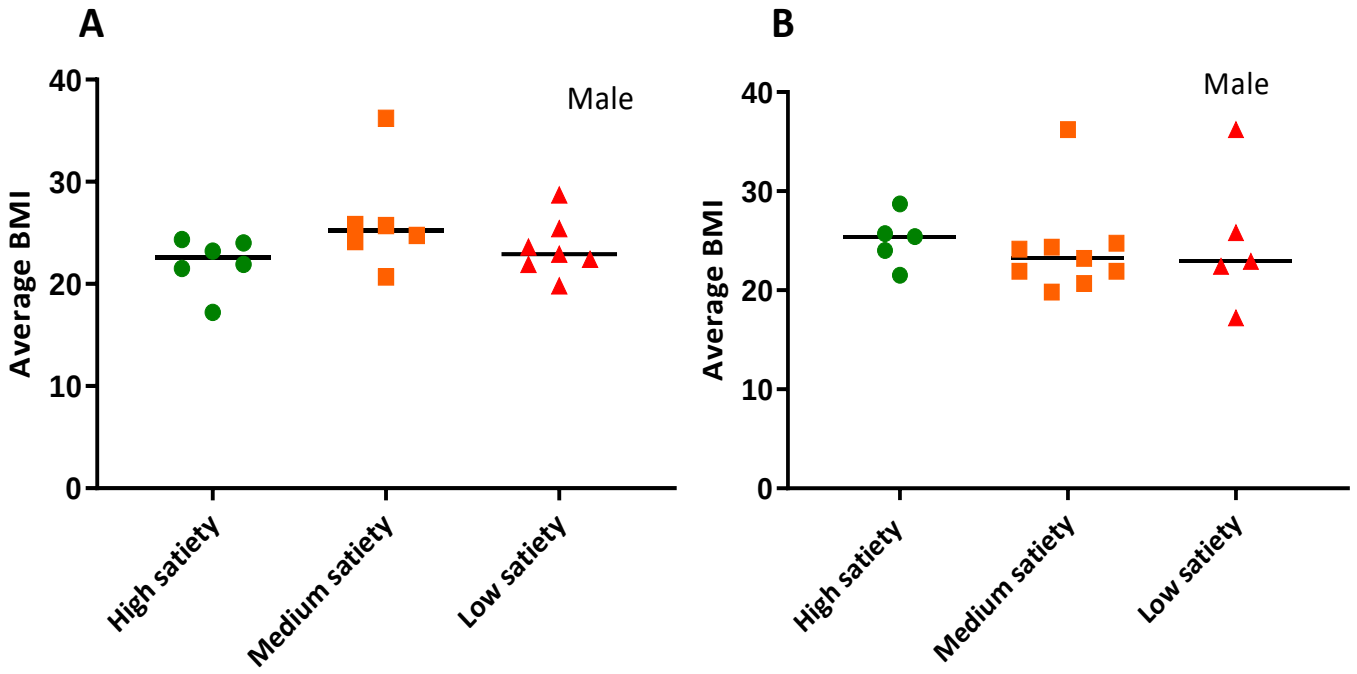

Figure 4-12: Average BMI male based on *ad libitum* lunch intake by using (A) method one (B) method two.

4.3.9.2 Participants average BMI for low, medium and high satiety based on TEI

Figure 4.13 (A) presents average BMI of females based on TEI using method one. One-way ANOVA showed no significant differences between low, medium and high satiety females in average BMI (F= 1.079) (p=0.357). (B) presents average BMI of females based on TEI using method two. One-way ANOVA showed no significant differences between low, medium and high satiety females in average BMI (F= 0.210) (p=0.811). Figure 4.14 (A) present average BMI of males based on TEI using method one. One-way ANOVA showed no significant differences between low, medium and high satiety males in average BMI (F= 0.597) (p=0.560). (B) presents average BMI of males based on TEI using method two. One-way ANOVA showed no significant differences between low, medium and high satiety males in average BMI (F= 0.4055) (p=0.672).

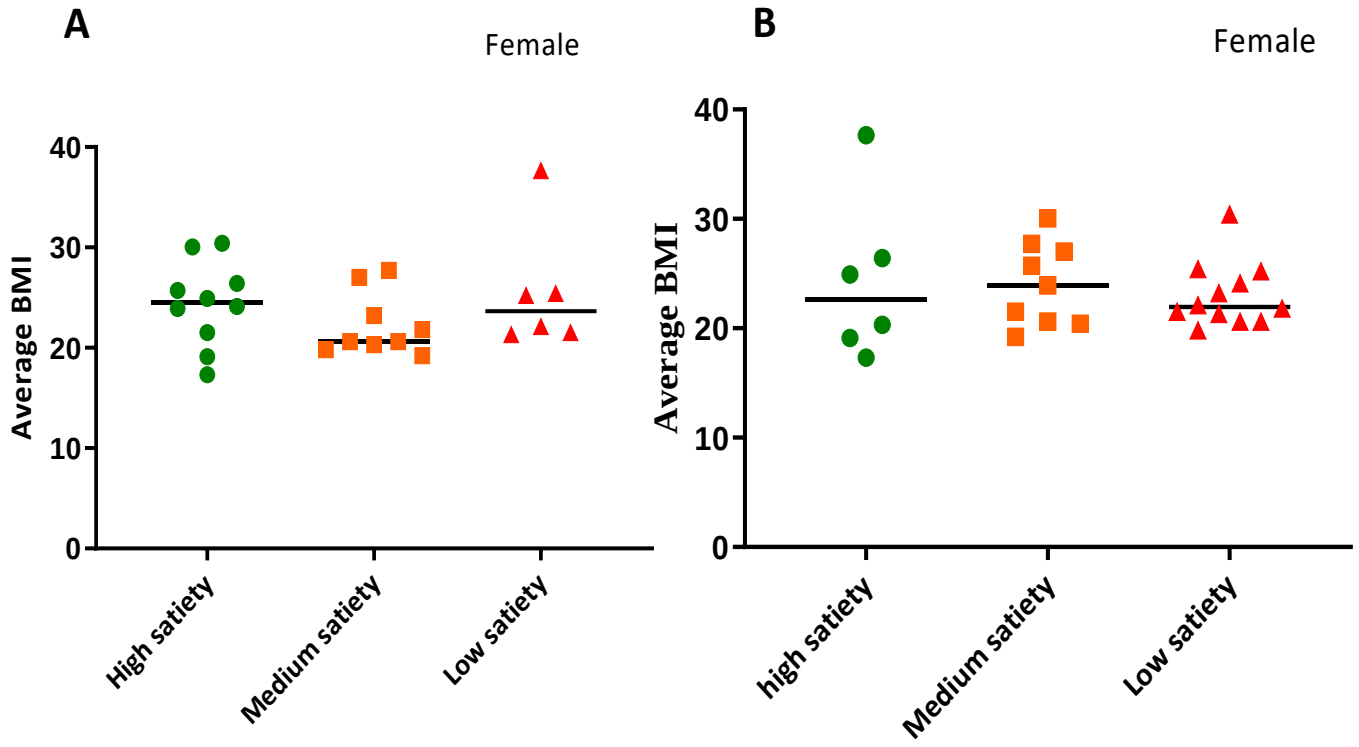

Figure 4-13: Average BMI of female based on total energy by using (A) method one (B) method two.

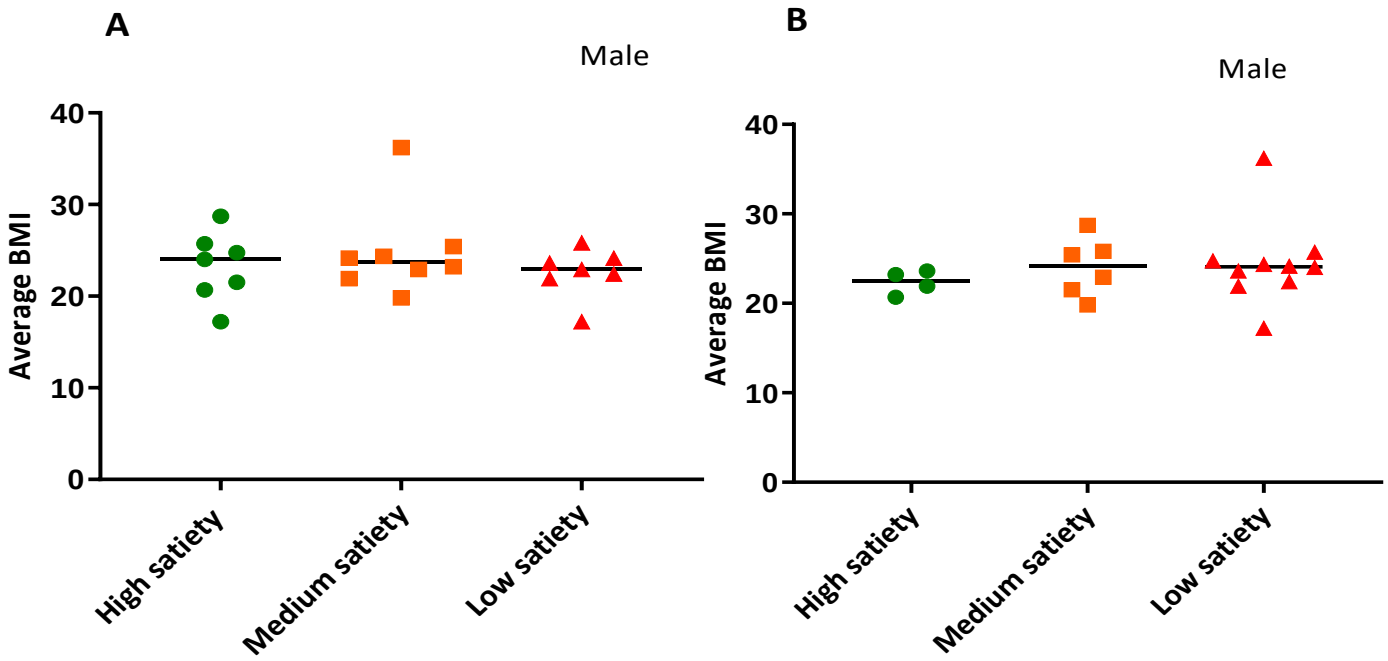

Figure 4-14: Average BMI male based on TEI in males by using (A) method one (B) method two.

4.3.10 Average TFEQ subscales in low, medium and higher satiety participants based on TEI and *ad libitum* lunch intake.

4.3.10.1 Participants TFEQ subscales for low, medium and high satiety based on TEI.

Figure 4.15 (A) presents TFEQ subscales in low, medium and high satiety participants based on TEI for females using method one. One-way ANOVA showed no significant differences between the three groups; TFEQ Restraint (F= 0.365) (p= 0.698), Hunger (F= 0.424) (p= 0.661) and TFEQ Disinhibition (F= 4.366) (p= 0.026).

(B) represents TFEQ subscales in low, medium and high satiety female participants, based on TEI using method two. One-way ANOVA showed no significant differences between the groups; TFEQ Restraint (F=1.057) (p= 0.364), Hunger (F= 0.809) (p= 0.458) and TFEQ Disinhibition (F= 2.292) (p= 0.124). Figure 4.16 (A) represents TFEQ subscales in low, medium and high satiety male participants based on TEI using method one. One-way ANOVA showed no significant differences between the three groups; TFEQ restraint (F= 0.365) (p=0.698), TFEQ Hunger (F=1.703) (p=0.205) and TFEQ Disinhibition (F=2.292) (p=0.124). (B) presents TFEQ subscales in low, medium and high satiety male participants based on TEI using method two. One-way ANOVA showed no significant differences between low, medium and high satiety females in TFEQ Restraint (F=0.273) (p=0.764), Hunger (F=0.0750) (p= 0.928) and TFEQ Disinhibition (F= 1.407) (p= 0.279).

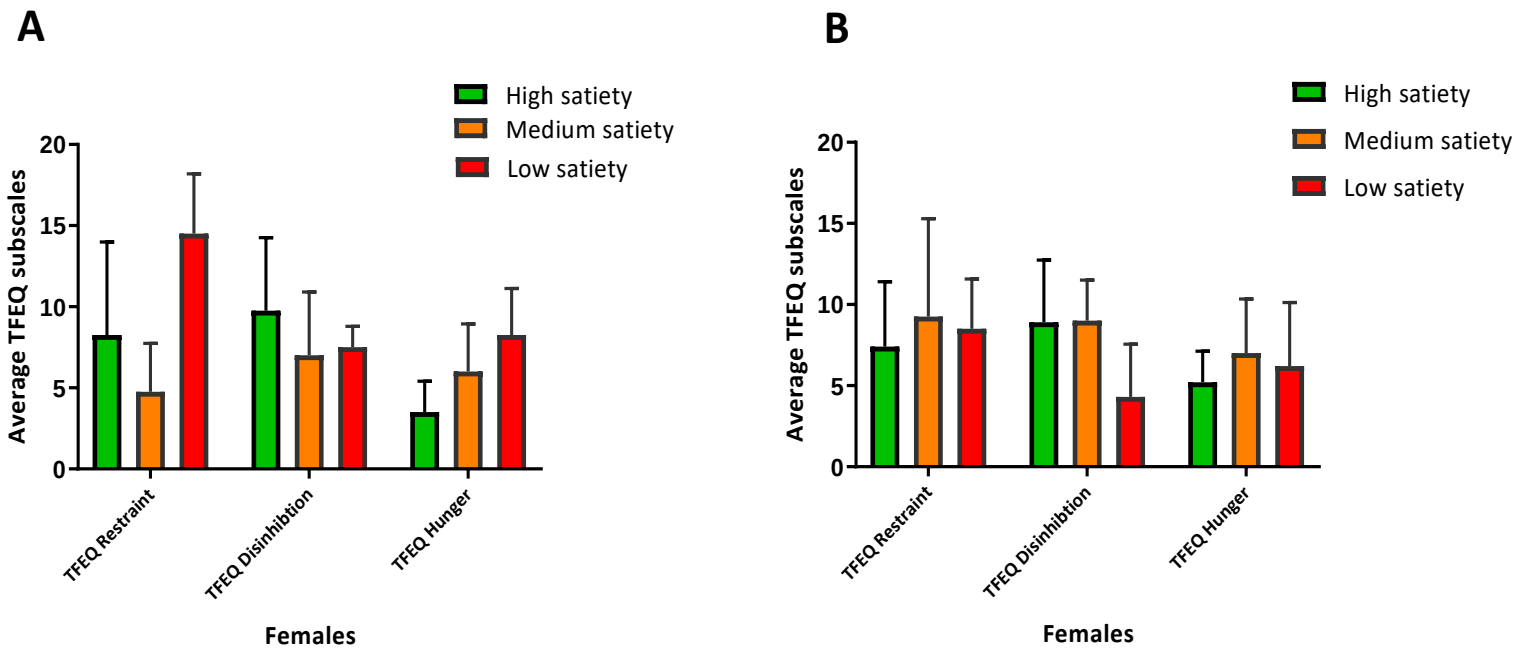

Figure 4-15: Average TFEQ subscales of female based on TEI using (A) method one (B) method two.

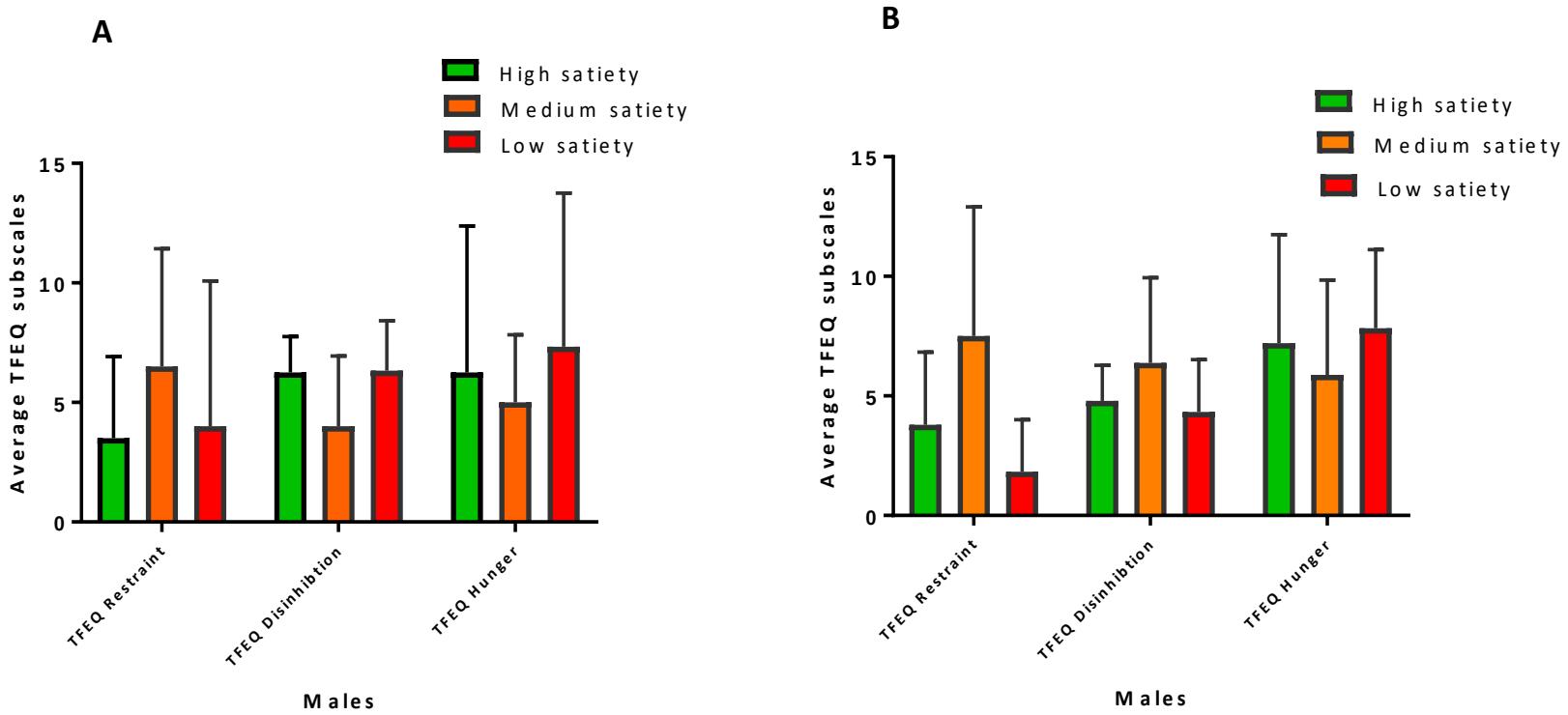

Figure 4-16 : Average TFEQ subscales of male based on TEI using (A) method one (B) method two.

4.3.10.2 Participant average TFEQ subscales for low, medium and high satiety based on *ad libitum* lunch intake.

Figure 4.17 (A) represents TFEQ subscales in low, medium and high satiety of female participants based on *ad libitum* lunch intake according to divide females by using method one. One-way showed no significant differences between low, medium and high satiety females in TFEQ Disinhibition (F=2.6) (p= 0.098) and Hunger (F=2.43) (p= 0.112). There was a significant difference between the 3 groups in TFEQ Restraint (F=3.621) (p= 0.044). (B) presents TFEQ subscales in low, medium and high satiety female participants based on *ad libitum* lunch intake using method two. One-way ANOVA showed no significant differences between low, medium and high satiety females in TFEQ Restraint (F=0.2429) (p= 0.786), Hunger (F=1.679) (p= 0.209) and TFEQ Disinhibition (F=0.555) (p= 0.581).

Figure 4.18 (A) displays TFEQ subscales in low, medium and high satiety male participants based on *ad libitum* lunch intake using method one. One-way ANOVA showed no significant differences between low, medium and high satiety females in TFEQ Restraint (F=0.609) (p=0.555), Hunger (F=2.891) (p= 0.083) and TFEQ Disinhibition (F=0.156) (p= 0.856). (B) shows TFEQ subscales in low, medium and high satiety male participants based on *ad libitum* lunch intake using method two. One-way ANOVA showed no significance differences between low, medium and high satiety females in TFEQ Disinhibition (F=0.354) (p= 0.707), Hunger (F=1.034) (p= 0.378) and TFEQ Restraint (F=0.300) (p= 0.744).

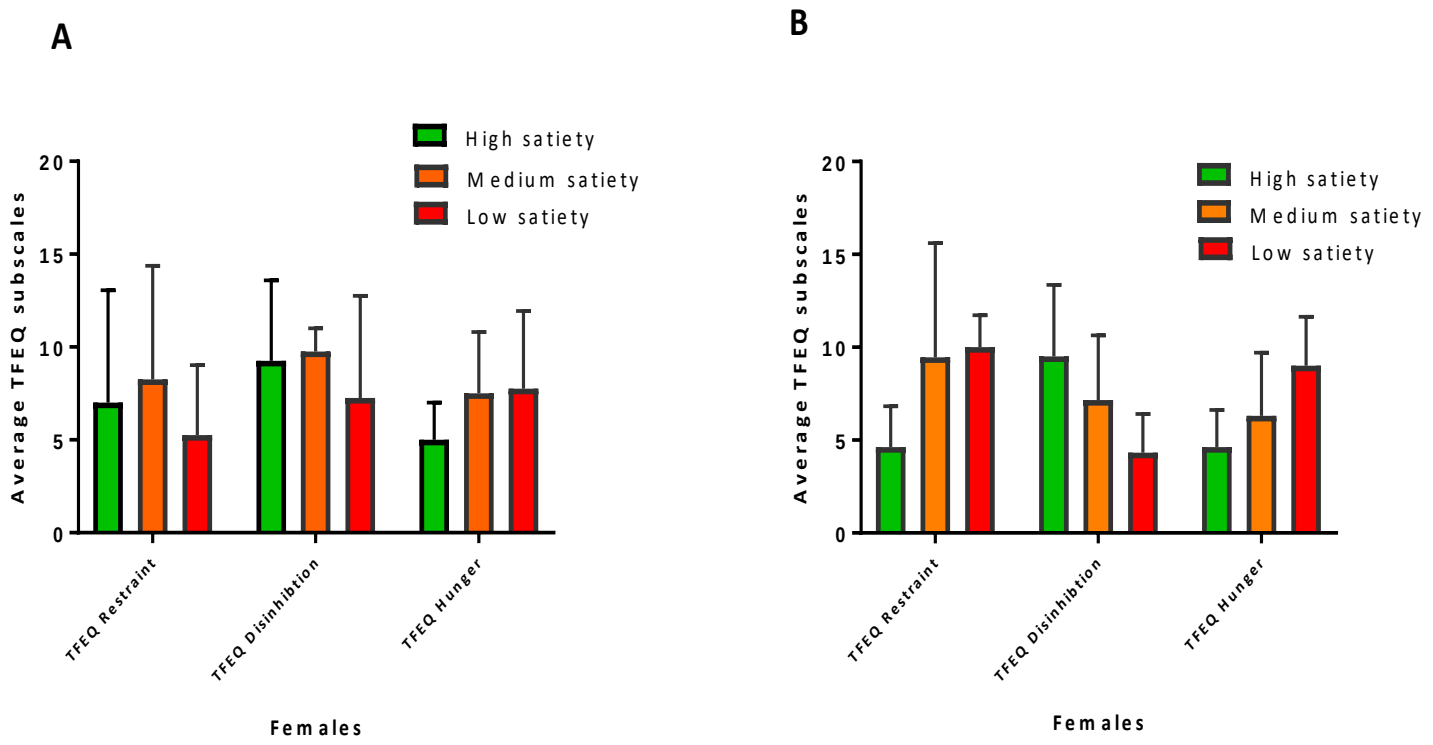

Figure 4-17 : Average TFEQ subscales of females based on *ad libitum* lunch intake by using (A) method one (B) method two.

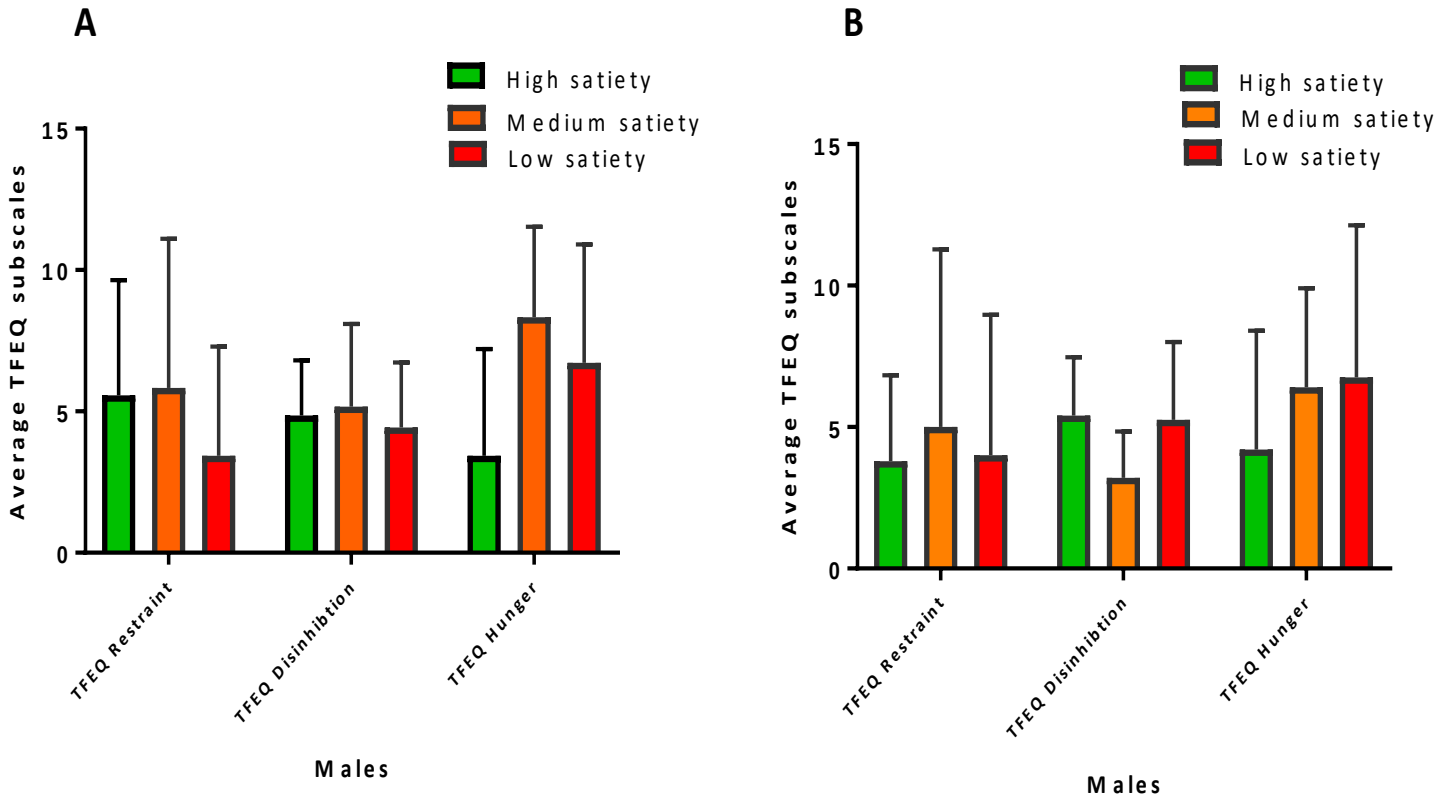

Figure 4-18: Average TFEQ subscales of males based on *ad libitum* lunch intake by using (A) method one (B) method two.

4.4 Discussion

Some people have a low satiety response to food, and therefore have a tendency to overconsume when feeding, so the present study aimed to identify satiety phenotypes in individuals, which can then be used to identify those at risk of overeating (Dalton *et al.*, 2015). The methods used in this chapter have enabled the development of heat maps to identify satiety phenotypes. The potential application of this developed method is to help professionals working in weight-loss programmes, as will be described in chapters 5 and 6. A heat map can be used as a grid to score each participant for a set of variables as 'high', 'medium' or 'low' satiety against predetermined ranges and an overall score determined. This can then be used to identify people prior to the start of a weight loss programme who may struggle to lose weight due to their reduced satiety phenotype and allow for additional specific advice to support these people.

The aim of this chapter was to develop this heat map concept, including identifying predictive variables and determining the ranges for each of these variables to use in the heat map. Potential variables were studied to determine their relationship with food intake in a controlled laboratory setting (*ad libitum* pasta lunch) and in the free-living environment (calorie intake measured by a 3-day food diary). The variables investigated were VAS appetite scores in response to a test breakfast, gut hormone concentrations and TFEQ subscale scores.

4.4.1 Participant characteristics at baseline

The current study showed males consumed more fat than females and that females tended to crave CHO more than males. These results are in line with Kiefer, Rathmanner and Kunze (2005) who reported the same findings. Females had significantly higher scores for Restraint and Disinhibition in this current study, which has been described previously by Provencher *et al., (2003*). The possible explanation for this may be that women in our society have more often greater concern about dieting and their body weight than men. It appears that women consciously restrict their food intake more than men to control their body weight or to promote weight loss, even if their BMI is in the normal range (Carmody *et al.,* 1995).

4.4.2 VAS, TEI, macronutrient intake and *ad libitum* lunch intake

4.4.2.1 Relationships of fasting, post-breakfast, thirty minute and AUC of VAS and TEI

In the present study based on the different assessment time points of the VAS appetite score measures, people who had higher scores for VAS fasting prospective food intake and AUC fasting composite appetite score consumed more energy according to their food diaries. All VAS measures (fasting prospective food intake, AUC fasting composite, post-breakfast hunger, 30 min hunger and 30 min desire to eat) were strongly correlated with TEI in females, but this effect was not as strong in men. Although the data showed that VAS scores in females at the fasting, post breakfast and 30 min time points were significantly correlated with TEI, there was no difference in correlation between males and females in VAS post-breakfast hunger and 30 min fullness.
These finding agree with Drapeau *et al.,* (2019) who found people with low satiety consumed more TEI measured by their food diary.

These findings are partly in accordance with a previous observation by Drapeau *et al.,* (2007) that reported fasting hunger, fullness and desire to eat scores did not correlate with TEI in females. However, they found no significant correlation between fasting prospective food intake scores and TEI in females. Moreover, VAS fasting for 4 questions was correlated with TEI in males, in this current study again in agreement with findings by Drapeau *et al.,* (2007). The possible explanation of differences could be sample size, which was larger in the Drapeau *et al.,* (2007) study with 139 females, whereas in the present study it was 26. Another explanation could be the total energy consumption according to the food diary; in Drapeau *et al.,* (2007) the average total energy consumed was 2,375 kcal, whereas in the present study it was 1,532 kcal. A further explanation may be that the participants were classified as obese in the Drapeau *et al.,* (2007) study and in the present study participants were normal weight or overweight. Obese and normal weight individuals have different eating behaviours, obese people usually under report consumption but in the present study normal weight participants showed under reporting compared with the Drapeau *et al.,* (2007) study, the reason behind this may be that teenagers focus on their body shape that could explain the differences.

In contrast to the present study Doucet *et al.,* (2003) reported that fasting hunger, fullness, desire to eat and prospective food consumption did not predict reported EI. The possible explanation may be body weight status in the participants in the Doucet *et al.,* (2003) study, which included 19 people with obesity, whereas the present study typically recruited people who were normal or overweight. Further, age may be an influencing factor in which the average age for men was 44 years and 39 years for women in Doucet's study, compared to the average age for men was 30 and women was 21 in this study. Older adults have been reported to experience less hunger than their younger adult counterparts (Chapman *et al.,* 2002; Hays and Roberts, 2006).

When the VAS scores for the assessments over the whole morning were combined as the AUC, similar results were found with people who have high hunger scores typically eating more energy as reported in the food diaries. AUC VAS appetite scores were strongly correlated with TEI in females, but this effect was not as strong in men. Two previous studies agreed with this finding where Drapeau *et al.,* (2007) included men (n =176) and women (n = 139) found that there was a strong positive correlation between AUC desire to eat and AUC prospective food and TEI as measured using the food diary in females. Douce*t et al.,* (2003) showed that AUC 'hunger' AUC 'fullness' 'desire to eat' and AUC 'prospective food consumption' were not significantly correlated with reported 24 hour EI in males.

4.4.3 Relationship of SQ, VAS and TEI

The SQ was investigated as a measure in this current study, as other published studies have used this approach and it is less time consuming than the AUC measurements. SQ is a measure of the satiating effect of foods relative to their energy content. The current study showed no correlation between SQ for each of the four questions as well as the SQ composite score and TEI. This agrees with Drapeau *et al.,* (2007) who also found no significant correlation between SQ for each question and TEI reported in food diaries. McNeil *et al.,* (2014) showed the SQ of prospective food consumption and fullness predicted TEI and percentage CHO intake, respectively, when compared with food diary records. The r values in this study compared to the results reported here, but the McNeil *et al.*, (2014) results were significant and in the present study they were not due to it being under powered with a lower participant number.

4.4.4 Relationship between fasting VAS and macronutrient intake

In the current study, people who were hungrier reported a lower percentage of fat in their diet. There was a negative correlation between fasting scores for all 4 questions and fat intake in females and males. Males who were hungry had a higher percentage of CHO in their diet. This finding agrees with Beasley *et al.,* (2009) who found appetite ratings are higher on a CHO diet compared with a protein diet.

4.4.5 Relationships SQ of VAS macronutrient intake of food diary

In the present study SQ was not related to the percentage CHO intake. Based on the published literature only one study examined SQ and macronutrient intake. This finding of the current study agrees with McNeil *et al.,* (2014) who showed that SQ 'prospective food consumption' and 'fullness' predicted TEI and percentage CHO intake, respectively, when measured with food diaries.

4.4.6 VAS and *ad libitum* lunch intake

4.4.6.1 Fasting, post-breakfast and thirty minute VAS and *ad libitum* lunch intake

In the current study, people who were hungrier based on their VAS appetite measures at the different time points consumed more at the *ad libitum* lunch. VAS scores were strongly correlated with *ad libitum* lunch intake in females but this effect was not as strong in men and did not reach significance. Females who had low scores on the VAS 30 min fullness measure, consumed a lower amount of *ad libitum* lunch intake which was also seen in males. The interpretation of this difference could be the small sample size, such that the study is underpowered. The results of the present study corroborate a previous study highlighting that prospective food consumption predicted forthcoming EI (Barkeling *et al.,* 1995). In addition, the findings also show 'desire to eat' scores predicted forthcoming EI that was also found by Barkeling *et al.*, (1995).

Gibbons *et al.,* (2013) agreed with the present study where they found changes in ghrelin and hunger immediately before the lunch meal (at 180 minutes), which was positively associated with EI (hunger, high-fat/low-CHO: r= 0.461, P=0.073 and high CHO/ low-fat: r = 0.550, $P < 0.05$) However, there was no difference in *ad libitum* energy intake at the standard lunch meal after either the high-fat/low-CHO (947 kcal)

or high-CHO/low-fat (939kcal) breakfasts. A greater suppression of ghrelin and hunger was associated with the smaller meal size.

The current study demonstrated that there was a strong correlation between AUC VAS scores and *ad libitum* lunch in females. Females consumed more based on AUC hunger and AUC prospective food intake, this effect was not as strong in men. The AUC composite score did not correlate with *ad libitum* lunch intake in females and males. These results are in contrast with those of Deighton, Frampton and Gonzalez (2016) who found AUC composite appetite scores were more strongly correlated with *ad libitum* lunch intake in males. A possible explanation for differences in results may again be due to sample size as the in Deighton, Frampton and Gonzalez study it was 10 participants, which was smaller than in the current study.

4.4.7 SQ of VAS and *ad libitum* lunch intake

The present study revealed that there was no significant correlation between *ad libitum* lunch intake and SQ composite score and SQ of each of the four questions in the VAS appetite score. These results agree with McNeil e*t al.,* (2014) who reported that SQ fullness and SQ prospective food consumption were better predictors for subsequent EI. They found an increase in 'fullness' and 'prospective food consumption' predicted overall decreases in energy at lunch at 3 and 5 years.

The present findings contrast with previous research that found that SQ for fullness was the best predictor of *ad libitum* energy intake, compared to 1 h AUC (Drapeau *et al.,* 2005 and Drapeau *et al.,* 2007). A possible explanation for SQ related to findings in this current study did not predict *ad libitum* lunch intake, was that the current study was carried out on normal weight and overweight participants and Drapeau *et al.,* (2007) study included people with obesity as well as there being a difference in ages in the two study cohorts with Drapeau *et al.,* (2005) and Drapeau *et al.,* (2007) studies including subjects with 20-50 age range, whereas the present study majority of participants were students, which could explain these differences. Lastly one explanation for the differences may be the calorie content in the breakfast test meal, males were given different quantities compared to females, in both studies. Males and females in Drapeau *et al.,* (2005) and Drapeau *et al.,* (2007) consumed a higher calorie

breakfast test meal than in the present study and both sexes consumed different amounts of energy. In Drapeau *et al.,* (2005) and Drapeau *et al*., (2007) the calories for breakfast were 598·7 kcal for women and 733·1 for men. Whereas in the current study males and females consumed the same energy, which was 327kcal. The interpretations for this could be a high energy test meal can affect SQ scores because SQ measures the satiating effect of foods relative to energy content. This high EI may make participants feel less hungry for a longer time period than the lower energy consumed in the present study.

4.4.8 The correlation between subscales of TFEQ and food intake (*ad libitum* lunch and TEI and macronutrient intake)

4.4.8.1 Subscales of TFEQ and TEI and macronutrient intake

A limited number of studies have reported interactions between Disinhibition and Restraint scores in association with EI. Only a few studies have examined TFEQ Hunger for predicting *ad libitum* lunch intake or macronutrient intake from food diaries. The present study found no significant correlation between TFEQ Hunger and TFEQ Disinhibition and TEI, measured by a food diary in either females or males, however, females who had high scores of TFEQ Restraint consumed less total energy. In contrast to the results of current study, French *et al.,* (2014) reported a strong correlation between TFEQ Hunger, TFEQ Disinhibition and TFEQ Restraint and TEI. The interpretation as to why French *et al.,* (2014) study results differed from the current study was the sample size, which in the French study was over 2000 and allowed detection of people with different satiety levels. Another potential interpretation was age, French *et al.,* included older people, which affects food consumed by participants as food intake reduces with age as mentioned in earlier sections.

Moreover, a previous study has observed positive associations between hunger and EI (Provencher *et al.,* 2003). They reported that people with chronically high levels of hunger are more susceptible to overeating compared with those who do not report being often hungry. The mean for age, BMI, weight, percentage of macronutrients and daily energy were larger than the present study which may have led to different results.

The explanation why the present study differs from the finding of Provencher *et al.,* (2003) was the sample size was much bigger than the present study with 401

participants. Another explanation was mean age, BMI, weight and percentages of macronutrients and daily energy was larger than the present study, which may lead to different results.

4.4.8.2 Subscales of TFEQ and macronutrient intake

The findings of the current study agree with Lindroos *et al.,* (1997) who showed no correlation between TFEQ Hunger and daily EI of food diary and percentage of macronutrients (protein, CHO and fat) in a non-obese group. However, in the same study there was a positive association between TEI and hunger in the obese group. Moreover, there was a strong relationship between TFEQ Disinhibition and TEI based on food diaries. A potential explanation may be that there were 179 people with obesity in Lindroos *et al.,* (1997) study compared to much lower numbers in the current study. Disinhibition has been related to higher BMI and EI (Provencher *et al.,* 2003; Bellisle *et al.,* 2004; Dykes, *et al.,* 2004; Bryant *et al.,* 2008). Moreover, it has widely been reported that people with obesity and overweight individuals have higher disinhibition scores compared to normal weight individuals (Provencher *et al.,* 2003). Normal BMI in participants in the present study affects TFEQ subscales to predict macronutrient intake.

4.4.8.3 The correlation of subscales of TFEQ and *ad libitum* lunch intake

Findings of the current study found no correlation between any of TFEQ subscales and *ad libitum* lunch intake. This observation is concordant with previous studies that showed no association between TFEQ Disinhibition and increase *ad libitum* lunch intake (Dalton *et al.,* 2015). One reason for the lack of correlation in the present study could be BMI, most participants had normal BMI. It has been reported that high BMI is correlated with high TFEQ Hunger and Disinhibition and food intake (Provencher *et al.,* 2003; Bellisle *et al.,* 2004), therefore a similar study carried out in people with obesity might find a relationship between TFEQ scores and lunch intake. The present study agreed with Bellisle and Dalix (2001) which was carried out on 41 healthy women with normal weight. They reported TFEQ Hunger and TFEQ Disinhibition did not correlate with lunch intake.

In contrast to the present study, a lab based experimental study carried out by Westerhoefer *et al.,* (1994) found high disinhibition with high restraint was associated with higher EI in an ice cream *ad libitum* test and 200 ml of milkshake. This difference

in results could be explained by sample size in Westerhoefer *et al.,* (1994) study which was 141. Another explanation may be the palatability of the food. In Westerhoefer *et al.,* (1994) study *ad libitum* test was ice cream and 200 ml of milkshake and it was highly palatable food and high fat and sugar, whereas in the present study it was pasta.

4.4.9 The correlation of hormones and food intake (*ad libitum* lunch intake and TEI and macronutrient intake)

4.4.9.1 Gut hormones and TEI and macronutrient intake of food diary

The results of the present study showed that females with higher scores of PYY reported lower TEI in their food diaries and males who had higher PP consumed less energy. The present study also reported that males with higher PP had a lower percentage of protein and higher percentage of fat in their diet. Fasting ghrelin and fasting GLP-1 showed no significant correlation with total energy and macronutrient intake in both sexes. In contrast to the present study, Ratliff *et al.,* (2010) found that there was no correlation between reduction in macronutrient intake and PPY and GLP-1. The explanation may be age and gender of cohort as Ratliff *et al.,* (2010) included only males aged 20-70 years. Several studies have suggested that age decreases the circulating acyl ghrelin levels (Rogers *et al.*, 2016).

4.4.9.2 Gut hormones and *ad libitum* lunch intake

The present study data indicated that there was no significant correlation between fasting PP, ghrelin and GLP-1 and PYY and *ad libitum* lunch intake. Moreover, AUC of gut hormones (AUC PP, AUC ghrelin and AUC GLP-1 and PYY) were not significantly correlated with *ad libitum* lunch intake. The finding of the current study is in accordance with Chowdhury *et al.,* (2016) who observed no significant correlation between fasting ghrelin at baseline and *ad libitum* lunch intake. They indicated that fasting reduced satiety hormone responses to a subsequent lunch meal but counterintuitively also reduced concentrations of the appetite-stimulating hormone ghrelin during the afternoon, relative to lunch consumed after breakfast. This may be explain why fasting ghrelin in the present study did not predict *ad libitum* lunch intake. Moreover, partially, this finding agrees with a previous study carried out by Votruba *et al.,* (2009) who found daily fasting ghrelin concentration did not predict *ad libitum* lunch intake over 3 days. The explanation of why fasting ghrelin concentration did not

predict *ad libitum* lunch intake may be because plasma ghrelin levels rise before the start of a meal and fall again rapidly after the initiation of the meal in humans (Cummings *et al.,* 2004; Cummings *et al.,* 2001).

In contrast to the present study, Ratliff *et al.,* (2010) used a crossover design with each participant having two different breakfasts in a randomized and balanced order separated by 1 week. Both test breakfasts were isocaloric; the egg-based breakfast (EGG) consisted of 3 scrambled eggs and 1.5 pieces of white toast. The bagel-based breakfast (BAGEL) consisted of 1 white bagel, 1/2 tablespoon of low-fat cream cheese, and 6 oz of low-fat yogurt. This study reported that reduction in ghrelin and ghrelin AUC after an egg-based breakfast, which consisted of 3 scrambled eggs and white toast, resulted in reduced EI at an *ad libitum* lunch buffet. The possible explanation for differences of both studies could be the breakfast test meal which contained high protein, as previous studies showing that high protein decreases the concentration of ghrelin, which is as satiety effect (Veldhorst *et al.,* 2009). In contrast to results of the present study, Salbe *et al.,* (2004) found that there was a negative correlation between fasting ghrelin and *ad libitum* food intake. Salbe *et al.,* (2004) provided butter, peanut butter, cream cheese, jams, salad items and dressings, crackers, bread, tortillas, Indian fried bread, spices and salsa, orange juice, apple juice, milk, and a six-pack of soda for 3 days as food intake. The possible explanation could be palatability of food and variety in food. *Ad* libitum food intake in Salbe *et al*., (2004) was much more palatable and varied than the present study, so participants can eat without becoming bored with the food.

In contrast to the present study, Gibbons *et al.,* (2013) found that GLP-1 and PYY increased more after a high-fat breakfast meal. GLP-1 was negatively associated with EI but the PYY profile was not associated with food intake. A possible explanation as discussed in previous section was sample size in the Gibbons *et al.,* (2013) study which was 16 participants, including 11 females; it was smaller than present study. Moreover, the test meal consisted of yogurt, honey and fruit accompanied by a choice of tea or coffee (about 590 kcal), which could make participants not feel hungry until lunch time. Moreover, Gibbons *et al*., (2013) study included overweight and obese individuals and in the present study participants had normal weight. A decrease in total fasting PYY, or

no changes in fasting, or impaired postprandial PYY have been reported in overweight people (Moran *et al.,* 2007). This may explain the differences with the current study.

4.4.10 Food craving, TEI and macronutrient intake and *ad libitum* lunch intake

In the present study, food cravings did not predict *ad libitum* lunch intake. However, food craving scores were correlated with intake of some macronutrients, as measured by the food diary. Male participants with lower scores for fast food craving consumed more energy. Moreover, males with higher scores of sweet food craving consumed more protein. The interpretation could be related to sex because of differences in food cravings with men reporting higher craving for savoury foods (e.g. meat, fish, eggs), whereas women report more craving for sweet foods (e.g. chocolate, pastries, ice cream. Several studies have shown that men may crave different types of sweets than women do (e.g. sugar sweetened beverages, but not chocolate) (Zellner *et al.,* 1999; Weingarten and Elston, 1991).

These findings contrast to those reported by Chao *et al.,* (2014) who found significant positive relationships between specific categories of food cravings and habitual intake of those foods, fast food craving was association with sweet, high fat and CHO. Cravings for sweets were significantly associated with intake of sweets and high fat and fast food.

The potential explanation could be body weight as Chao *et al.,* (2014) included overweight and obese participants and there is evidence that people with obesity have higher frequencies of food cravings than normal weight individuals (Abilés *et al.,* 2010).

4.4.11 Heat map

Previous studies have been studied low satiety phenotypes. Drapeau *et al.,* (2005) used 1h post meal AUC and the SQ as predictors of EI and Drapeau *et al.,* (2007) also used VAS (fasting, fullness, desire and prospective) to identify low satiety phenotype. Dalton *et al.,* (2015) also examined SQ to identify low satiety phenotype. They found that a low SQ was associated with a greater implicit wanting for high fat foods and higher scores on the TFEQ Disinhibition subscale.

In the current study, a heat map based on food diaries predicted hunger of participants more than *ad libitum* lunch by method one. The heat map was used in this chapter to identify individual phenotypes with different independent variables. Scores of these variables were used in chapters 5 and 6 to predict phenotype in a clinical population and to help professionals at these clinics with a visual presentation sheet to identify reduced satiety people. In this chapter a heat map designed with independent variables used linear regression to predict which variables have the larger r value and those variables will be applied in chapters 5 and 6. These variables are fasting of VAS for4 questionnaire, post breakfast desire to eat, TFEQ hunger, SQ composite of appetite score and fasting PP.

The key findings of the heat maps, heat map based on food diaries predict more low satiety people than heat map based on the *ad libitum* by using method one. There was no significant difference in average BMI in males and females by using methods one and two. There were no significant differences in average TFEQ scores for low, medium and high satiety participants based on TEI and *ad libitum* lunch, except TFEQ disinhibition scores females based on TEI and TFEQ Restraint scores females based on *ad libitum* lunch. In heat map participants nut31 and N26 who were high satiety ate less energy in *ad libitum* and food diary.

4.4.12 Summary

In conclusion, in the current study VAS appetite measures are the best tool to predict *ad libitum* food intake and food diary intake in a laboratory setting. Because these measures showed a strong correlation with *ad libitum* food intake and food diary intake. Gender impacted on the results of this study; females' VAS scores showed a greater correlation with total energy, macronutrient intake and *ad libitum* than males. TFEQ subscales did not predict TEI and macronutrient intake, measured by food diary and *ad libitum* food intake. The results of the present study showed that females with higher levels of PYY reported lower TEI in their food diaries. Males who had higher PP consumed less energy and reported less percentage of protein and higher percentage of fat in their diet. Food cravings scores in males were negatively correlated with TEI. Sweet food cravings were positively correlated with protein intake and fast food

cravings was negatively correlated with fibre intake in males. Food craving data results did not correlate with *ad libitum* food intake.

The possible explanation why fasting ghrelin, PP and GLP-1 did not predict *ad libitum* pasta intake may be the characterisation of the low satiety phenotype as realized in the laboratory environment, which is clearly different from natural real-life situations. As a result, it is possible that environmental factors could play a role in the expression of the low satiety. The duration of the trial day of this study was approximately 3 hours to achieve all blood sample, with more participants VAS and *ad libitum* lunch meal. Results of this chapter show that no additional information was gained by using AUC compared to measures that take a shorter time, thus in the clinical study, a shorter duration with one fasting sample collected would allow more participants to be recruited, if they knew just one blood sample would be collected and a short waiting time. The fasting sample of VAS shows best predicted for food intake to assess study phenotype. Moreover, even the AUC alone shows a strong prediction with linear regression analysis but it was excluded from the heat map because duration of setting for trail in clinic is less than 3 hours and this duration may make people who are waiting for 3 hours feel more hunger than setting shorter.

4.4.13 Study strengths and limitations

This study had strengths and limitations. The strength of the study was that it used multiple measurements to predict satiety phenotype. The limitations of the study were the small number of participants and the study sample consisted of students so the age range was limited. The results could differ in other populations because people eat less and make different food choices as they get older (Drewnowski and Shultz, 2001). The other limitation is that only 1-day assessments of all measurements were taken, which does not account for normal day-to-day variations in these measurements.

Chapter 5

Evaluation of visual analogue scale, fasting plasma gut hormones and eating behaviour traits prior to participation in a structured Tier 3 weight-loss programme

5 Introduction

Overweight and obesity are major public health concerns worldwide and more than forty percent of all adults struggle to control their body weight with the use of weight loss strategies (Santos *et al.,* 2017). Many researchers have focussed on identifying effective weight loss treatments to benefit adults who are overweight or obese. Despite this effort, long-term outcomes vary substantially across individuals. No single intervention works well for everyone, thus the identification of individual factors that predict successful weight loss could help to enhance personalised interventions. Assessment of these factors at baseline before the intervention could allow for tailored delivery of extra support for individuals who are less responsive to treatment (James *et al.*, 2018).

Recently a review by Carraça *et al.,* (2018) summarised the evolution of research on pre-treatment predictors of weight loss over the last decade. The most consistent pre-treatment predictor of weight loss, according to the literature, was fewer previous weight loss attempts. In this review several potential factors were identified as non-significant predictors including: binge eating, eating self-efficacy or cognitive eating restraint, suggesting that overweight/obese individuals might successfully manage their weight, even if initially presenting unfavourable scores on these predictors. However, more high-quality research is required to draw more reliable conclusions about the role of these factors.

Considering the lack of success in the long term treatment of obesity, there is still a need to investigate more reliable markers of predictors of body weight loss (Drapeau *et al.,* 2007).

5.1 Factors previously studied in predicting weight loss

5.1.1 VAS to measure subjective ratings of appetite

Subjective ratings of appetite, as measured using VAS, have been shown in some studies to predict subsequent EI and changes in body weight and are frequently used in studies assessing appetite (Flint *et al.,* 2000; Drapeau *et al.,* 2007).

5.1.2 Eating behaviours

Behavioural traits are another variable which have been reported to predict weight loss. Eating behavioural traits assessed by tools such as the TFEQ have been studied in weight loss intervention trials. Three eating behaviour traits: Restraint, Disinhibition and Hunger have been used as predictors of weight loss. The definition of these traits was discussed in chapter 1. Stunkard and Messick (1985) have shown an interaction between Restraint and Disinhibition and predicted BMI, high BMI associates with lower Restraint and higher Disinhibition scores. Disinhibition has been shown to be the strongest predictor of increased BMI and weight gain over time and the development of obesity (Bryant, King and Blundell, 2008; Williamson *et al.,* 1995).

Previous studies have focused on eating behaviours in short- and long-term weight loss programmes in several environments, including research environments (Teixeira *et al.,* 2010; Foster *et al.,* 1998), clinic-based (Byrne, Barry and Petry, 2012; Dalle *et al.,* 2009), and community based programmes (McGuire *et al.,* 2001). In most studies, the higher scores at baseline of restraint were positively correlated with greater weight loss. In addition, it has also been shown that in individuals taking part in weight loss treatments, restraint increases with weight loss. Disinhibition has been shown to be inversely associated with weight loss, with greater reductions in disinhibition associated with greater weight loss (Batra *et al.,* 2013) However, examining the Hunger subscale of the TFEQ for predicting body weight loss has had little attention in the literature. A negative correlation with weight loss has been shown with individuals who had higher scores on the TFEQ Hunger scale who were struggling to lose weight and lower baseline Hunger subscale scores were associated with greater weight loss (Batra *et al.,* 2013). This subscale of TFEQ refers to the desire to eat in response to physiological signals and external cues and higher scores on this subscale may indicate individuals who have a low satiety phenotype, leading to increased hunger and thereby identify those who may struggle to lose weight.

5.1.3 Gut hormones

Another variable studied to predict weight loss is the concentration of plasma gut hormones. Body weight is controlled by complex physiologic systems in which hormones signal body energy stores and nutrient intake to the CNS pathways

controlling energy homeostasis. Gut hormones play an important role in the regulation of body weight (Sumithran *et al.,* 2011) and may be involved in the pathogenesis of obesity (Le Roux *et al.,* 2006; Batterham *et al.,* 2003a). Much research has focussed on gut hormones including ghrelin, GLP-1, PYY and PP that fluctuate episodically and are believed to have contrasting actions on appetite control (Cummings *et al.,* 2002; Batterham *et al.,* 2002). Episodic hormones are those that change during the day, particularly before and after meals. Fasting ghrelin concentrations have been shown to be lower in people with obesity, when compared with normal weight controls (Cummings *et al.,* 2002). This lower in plasma ghrelin level may contribute to increased appetite and weight gain (Cummings *et al.,* 2002). It has been shown that the concentration of plasma PP increases following a meal and the action of PP involves a reduction in food intake (Troke, Tan and Bloom, 2014). As a result, it plays a vital role in the regulation of body weight (Karra and Batterham, 2010) and has been shown to be reduced in people with obesity. GLP-1 is of relevance to appetite and weight maintenance because it has actions on the GI tract, as well as being involved in the direct regulation of appetite, decreasing hunger (Troke, Tan and Bloom, 2014). PYY has been reported to reduce food intake and prompt weight loss (Mishra, Dubey and Ghosh, 2016) and is reduced in overweight people in total fasting (Moran *et al.,* 2007).

Several studies have examined these gut hormones in blood to predict weight loss. Koska *et al.,* (2004) demonstrated that fasting PP levels were negatively associated with weight loss and Hainer *et al.,* (2008) found there was an inverse correlation between baseline PP levels and body weight change. In contrast, Polsky *et al.,* (2013) have observed that baseline plasma ghrelin and PP and GLP-1 did not predict weight loss in subjects who participated in a 16-week intervention.

The present study was designed to examine subjective measures of appetite, TFEQ scores and plasma gut hormone concentrations prior to a weight loss programme in order to identify people who were less likely to succeed in weight loss. If it is possible to identify people with a low satiety phenotype at the beginning of an intervention, additional support and strategies can be used to improve the weight-loss outcome for this subgroup.

5.1.4 Rotherham Institute for Obesity

The current study was carried out in The Rotherham Institute for Obesity (RIO), which is a unique and specialist centre for the management of weight problems with a multidisciplinary approach to reducing and maintaining weight loss. RIO brings together all the National Health Service (NHS) approved and evidence-based methods for weight loss into one primary care based centre that maximises the chances for successful weight loss.

RIO delivers Tier 3 multidisciplinary weight management services to manage weight problems via providing specialists that can offer different approaches. This includes dedicated Obesity Specialist Nurses, Healthcare Assistants, Dietetics input for complex dietary needs, and "Rotherham Cook and Eat" skills education, Talking Therapies including psychological input and support, Exercise Therapists to offer a personalised training programmes and facilities to provide group work for exercise, therapies and nutritional advice.

5.1.5 Aims

The main aim of the present study was to develop methods to identify individuals who may struggle to lose weight on a weight management programme due to their reduced satiety.

Specific objectives were:

1- To investigate the relationship between VAS appetite rating scores and subsequent weight loss.

2- To investigate the relationship between fasting concentrations of plasma gut hormone concentrations and subsequent weight loss.

3- To investigate the relationship between TFEQ subscales and subsequent weight loss.

4- To combine these data to investigate whether it is possible to identify individuals who struggle to lose weight due to reduced satiety.

5.2 Materials and methods

Study design, setting, anthropometry, questionnaires, test meal, assessments of appetite, blood sample collection, laboratory methods, gut hormone analysis are described in the relevant sections in the general methods chapter 2.

The timing and frequency of completed VAS appetite scores in chapter 4 was 3 hours and 5 times, whereas in this study the timing was one hour and 15 minutes and VAS appetite scoring completed respectively four times. This was chosen for 3 reasons 1) Fasting, post and 30 min VAS appetite scores showed the strongest correlation with total energy and *ad libitum* intake 2) Following previous work by Dalton *et al*., (2015) measurement of the SQ in 75 minutes post-breakfast was established and 3) Reduced participant burden making the study more convenient for participants.

The results from chapter four showed that the fasting blood sample provided the most useful information, therefore in this study just one finger prick blood sample was taken. The advantage of this was that it reduced the cost of the analysis and made it more likely that people would be willing to take part as they only had to give one blood sample. It also fitted well with the as participants at RIO do not provide venous blood samples as part of their routine care.

5.2.1 Participants and recruitment

Thirty-four people with obesity and overweight were enrolled in this study prior to starting a weight loss programme. Eight participants dropped out from the study; of these, four participants signed up for the RIO programme on the first day but did not attend, and four took questionnaires to complete at home and made an appointment to give a blood sample but then did not come to the appointment. So, this study included twenty-six (17 females 9 males) participants. Twenty two of the participants gave blood samples for hormone analysis. Four participants just completed the questionnaires and did not agree to blood sample collection. Twenty six participants completed up to one month and thirteen participants carried on for three months.

Exclusion criteria were if participants were unwilling or unable to provide informed consent to undertake the study and if they disliked or were allergic to the foods used in the study. Participants were aged between 27-77 years (56.6 ± 12.1). All participants

were recruited from RIO, Rotherham, UK. Ethical approval for the study was granted by the Integrated Research Approval System (IRAS) (ID/208879) (Appendix 14).

5.2.2 Blood sample and VAS

A fasting blood sample was collected when participants first arrived in the morning at RIO after a 12 hour overnight fast. Participants filled out VAS appetite scoring before consuming a test breakfast and another VAS appetite score was completed at 15, 45 and 75 minutes post consumption of breakfast.

The test breakfast consisted of cornflakes, milk and juice as described in the methods section in chapter 2. The VAS appetite questions were used to evaluate sensations of appetite measured hunger, fullness, desire to eat and prospective food intake. Blood samples were collected into ice-chilled tubes containing protease inhibitors after centrifugation and 100 µL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Plasma concentrations of acylated ghrelin, PYY, PP and GLP-1 concentrations were measured as described in chapter 2.

5.2.3 Statistical analysis

All data were analysed using the Statistical Package for the Social Science (SPSS) software version 24.0 (IBM) for Windows (SPSS Inc., Chicago, IL, U.S.A.) and Prism version 7.03 (GraphPad Soft-ware Inc., La Jolla, CA). Correlation coefficients were used to assess relationships between variables. T-tests were used to assess sex differences in age, height, body mass, BMI and TFEQ. Repeated measures one way ANOVA was used to determine whether there were any statistically significant differences between females and males for VAS appetite scores over time.

AUC composite appetite scores were calculated using the equation hunger score+ desire score + prospective food intake score + (100-fullness score) (Hill, Blundell, 1982).

5.3 Results

5.3.1 Baseline characteristics of the study participants

The baseline characteristics of all the participants are displayed in table 5.1. There was a significant difference in TFEQ restraint between males and females, with females having higher scores on this measure.

Table 5-1 : Baseline characteristics of the study participants

Variables	All (n=26) Mean ± SD	Females (n=17) Mean ± SD	Males (n=9) Mean ± SD	t	*P*	d
Age (y)	56.7±12.5	53.7±14.1	61.3±7.1	-1.517	0.142	0.685
Height (m)	166.8±10.1	163.5±9.7	173±7.9	-2.407	**0.018**	1.080
Body mass (kg)	114.8±17.5	113.6±16	117.00±21	-0.457	0.652	0.179
BMI (kg/m^2)	41.1±5	42. 4±4.7	39.4±5.8	1.465	0.165	0.532
WC (cm)	124.6±11.5	123.5±10.7	126.7±13.2	-0.668	0.510	0.265
TFEQ Hunger	5.4±3.9	5.3±4.1	5.4±3.7	-0.013	0.989	0.005
TFEQ Restraint	9.4±3.2	10.4±3.2	7.1±1.9	2.481	**0.007**	1.245
TFEQ Disinhibition	7±3.7	7.1±4.1	6.9±2.85	0.158	0.876	0.078

Values are mean ± standard deviation, n=26, WC = waist circumference, BMI = body mass index, $p < 0.05$ for the differences by between males and females. d= Cohen's d.

5.3.2 Percentage weight loss after one month

Figure 5.1 shows individual percentage weight loss after one month. Twenty four of the participants lost weight (17 female and 7 males) and two males gained weight. There were no differences in average weight loss between females and males. Average weight loss for females was 2.10 kg and SD=2.87, average weight loss for males 2.11kg and SD=1.89.

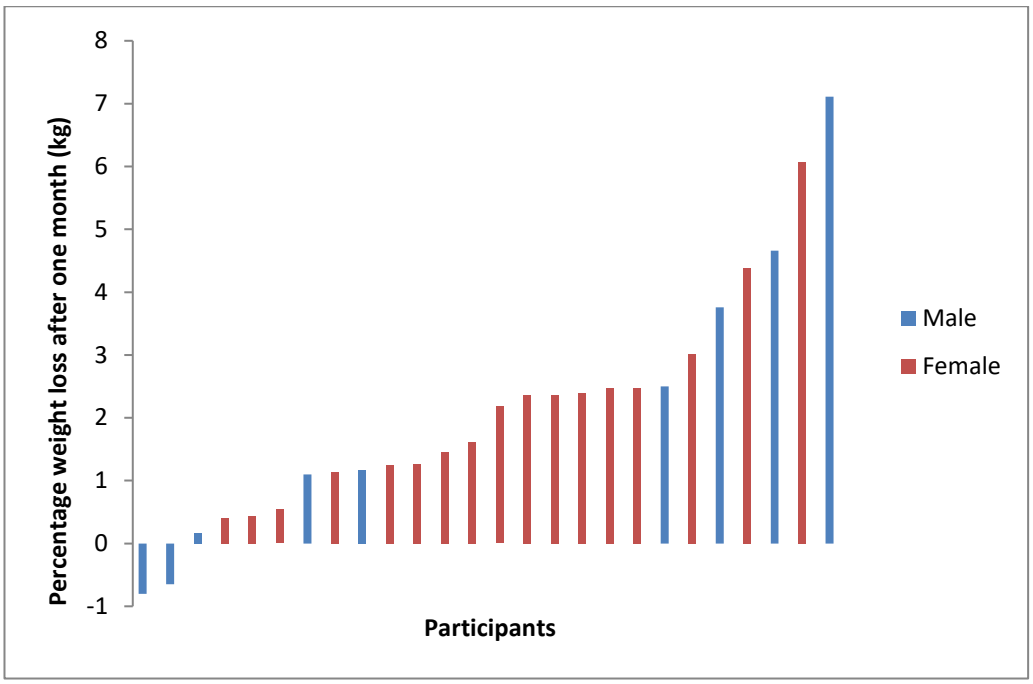

Figure 5-1: Individual percentage weight loss and weight gained after one month on Advice diet and regular exercise.

5.3.3 Percentage weight loss after three months

Figure 5.2 shows percentage weight loss after three months. 13 participants lost weight (9 female and 4 males) over three months. Average weight loss for females was 4.731 kg and SD= 3.21, the average weight loss for males was 2.51kg and SD= 0.97.

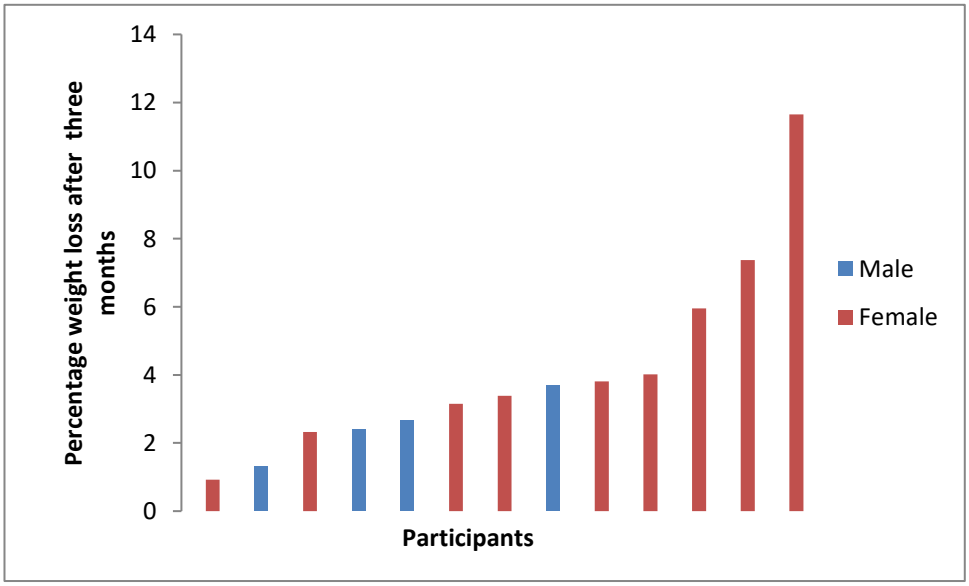

Figure 5-2 : Percentage weight loss after three months on Advice diet and regular exercise.

5.3.4 VAS scores versus time post breakfast, during the test morning.

Figure 5.3 shows mean and SD of VAS appetite scores for females and males. Based on repeated measure one way ANOVA there were no significant differences in mean VAS scores between females and males.

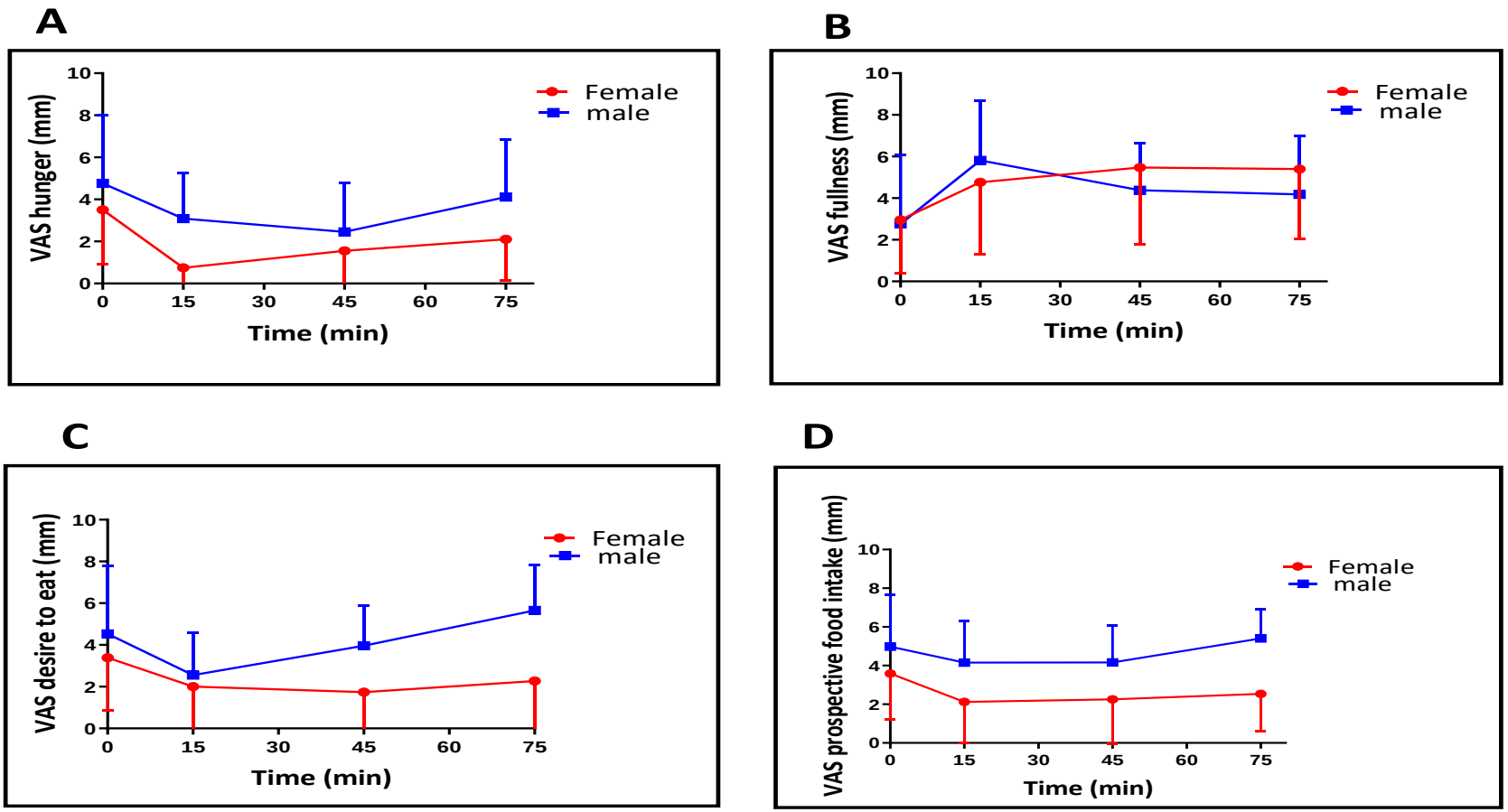

Figure 5-3 : The VAS scores for hunger (A), fullness (B), desire to eat (C) and prospective food intake (D) versus time post breakfast, during the test morning. p= 0.07 for hunger, p= 0.456 fullness, p= 0.190 desire to eat and p=0.083 prospective food intake.

5.3.5 VAS appetite scores and subsequent weight loss

5.3.5.1 Relationship between VAS scores (different time points, AUC and SQ) and percentage weight loss after one month

Table 5.2 shows the relationship between VAS scores (different time points, AUC and SQ) and percentage weight loss after one month of all participants. None of the scores at individual time points, AUC or SQ of VAS were significantly correlated with percentage weight loss after one month on advice diet and regular exercise. There was an inverse correlation between the 75 min desire to eat and percentage weight loss after one month and a positive correlation between SQ desire to eat score and the percentage weight loss after one month, but these did not reach significance. In females after adjusting for age, SQ hunger (r=0-.590*, p=0.034) and SQ fullness (r=-0.555*, p=0.049) were significantly negatively correlated with percentage weight loss after one month.

When the participants were split by gender (shown in figure 5.4 (A)) the analysis showed a negative correlation in females between fasting hunger scores (r=-0.561*, p=0.037) and percentage weight lost after one month. Whereas, there was no correlation in males between fasting hunger scores and percentage weight loss after one month. Moreover, in figure 5.4 (B) there was a significant inverse correlation between VAS 30 min fullness (r=-0.574, p=0.032) and percentage weight loss after one month in females, while males showed no correlation.

Table 5-2 : Relationship between VAS scores (different time points, AUC and SQ) and percentage weight loss after one month for all participants. (N=22)

Percentage weight loss after one month	**Fasting hunger**	**Fasting fullness**	**Fasting desire to eat**	**Fasting prospective food intake**	
Spearman correlation	-.0234	-0.259	0.028	-0.015	
Sig. (2-tailed)	0.295	0.244	0.902	0.948	
Percentage weight loss after one month	**15 mins hunger**	**15 mins fullness**	**15 mins desire to eat**	**15 mins prospective food intake**	
Spearman correlation	--0.330	-0.101	-0.371	-0.254	
Sig. (2-tailed)	0.133	0.655	0.089	0.243	
Percentage weight loss (after one month)	75 mins hunger	**75 mins fullness**	**75 mins desire to eat**	**75 mins prospective food intake**	
Spearman correlation	-0.318	0.205	-0.367	0.019	
Sig. (2-tailed)	0.149	0.359	0.093	0.932	
Percentage weight loss (after one month)	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite appetite score**
Spearman correlation	-0.171	0127	-0.185	-0.118	-0.268
Sig. (2-tailed)	0.445	0.574	0.409	0.602	0.228
Percentage weight loss after one month	**SQ hunger**	**SQ fullness**	**SQ desire to eat**	**SQ prospective food intake**	**SQ composite of appetite score**
Spearman correlation	-0.175	-0.213	0.393	-0.043	-0.008
Sig. (2-tailed)	0.436	0.342	0.071	0.849	0.973

5.3.5.2 Relationship between VAS scores (different time point, AUC and SQ) and percentage weight loss after three months.

Table 5.3 represents the relationships between VAS (different time points, AUC and SQ) and percentage weight loss after three months for all participants. There was a negative correlation between the 15 mins prospective food intake (r=-0. 615*, *p*=0.044) and percentage weight loss after three months. Fasting hunger, fasting fullness, ratings at 15 mins after breakfast for hunger, fullness and desire to eat questions, 75 min desire to eat for 4 questions and AUC composite of appetite score were negatively correlated with percentage weight loss after three months and SQ desire to eat was positively correlated with percentage weight loss after three months, but none of these correlations reached significance. There were no correlations between AUC and SQ of VAS and percentage weight loss after three months.

Table 5-3 : Relationship between VAS scores (different time points, AUC and satiety quotient) and percentage weight loss after three months for all participants. (N=11)

Percentage weight loss after three months	Fasting hunger	Fasting full	Fasting desire to eat	Fasting prospective food intake	
Spearman correlation	-0.312	-0.457	-0.137	-0.034	
Sig. (2-tailed)	0.350	0.158	0.689	0.920	
Percentage weight loss after three months	**15 mins hunger**	**15 mins fullness**	**15 mins desire to eat**	**15 mins prospective food intake**	
Spearman correlation	-0.538	-0.303	-0.557	-0. 615*	
Sig. (2-tailed)	0.088	0.365	0.075	0.044	
Percentage weight loss after three months	**75 mins hunger**	**75 mins fullness**	**75 mins desire to eat**	**75 mins prospective food intake**	
Spearman correlation	-0.155	0.102	-0.366	-0.041	
Sig. (2-tailed)	0.649	0.766	0.268	0.905	
Percentage weight loss after three months e	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite of appetite score**
Spearman correlation	-0.509	0.022	-0.291	-0.270	-0.391
Sig. (2-tailed)	0.110	0.949	0.386	0.422	0.234
Percentage weight loss after three months	**SQ hunger**	**SQ fullness**	**SQ desire to eat**	**SQ prospective food intake**	**SQ composite of appetite score**
Spearman correlation	-0.299	-0.231	0.452	0.013	0.034
Sig. (2-tailed)	0.371	0.495	0.163	0.969	0.921

5.3.5.3 Relationship between VAS (different time points, AUC and SQ) and BMI change

Table 5.4 displays relationships between VAS scores (different time points, AUC and SQ) and BMI change in all participants. None of the measures at different time points, AUC and SQ of VAS correlated with percentage weight loss after one month. Fasting hunger, 75 min desire to eat and SQ desire to eat and SQ composite of appetite score were negatively correlated with BMI change and SQ fullness was positively correlated with BMI, but these correlations did not reach significance. In females, after adjusting for BMI, SQ hunger values were negatively correlated (r=0-.641, p=0.018) with BMI change. When the participants were split by gender (figure 5.5 (A)) the analysis showed a significant negative correlation between fasting hunger (r=-0.552, p=0.04) and BMI change in females. Whereas, there was no correlation found in males between VAS fasting hunger and BMI change. Moreover, in figure 5.5 (B) there was no correlation between SQ hunger and BMI change in both females and males.

Table 5-4: Relationship between VAS scores (different time points, AUC and SQ) and BMI change for all participants. (N=22)

BMI change	**Fasting hunger**	**Fasting fullness**	**Fasting desire to eat**	**Fasting prospective food intake**	
Spearman correlation	-0.354	-0.265	-0.036	-0.036	
Sig. (2-tailed)	0.106	0.233	0.873	0.875	
BMI change	**15 mins hunger**	**15 mins fullness**	**15 mins desire to eat**	**15 mins prospective food intake**	
Spearman correlation	-0.284	-0.184	-0.276	-0.189	
Sig. (2-tailed)	0.200	0.411	0.214	0.399	
BMI change	**75 mins hunger**	**75 mins fullness**	**75 mins desire to eat**	**75 mins prospective food intake**	
Spearman correlation	-0.229	0.148	-0.349	-0.005	
Sig. (2-tailed)	0.305	0.510	0.111	0.982	
BMI change	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite of appetite score**
Spearman correlation	-0.240	0.063	-0.182	-0.120	-0.268
Sig. (2-tailed)	0.282	0.782	0.416	0.596	0.228
BMI change	**SQ hunger**	**SQ full**	**SQ desire to eat**	**SQ prospective food intake**	**SQ composite of appetite score**
Spearman correlation	-0.239	0.308	0.009	-0.218	-0.310
Sig. (2-tailed)	0.284	0.163	0.968	0.329	0.161

5.3.5.4 VAS (different time points, AUC and SQ) and waist circumference change.

Table 5.5 presents relationships between VAS scores (different time points, AUC and SQ) and waist circumference change of all participants. None of the measures at different time points, AUC and SQ of VAS scores were correlated with waist circumference change. There was a negative correlation between 75 min desire to eat and waist circumference change and a positive correlation between SQ desire to eat and SQ composite of appetite score and waist circumference change, but these did not reach significance However, after adjusting for age there was a negative correlation between SQ composite appetite scores (r=0-.444*, p=0.044) and waist circumference change of all participants. Moreover, after adjusting for age, SQ composite appetite score was strongly negatively correlated (r=-0.688*, p=0.009) with waist circumference change in females. After adjusting for BMI, SQ desire to eat was positively correlated (r=0.559*) (p=0.047) with waist circumference change in females. Furthermore, in males after adjusting for BMI, SQ desire to eat was negatively correlated (r=0-.930*, p=0.002) with waist circumference change and AUC hunger (r=-.785*, p=.036) and AUC composite appetite scores (r=-0.802*, p=0.030) were also inversely correlated with waist circumference change. In figure 5.5 (c), when the participants were split by gender the analysis showed no correlation between SQ desire to eat and waist circumference measurement change.

Table 5-5 : Relationship between VAS scores (different time points, AUC and SQ) and waist circumference change for all participants. (N=22)

Waist circumference change	**Fasting hunger**	**Fasting fullness**	**Fasting desire to eat**	**Fasting prospective food intake**	
Spearman correlation	-0.037	-0.018	0.088	0.089	
Sig. (2-tailed)	0.869	0.936	0.698	0.693	
Waist circumference change	**15 mins hunger**	**15 mins fullness**	**15 mins desire to eat**	**15 mins can eat more**	
Spearman correlation	-0.092	0.183	-0.150	-0.107	
Sig. (2-tailed)	0.684	0.416	0.505	0.635	
Waist circumference change	**75 mins hunger**	**75 mins fullness**	**75 mins desire to eat**	**75 mins can eat more**	
Spearman correlation	-0.217	0.075	-0.308	-0.026	
Sig. (2-tailed)	0.332	0.740	0.163	0.909	
Waist circumference change	**AUC hunger**	**AUC fullness**	**AUC desire to eat**	**AUC prospective food intake**	**AUC composite of appetite score**
Spearman correlation	-0.042	0.198	-0.142	-0.090	-0.214
Sig. (2-tailed)	0.853	0.378	0.528	0.692	0.339
Waist circumference change	**SQ hunger**	**SQ fullness**	**SQ desire to eat**	**SQ prospective food intake**	**SQ composite of appetite score**
Spearman correlation	0.134	-0.053	0.369	0.152	0.282
Sig. (2-tailed)	0.552	0.816	0.091	0.500	0.216

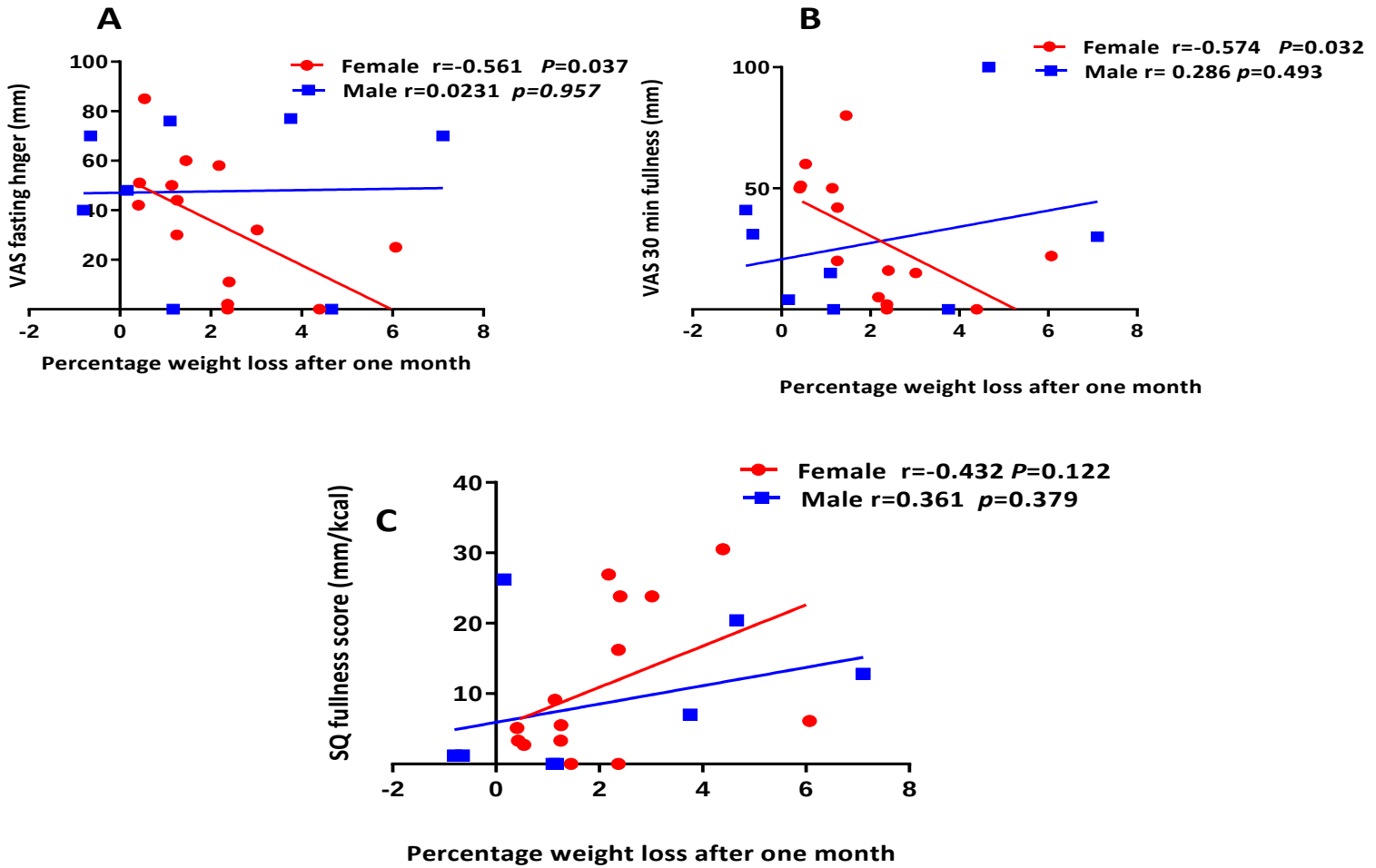

Figure 5-4: Relationship between VAS and percentage weight loss after one month. (A=VAS fasting hunger B= VAS 30 min fullness and C= SQ fullness scores).

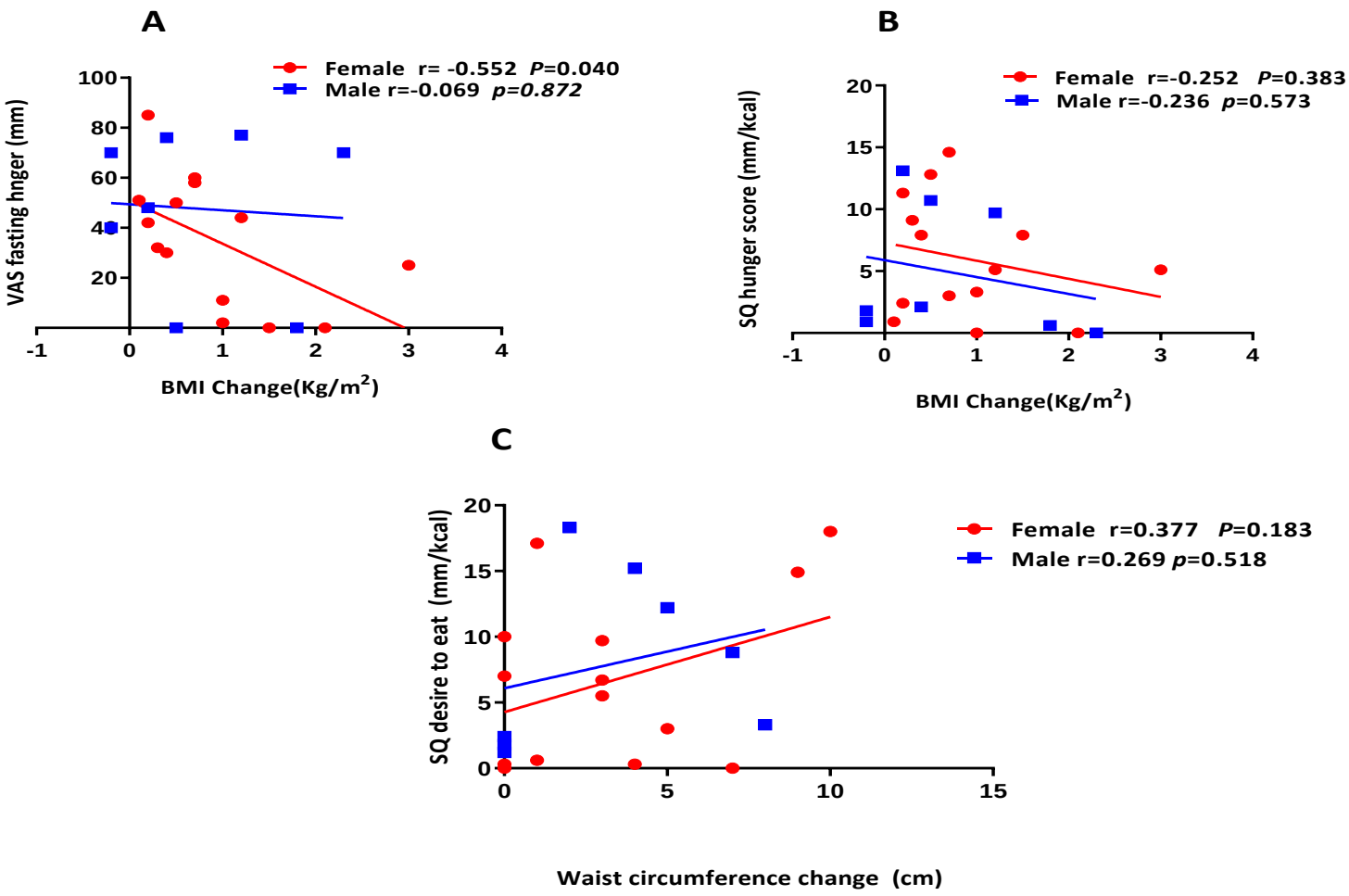

Figure 5-5 : Relationship between VAS scores and BMI and waist circumference change. (A=VAS fasting hunger B= SQ hunger scores and C= SQ desire to eat scores).

5.3.6 The correlation between TFEQ subscales and subsequent weight loss

Table 5.6 displays relationships between the scores for the TFEQ subscales and subsequent weight loss. There was no correlation between the TFEQ subscales and subsequent weight loss. TFEQ Hunger and TFEQ Disinhibition were inversely correlated with percentage weight loss after one month, but this did not reach significance.

Table 5-6 : Correlation between TFEQ subscales and subsequent weight loss

Percentage weight loss after one month	**TFEQ Hunger**	**TFEQ Restraint**	**TFEQ Disinhibition**
Spearman correlation	-0.311	-0.145	-0.385
Sig. (2-tailed)	0.130	0.490	0.057
N	25	25	25
Percentage weight loss after three months	**TFEQ Hunger**	**TFEQ Restraint**	**TFEQ Disinhibition**
Spearman correlation	0.152	0.033	0.012
Sig. (2-tailed)	0.620	0.914	0.970
N	13	13	13
BMI change	**TFEQ Hunger**	**TFEQ Restraint**	**TFEQ Disinhibition**
Spearman correlation	-0.144	-0.063	-0.242
Sig. (2-tailed)	0.493	0.766	0.245
N	25	25	25
Waist circumference change	**TFEQ Hunger**	**TFEQ Restraint**	**TFEQ Disinhibition**
Spearman correlation	-0.084	0.040	-0.142
Sig. (2-tailed)	0.689	0.850	0.499
N	25	25	25

5.3.7 Fasting plasma gut hormones concentrations and subsequent weight loss

Table 5.7 presents the relationships between fasting plasma concentrations of ghrelin, PP, PYY and GLP-1 and subsequent weight loss for. PYY levels showed a positive correlation (r=0.432*, p=0.045) with percentage weight loss after one month. Moreover, PYY was positively correlated with BMI change and negatively correlated with percentage weight lost after three months, but these correlations did not reach significance. In figures 5.6, 5.7 and 5.8 when the participants were split by gender, analysis highlighted that no correlations were seen between gut hormone concentrations and subsequent weight loss.

Table 5-7 : The correlation between fasting plasma gut hormone concentrations and weight loss subscales

Percentage weight loss after one month	**ghrelin**	**PP**	**PYY**	**GLP-1**
Spearman correlation	0.035	0.198	0.432*	-0.025
Sig. (2-tailed)	0.878	0.378	0.045	0.911
N	22	22	22	22
Percentage weight loss after three months	**ghrelin**	**PP**	**PYY**	**GLP-1**
Spearman correlation	-0.263	0.086	-0.343	0.078
Sig. (2-tailed)	0.434	0.803	0.302	0.819
N	11	11	11	11
BMI change	**ghrelin**	**PP**	**PYY**	**GLP-1**
Spearman correlation	-0.088	0.218	0.383	-0.029
Sig. (2-tailed)	0.696	0.330	0.079	0.897
N	22	22	22	22
Waist circumference change	**ghrelin**	**PP**	**PYY**	**GLP-1**
Spearman correlation	0.123	0.080	0.207	-0.223
Sig. (2-tailed)	0.584	0.722	0.356	0.319
N	22	22	22	22

*Correlation is significant at p< 0.05 level (2-tailed).

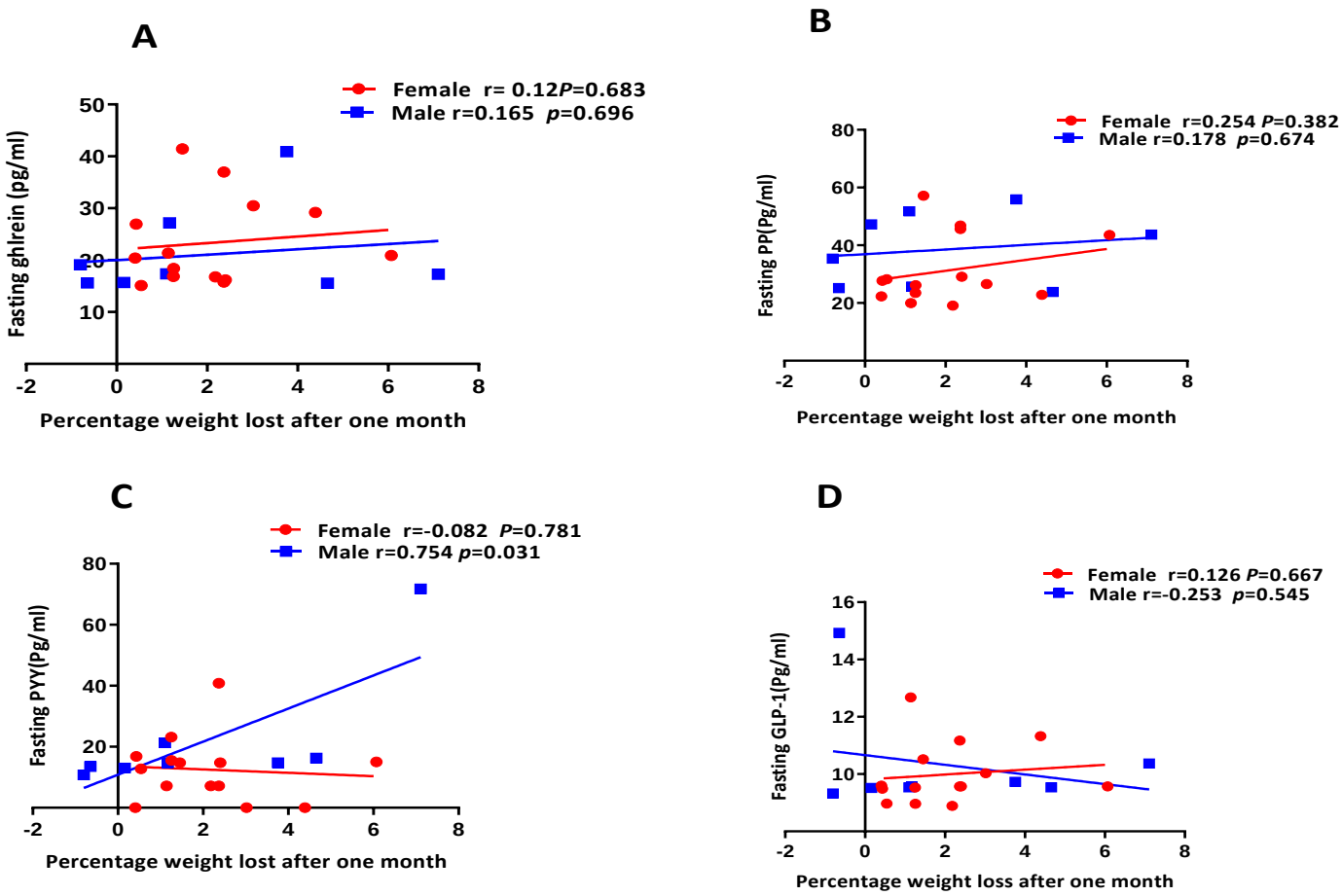

Figure 5-6: Relationships between fasting plasma gut hormones and percentage weight loss after one month (A= ghrelin, B= PP C= PYY and D= GLP-1).

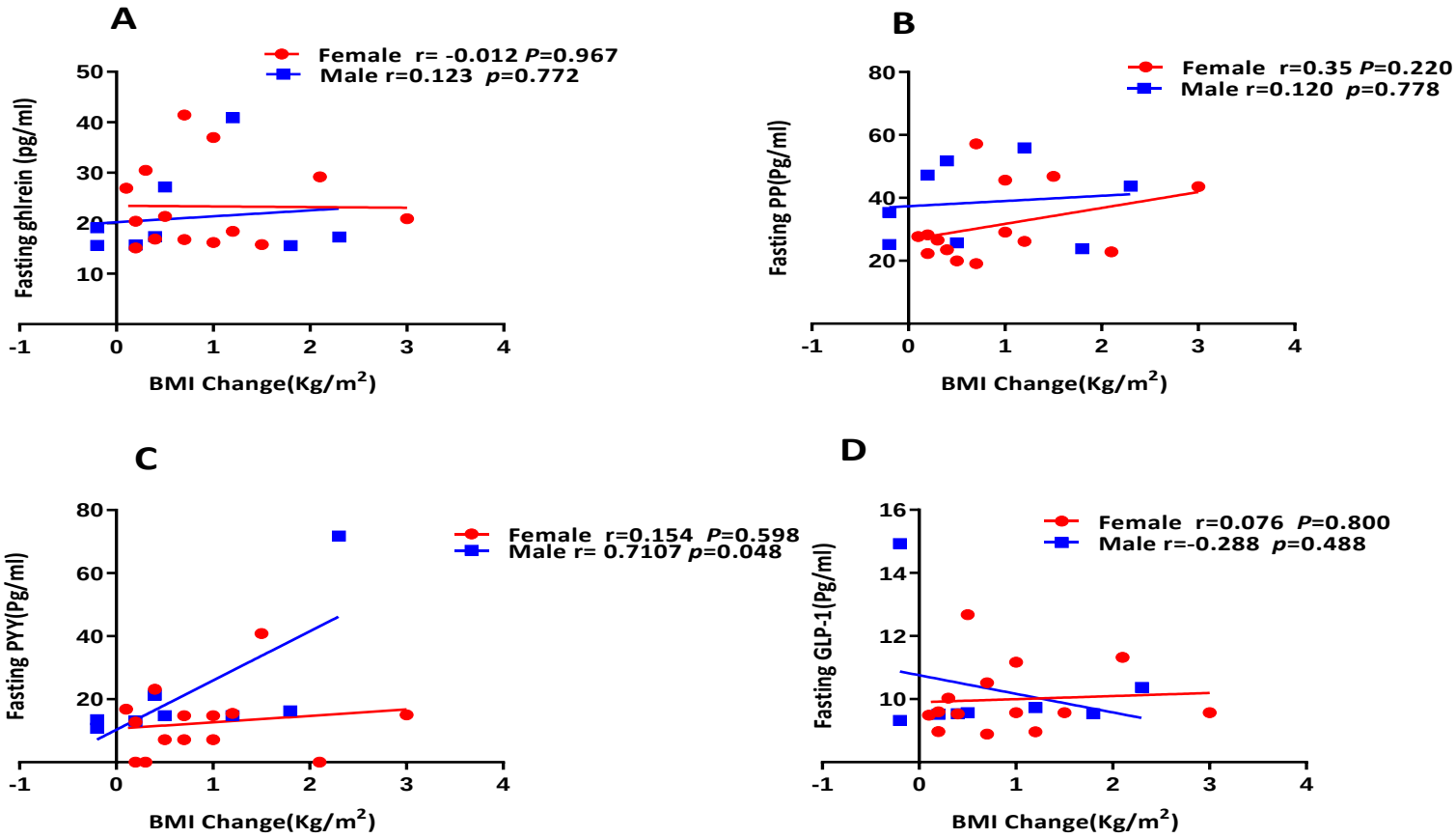

Figure 5-7: Relationships between plasma gut hormones and BMI change (A= fasting ghrelin B= fasting PP C= fasting PYY and D= fasting GLP-1).

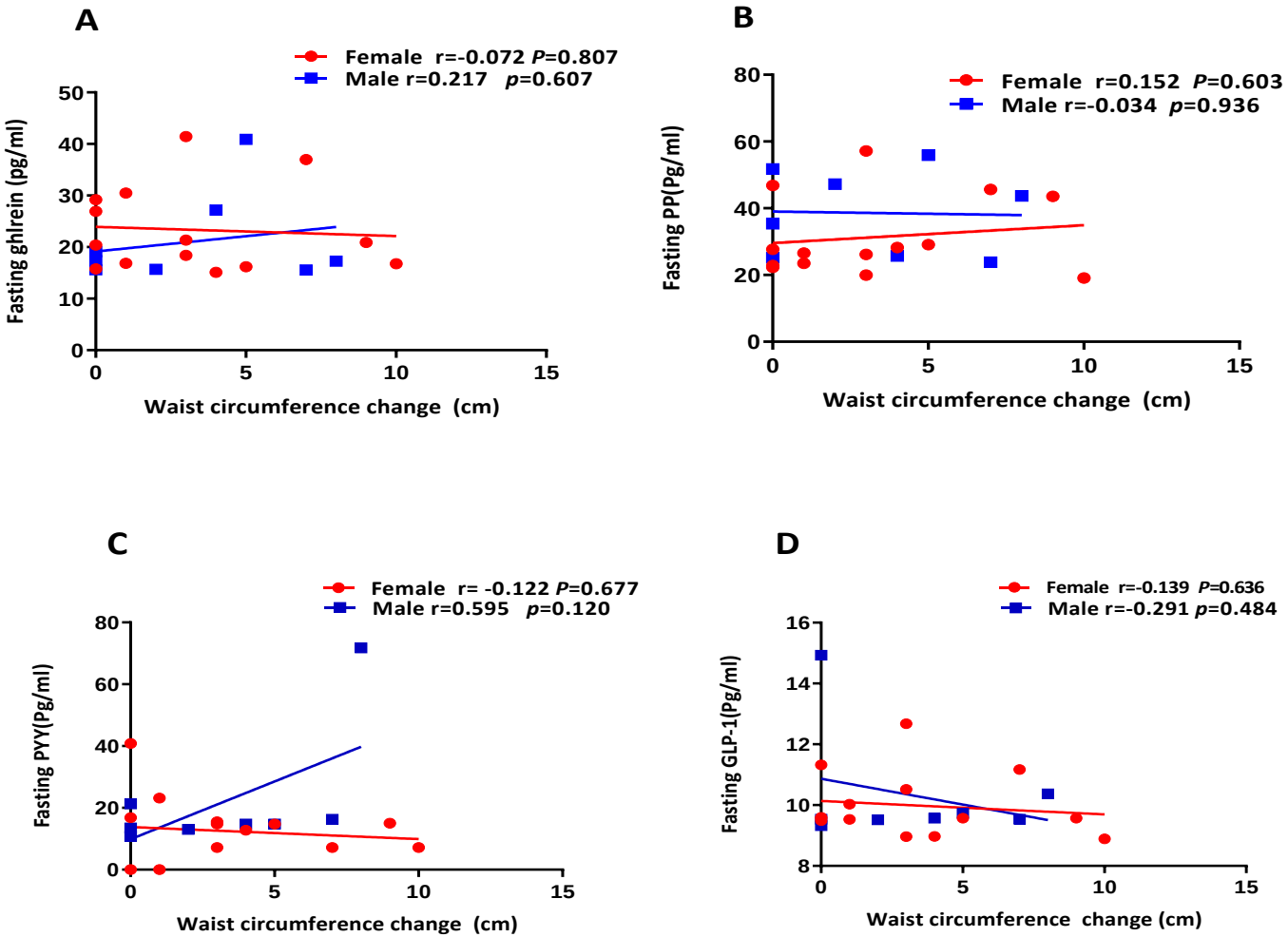

Figure 5-8: Relationships between fasting plasma gut hormones and waist circumference change (A= ghrelin, B= PP, C= PYY and D= GLP-1).

5.3.8 Identification of individual satiety phenotypes

The independent variables selected in chapter 4 were used to assess satiety phenotype in the present study to determine whether satiety phenotype predicted weight loss. As the participant numbers in this part of the study were low, the heat map included all participants rather than splitting them by gender.

Table 5.8 presents the heat map and satiety phenotypes determined for all participants. Using the scoring system developed in chapter 4, this identified that seven people had a low satiety phenotype, eleven people with a medium satiety phenotype and four people with a high satiety phenotype.

Table 5-8: Heat map of variables predicting satiety phenotype

Participant number	Fating PP	TFEQ Hunger	SQ composite appetite score	Fasting hunger	Fasting fullness	Fasting desire to eat	Fasting can eat more	post breakfast desire to eat	Hunger score	Phenotype
1									9	Medium satiety
2									10	Low satiety
5									5	High satiety
8									4	High satiety
10									8	Medium satiety
11									8	Medium satiety
13									6	Medium satiety
15									13	Low satiety
18									11	Low satiety
19									2	High satiety
20									4	High satiety
21									7	Medium satiety
22									9	Medium satiety
23									7	Medium satiety
24									5	High satiety
25									4	High satiety
26									11	Low satiety
28									6	Medium satiety
30									5	High satiety
31									6	Medium satiety
32									8	Medium satiety
33									9	Medium satiety

5.3.9 Weight loss of participants by satiety phenotype

Figure 5.9 A, B and C presents the average subsequent weight loss of all participants divided by their phenotype of low, medium and high satiety. As can been seen in figure (A), (B) and (C) there were no differences between the 3 groups based on one way ANOVA. Average BMI change p=0.523, average waist circumference change p=0.6496 and average body weight change p= 0.6739. In general people who had low satiety, had lower average weight loss and lower average BMI change, but there was wide variability so this was not significant.

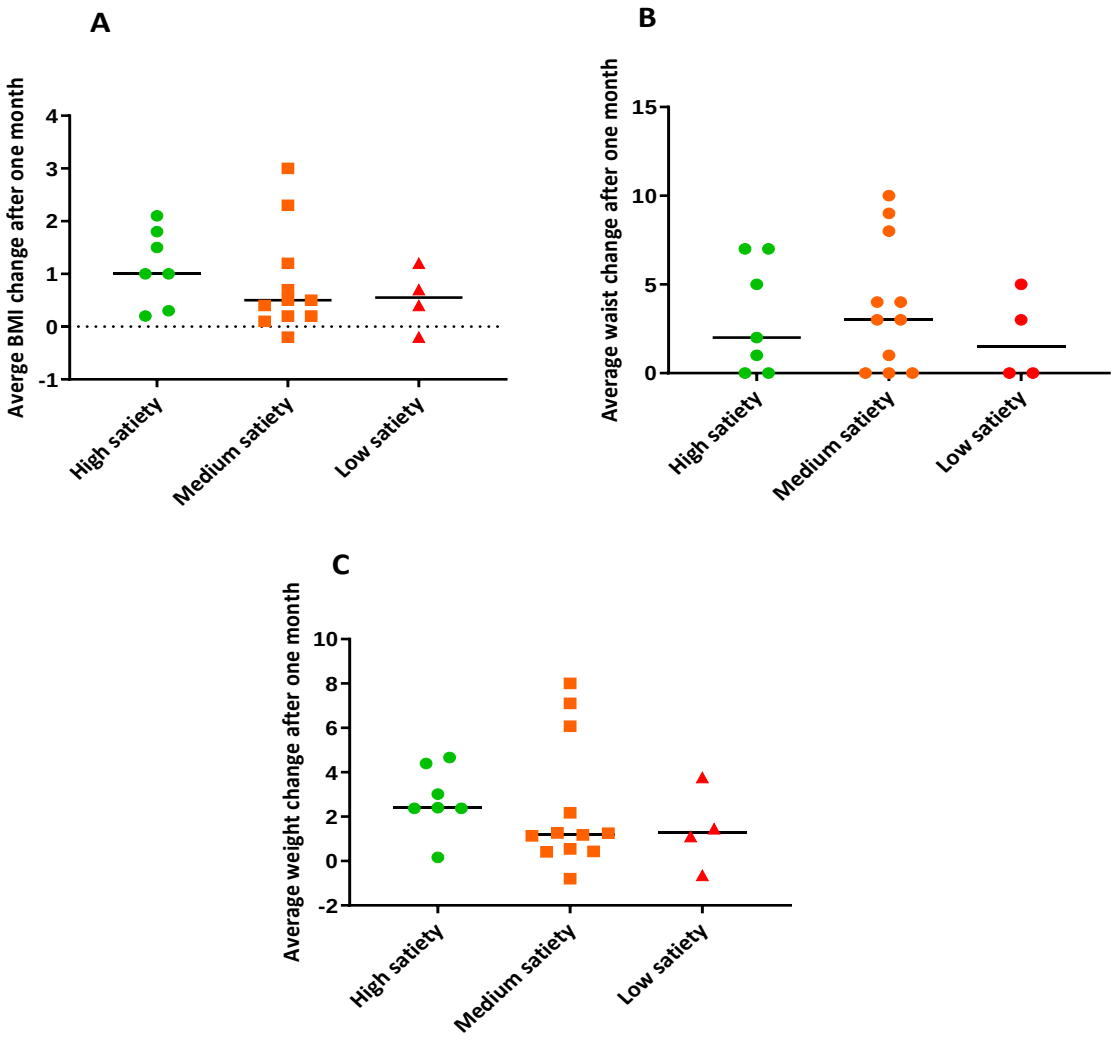

Figure 5-9 : Average subsequent weight loss of all participants one month on a diet (A= average BMI change, B= average waist change and C= average weight change)

5.3.10 Average TFEQ subscales and subsequent weight loss

Figure 5.10 represents the average TFEQ subscale and subsequent weight loss after one month on Advice diet and regular exercise. Based on one way ANOVA there were differences between low, medium and high satiety groups in TFEQ Restraint scores p= 0.0120 and TFEQ Hunger scores p=0.0014. However, there were no significant differences between low, medium and high satiety groups for TFEQ Disinhibition scores p= 0.2146.

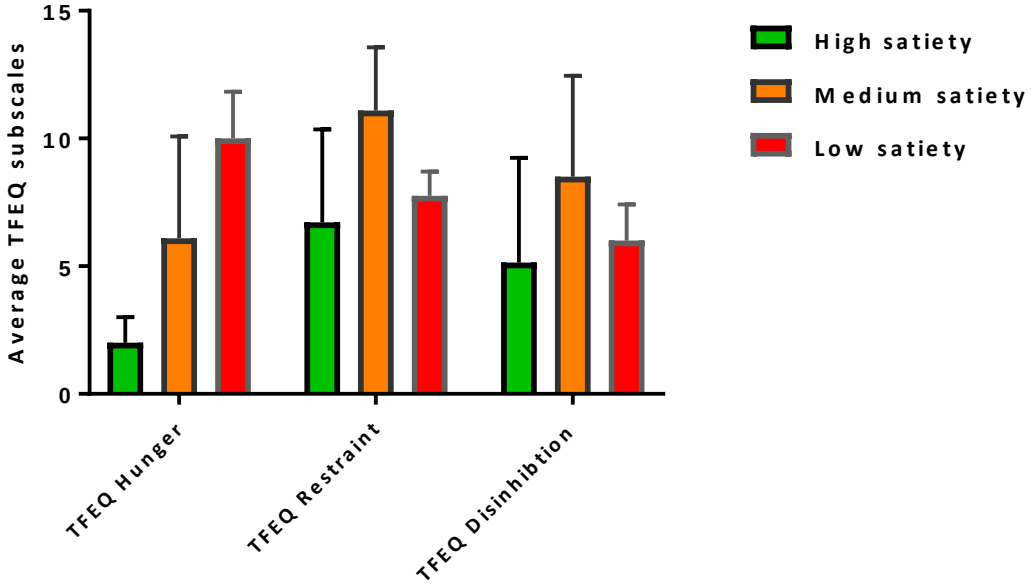

Figure 5-10 : Average TFEQ subscales and satiety phenotype

5.4 Discussion

Gaining a better understanding of and identifying the factors that influence weight change prior to weight loss programmes is important to encourage successful weight loss (James *et al.,* 2018). The main aim of this study was to investigate variables which might predict weight loss and thus identify those individuals, prior to their participation in a weight management programme, who may struggle to lose weight due to impaired satiety.

5.4.1 Baseline characteristics of participants

Females showed higher scores of TFEQ Restraint than males at baseline, which is in agreement with previous reports that Restraint and Disinhibition scores were higher in women compared to men (Bellisle *et al.,* 2009; Lesdema *et al.,* 2012; Westenhoefer, Stunkard and Pudel, 1999).

5.4.2 VAS scores at different time points and percentage of weight loss after one month

The present study indicates that people who had high VAS hunger scores at different time points, measured by AUC and SQ subsequently struggled to lose weight. Taking gender of participants into account, the present study showed that females with higher fasting hunger scores lost less percentage of weight after one month (r=-0.561*, p=0.037). Whereas, fasting hunger scores in males did not predict percentage weight lost after one month. The current study's findings are in accordance with those recently observed in another weight loss intervention where Sayer *et al.,* (2018) reported that higher baseline hunger VAS scores were inversely associated with weight loss at 16 and 24 weeks in 120 participants.

The finding of the current study showed VAS fullness score did not predict weight change in males. Similarly, Sayer *et al.,* (2018) found that fullness ratings were not associated with changes in body weight in all participants. Furthermore, the findings of the current study reported that females who were less full at 30 min (r=-0.554, p=0.032) based on fullness VAS, lost more percentage of weight after one month. Whereas, fasting hunger VAS in males and SQ fullness scores in females and males did not predict percentage weight loss after one month. The explanation for this result could be low sample numbers. Adam *et al.*, (2006) also reported a similar outcome

after, an 8% weight loss. Moreover, Drapeau *et al.,* (2007) reported that they did not find a correlation between SQ fullness and weight change in males.

In agreement with the present study, Nymo *et al., (*2017) examined relationships between VAS scores and weight loss and reported that hungrier people found it harder to lose weight at baseline in 31 participants. Two previous studies by Drapeau *et al.,* (2007) and Doucet *et al.,* (2000) reported that higher fasting hunger scores were associated with a lower body weight loss in men, a possible explanation for the present study not finding a relationship, may be the smaller sample size and the study did not have the power to show any relationship.

5.4.2.1 SQ of VAS and percentage of weight loss after one month

The present study indicates that SQ did not significantly correlate with percentage weight loss after one month. This finding agrees with an earlier study by Drapeau and colleagues who found the baseline of SQ did not correlate with weight loss in men (n = 176) and women (n = 139) (Drapeau *et al.,* 2007).

5.4.2.2 SQ of VAS and BMI change

In females, after adjusting for BMI, SQ hunger was negatively correlated (r=-0.641, p=0.018) with BMI change. The present study showed that females who had lower fasting hunger scores (r=-0.552*, p=0.040) had a reduced BMI after one month, while fasting hunger and SQ hunger scores in males did not predict BMI change after one month.

5.4.2.3 SQ of VAS and waist circumference change

Based on the literature, few studies have focused on the SQ to predict weight change. The present study's findings are in agreement with McNeil *et al.,* (2014) who reported SQ desire to eat was negatively correlated with waist circumference change.

5.4.3 Fasting plasma gut hormones and percentage weight loss after one month

The present study indicated that participants who had higher fasting concentrations of plasma PYY showed the greatest percentage weight loss after one month. This finding is in accordance with those observed in a weight loss intervention, reported by Roth *et al.,* (2005) which indicated that higher baseline fasting PYY levels were associated with weight loss, and the increase in PYY is positively related to the extent of weight loss.

The finding of the present study showed that ghrelin did not predict weight loss after one month. Similarly, Polsky *et al.,* (2013) also observed that baseline ghrelin and PP did not predict weight loss in 61 subjects participating in a 16-week intervention for weight loss. However, this finding is in contrast with Williams *et al.,* (2016) who found that a higher baseline concentration of unacylated ghrelin predicted greater weight loss at six months in males and females. However, the types of ghrelin measured was different in both studies, Williams *et al.,* measured baseline unacylated ghrelin for predicting weight loss whereas in the current study acylated ghrelin was measured to predict weight loss. Acylation is thought to be essential for ghrelin to bind to the GHS-R1 and to cross the BBB. In humans, unacylated ghrelin does not possess the pituitary and pancreatic activity of acylated ghrelin. Therefore, unacylated ghrelin is considered to be unimportant for appetite regulation (Broom *et al.*, 2007) and it is recommended that acylated ghrelin is studied, as in the current study.

Furthermore, Sumithran *et al.,* (2011) observed that high baseline ghrelin in 34 women was associated with weight loss at 12 months after caloric restriction and exercise. The current study included both sexes, whereas the Sumithran *et al.,* study included only females. A potential explanation why ghrelin predicted weight loss in the Sumithran *et al.,* study is the weight loss period was shorter in this present study (one month and 3 months) than in Sumithran *et al.,* (2011) which was 62 weeks. Sumithran *et al.,* (2011) only included females, it has also been suggested that females have higher baseline ghrelin (Makovey *et al.,* 2007) which could explain the differences between the studies.

In the present study, baseline PP concentration was not significantly correlated with percentage weight loss after one month in female participants. This finding is in contrast with Hainer *et al.,* (2008) who reported that PP was inversely correlated with BMI change in women. A potential explanation for differences in the result may be sample size. Hainer *et al.,* (2008) included sixty seven women whereas the current study included only seventeen.

In the present study, GLP-1 hormone levels did not correlate with subsequent weight loss. The present study found that baseline GLP-1 levels did not predict weight loss percentage after one month. A previous study by Polsky *et al.,* (2013) showed GLP-1 did not predict weight loss, which agrees with the present study.

5.4.3.1 Plasma gut hormones and BMI change

The findings of the present study showed no correlation between baseline ghrelin and BMI change. However, other authors have found higher baseline ghrelin concentrations were inversely correlated with BMI changes at 12 months (Garcia, *et al.,* 2006). The difference is likely to be due to the longer period of the intervention. It has also been reported that there are sex differences in the plasma concentrations of ghrelin, overweight females having higher fasting concentrations (Makovey *et al.,* 2007) than overweight males.

In the present study, baseline plasma levels of PP hormone did not predict BMI change in female participants. This finding is in contrast with Hainer *et al.,* (2008) who reported that PP was inversely correlated with BMI change in 67 women.

5.4.3.2 Plasma gut hormones and waist circumference change

In the current study, there was no correlation between baseline PP and change in waist circumference. The finding of the current study differs from a study by Koska *et al.,* (2004) who demonstrated that fasting PP level was negatively associated with waist circumference change. However Koska's study was carried out in Pima Indians who have a genetic propensity to weight gain.

In the current study there was no correlation between baseline measurement of plasma ghrelin and waist circumference and percentage of weight lost change after one month. Similarly, Santosa *et al.,* (2007) showed initial level of ghrelin did not predict weight loss in 35 women.

There was no correlation between baseline PP and percentage of weight loss change after one month and BMI. This agrees with Dixon *et al.,* (2011) who have also indicated that fasting level of PP did not predict weight loss.

When split by gender of participants, plasma level gut hormones did not predict subsequent weight loss. This finding is in agreement with a previous study of a weight management programme where Hainer *et al.,* (2008) found no significant correlation between baseline levels of PYY and weight loss after a 3-week weight management in 67 women with obesity.

When split by gender of participants, plasma levels PP hormone did not predict subsequent weight loss. However, findings of Hainer *et al.,* (2008) contrasts with the

findings of the present study as they reported baseline PP concentrations were inversely related to waist circumference change in women.

5.4.4 TFEQ subscales and subsequent weight loss

The results of the current study reported that people with low scores of hunger (TFEQ Hunger and TFEQ Disinhibition) lost more weight. Previous research showed a similar pattern in which baseline TFEQ Hunger scores were negatively correlated with weight change (R= - 0.25, *p* = 0.03) at 6 months (Batra *et al.,* 2013). Moreover, other previous findings are also in agreement with the present study result, Bas and Donmez, (2009) reported that lower TFEQ Hunger and TFEQ Disinhibition scores show associations with weight loss at 20 weeks. A previous study by Hay*s et al*., (2006) was in partial agreement with the present study finding, which showed low hunger scores at baseline predict weight loss whereas TFEQ Restraint did not predict weight loss. However, it was in contrast to the current study finding where reported disinhibition did not predict weight change. The potential explanation for these differences may be longer duration of weight loss interventions. Larger samples of females in Hay*s et al*., (2006) could also explain these differences.

The findings of the current study are in contrast to Teixeira *et al.,* (2010) who reported TFEQ Hunger and TFEQ Disinhibition did not predict weight change at 12 months in 225 overweight and obese women. This difference in findings may be duration of weight loss programme in Teixeira *et al.,* (2010) which was 12 months.

In contrast to the present study, James *et al.,* (2017) indicated that lower baseline TFEQ restraint predicted greater weight loss over the year and baseline levels for all other subscales were not significantly related to weight change over one year. The differences in findings could be explained by the large sample of females in James *et al.,* (2017) which was 186 females, whereas the present study only had 17 females. Duration of weight loss could also explain the differences with the duration study of James *et al.,* (2017) being one year and present the study one month.

The findings of the current study showed TFEQ Hunger did not predict BMI and waist circumference change. This is in contrast with Provencher *et al.*, (2003) who showed a positive association between susceptibility to hunger and BMI and waist circumference. The results of Provencher *et al.,* (2003) differ from the current study in sample size as

their study included 596 participants and mean weight, BMI and waist circumference were lower than the present study, which may explain the different result. Another explanation may be age, as in the current study the average age was 27-77 years which is higher than the age of participants in the previous study mentioned above and people eat less and make different food choices as they get older and it could explain the differences (Drewnowski and Shultz, 2001).

5.4.5 Identification of satiety phenotype

The heat map was constructed using the methodology developed in the previous chapter. Using this method, the satiety phenotype of twenty two participants was determined, seven participants were identified with a high satiety phenotype and four participants with a low satiety phenotype and the remainder with a medium satiety phenotype. The findings of the present study indicated that people with the high hunger phenotype on average had a lower change in BMI, percentage of weight loss after one month and waist circumference change compared to the other groups. Moreover, low satiety phenotype participants had the highest TFEQ Hunger scores at baseline compared to people with the lower and medium hunger phenotypes and had medium TFEQ-R and low TFEQ-D, although this is not surprising as the TFEQ hunger score was one of the variables used to build the heat map.

The heat map is a visual representation of data and is designed to make an easy score sheet that could be used in the clinic to determine someone's satiety phenotype. This method of using a heat map could allow clinical staff to predict satiety phenotypes of individuals prior to their participation in a weight loss programme. This heat map method will direct clinical staff to make plans for personalised programmes to facilitate weight loss for each participant.

5.4.6 Summary

The key findings of this chapter are that people who had high hunger scores at different times points, measured by AUC and SQ of VAS subsequently struggled to lose weight. VAS fullness did not predict weight change in males. In the present study females who had higher fasting hunger scores, lost a lower percentage of weight after one month. Whereas, fasting hunger in males did not predict percentage weight loss after one month. Changes in waist circumference were positively associated with desire to eat SQ in females. Fasting plasma gut hormones did not correlate with

subsequent weight loss. The results of the current study report that, people with low scores of hunger (TFEQ Hunger and TFEQ Disinhibition) had more weight loss subsequently.

Based on the findings of the current study, the VAS measure (fasting hunger and 30 min fullness) was the best measure to predict weight loss of people with obesity prior to starting on a weight management program. The findings of the present study indicated that people with the low satiety phenotype on average had a lower change in BMI and lower percentage weight loss after one month and lower waist circumference change compared to the other groups and had the highest TFEQ Hunger scores at baseline, compared to people with than low and medium hunger phenotypes and had medium TFEQ Restraint and low TFEQ Disinhibition.

In chapter 4 the results showed differences between women and men for the TFEQ scores therefor in chapter 6 the data was split into 2 groups only as the distribution of the participants meant that one group would only have 1 male participant which is not possible to analyse statistically.

5.4.7 Study strengths and limitations

The present study has strengths and limitations. One strength is that it included both males and females, which helped to provide more data regarding gender and weight loss, as males are a commonly under represented group in these types of interventions. Moreover, it has used questionnaires, VAS hunger at different point and fullness SQ scores and PPY hormone measurements to predict people who struggle to lose weight. The current study presented data for one month, which is valuable to make predictions in the short term prior to initiating a weight loss programme as many studies predicted weight loss over 6 months or more. A major limitation was the small sample size, especially for males at the three month weight loss time point.

Chapter 6

Evaluation of food cravings, hunger and food intake as predictors of successful weight-loss in a Tier 2 setting

6 Introduction

It is well known that imbalance between EI and energy expenditure leads to weight gain. There are numerous proven methods to lose weight, including lifestyle modification, pharmacotherapy and bariatric surgery. Despite these methods, many individuals who attempt weight loss are not successful. One thing that could help in improving effective weight loss strategies would be a better understanding of the factors that predict the ability to lose weight (Polsky *et al.,* 2013).

6.1.1 Factors reported to predict successful weight loss

6.1.1.1 Food cravings

Food cravings have been recognised as one possible precursor to overeating (Gendall *et al.,* 1998). Food cravings are a powerful physiological and psychological urge that induce the seeking and eating of a food (Cepeda-Benito *et al.,* 2000). Thus, cravings differ from hunger in that they usually concentrate on specific individual foods that are often high energy foods (Christensen, 2007; Gilhooly *et al.,* 2007), whereas hunger is relieved by a variety of foods (Martin, O'Neil and Pawlow, 2006) that differ in caloric density. Previous research has reported that individuals with higher frequencies of food cravings have higher BMI than normal weight individuals (Abiles *et al.*, 2010; Chao *et al*., 2014). Experiencing food cravings has been identified to be a common phenomenon (Dalton *et al.,* 2017). However, the frequency and intensity of food cravings appear to be more prevalent and intense in people with obesity and are associated with excess EI, weight gain, lack of success in weight loss and early drop-out from obesity treatment programmes (Delahanty *et al.,* 2002: White *et al.,* 2002). This raised level of food cravings may explain why some people with obesity struggle to lose weight. Measurement of initial food craving scores for people prior to weight loss programmes may improve weight intervention strategies and help participants to succeed in weight loss.

There are gender differences in the risk for, and consequences of obesity. Gender is a known risk factor for obesity and being female doubles the chance of becoming overweight (Martin and Ferris, 2007). Globally, females are three percent more likely to become overweight or obese than men (WHO, 2010). Because females are increasingly affected by obesity, there may be a gender-related mechanism that

underlies these differences. Given the role of craving in obesity, one mechanism underlying gender-based health disparities in obesity could relate to gender differences in craving; it has been shown that men and women have different experiences of craving and different behavioural responses to it (Hallam *et al.,* 2016).

6.1.1.2 Measurement of food cravings

It has been shown that the FCI is a reliable and valid measure of general and specific food cravings (White *et al.,* 2002), which can be used in research related to overeating and binge eating. Moreover, the FCI may be useful and valuable in treatment studies that target obesity and/or food cravings (White *et al.,* 2002). Many studies have examined the relationship between food cravings and weight change. Ferguson *et al.,* (1992) and Elfhag and Rössner, (2005) suggested that reducing and managing food cravings is a key component in the management of obesity and successful weight loss maintenance. Moreover, previous findings reported that initial high levels of food craving may be related to less successful weight loss (Abilés *et al.,* 2010; Chao *et al.,* 2014; Franken and Muris, 2005). In contrast, a study by Batra *et al.*, (2013) reported a positive correlation between food craving and baseline BMI, which demonstrated that there was no significant relationship between baseline food craving and weight change.

6.1.2 Eating behaviours

Individual differences in stable eating behaviours have a significant impact on food choices, EI and BMI. Most people will overeat to a certain extent in a high-risk food environment. However, some people are likely to be more susceptible than others and are at higher risk for excess EI and weight gain (French *et al.,* 2012; Blundell and Cooling, 2000; Blundell *et al.,* 2005). The TFEQ is one method of measurement that has been developed to measure individual differences in eating behaviours related to EI and body weight. The TFEQ has been widely applied as a tool in weight loss research to measure eating behaviour traits in people with obesity. It has been used to predict weight loss in clinical patients, and to monitor changes throughout treatment (Stunkard and Messick, 1985; Bryant, King, and Blundell, 2008). Previous studies have focused on two eating behaviours traits (Restraint and Disinhibition) in weight loss intervention studies. However, examination of the hunger subscale of TFEQ to predict weight loss has had little attention in the literature.

Two studies have shown that TFEQ Hunger is inversely correlated with weight loss and those individuals who had higher baseline scores of TFEQ Hunger struggled to lose weight. Bas and Donmez, (2009) have reported that there was a negative correlation between baseline hunger and weight change. Batra *et al.,* (2013) have shown that reduction in baseline hunger subscale scores was associated with greater weight loss.

6.1.3 Food diaries

Dietary records or food diaries are a prospective, open-ended survey method where the subjects are given a recording form to collect data about their foods and beverages consumed over a previously specified period of time. They can be useful to estimate the current diet of individuals and population groups, as well as to identify groups at risk of inadequacy. Food diaries have been used in epidemiological and in clinical studies (Ortega, Pérez-Rodrigo and López-Sobaler, 2015). Previous research has reported that dietary records have high validity and precision when used following adequate procedures and over a sufficient number of days (Yang *et al.,* 2010; Schlundt, 1998) (More details on food diaries are discussed in chapter one). Macronutrients play a role in satiety. This has culminated in a macronutrients hierarchy of satiating power, with protein having the highest potency (protein > CHO > fats) (Blundell and Macdiarmid, 1997).

An understanding of macronutrient composition i.e. the relative amounts of protein, fat, and CHO on short-term food intake (FI) may assist in the design of longer trials aimed at achieving healthier body weights. There is considerable confusion, for example, about the dietary mechanism that supports long-term weight reduction and the maintenance of reduced body weight. For this reason, a better understanding of diet and the short-term effects of its composition may offer approaches and insights into the design of strategies and meal plans for successful long-term weight reduction (Bellissimo and Akhavan, 2015). Several studies have used food diaries to examine macronutrient intake in predicting weight loss. Buscemi *et al.,* (2017) reported that low initial levels of caloric intake were positively associated with changes in BMI. Moreover, Hays *et al.,* (2006) indicated that reported fibre intake was significantly positively associated with weight change, whereas CHO and protein intakes were significantly inversely associated with weight change. Conversely, Savage, Marini and

Birch (2008) found that fibre and caloric beverage intakes were not significant predictors of weight change.

6.1.4 Shape-Up

The current study was carried out at Rotherham Leisure Complex, Rotherham, UK as part of a tier 2 services called Shape-Up. It is a multicomponent lifestyle intervention that has been recommended by National Institute for Health and Care Excellence (NICE) to manage obesity. Tier 2 programmes include dietary advice, physical activity and behavioural therapies and are generally delivered over 10 weeks (Read and Logue, 2016). These programmes aim to support adults who are classified with BMI of ≥25 kg/m^2 (without comorbidities or managed comorbidities) to lose weight and sustain their weight loss through a multicomponent programme (Ells *et al.,* 2018). Often Tier 2 programmes are offered within a primary care setting, however some areas have commissioned commercial organisations to deliver weight management services in the community (Read and Logue, 2016). The Shape-Up Programme is a unique programme that gives participants the opportunity to go through the self-help manual together as a group. It consists of 10 week sessions with groups run for 2 hours in the morning and evenings at times to suit a range of people. Each session covers a topic including: how to limit further weight gain**,** how to achieve modest weight loss at a healthy, sustainable rate**,** getting into a regular eating pattern and how to balance the types of food that participants eat. The Shape-Up programme combines expertise in psychology, nutrition, exercise and health promotion to maximise the participants' chance of successful weight loss.

6.1.5 Aims

The main aim of the present study was to identify at baseline, individuals with a reduced satiety phenotype who may struggle to lost weight on a weight management programme.

Objectives:

1- To investigate the relationship between TFEQ subscales and weight loss.

2- To investigate the relationship between 3- day food diary content and weight loss.

3- To investigate the relationship between food craving scores and weight loss.

4 – To combine these data to investigate whether it is possible to identify individuals with a reduced satiety phenotype.

6.2 Materials and methods

6.2.1 Participants and recruitment

Forty-nine people (37 female and 12 male) with either obesity or overweight were recruited from Rotherham Leisure Complex, Rotherham, UK. All participants were on a 10 week weight loss programme (advice diet and regular exercise). Of those, thirty-nine participants (29 females and 10 males) completed the study. Ten were excluded from analysis as five of them signed up but then changed their minds, two did not meet the inclusion criteria of the study due to having diabetes and three participants dropped out after only 3 sessions of the programme. Ethical approval for the study was granted by Rotherham Council (ID/ 208879)(Appendix 15).

Participants were excluded if they had any chronic disease such as heart disease diabetes or hypertension. Participants were aged between 23-81 years (Mean ± SD 50.69±13.82). Details of the study design, study setting, anthropometry and questionnaires (TFEQ, food craving and food diary questionnaires) were used in this study as previously described in the relevant sections in the general methods (chapter 2).

6.2.2 Questionnaires

Participants were recruited through two visits by the researcher on two consecutive weeks. A presentation was made about the study for participants at the Rotherham Leisure Complex during the first session of their programme. They were provided with information and a description of the study protocol and study packs were given to anyone interested in taking part. The packs included the FCI questionnaire and TFEQ-51. Participants were also asked to complete a 3-day food diary during the week. After the session in week 1, researchers returned the following week to collect the questionnaires. Participants completed and signed the consent form. Food diaries were analysed using recorded data to assess total energy and macronutrient intake as described previously.

6.2.3 Statistical analysis

All data were analysed using SPSS software version 24.0 (IBM) for Windows (SPSS Inc., Chicago, IL, U.S.A.) and Prism version 7.03 (GraphPad Soft-ware Inc., La Jolla, CA). Nutritics software (Libro, Dublin, UK) (version 1.8) was used to calculated macronutrients. Correlation coefficients were used to assess relationships between variables. T-tests were used to assess gender differences in age, height and body mass. T-tests were also used to assess difference in mean of BMI and TFEQ subscales, macronutrients and food craving scores.

A heat map was used to identify individual hunger phenotypes in the participants using the methodology developed in chapter 4. Participants were split into two groups high and low hunger based on their TFEQ Hunger subscale. Satiety phenotype was determined using the Hunger scores. The Hunger scores were from 0 to 13 with 0 being least hungry and 13 most hungry. Low hunger participants had a range of scores from 0-6 and high hunger participants scores were from 7-13. Data are represented as mean ± SD, unless otherwise indicated, a p value < 0.05 was considered to be statistically significant.

6.3 Results

6.3.1 Baseline characteristics of the study participants

The baseline characteristics of all the participants are shown in table 6.1. There was a significant difference in waist circumference and TFEQ Restraint between females and males. Males had a larger mean waist circumference than females and females had higher scores for TFEQ restraint than males.

Table 6-1: Baseline characteristics of the study participants

Variables	All (n=39) Mean ± SD	Female (n=29) Mean ± SD	Male (n=10) Mean ± SD	t	*p*	d
Age (y)	50.7±13.8	49.1±13.9	55.2±13.32	-1.203	0.237	0.447
Weight(kg)	104.5±19	101.1±19	114.2±17.8	-1.917	0.063	0.715
BMI (kg/m^2)	37.1±6.4	37±6.8	37.3±5	-0.134	0.894	0.053
WC (cm)	118.5±15.7	115.5±16.5	127.0±9.6	-2.077	**0.045**	0.853
TFEQ Hunger	4.6±4	4.5±4.3	4.7±3.3	-0.123	0.903	0.048
TFEQ Restraint	9.1± 3.8	9.9±3.4	6.6±4.2	2.526	**0.016**	0.872
TFEQ Disinhibition	6.8±4.5	6.8±4.9	6.8±3.5	.016	0.987	0.007

Values are mean± standard deviation, t= t-test and d Cohen's d. BMI: body mass index. WC: Waist circumference SD: standard deviation. $p < 0.05$ for the differences between males and females.

6.3.2 Food diary results and food craving scores of participants

Table 6.2 shows the TEI and macronutrient intake recorded based on food diary and food craving scores for all participants and then split into females and males. No difference was found between males and females for any measure in the food diary, or for food cravings.

Table 6-2: The mean ± SD of TEI and macronutrient intake based on food diaries and food craving.

Variables	All (n= 39) Mean ± SD	Females (n= 29) Mean ± SD	Males (n= 10) Mean ± SD	t	*p*	d
Food diary-TEI (Kcal)	1054.5±301.3	1084.6±289.6	960.5±337.7	1.014	0.318	0.394
CHO (% of TEI)	46.7±10.6	47.3±9.3	44.9±14.5	0.445	0.667	0.198
Protein (% of TEI)	20.1±5.7	20.3±6.1	18.99±4.66	0.290	0.774	0.240
Fat (% of TEI)	31.5±9.4	32.4± 9	30.5±10.6	0.249	0.805	0.189
Fibre (g)	1.4±0.7	1.3±0.5	1.55±1.1	-0.651	0.534	0.309
Fast food craving	6.5±5.4	5.9±4.8	8.2±6.9	-1.148	0.258	0.381
Sweet craving	9.4±6.6	10.1±6	7.4±8.36	1.114	0.272	0.372
Fat craving	5.9±4.4	5.1±4.3	8±3.9	-1.846	0.073	0.695
Carbohydrate craving	11.1±6.7	10.3±6.2	13.6±7.7	-1.361	0.182	0.471

Values are mean ± standard deviation, t= t-test and d Cohen's d. SD: standard deviation. TEI and macronutrient intake estimated from 3-day food records. g = gram, Kcal = Kilocalories

6.3.3 Weight loss of all participants after ten weeks

Figure 6.1 presents weight loss of all participants after ten weeks. 38 participants lost weight and one participant did not lose weight. Most participants lost between 2- 5 kg and the remainder lost more than 6 kg over 10 weeks.

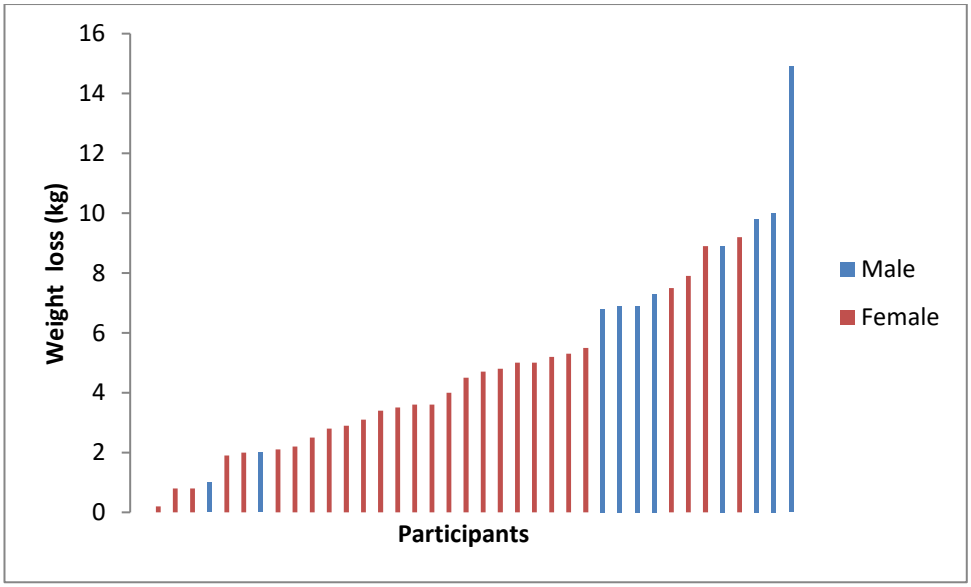

Figure 6-1 : Weight loss of all participants after ten weeks on the Shape-up programme

6.3.4 TEI, macronutrient intake of food diary and baseline anthropometric measures

Table 6.3 shows no significant correlation between TEI, macronutrient intake measured by food diary and body mass, BMI or WC. Fat intake was negatively correlated with all of these baselines anthropometric measurements but this did not reach significance.

6.3.5 Food craving and baseline anthropometric measures

As can be seen in table 6.3 there was a positive correlation between craving for high fat and the baseline measures of body mass ($r=0.352^{*}$, p=0.028) BMI ($r=0.379^{*}$, p=0.017) and WC ($r=0.518^{**}$, p=0.001). Moreover, craving for CHO was positively correlated with body mass (r= 0.328*, p=0.042) and WC (r=0.399*, p=0.012). Cravings for fast food were positively correlated with baseline anthropometric measures, but this did not reach significance. There was no correlation between sweet food cravings and baseline anthropometric measures. The data indicated that people who crave higher fat, CHO and fast food had higher baseline anthropometric measures. Figure 6.2 presents all correlation data between food craving scores and anthropometry measures split by gender. A positive correlation was observed between fat food craving and waist circumference in females (r=0.617, p=0.004) in contrast, a negative correlation was found in males (r=-0.766, p=0.044). Furthermore, fat food cravings were positively associated with body mass (r=0.462, p=0.012) BMI (r=0.472, p=0.009) in females, but in males, fat craving scores were not significantly correlated with body mass (r=-0.207, p=0.564) or BMI (r=-0.150, p=0.679). CHO food craving was positively correlated with waist circumference in females (r=0.444, p=0.016). No significant relationship was found between CHO craving and waist circumference in males (r=0.055, p=0.881).

6.3.6 The TFEQ subscales and baseline anthropometric measures

TFEQ Hunger, Cognitive restraint and Disinhibition did not correlate with baseline body mass, BMI and WC. TFEQ Hunger was negatively correlated with WC and Cognitive restraint was positively correlated with WC, but these correlations did not reach significance.

Table 6-3 : Relationships between food craving, macronutrient intake food diary, TFEQ subscales and baseline anthropometric measures

Correlation	Body mass (kg)	BMI (kg/m^2)	WC (cm)
Food diary-Energy	0.011	0.117	-0.021
Food diary-CHO	0.069	0.171	0.066
Food diary-Protein	0.034	0.190	0.094
Food diary-Fat	-0.211	-0.271	-0.218
Food diary-Fibre	-0.311	0.021	-0.127
Fast food craving	0.288	0.254	0.307
Sweet craving	-0.101	-0.026	-0.098
Fat craving	**0.379***	**0.352***	**0.518****
CHO craving	**0.328***	0.271	**0.399***
TFEQ Hunger	0.176	0.167	0.293
TFEQ Restraint	0-.073	-0.008	-.0279
TFEQ Disinhibition	0.223	0.268	0.230

Values represent Pearson correlation coefficient, r values

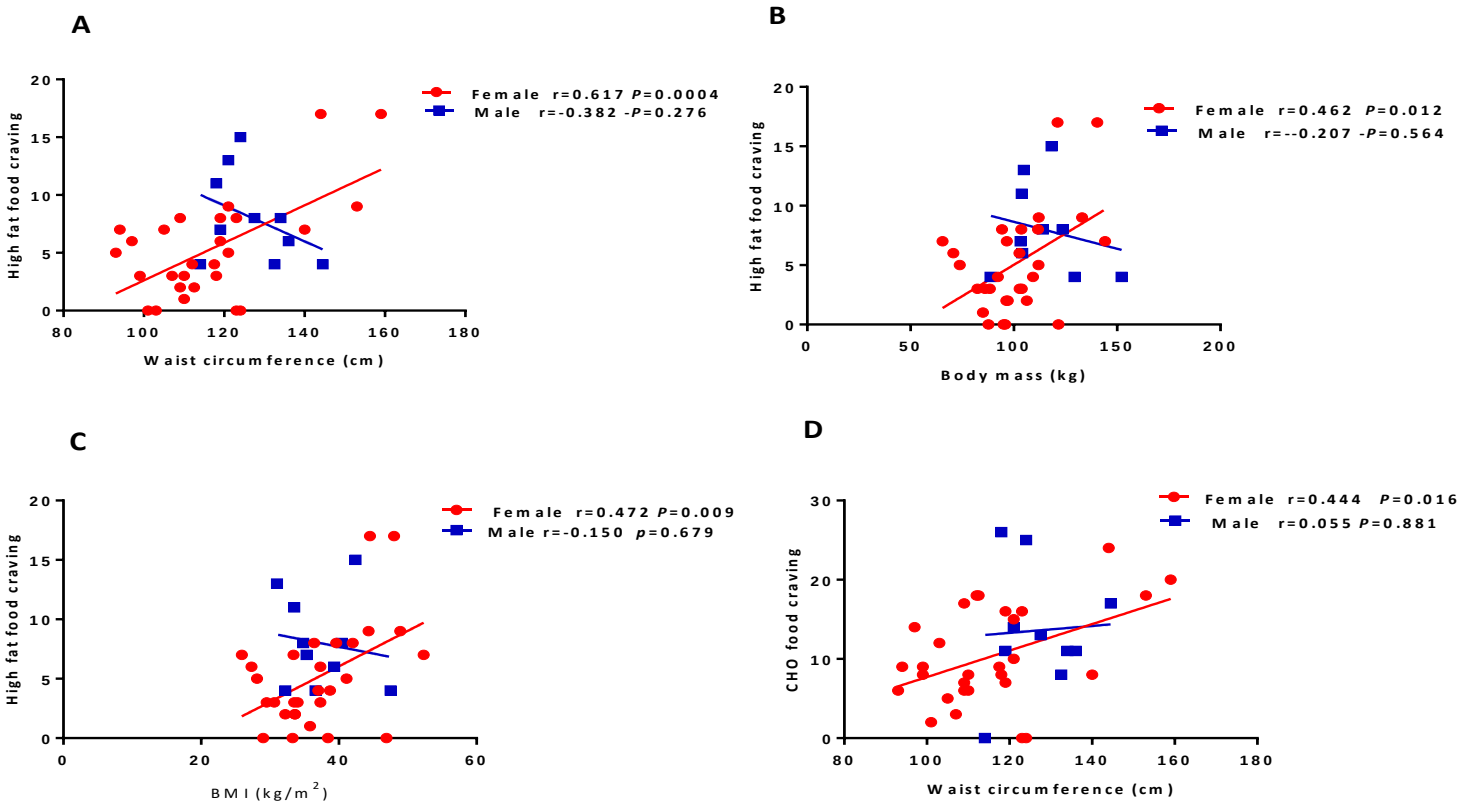

Figure 6-2: Relationship between food craving and baseline of anthropometry measurements for all participants (A= high fat food craving, B= high fat food craving, C= high fat food craving and D= CHO food craving

6.3.7 TEI, macronutrient intake food diary, TFEQ subscales and subsequent weight loss (all participants)

Table 6.4 displays correlations between TEI and macronutrient intake based on food diaries, TFEQ subscales and subsequent weight loss of all participants. No correlation was found between TEI, macronutrient intake measured by food diary, TFEQ subscales and subsequent weight loss in the participants. CHO was negatively correlated with body mass change and BMI change and fibre was positively correlated with body mass change but these relationships did not reach significance.

Table 6-4 : The relationship between TEI, macronutrient intake based on food diary, TFEQ subscales and subsequent weight change of all participants

Correlation	TEI (kcal)	% CHO	% Protein	% Fat	Fibre (g)	TFEQ Hunger	TFEQ Restraint	TFEQ Disinhibition
Body mass change	0.041	-0.321	0.156	-0.051	0.142	0.083	-0.125	0.119
BMI change	0.143	-0.324	0.128	0.053	0.073	0.015	-0.033	0.140
WC change	0.061	-0.112	0.115	-0.030	-0.102	0.085	-0.054	-0.042

Values represent Pearson correlation coefficient, r value

6.3.8 Food craving and subsequent weight loss

Table 6.5 shows data for food craving scores and subsequent weight loss values for the participants. The data indicate no relationship between food craving scores and subsequent weight loss. CHO craving was negatively correlated with body mass and BMI change but this did not reach significance and no correlation was found between CHO craving and WC change.

Table 6-5: The relationship between food craving and subsequent weight loss

Correlation	Fast food craving	Sweet craving	Fat craving	CHO craving
Body mass change	-0.003	-0.249	-0.043	-0.310
BMI change	-0.060	-0.132	-0.092	-0.306
WC change	-0.184	-0.018	0.098	-0.169

Values represent Pearson correlation coefficient, r values

6.3.9 TEI, macronutrient intake based on food diaries, TFEQ subscales and subsequent weight loss

Tables 6.6 and 6.7 below present the relationships between TEI, macronutrient intake, recorded by food diaries, TFEQ subscales and subsequent weight loss in females and males. No correlation was found between TEI, macronutrient intake, TFEQ subscales and subsequent weight loss of female participants (Table 6.6) or male participants (Table 6.7). CHO was negatively correlated with body mass change in males, but this relationship did not reach significance. Fat and cognitive restraint were negatively correlated with WC change in males, but this did not reach significance. Moreover, fibre was positively correlated with BMI change in males but this did not reach significance

Table 6-6 : The relationship between TEI, macronutrient intake based on food diaries, TFEQ subscales and subsequent weight loss in females.

Correlation	TEI (kcal)	% CHO	% Protein	% Fat	fibre (g)	TFEQ Hunger	TFEQ Restraints	TFEQ Disinhibition
body mass change	0.146	-0.040	0.247	-0.157	0.261	0.020	0.173	0.085
BMI change	0.209	-0.139	0.149	0.008	0.138	0.114	0.158	0.082
WC change	0.072	-0.116	0.102	0.067	0.112	0.012	0.038	0.032

Values represent Pearson correlation coefficient, r values

Table 6-7 : The relationship between TEI, macronutrient intake based on food diaries, TFEQ subcategory and subsequent weight loss in males.

Correlation	TEI (kcal)	% CHO	% Protein	% Fat	fibre (g)	TFEQ Hunger	TFEQ Restraint	TFEQ Disinhibition
body mass change	0.220	-0.688	-0.230	0.150	0.108	0.055	-0.069	0.547
BMI change	0.448	0.038	-0.022	-0.064	0.462	0.102	0.011	0.012
WC change	0.025	-0.120	0.185	-0.499	-0.131	-0.179	-0.400	-0.171

Values represent Pearson correlation coefficient, r values

6.3.10 Food craving and subsequent weight loss

Tables 6.8 and 6.9 below show the relationships between food craving and subsequent weight loss in participants. There was no relationship between high fat, sweet and fast food cravings and subsequent weight change in female participants (table 6.8).

However, CHO craving was negatively correlated with body mass change (r=-0.413*, p= 0.026) in females. Table 6.9 presents the relationship between food cravings and subsequent weight loss in male participants. There was no significant relationship between high fat, sweet and fast food and subsequent weight loss in male participants. However, CHO craving was inversely correlated with BMI change (r= -0.0647, p=0.043) in males.

Table 6-8: The correlation between food craving and subsequent weight loss in females.

Correlation	Fast food craving	Sweet craving	Fat craving	CHO craving
body mass change	-0.014	-0.040	-0.155	**-0.413***
BMI change	0.083	0.082	-0.277	-0.298
WC change	0.093	0.081	-0.217	-0.198

Values represent Pearson correlation coefficient, r values

Table 6-9 : The correlation of food craving scores and subsequent weight loss in males.

Correlation	Fast food craving	Sweet craving	Fat craving	CHO craving
body mass change	-0.445	-0.385	-0.304	-0.611
BMI change	-0.495	-0.371	-0.273	**-0.647***
WC change	-0.023	-0.307	-0.260	-0.083

Values represent Pearson correlation coefficient, r values

6.3.11 Identification of satiety phenotypes

The only independent variable studied in chapter 4 that was also assessed in this part of the study was TFEQ Hunger. The timing of the Shape-Up sessions meant it was not possible to carry out the test breakfast part of the study or to collect fasting blood samples. Therefore the participants were split into 2 groups based on their TFEQ scores. In chapter 4 the results showed differences between women and men for the TFEQ scores, therefore in this chapter the data was split into only 2 groups, low (0-6) and high (7-13) not 3 groups as in chapter 4. This was because when participants were allocated to the 3 groups using the range of TFEQ hunger scores, 0-4 for low, medium 5-9, and 10-13 for high satiety, the high satiety group only had one male participant.

Table 6.10 presents participants split by TFEQ hunger scores. As can be seen in the table there were 27 people with high satiety and 12 with low satiety ratings.

Table 6-10 : Heat map of TFEQ Hunger and satiety phenotype

PN	Gender	TFEQ Hunger	Phenotype
56	Female	0	High satiety
82	Female	0	High satiety
115	Female	0	High satiety
77	Female	0	High satiety
96	Female	0	High satiety
116	Female	0	High satiety
103	Female	0	High satiety
67	Female	0	High satiety
31	Female	1	High satiety
34	Male	1	High satiety
23	Male	2	High satiety
47	Male	2	High satiety
19	Female	2	High satiety
94	Female	2	High satiety
151	Female	2	High satiety
163	Male	2	High satiety
117	Female	2	High satiety
55	Female	3	High satiety
72	Female	4	High satiety
120	Female	4	High satiety
59	Female	5	High satiety
71	Male	5	High satiety
97	Male	5	High satiety
33	Female	5	High satiety
73	Female	6	High satiety
29	Female	6	High satiety
100	Male	6	High satiety
27	Female	7	Low satiety
86	Female	7	Low satiety
2	Female	7	Low satiety
85	Female	7	Low satiety
121	Male	7	Low satiety
92	Female	8	Low satiety
159	Female	9	Low satiety
106	Female	10	Low satiety
22	Male	12	Low satiety
54	Female	13	Low satiety
28	Female	13	Low satiety
99	Female	13	Low satiety

PN=Participant number

6.3.12 Average subsequent weight loss after 10 weeks on the programme

Figure 6.3 A, B and C present the average subsequent weight loss of all participants split into the 2 groups by low and high satiety. As can been seen in figures (A) and (B) people with high satiety have a greater BMI change and weight change after one month, but the difference is not significant. Figure (C) shows that the reverse is true for average WC change after one month. Based on a t-test there were no statistically significant differences between the low and high satiety groups.

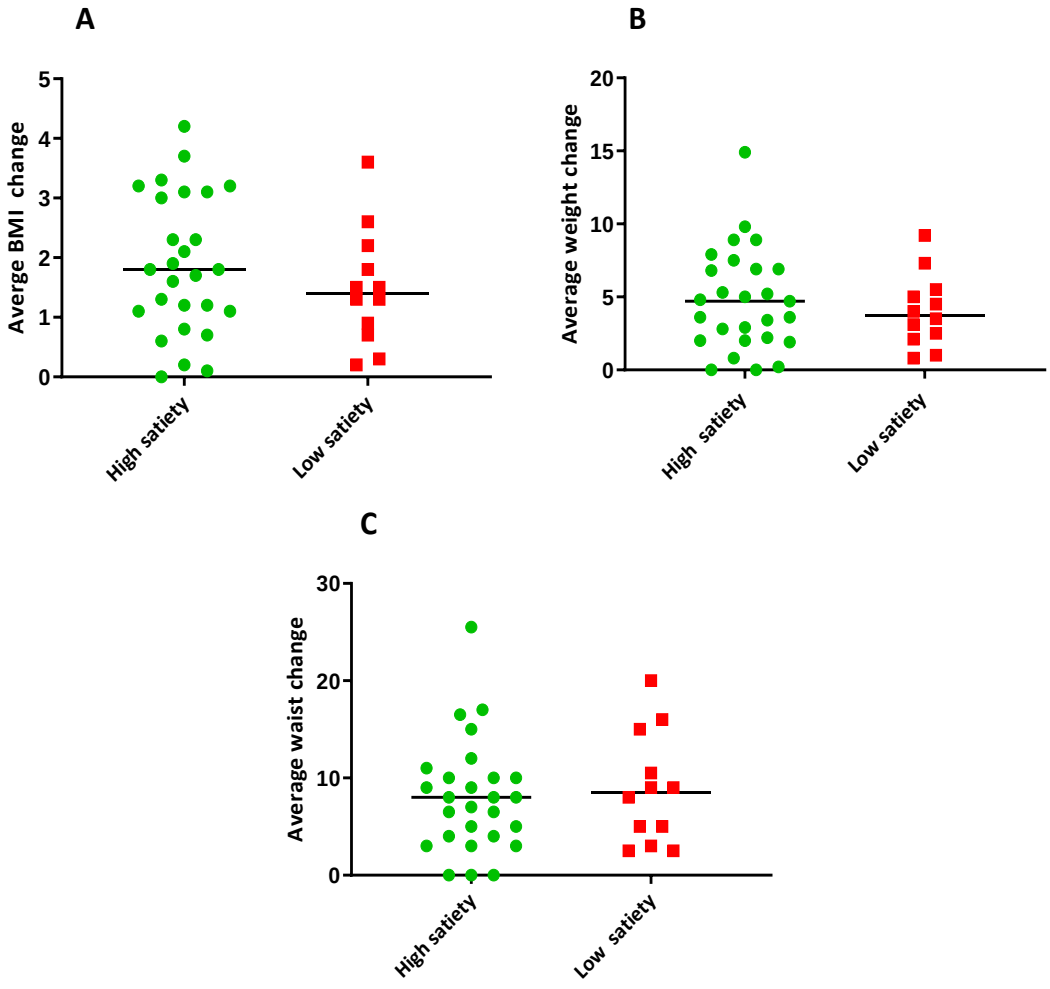

Figure 6-3 : Average subsequent weight loss of all participants (A= average BMI change, B= average weight change and C= average waist change

6.3.13 Average TFEQ subscales for low and high satiety participants

Figure 6.4 represents the average TFEQ subscale score and subsequent weight loss split by low and high satiety groups. Based on a t-test there was no statistical difference between low satiety and high satiety groups in TFEQ subscales p= -0.1480 Hunger and p=0.2483 restraint. There was a significant difference between low and high satiety groups in TFEQ disinhibition p= 0.043.

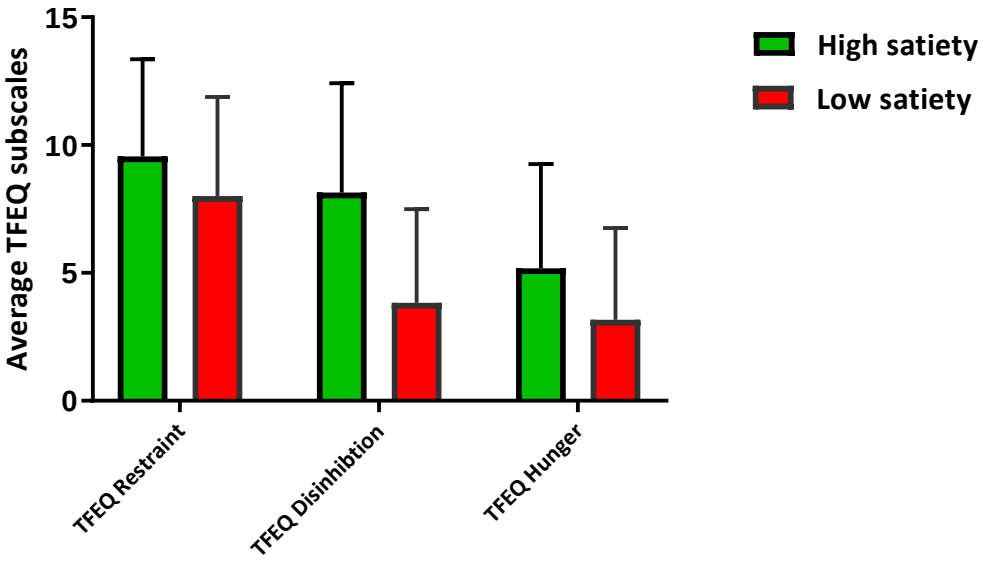

Figure 6-4 : Average TFEQ subscale scores for low and high satiety participants

6.3.14 Average macronutrient intake in low and high satiety participants

Figure 6.5 displays the average macronutrient intake in low and high satiety participant groups. T-test analysis shows that there was a significant difference between low and high satiety groups in CHO intake, p= 0.049. There was no difference between the 2 groups in fat, p= 0.0647and protein p=0.260 intake, based on food diaries.

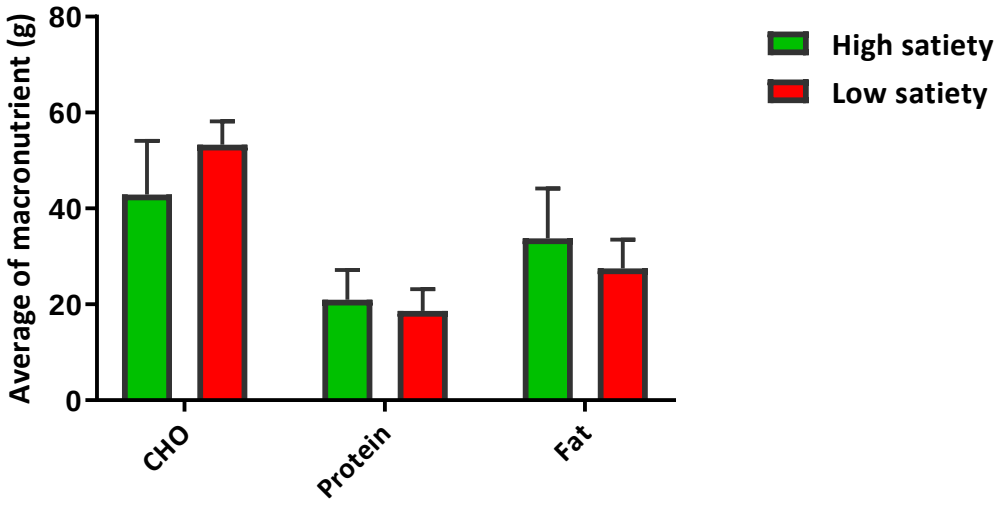

Figure 6-5: Average macronutrient intake in low and high satiety participants

6.3.15 Average food craving values in low and high satiety participants

Figure 6.6 presents the average food cravings in low and high satiety participants. T-test analysis shows that there were significant differences between low and high satiety groups in fast food p= 0. 033, sweet food, p=0.007 and CHO food intake, p=0.08. There was no difference between the 2 groups in fat craving scores, p= 0.2975.

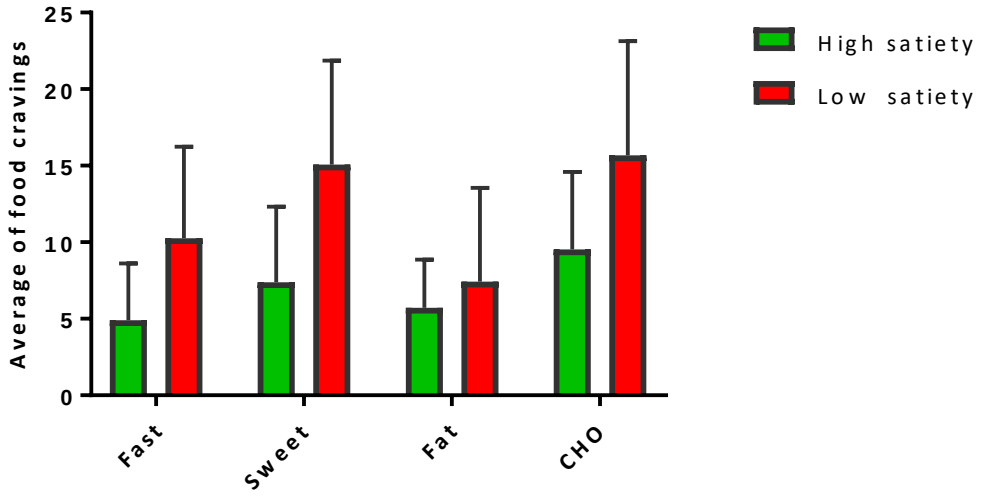

Figure 6-6: Average food cravings in low and high satiety participants

6.4 Discussion

Some individuals are more vulnerable to weight gain than others and are at higher risk of excess EI. Successful weight loss varies with some people responding well to interventions and others finding success more difficult. Methods to predict those individuals who struggle to lose weight prior to starting a weight loss programme could help them to success fully lose weight with additional support (Dalton *et al.,* 2015; Polsky *et al.,* 2013). The main aim of the present study was to develop methods to identify predictors of weight loss, in order to identify individuals, prior to participation in a weight management programme, who struggle to lost weight due to reduced satiety.

6.5 Baseline of food craving scores, food diaries data and TFEQ subscales of participants

In the current study, food cravings and macronutrient intake, measured by a food diary, and eating behaviour were investigated as variables that may predict weight loss. There were significant differences between males and females in baseline waist circumference and TFEQ Restraint. The current study showed no differences between males and females in scores of TEI, macronutrient intake as measured by food diaries, and food craving questionnaires.

6.5.1 TEI, macronutrient intake based on food diaries and baseline anthropometric measures

In the current study TEI and macronutrient intake based on a food diary did not correlate with baseline anthropometric measures. The findings of the present study contrasts with those of Ma *et al.,* (2005) who found a positive correlation between the percentage of calories from fat and TEI based on food diaries and higher BMI in females. However, in their study the percentage CHO intake showed no correlation with BMI. This study had a larger sample of 572, which could explain the difference as the present study only had 39 participants.

In the current study, when the data was split by gender, in females, high fat intake scores were significantly correlated with WC, body mass and BMI. Moreover, CHO intake was significantly correlated with WC. However, in males high fat and CHO intake

did not significantly correlate with WC, body mass and BMI. The potential interpretation of why these results was observed in females but not in males, may be the small sample size of males in the current study.

6.5.1.1 Food craving and baseline anthropometric measures

The results of the present study are partially in accordance with Batra *et al.,* (2013) who reported there was a positive correlation between food craving and baseline BMI. However, Batra *et al*., found this correlation in all participants and the present study showed this result only in females. Another finding from Chaudhari and Huerta-Franco (2017) also agrees with the current study. They showed that 98.7% of women had food craving behaviours and that food craving was a positive predictor of BMI in females. The explanation for this may be food craving behaviour is generated primarily by leisure activities and negative moods such as food psychological reactance, followed by depression (sadness), anxiety/stress and less frequently anger. Therefore, the study of food craving behaviour may be the key to identify possible eating behaviours that lead to increased weight and body fat or failure in eating behaviour disorders.

6.5.2 TEI and macronutrient intake and subsequent weight loss

TEI and macronutrient intake from a 3 days food diary did not predict people who would struggle to lose weight prior to participation in a structured weight-loss programme. A possible explanation for this result may be that people do not always fully report their habitual food intake and they feel embarrassed or guilty about recording their diet and so leave out more calorie dense items or snacks (Macdiarmid and Blundell, 1997). Another explanation may be that there is no relationship between food intake measured by a 3 day food diary, prior to starting a weight loss programme and subsequent weight loss and that is why this measure did not predict weight loss.

The findings of the present study are in contrast with previous study findings where Buscemi *et al.,* (2017) used a 3 day food diary record and found that low initial levels of caloric intake at baseline were positively associated with changes in BMI, but they did not find a relationship at average and high levels of initial caloric intake with BMI change. The explanation of why the results of this study differ from the current study may be the duration of the intervention in this study which was 18 months. Moreover,

the larger sample could be another explanation; there which were 202 participants in the Buscemi *et al.,* (2017) study.

After the data was spilt by gender, the present study showed no relationship was found between TEI based on a 3 day food diary data prior to starting the weight loss programme and subsequent weight loss. This finding is supported by Ledikwe *et al.,* (2006) who reported no correlation between total energy density and BMI change in males. However Martí-Henneberg *et al.,* (1999) also found no correlation between total energy density and BMI in 348 females.

6.5.2.1 Macronutrient intake and subsequent weight loss

The results of the present study showed no relationship between macronutrient intake based on the food diary and subsequent weight loss in all participants.

Linde *et al.,* (2006) showed that at baseline, higher fibre intake was associated with reduction in BMI at 24 months. This difference may be due to the large sample size in Linde *et al.,* (2006) with 508 men and 1293 women or the duration of study which was 24 months in Linde *et al.,* (2006) , whereas here it was 10 weeks. A further study from Du *et al.,* (2009) showed total fibre intake was inversely associated with weight and waist circumference. However, this study used a food frequency questionnaire to assess nutrients and the duration of the study follow up was 6.5 years. A previous study by Champagne, (2011) examined the relationship between dietary intake and weight loss. The findings of this study, in contrast with the present findings, indicated that lower fat percentage intake was associated with weight loss. The potential explanation for the differences in finding may be the larger sample size in the study by Champagne (2011) which was 828 and assessment of nutrient intake could be another explanation as in the Champagne (2011) study, food frequency questionnaires were used and the present study used a food diary. Food frequency questionnaires are often filled out over longer time periods e.g. a month, and in that case they probably would not give the same answer as a food diary.

Moreover, when the data was split by gender, the TEI and macronutrient intake measured on the food diaries did not correlate with subsequent weight loss. This finding is in accordance with Savage, Marini and Birch (2008) who used three day recalls to assess TEI and macronutrient intake and they reported dietary fibre and caloric beverage intakes were not significant predictors of weight change.

In contrast to the current study Hays *et al.,* (2006) indicated that dietary percentage of CHO and dietary protein was inversely correlated with weight loss and fibre was positively correlated with weight loss. However, the present study finding agrees with Hays *et al*., (2006) that dietary fat percentage did not correlate with weight loss. An explanation of this difference could be the body weight of the participants in Hays *et al.,* (2006) who were normal weight, while the present study consisted of people with obesity. It has been reported that people with obesity intentionally underestimate food intake to improve their self-esteem as a form of self-deception or self-presentation because they want to present themselves in a positive light to others (Muhlheim *et al.,* 1998).

6.5.3 TFEQ subscales and subsequent weight loss of participants

The current study evaluated TFEQ subscales to investigate whether people who were hungrier were less likely to succeed with weight loss when participating in a structured weight-loss programme. The results of the present study indicated that there was no correlation between the TFEQ subscale scores and weight loss in all participants or when the data was spilt by gender.

In agreement with the present study finding, Hays *et al*., (2006) included 76 females. They showed cognitive restraint and disinhibition measures did not predict weight loss.

In contrast to the current study, previously reported findings by Batra *et al.,* (2013) have found that baseline TFEQ hunger scores predicted greater weight change (R= -0.25, p = 0.03). The potential explanation for these different results was duration of study, which was 6 months in Batra *et al., (*2013) study and 10 weeks in the present study. Sample size could be another explanation for the different finding. Moreover, the mean scores of hunger in the Batra *et al,* (2013) study were higher than in the present study which could explain differences.

In contrast to the present study, James *et al.,* (2017), Buehler *et al.,* (2017) and Foster *et al.*, (1998) reported that lower baseline restraint predicted greater weight loss, while baseline Disinhibition and Hunger scores were not related to weight change. This difference may be due to the duration of the study with one year in both the James *et al.,* (2017) and Foster *et al*., (1998) studies. Participants in all studies were female and larger numbers were studied than in the current study. Differences in statistical

analysis in both studies could contribute to the differences. However, Bas and Donmez, (2009) have reported that reduced TFEQ Hunger and TFEQ Disinhibition score did show associations with weight change at 20 weeks.

In contrast to the present finding Rock *et al.,* (2017) found only TFEQ restraint was associated with weight loss. A possible explanation for this difference may be the larger sample In Rock *et al.,* (2017) which included 100 participants and a 6 month duration study in Rock *et al.,* (2017) could be another explanation compared with 39 participants studied over 10 weeks in the current study.

6.5.4 Food craving and subsequent weight loss

In the present study food craving scores did not correlate with subsequent weight loss values of all participants. However, although there was no significant correlation, results indicated that people who had lower food cravings subsequently lost more weight

This result agrees with current findings of Sayer *et al.,* (2018) who reported that the baseline food craving was linearly and inversely associated with weight loss at week 16 ($r = - 0.23$, $p = 0.037$). The people who had higher scores of food craving struggled to lose weight. Moreover, Buscemi *et al.,* (2017) reported that higher initial food cravings were associated with a more gradual reduction in BMI from baseline to 6 months. The findings of the present study are consistent with the existing evidence which shows that there was negative correlation between all food cravings at baseline and weight change at 6 months (Batra *et al.,* 2013).

Furthermore, previous studies have found a positive correlation between increased level of cravings and BMI, they reported that initial high levels of food craving may be related to less successful weight loss (Abilés *et al.,* 2010; Chao *et al.,* 2014; Franken and Muris, 2005). Targeting food cravings clinically might be beneficial for individuals enrolled in weight loss programmes who report higher levels of food cravings at baseline.

Moreover, the findings of the present study indicated that CHO food craving was inversely correlated with body mass change in females and BMI change in males. In contrast to the present study Gilhooly *et al.,* (2007) reported participants with a higher percentage of weight loss, craved foods with higher energy densities compared to

those who lost a lower percentage of weight. However, this study examined changes of food craving during 6 months and the present study examined food craving at baseline.

6.5.5 Identifying a satiety phenotype

The heat map used the TFEQ Hunger scores to identify satiety phenotypes, twenty-seven participants had a high satiety and twelve participants had a low satiety phenotype. The findings of the present study indicate that people with a high satiety phenotype on average lose less weight and BMI has a smaller change. The independent variable used in the heat map is the TFEQ Hunger score. It was chosen based on findings in chapter 4, because it predicts an individual phenotype of low and high satiety spilt by TFEQ hunger scores.

6.5.6 Summary

The key findings of this chapter are that baseline TEI and macronutrient intake based on food diaries did not predict people who would struggle to lose weight, prior to participation in a structured weight-loss programme. The results of the present study indicated that there was no correlation between the TFEQ subscale and subsequent weight loss. Food craving did not predict subsequent weight loss in all participants but when data was spilt by gender, CHO food craving was inversely correlated with body mass change in females and BMI change in males.

6.5.7 Study strengths and limitations

The strength of the study was that both genders were included. The current study presented data from participants on a 10-week weight loss programme. It would be valuable to be able to predict in the short term, prior to starting a weight loss programme, factors which indicate whether the programme would be successful. Many studies predicted weight loss with regards to a 6 month weight loss programmes. One limitation in the current study was the small sample size.

Chapter 7

General discussion

7 General discussion

The overall aim of the studies reported in this thesis was to test methodologies and to develop a protocol to identify high hunger and low satiety phenotypes in individuals. Such a protocol could potentially help healthcare professionals working in weight-loss programmes to identify people at baseline who have low satiety prior to participating in a weight management programme.

7.1 Background to the investigations

Appetite control plays an important role in energy balance, eating behaviour and body weight control (Finlayson *et al.,* 2007). Research has shown large variability in appetite control capacities in people with obesity as well as in normal weight individuals**.** There are different methods to assess satiety and hunger in individuals. The use of VASs to characterise appetite sensations has been shown to provide a good degree of within-subject reliability and validity, in that these sensations predict meal initiation and the amount of food eaten (Stubbs *et al.,* 2000). Drapeau and colleagues examined fasting VAS for 4 questions, AUC and SQ to measure satiety phenotype in people with obesity (Drapeau *et al.,* 2005; Drapeau *et al.,* 2007). Their research indicated that high scores of fasting hunger, desire to eat and prospective food intake were associated with lower body weight loss. Moreover, they showed that AUC of all appetite sensation and SQ fullness were predictive of an *ad libitum* test lunch EI in women. Drapeau *et al.,* (2005) also reported that AUC fullness and SQ fullness predicted TEI measured by food diary in the Drapeau studies. McNeil *et al.,* (2014) found that prospective food consumption and fullness SQ predicted EI and CHO intake, measured by a food diary in agreement with the findings of Drapeau *et al.*

TFEQ is another measure that can be used in assessment of food intake and weight management. People with obesity with weak satiety responsiveness had higher scores on TFEQ Disinhibition and Hunger compared to controls (Barkeling *et al.,* 2007), which are eating behaviour traits linked to overconsumption and, a higher BMI (Bryant, King and Blundell, 2008).

Gut hormone levels (ghrelin, CCK, GLP1, PP and PYY) are another measure that is used in assessing food intake and body weight regulation. These hormones have a vital role in the short-term control of appetite. Numerous studies have shown that intravenous

administration of ghrelin in both lean people and people with obesity can increase feelings of hunger and EI from buffet meals (Druce and Bloom, 2006; Druce *et al.,* 2005; Wren *et al.,* 2001). IV administration of PYY (3-36) has also been demonstrated to lead to a reduction in appetite and an almost 30% restriction in caloric intake in lean people and people with obesity (Batterham *et al.,* 2003a). A number of studies have observed that the infusion of PP at 5 pmol/kg min following a buffet meal, reduced EI by 11% compared with saline control, together with a decrease in hunger as analysed by VAS (Jesudason *et al.,* 2007). Various studies have confirmed that systemic administration of GLP-1 hormone decreases food intake slows gastric emptying and reduces body weight (Marathe *et al.,* 2011; Van Bloemendaal *et al.,* 2014).

Food craving is one of the measurements that is used in predicting weight management success. Martin *et al.,* (2008) found significant correlation between consumption of corresponding types of foods and weight loss. Ferguson *et al.,* (1992) and Elfhag and Rössner, (2005) suggested that reducing and managing food cravings is important in the management of obesity and successful weight loss maintenance.

Finally, food diary records have been used in relationship between macronutrient intake and weight management. Johnstone *et al.,* (2008) reported that people who had a lower percentage of CHO in their diet had higher scores for hunger using the VAS. Buscemi *et al.,* (2017) indicated that low initial levels of caloric intake were positively associated with changes in BMI. Moreover, Hays *et al.,* (2006) reported that fibre intake was significantly positively associated with weight loss.

Although these studies have measured food intake and weight regulation, they did not focus on using a baseline satiety phenotype in a clinical setting to predict whether people with low satiety found it more difficult to lose weight. As a result, due to this gap in the literature the studies in this thesis focused on the baseline satiety phenotype.

7.2 Key findings

The table below shows all measures and the key findings of this thesis.

Table 7-1 : Comparison of variables as predictors in the different studies undertaken in this thesis

Variables	**Pilot study**	**Prediction satiety phenotype in individuals with normal weight study**	**Tier 3 study**	**Tier 2 study**
VAS	Low satiety participants on VAS (fasting hunger and prospective food intake) measurement ate more calories measured by food diary	Low satiety participant on VAS measurement ate more calories measured by food diary	People who had low satiety measured by VAS struggled to lose weight	No VAS measurements used
Gut hormones	Did not predict TEI measured by food diary	Did not predict TEI measured by food diary	People who had higher levels of PYY lost more weight at one month	No gut hormone analysis used
TFEQ subscales	Did not predict TEI measured by food diary	TFEQ Restraint measure was negatively correlated with fat intake in women and TEI in males, but did not predict *ad libitum* lunch intake	Did not correlate with weight loss	Did not correlate with weight loss
Food craving	Obese and overweight individuals who have high scores for food craving ate less protein although this correlation did not reach significance.	A strong negative correlation between fast food cravings and TEI in men. Sweet food craving was positively correlated with protein intake, but did not predict *ad libitum* lunch intake in both genders	No food craving measured	Women who crave more CHO struggled to lose weight and men who crave more CHO struggled to reduce their BMI

7.3 VAS measures in the laboratory reflect real life

The results for the pilot group (chapter 3) and the normal healthy student group study (chapter 4) in the laboratory reflected participant food intake in real life. In the pilot study, people who had high hunger indicated by measuring (fasting hunger and prospective food intake in the laboratory ate more calories in their real life according to their food diaries. Moreover, they ate a lower percentage of CHO in their diets. Those, people who had higher scores at fasting and AUC hunger had a lower percentage of protein in their daily food intake.

In the normal healthy weight student study, those who had high scores for hunger at fasting, post breakfast, 30 min and AUC VAS ate more calories in their real life. These results applied to women, whereas men had higher scores at fasting desire to eat, prospective food intake and AUC composite of appetite scores, they had a lower percentage of CHO in their food diaries. These results show that VAS in the laboratory measurements can reflect real life satiety phenotype in different groups (people with obesity and healthy weight). This finding could be applied in a community setting, using food diaries to identify people who are more known to have a more hungry phenotype before they start a weight loss programme may increase the success rate of the programme.

7.4 Optimal measurements of VAS (fasting, SQ and AUC) in prediction of a satiety phenotype

In this thesis VAS measurements (fasting, SQ and AUC) have been used in three studies in different groups of people including individuals who were healthy weight, overweight or obese and in different settings i.e. In a university laboratory or a community, to investigate which measure of VAS is the best to identify a satiety phenotype.

In the pilot and healthy student studies the fasting VAS scores showed strong correlation with EI measured by a food diary and AUC showed some correlation with EI. However, in contrast to previously published papers (McNeil *et al., (*2014) and Dalton *et al.,* (2015) SQ was not a good predictor of satiety phenotype in the laboratory setting, as a result, the protocol of VAS measurements in the community was changed. The timing and frequency of completed VAS appetite scores in the

laboratory setting were 3 hours and 5 assessment times within this period, whereas in the community setting, this was changed to one hour and 15 minutes and the VAS appetite scoring completed respectively four times. As the results from the pilot study showed that you can get similar information about someone's hunger or satiety by only measuring VAS scores for hunger and appetite for a shorter time period.

7.5 Plasma gut hormone levels as a measure to predict a satiety phenotype

Gut hormones were used in the laboratory setting study, and in the community to investigate their predictive value for satiety phenotypes in participants. In this thesis four gut hormones were investigated using five blood samples taken at different time points in the laboratory setting to identify the optimal hormones to measure, as well as the timing of blood sampling to identify satiety phenotypes. These findings were then used to inform the studies based in the community settings. The results from chapters 3 and 4 showed that fasting PYY and PP hormones predicted a satiety phenotype. Females who had low levels of PYY consumed more calories measured by a food diary and males with low level of PP consumed more calories. The fasting blood sample provided the most useful information in the laboratory setting, therefore in the community setting just one fingerpick blood sample was taken after the participant fasted. The advantage of this was that it reduced the cost of the analysis and made it more likely that people would be willing to take part as they only had to give one blood sample.

7.6 TFEQ

TFEQ measurement was used in all studies in this thesis but this measure did not predict satiety phenotype in any. Some subscales showed a correlation with EI and macronutrient consumption, but this correlation did not reach significance. Although in some reports this measure showed good correlation with satiety (Bryant, King and Blundell, 2008; Barkeling *et al.,* 2007), the small samples size in the studies carried out in this thesis affected the result.

7.7 Food craving

In the pilot study people with obesity and overweight who crave fast food, CHO or fat food, ate less protein, even though this correlation did not reach significance. In the student study where participants had a healthy weight, food cravings did not predict *ad libitum* lunch intake in either women or men. However, men who had higher fast food cravings consumed less calories measured by a food diary and if participants craved sweet food, then they consumed more protein as evidenced by their food diaries. In the Tier 2 community setting food craving predicted weight loss. In this study CHO food craving was the best measure for predicting weight loss.

7.8 Gender

In this thesis the numbers of female participants was higher than males and in many studies in the literature it has been shown that females predominantly participate in weight loss trials (Ahern *et al.,* 2017).

In the student study, participants with a healthy weight showed that some measures are more significant in females compared to males. For example, fasting hunger at different time points and AUC VAS predicted calorie intake measured by a food diary in females. Whereas, VAS measures (fasting desire to eat and prospective food intake) predicted percentage of fat intake recorded in the food diary. TFEQ Restraint measure showed a negative correlation with fat intake in females and TEI in men. Fasting and AUC PYY showed an inverse correlation with TEI in females. Whereas, in men there was a negative correlation between fasting PP and TEI. Moreover, fasting ghrelin was negatively correlated with the percentage protein intake in males and there was no correlation between macronutrient intake measured by food diaries and gut hormone levels in females.

Food craving did not predict TEI or macronutrient intake in females whereas there was a strong negative correlation between fast food cravings and TEI. Moreover, sweet food craving was positively correlated with protein intake in males.

This study showed a strong correlation between VAS (fasting hunger, prospective food intake and AUC) and *ad libitum* lunch intake in females. Whereas, in males there was no significant correlation between VAS and *ad libitum* lunch intake, this may be

because of the low number of male participants. Other measurements such as TFEQ, gut hormone levels and food craving were not significantly correlated with *ad libitum* lunch intake in either gender.

In the Tier 2 community study, VAS measures showed a strong correlation with weight loss. Females who had a higher score for fasting hunger struggled to lose their body weight at one month, whereas VAS measures did not predict weight loss in men. TFEQ and gut hormone levels did not predict weight loss in either gender.

In the Tier 3 community setting study, food diaries and TFEQ did not predict weight loss in either gender whereas CHO food craving showed a significant correlation with weight loss. Females who craved more CHO struggled to lose weight and males who craved more CHO struggled to reduce their BMI.

7.9 Heat map

The methods used in chapter 4 in a laboratory setting with healthy weight people enabled the development of heat maps to identify satiety phenotypes. The potential application of this developed method is to help professionals working in weight-loss programmes. The result of the heat map showed that a heat map based on food diary records better predicted hunger of participants than the *ad libitum* lunch using method one. In method one, heat maps of participants spilt by gender were analysed. For each variable, participants were ranked in order from small to large values of the variable. The participants were then split into tertiles according to the value of the variable. Each participant was allocated a colour for that variable: i.e. green cells for low scores, orange for medium and red for high scores for variables, where a low score was associated with satiety e.g. ghrelin, or hunger and a high score associated with hunger or low satiety. For other measures e.g. fullness or PP hormones, large values were green and small values red. This process was repeated with each variable so that each participant was allocated a colour for each variable and this was used to build a heat map for each person. After that the heat map was reordered by *ad libitum* lunch intake and the same process was used to rank by food diary TEI.

The heat map was applied in a Tier3 community setting to help to identify satiety phenotypes and those participants that would struggle to lose weight. The findings of the present study indicated that people with the high hunger phenotype on average

had a lower change in BMI, percentage of weight loss after one month and WC change compared to the other groups. Moreover, low satiety phenotype participants had the highest TFEQ Hunger scores at baseline, compared to people with the lower and medium hunger phenotypes and had medium TFEQ Restraint and low TFEQ Disinhibition, although this is not surprising as the TFEQ Hunger score was one of the variables used to build the heat map.

In the Tier 2 study in a community setting the heat map used the TFEQ Hunger scores to identify satiety phenotypes. The findings of this study indicated that people with a high satiety phenotype on average lose less weight and had a reduced change in BMI. The independent variable used in the heat map is the TFEQ Hunger score. It was chosen based on findings in chapter 4 because it predicts an individual phenotype of low and high satiety spilt by TFEQ hunger scores. The heat map results in the Tier 3 community setting showed good results with VAS and TFEQ which can help to identify people prior to the start of a weight loss programme who may struggle to lose weight due to their reduced satiety phenotype and allow for additional advice to support these people.

7.10 Future work

To apply this protocol in the future in a clinical setting or community, the focus should be on certain measures. VAS provide the best measurement for professionals working in community weight-loss programmes to focus on to identify people who have low satiety at baseline prior to participation in a weight management programme. They also have the advantage of being one the cheapest tools studied in this thesis.

When gut hormones were studied, fasting samples showed the best predictive value especially fasting PPY and PP. TFEQ Hunger and food diaries are important to understand the eating behaviour of people who want to lose weight. CHO appeared to be the best items of food craving to study satiety phenotype in the community setting. Applying gut hormone level measurements in a Tier 2 study would be useful to predict high hunger phenotype people because they gave good results in the laboratory and the method can be applied by dividing people into groups following overnight fasting in the morning and only one finger prick sample was required.

7.11 Limitations of the study

Assessments of all measurements should be repeated on several days as studying only on one day does not account for normal day-to-day variations in these measurements. Another limitation was participants on a high protein diet were lost to follow up. In the pilot study people found the high protein diets hard to follow, which may be related to the recipes provided and the cost of a high protein diet.

The results of the *ad libitum* lunch meal could be validated in future studies taking in to account whether people like or dislike the food, as the results in the current study may depend on whether people liked pasta, influencing the amount they ate.

A larger sample population is needed in all future studies.

In chapter 4, the original method development study could have varied the breakfast food amount by BMI to control for this. A high protein diet could be tried in a randomised control trial as a personalised diet for low satiety people. People could be split into two groups and allocated two diets, high protein diet and normal care diet, this will help to identify whether the high protein diet is the best diet for low satiety people or not.

7.12 Final conclusion

This study has developed a methodology that could be used to identify people with a low satiety phenotype in a way that could be reproduced in a clinical setting. This method is cheap and easy to apply without the use of complex data analysis. Further studies are required to refine the choice of variables and to test the validity of the method in a clinical setting with a large cohort of people who are overweight or suffer from obesity.

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9 APPENDICES

9.1 Appendix 1 Pre-Test Medical Questionnaire

Sheffield Hallam University

Faculty of Health and Wellbeing Research Ethics Committee

Sport and Exercise Research Ethics Review Group

Pre-Test Medical Questionnaire

Name: _____

Date of Birth: _____ Age: _____Sex: _____

Telephone/Mobile: _____ E-mail: _____

Please answer the following questions by putting a circle round the appropriate response or filling in the blank.

1. How would you describe your present level of activity?

   Sedentary / Moderately active / Active / Highly active

2. How would you describe you present level of fitness?

   Unfit / Moderately fit / Trained / Highly trained

3. How would you consider your present body weight?

   Underweight / Ideal / Slightly over / Very overweight

4. Did your weight fluctuate in the past 6 months? Yes / No

4.1 If yes, how much?.....

5. Are you currently trying to lose or gain weight? Yes / No

5.1 If yes, how? ……..

6. Smoking Habits Are you currently a smoker? Yes / No

How many do you smoke …….. per day

Are you a previous smoker? …… Yes / No

How long is it since you stopped? ..... years

Were you an occasional smoker? …….. Yes / No

Were you a regular smoker? ……Yes / No

7. Do you drink alcohol? Yes / No

If you answered **Yes**, do you usually have?

An occasional drink / a drink every day / more than one drink a day?

8. Have you had to consult your doctor within the last six months? Yes / No

If you answered **Yes**, please give details……

……

……

9. Are you presently taking any form of medication? Yes / No

If you answered **Yes**, please give details…….

10. As far as you are aware, do you suffer or have you ever suffered from:

a Diabetes? Yes / No **b** Asthma? Yes / No

c Epilepsy? Yes / No **d** Bronchitis? Yes / No

e *Any form of heart complaint? Yes / No **f** Raynaud's Disease? Yes / No

g *Marfan's Syndrome? Yes / No **h** *Aneurysm/embolism? Yes / No

I Anaemia Yes / No

11. ***Is there a history of heart disease in your family? Yes / No

12. ***Do you currently have any form of muscle or joint injury? Yes / No

If you answered **Yes**, please give details…….

13. Have you had to suspend your normal training in the last two weeks? Yes / No

If the answer is **Yes** please give details…….

If blood is not being taken from you please disregard Section 14. below.

14. * Please read the following questions:

a) Are you suffering from any known serious infection? Yes / No

b) Have you had jaundice within the previous year? Yes / No

c) Have you ever had any form of hepatitis? Yes / No

d) Are you HIV antibody positive Yes / No

e) Have you had unprotected sexual intercourse with any person from an HIV high-risk population? Yes / No

f) Have you ever been involved in intravenous drug use? Yes / No

g) Are you hemophiliac? Yes / No

15. As far as you are aware, is there anything that might prevent you from successfully completing the tests that have been outlined to you? Yes

IF THE ANSWER TO ANY OF THE ABOVE IS YES THEN:

a) **Discuss the nature of the problem with the Principal Investigator.**
b) **Questions indicated by (*) Allow your Doctor to fill out the 'Doctors Consent Form provided.**

As far as I am aware the information I have given is accurate.

Signature: ……

Date: ……/……/…

9.2 Appendix 2

Sheffield Hallam University

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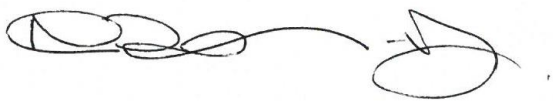
May 18, 2015

Dear Dr. Dalton & Ms. Abdella-Hameida Elfarssi

Thank you second version of your project entitled: Pilot study to investigate the effectiveness of personalised diet plans based on hormonal and genetic analysis. Following re-review we are delighted to give you ethic permission to undertake the project. However please note that on your PIS you have David Binney as the head of ethic, this is in fact Peter Allmark. Please can you make this small change. Apart from this, you may get started.

Yours sincerely,



Dr. Nicola Jordan-Mahy
Chair of the BMRC Research Ethics Committee.

9.3 **Appendix 3** Participant information sheet 1

Faculty of Health and Wellbeing Research Ethics Committee

Biomedical Research Centre Review Group

Participant Information Sheet

Project Title	Pilot study for developing methods to identify individuals with blunted satiety by measured hormone, appetite and eating behaviour

Supervisor/Director of Studies	Dr. Caroline Dalton

Principal Investigator	Hameida El Farssi

Principal Investigator telephone/mobile number	0114 225 3695

Purpose of Study and Brief Description of Procedures

You are being invited to take part in a research study. Before you decide if you wish to take part it is important for you to understand why the research is being undertaken and what it will involve for you. Please take time to read the following information carefully. The investigators will go through this information sheet with you and answer any questions you have. Please ask if there is anything that is not clear or if you would like more information. Take your time to decide if you would like to take part.

Procedures

Read participant information sheet
Consider study, ask questions
Complete and sign consent form
Height and weight measurements
Completion of eating behaviours and food cravings questionnaires
Collection of a cheek cell sample. The process to take the cheek cell sample is entirely painless; it is very similar to gently brushing the inside of your cheek with a toothbrush. You would carry out this process yourself with help from our researcher (if required).

Benefits of the study
The study will provide important information regarding eating behaviours and food cravings and the influence of genetics on these effects. The study is anonymised, so we will not be able to give you any individual feedback on your results, but we will be happy to discuss the overall findings

of the study with you.

What will happen to the results at the end of the study?

The results will be published as academic reports in scientific journals. There will be no identifying information published. We will also write a summary report of anything we discover which we will send to any of the people who participated in the study who want to see it. This will not contain any identifying information.

Will my taking part in this study be kept confidential?

Yes, no one will know you have taken part in the study except the members of the research team. Your consent form will be placed by you in a sealed envelope separate from the questionnaires and the DNA sample. You will be allocated a participant number, and this will not be linked to your consent form therefore the data we collect from you will be completely anonymous and cannot be traced back to you.

What will happen to my DNA sample?

Your DNA sample will be stored in our laboratories which have restricted access. The samples will be coded with a participant number which cannot be traced back to you. We will only use the sample to study genes involved in the topics covered by this ethics application and we will not allow access to your dna sample by any third party.

Has this study got ethical approval?

Yes, this study was approved by the local Ethics Committee. This means that experts who are not involved in the study have carefully considered the aims and methods of the study and agreed that they meet their guidelines. You can find out more about their work on their website

www.sth-research.group.shef.ac.uk/research/index.html

Right to Withdraw

Remember that you always have the right to withdraw from the study at any time.

It has been made clear to me that, should I feel that these Regulations are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform Dr Nikki Jordan-Mahy, Chair of the Faculty of Health and Wellbeing Research Ethics Committee (Tel: 0114 225 3120) who will undertake to investigate my complaint.

9.4 **Appendix 4 :** Informed consent form

INFORMED CONSENT FORM	
TITLE OF PROJECT: Study to investigate the factors influencing body weight plan IRAS number: 208879	
Please read the questions below and circle your answer. If you do not understand any of the questions, please ask the researcher.	
Have you read and understood the Participant Information Sheet? (version 3 January 2017)	YES/NO
Have you had an opportunity to ask questions and discuss this study?	YES/NO
Have you received satisfactory answers to all of your questions?	YES/NO
Have you received enough information about the study?	YES/NO
To whom have you spoken? ..... Do you understand that you are free to withdraw from the study? □ at any time □ without having to give a reason for withdrawing	YES/NO
Do you agree to the secure storage of your anonymised data?	YES/NO
Do you agree to provide blood samples and a DNA sample; these will also be stored securely?	YES/NO
Do you agree that your samples and data can be used in future studies?	YES/NO
Do you agree that medical notes and data collected during the study can be looked at by individuals from regulatory authorities or sponsors, where it is relevant to you taking part in the research?	YES/NO

Do you agree to take part in this study?	YES/NO
Signed..... Date ..... (NAME IN BLOCK LETTERS) ..... Original to be filed in the Investigator site file, 1 copy for patient, 1 copy to be kept in the patient's medical records at RIO.	

9.5 **Appendix 5** Dieting and Weight History Questionnaire

Participant number ……..

1. What is the most you have ever weighed since reaching your full height? (Do not count any weight gain due to medical conditions or medications).

   The most I have weighed since reaching my full height is ……pounds

2. What is the least you have ever weighed since reaching your full height? (Do not count any weight loss due to medical conditions or medications).

   The least I have ever weighed since reaching my full height is…….pounds

3. What is your current weight?.....pounds

4. If the difference between the answer to question 1 and question 3 is less than 5lbs please go to question 5. If this difference is 5lb or more please circle the statement below that best describes this difference:

a. The difference between my highest weight and my current weight is due to weight that I lost on purpose.
b. The difference between my highest weight and my current weight is due to weight I lost even though I wasn't trying to
c. I'm not sure why I weigh less than I once did

5. For how long have you been at or close to (within 2lbs) your present weight……months

6. Which of these statements best describes what has happened to your weight during the past 6 months? (please circle one)

a. My weight has stayed about the same
b. I've been losing weight
c. I've been gaining weight
d. My weight has fluctuated a lot

7. Are you currently on a diet? YES/NO if No please go to Q13

8. Are you currently dieting to lose weight or to avoid gaining weight? (Please circle one)
   To lose weight To avoid gaining weight

9. How long have you been on your current diet? ……..weeks

10. How much longer do you anticipate being on your diet?.....weeks

11. How much weight (if any) have you lost on your current diet?.....pounds

12. How much more weight do you intend to lose on your current diet?.....pounds

13. Have you ever been on a diet to lose weight (not including a current diet)? YES/NO
    If your answer is No skip Q14-17
14. About how long ago were you last on a diet to lose weight (if you are on a diet refer to the most recent previous diet)? ……..weeks

15. About how old were you when you went on your first diet?.....years old

16. Please estimate as best you can the numbers of times in your life you have dieted and purposely lost the amount of weight listed
    How many times in your life have you dieted and lost:

    1-4 pounds……..times

    5-10 pounds……times

    11-20 pounds……..times

    21 or more pounds……..times

17. Please give details of any of the following diets have you previously tried?

Calorie counting	Atkins
Low fat	SlimFast
Low Carbohydrate	High Protein
Weight watchers	Slimming World
5:2	Other

Diet: How long you followed the diet: weeks

Weight loss:……pounds How long you kept the weight off……..weeks

Diet: How long you followed the diet:

Weight loss:……pounds How long you kept the weight off……..weeks

Diet: How long you followed the diet:

Weight loss:……pounds How long you kept the weight off……..weeks

18. What do you consider a good weight for yourself? …….pounds

19. Were you overweight as a child (12 years or below)? YES/NO

20. Were you overweight as a teenager (12-18 years? YES/NO

21. Was there a particular time in your life where you put on weight? Please give details:

.....

22. How often do you eat out? ……..

What types of meals?.....

10. Do you drink alcohol? Yes / No

If you answered **Yes**, how many drinks do you have a week?.....

What kind of drinks (beer, wine, spirits ?

12. Do you feel your weight accurately reflects the amount you eat? YES/NO

13. List any foods that you consider:

Bad to eat ……

Good to eat …….

14. How many hours of sleep do you get each night?

Less than 6 hours □ 6-7 hours □ 7-9 hours □ more than 9 hours □

15. Do you feel rested on most days? No □ yes □

16. How many meals a day do you eat? …….

17. Do you snack or eat between meals? If yes, on what, and when, where, and how much?

9.6 **Appendix 6** Food preferences

Diary	Like	Dislike	Definitely must be in my diet	Definitely must not be in my diet	Vegetables/ starches	Like	Dislike	Definitely must be in my diet	Definitely must not be in my diet	Fruits	Like	Dislike	Definitely must be in my diet	Definitely must not be in my diet
Milk-whole					Beetroot					Apples				
Milk-low Fat					Onions					Lemons				
Milk-Non fat					Green peppers					Pears				
Cottage-cheese					Garlic					Apricots				
Cheese					Green Beans					Oranges				
Other cheeses and cheese spreads					Green salad					Peaches				
Flavoured yogurt					Sweet corn					Grapes				
Meat	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my diet**	Carrots					Mandarins/ Satsumas				
Roast beef					Celery					Fruit Cocktail				
Burgers					Spinach					Dried fruit				
Meatloaf					Cauliflower					Watermelon				
Hot Dogs					Broccoli					Pineapple				
Pork Chops					Asparagus					Bananas				
Pork Roast					Courgettes									

Ham					Squash					Strawberries				
Lamb					Cabbage					Prunes				
Veal					Peas					**Juices**	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my diet**
Liver					Tomatoes					Apples				
Sausages					Lettuce					Oranges				
Bacon					Mushrooms					Prunes				
Chicken-White Meat					Beans					Cranberry				
Chicken-Dark Meat					Lentils					Tomato				
Turkey-White Meat					Mashed potatoes									
Turkey-Dark Meat					Sweet Potatoes					**Desserts**	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my diet**
Eggs					Macaroni					Ice Cream				
Fish	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my diet**	Rice					Cheesecake				
Tuna fish					Pasta					Biscuits				
Shell fish e.g. mussels					Chips					Cakes				
Salmon					Roast potatoes					Pudding				
Haddock					Boiled potatoes					Custard				
Cod					Jacket potatoes					Fruit Pies				
White fish										Dessert with nuts				
Prawns														
Bread	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my**										

				diet										
White Bread														
Wholemeal Bread														
Rye Bread														
Bread with nuts and seeds														
Soups	Like	Dislike	Definitely must be in my diet	Definitely must not be in my diet										
Broth/Clear														
Creamy														
Vegetables														
Chicken														
Beef														
Tomato														
Potato														
Preferences Beverages	Like	Dislike	Definitely must be in my diet	Definitely must not be in my diet										
Regular soft drinks														
Beer														
Decaffeinated coffee														
Milk														
Milk in coffee or tea														
Tea														
Spirits														
Wine														
Juice														

Other														
Snacks	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my diet**										
Crisps														
Salted Nuts														
Unsalted nuts														
Savoury biscuits														
Sweet biscuits														
Chocolate bars														
Restaurant food	**Like**	**Dislike**	**Definitely must be in my diet**	**Definitely must not be in my diet**										
Italian food														
Thai food														Food allergies/ Intolerance
Chinese food														Shell fish
Indian food														Gluten
Fish and chips														Dairy Chocolate Nuts Other
Other														Culture/Ethnic/Religious food request
														None Yes----------
Signature					Title							Date		

Food and beverages preferences list

9.7 **Appendix 7** Food diary

FOOD DIARY

Instructions for the food diary:

Please fill out this food diary for 3 days, noting down what you have eaten as close as possible to the time you ate it (to help you to remember).

Please try to be as specific as possible about the amount of each item, what time you ate it and any brand names.

For example:

Breakfast 8am - 1 piece of Warburton's white bread with Olivio spread, 2 scrambled eggs, I large cup of coffee with 1 level teaspoon sugar.

Lunch 12.30pm - 1 small plate of lettuce and tomatoes, half a large tin (160g) of Princes tuna in brine, 2 small new potatoes, boiled, small portion of full fat salad dressing, 1 can Coke.

Evening meal 7pm - Beef chilli con carne with white rice (average portion). Cup of black coffee with 1 level teaspoon sugar. 1 medium slice Tesco own brand blackcurrant cheesecake.

Don't forget to include all drinks and any between meal snacks!

There is no need to change your diet in any way, all information is anonymised.

Day 1- Date:……..

Breakfast: Include all food and drinks (*example: 8am - 1 piece white toast and butter, 2 scrambled eggs, 1 cup of black coffee with 1 sugar*) Time:
Lunch : Include all food and drinks *(example: 12.30pm 1 small plate of lettuce and tomatoes, half a large tin (160g) of Princes tuna in brine, 2 small new potatoes, boiled, small portion of full fat salad dressing, 1 can Coke)* Time:
Evening meal: Include all food and drinks *(example: Beef chilli con carne with white rice (average portion). Cup of black coffee with 1 level teaspoon sugar)* Time:
Snack and drinks (not taken at mealtimes): *(example: 1 apple, 1 Mars bar, 1 small glass full fat milk, 1 plain biscuit, 1 large glass of white wine)*

Day2- Date:……..

Breakfast: Include all food and drinks (*example: 8am - 1 piece white toast and butter, 2 scrambled eggs, 1 cup of black coffee with 1 sugar*) Time:
Lunch : Include all food and drinks *(example: 12.30pm 1 small plate of lettuce and tomatoes, half a large tin (160g) of Princes tuna in brine, 2 small new potatoes, boiled, small portion of full fat salad dressing, 1 can Coke)* Time:
Evening meal: Include all food and drinks *(example: Beef chilli con carne with white rice (average portion). Cup of black coffee with 1 level teaspoon sugar)* Time:
Snack and drinks (not taken at mealtimes): *(example: 1 apple, 1 Mars bar, 1 small glass full fat milk, 1 plain biscuit, 1 large glass of white wine)*

Day 3- Date:……..

Breakfast: Include all food and drinks (*example: 8am - 1 piece white toast and butter, 2 scrambled eggs, 1 cup of black coffee with 1 sugar*) Time:
Lunch : Include all food and drinks *(example: 12.30pm 1 small plate of lettuce and tomatoes, half a large tin (160g) of Princes tuna in brine, 2 small new potatoes, boiled, small portion of full fat salad dressing, 1 can Coke)* Time:
Evening meal: Include all food and drinks *(example: Beef chilli con carne with white rice (average portion). Cup of black coffee with 1 level teaspoon sugar)* Time:
Snack and drinks (not taken at mealtimes): *(example: 1 apple, 1 Mars bar, 1 small glass full fat milk, 1 plain biscuit, 1 large glass of white wine)*

9.8 **Appendix 8** Food craving

FOOD CRAVING INVENTORY (FCI)

Name: _____Participant numbeR_____ Date: _____

Food craving is defined as an intense desire to consume a particular food (or food type) that is difficult to resist.

For each of the foods listed below (Items 1-28), please circle the appropriate letter using the following scale.

Over the past month, how often have you experienced a craving for the food?

A = Never

B = Rarely (once or twice)

C = Sometimes

D = Often

E = Always/almost every day

List of foods	Never	Rarely	Sometimes	Often	Almost everyday
Cakes	A	B	C	D	E
Pizza	A	B	C	D	E
Fried Chicken	A	B	C	D	E
Sausages	A	B	C	D	E
French Fries/Chips	A	B	C	D	E
Rice	A	B	C	D	E
Sausage Rolls	A	B	C	D	E
Gravy	A	B	C	D	E
Hamburger/Beefburger	A	B	C	D	E
Biscuits	A	B	C	D	E
Ice Cream	A	B	C	D	E
Pasta	A	B	C	D	E
Fried Fish	A	B	C	D	E
Cookies	A	B	C	D	E
Chocolate	A	B	C	D	E
Pancakes/Waffles	A	B	C	D	E
Bread Rolls/Bagels/Baps	A	B	C	D	E
Doughnuts	A	B	C	D	E
Sweets	A	B	C	D	E
Brownies/Muffins	A	B	C	D	E
Bacon	A	B	C	D	E
Steak	A	B	C	D	E

List of foods	Never	Rarely	Sometimes	Often	Almost everyday
Danish pastry	A	B	C	D	E
Baked Potatoes	A	B	C	D	E
Sponge Cake	A	B	C	D	E
Cereals	A	B	C	D	E
Sandwich Bread	A	B	C	D	E
Crisps	A	B	C	D	E

9.9 **Appendix 9** Three factor eating questionnaire

Participant number……. Date…….

We are interested in the reasons underlying peoples' attitudes to food and eating. Please indicate to what extent each of the following statements is true for you. Please note there are no right or wrong answers or trick questions. We simply want to know how you personally feels about food and eating. Please CIRCLE your response.

1. When I smell a sizzling steak or a juicy piece of meat (or vegetarian equivalent), I find it very difficult to keep from eating, even if I have just finished a meal.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

2. I usually eat too much at social occasions, like parties and picnics.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

3. I am usually so hungry that I eat more than three times a day.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

4. When I have eaten my quota of calories, I am usually good about not eating any more.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

5. Dieting is so hard for me because I just get too hungry.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

6. I deliberately take small helpings as a means of controlling my weight.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

9. When I feel anxious, I find myself eating.

   definitely true for me……quite true for me……occasionally true for me……definitely not true for me

10. Life is too short to worry about dieting.

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

12. I often feel so hungry that I just have to eat something.

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

14. I have a pretty good idea of the number of calories in common food.

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

16. It is not difficult for me to leave something on my plate.

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

17. A certain times of the day, I get hungry because I have got used to eating then.

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

20. When I feel blue, I often overeat.

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

    definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

definitely true for me……quite true for me……occasionally true for me……definitely not true for me

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

rarely I	sometimes	usually	always

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

not at all	slightly	moderately	very much

39. How often do you feel hungry?

only at mealtimes	sometimes between meals	often between meals	almost always

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

never	rarely	often	always

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

easy	slightly difficult	moderately difficult	very difficult

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

not at all	slightly	moderately	extremely

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

almost never	seldom	usually	almost always

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

unlikely	slightly unlikely	moderately likely	very likely

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

never	rarely	often	always

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

unlikely	slightly likely	moderately likely	very likely

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

almost never	seldom	at least once a week	almost every day

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

unlikely	slightly likely	moderately likely	very likely

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

never	rarely	sometimes	at least once a week

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

0	Eat whatever you want, whenever you want it
1	Usually eat whatever you want, whenever you want it
2	Often eat whatever you want, whenever you want it
3	Often limit food intake, but often 'give in'
4	Usually limit food intake, rarely 'give in'
5	Constantly limiting food intake, never 'giving in'

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

not like me	a little like me	pretty good description of me	describes me perfectly

9.10 **Appendix 10** Appetite Scale 1

Appetite Scale

Participant number……. **Pre breakfast**

How hungry do you feel?

I am not Hungry at all — I have never been more hungry

How satisfied do you feel?

I am Completely empty — I cannot eat another bite

How full do you feel?

Not at all full — Totally full

How much do you think you can eat?

Nothing at all — A lot

Participant number……. **After breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all hungry been more

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Participant number……. **1 hr after breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all hungry been more

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Participant number……. **2 hr after breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all been more

hungry

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Participant number……. **3 hr after breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all hungry been more

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

9.11 Appendix 11 Participant information sheet 2

version 3 Jan 2017

Project Title	**Study to investigate factors influencing the body a weight IRAS number: 208879**
Principal Investigator	Dr Caroline Dalton Biomolecular Sciences Research Centre Sheffield Hallam University
Principal Investigator telephone/email	0114 225 3695/c.f.dalton@shu.ac.uk

Purpose of Study and Brief Description of Procedures

You are being invited to take part in a research study. Before you decide if you wish to take part it is important for you to understand why the research is being undertaken and what it will involve for you. Please take time to read the following information carefully. One of our team is available to go through this information sheet with you and answer any questions you have. Please ask if there is anything that is not clear or if you would like more information. Take your time to decide if you would like to take part.

The project aims to establish whether it is possible to identify the biological and psychological factors that are associated with success on a weight management plan.

We think that there are differences in how people respond when they try to manage their weight. These individual differences may be due to genetic or hormonal variability, or they may be due to psychological differences. If we can identify factors that are associated with increase in body .we may be able to design better programmes to also help those people who find weight management more difficult.

If you choose to participate in this study, you will be asked to fill in some questionnaires about your attitudes to food and exercise and your feelings.

We also want to test your hormones and analyse your DNA, to do this we will need to take a DNA swab from your mouth.

Procedures:

Meeting 1 (after your 1st appointment at RIO, 30 minutes)

Read participant information sheet
Consider study, ask questions
Complete and sign consent form
Fill in questionnaires
Give a DNA sample by swabbing your mouth (this is painless and similar to using a toothbrush)

Meeting 2: **(2 hours, at RIO, a few days after meeting 1, or during the following week)**

Collection of blood sample. For the blood prick sample, you need to carry out a 12 hour overnight fast. A blood prick sample will be collected in the morning when you first arrive using a finger prick device (similar to that used by diabetics to test their insulin levels), this will be analysed for hormones.
Test breakfast and questionnaires. Once you have given the blood sample you will be asked to fill in a questionnaire about how hungry you feel, then you will be given a breakfast of cornflakes, milk and orange juice. If you are allergic to any of these items, we can offer an alternative e.g. rice krispies/soya milk. You will be asked to record how hungry you feel at time points during the next 1 hour and 45 minutes. During this time, you need to stay at RIO but are free to read or use your phone. At the end of this time the test will finish, and you can leave. You will be given £5 at the end of this session to cover your transport costs.

sible risks and discomforts:
The blood prick sample procedure is mildly uncomfortable, no other risks or discomforts are anticipated.

efits of the study:
The study will provide important information regarding whether It is possible to identify what factors predict success for people on a weight management programme. However, you will not be told your individual results.

What will happen to my DNA and blood sample?

Your DNA and blood samples will be stored in our laboratories which have restricted access. The samples will be coded with a participant number which cannot be traced back to you. We will not allow access to your samples by any third party.

What will happen to my samples and data at the end of the study?

We would like to store your samples and data so at the end of the study so that we can use them in similar research projects. If you would prefer that we did not do this, you can indicate this on the consent form and we will destroy your samples and remove your data from the database at the end of the study.

What will happen to the results at the end of the study?

The results will be published as academic reports in scientific journals. There will be no identifying information published. We will also write a summary report of anything we discover which we will send to any of the people who participated in the study who want to see it. This will not contain any identifying information. The report will be available in mid-2018. If you would like to have a copy of the report, please contact RIO nearer the time on 01709 720193.

Will my taking part in this study be kept confidential?

Yes, no one will know you have taken part in the study except the members of the research team. Your consent form will be placed by you in a sealed envelope separate from the questionnaires, the blood samples and the DNA sample. You will be allocated a participant number, and this will not be linked to your consent form therefore the data we collect from you

will be completely anonymous and cannot be traced back to you.

Has this study got ethical approval?

Yes, this study was approved by the local Ethics Committee. This means that experts who are not involved in the study have carefully considered the aims and methods of the study and agreed that they meet their guidelines.

Right to Withdraw:

Remember that you always have the right to withdraw from the study at any time.

If you feel that these Regulations are being infringed or that your interests are otherwise being ignored, neglected or denied, you should inform Dr Nikki Jordan-Mahy, Chair of the Faculty of Health and Wellbeing Research Ethics Committee art Sheffield Hallam University (Tel: 0114 225 3120 email n.jordan-mahy@shu.ac.uk) who will undertake to investigate your complaint.

9.12 **Appendix 12** Appetite Scale 2

Appetite Scale

Participant number……. **Pre breakfast**

How hungry do you feel?

I am not Hungry at all — I have never been more hungry

How satisfied do you feel?

I am Completely empty — I cannot eat another bite

How full do you feel?

Not at all full — Totally full

How much do you think you can eat?

Nothing at all — A lot

Participant number……. **After breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all hungry been more

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Participant number……. **1 hr after breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all been more

hungry

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Participant number……. **2 hr after breakfast**

How hungry do you feel?

I am not Hungry at all — I have never been more hungry

How satisfied do you feel?

I am Completely empty — I cannot eat another bite

How full do you feel?

Not at all full — Totally full

How much do you think you can eat?

Nothing at all — A lot

Participant number……. **3 hr after breakfast**

How hungry do you feel?

I am not _____ I have never

Hungry at all hungry been more

How satisfied do you feel?

I am _____ I cannot eat

Completely another bite

empty

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

9.13 Appendix 13 Example for high protein diet

Example 1 of high protein recipes for women

Day 6 calories 1399, protein 105g

Breakfast - Cheese and Veg Omelette.

Ingredients

2 large eggs, 30g Monterey jack cheese, 3 medium mushrooms, 1 plum tomato

Method

Spray the pan with cooking spray and place it on medium heat. Dice the tomatoes, and mushrooms. Crack the eggs in a separate bowl and add the water & salt and pepper to it. Beat with a fork or whisk until combined. Pour the egg mixture on the vegetables. Cook until sides are dry gently pivoting pan to ensure evenness. Flip omelette and cook on other side. Add shredded cheese to half. Put the cheese side on plate. Gently flip other side over to have half circle.

Egg and spinach tortilla

Ingredients

1 flour tortilla, 2 large eggs, 1 ounce cheddar cheese,2 cups fresh spinach, 1 tbsp. ground flaxseed

Method

Add each ingredient

Snack - Tuna crunch

Ingredients

Combine 100 g tuna (tinned in water), 60 g tinned sweetcorn, 2 celery stick, 2 tsp lemon juice.

Dinner - Chicken dish

Ingredients

3 Chicken Breasts 2 tbsp. Ground Garlic (Marinade)3 tbsp. Olive Oil (Marinade)3 tbsp. Balsamic Vinegar (Marinade)3 tbsp. Lemon Juice (Marinade)750g Sweet Potato Chopped into Wedges2 tbsp. Jerk Seasoning2 tbsp. Olive Oil.

Method

Add the ingredients for the marinade together in a bowl. Mix and add the chicken and leave covered in the fridge for 2-3 hours. Chop the sweet potato and add to a roasting tray with a drizzle of olive oil and the jerk seasoning. Mix through. Place chicken on a separate roasting tray and cover with foil. Place the sweet potato and chicken into the oven for 40mins at 200C or until cooked. Example 2 of high protein diet recipes for men

Example 2 of high protein recipes for men

Day 7 1905 calories, protein 140

Breakfast- Cottage cheese and strawberries

Mix together: 1 c Breakstone's 2% cottage cheese

3/4 c sliced strawberries

With -2 boiled egg.

Lunch- 8-Beef Sandwich

Ingredients

12 oz (lean beef roast) 4 sandwich thins or toast 1/4 c plain Greek yogurt 2 T soft cheese, 2 c green leaf lettuce 1/2 cup Onions

Method

Mix cheese and Greek yogurt. Layer between the bread the lettuce, beef, onions, and dressing. Makes 4 sandwiches

With your lunch take1 cup of skim milk

Snack- Feta and grapes

Ingredients

60 g feta cheese, 180 g grapes

With your snack take- ½ oz almond.

Dinner- Steak & blue cheese wrap

Ingredients

1 tbsp. olive oil, 1 x 140g/5oz sirloin steak, trimmed of fat and cut into strips, 1 small red onion, thinly sliced, ½ red pepper, deseeded and sliced, squeeze balsamic glaze, 25g Stilton or dolce latte, 1 small soft flour tortilla, handful rocket or baby spinach leaves

Method

Heat the oil in a hot frying pan, season the steak, then fry with the onion and pepper for 4 mins over moderate heat. Stir in the balsamic glaze, continue to cook for 1 min, then remove from the heat. Warm the tortilla. Slice the steak into thin strips, then tip back into the pan with any meat juices and mix with the veg. Spoon the mixture over the middle of the tortilla, crumble over the cheese, then scatter with rocket or spinach. Fold to make a wrap and serve straight away.

With your dinner take- tea with cup of skim milk

9.14 Appendix 14

NHS
Health Research Authority
North East - Tyne & Wear South Research Ethics Committee
HRA Jarrow
Rolling Mill Road
Jarrow
NE32 3DT

Telephone: 0207 1048084

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

24 January 2017

Dr Caroline Dalton
Biomolecular Sciences Research Centre
Sheffield Hallam University
Sheffield S1 1WB

Dear Dr Dalton

Study title:	**Study to investigate the psychological, hormonal and genetic factors influencing the success of a weight management plan**
REC reference:	**17/NE/0018**
IRAS project ID:	**208879**

The Proportionate Review Sub-committee of the North East - Tyne & Wear South Research Ethics Committee reviewed the above application by correspondence on 20 January 2017.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact hra.studyregistration@nhs.net outlining the reasons for your request. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

The PR Sub-Committee agreed that this was a well presented study with no material ethical issues.

On behalf of the Committee, the Sub-Committee gave a **Favourable** ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Recommendation: Consider providing the participants with an alternative to cornflakes for breakfast.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Additional condition specified by the REC:

- The participant information sheet needs to include further details regarding Meeting 2 - state the location and how long after the first visit this will take place.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity, e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [advert]	Version 2	30 November 2016
Covering letter on headed paper [Cover letter for ethics application]		
IRAS Application Form [IRAS_Form_10012017]		10 January 2017
Letter from funder [letter from funder]		03 January 2017
Letter from funder [letter from funder]		03 January 2017
Letter from funder [letter from funder]		03 January 2017
Letter from funder [letter from funder]		03 January 2017
Letters of invitation to participant [Letter of invitation]	Version 2	30 November 2016
Participant consent form [Informed consent form]	Version 2	30 November 2016
Participant information sheet (PIS) [Participant information sheet]	Version 2	30 November 2016
Referee's report or other scientific critique report [Sheffield Hallam University]		11 January 2017
Research protocol or project proposal [RIO protocol final version]	November 2016	03 January 2017
Summary CV for Chief Investigator (CI) [CV CF Dalton]		
Summary CV for student [CV Hamedia El Farssi]		
Summary CV for student [CV Hanan Abdella]		
Validated questionnaire [all questionnaires]	Version 2 November 2016	
Validated questionnaire [Three factor eating questionnaire]	November 2016	
Validated questionnaire [BREQ2 questionnaire]	November 2016	
Validated questionnaire [TAS-20 questionnaire]	November 2016	
Validated questionnaire [Visual analogue scale questionnaire to rate hunger]	November 2016	

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

17/NE/0018	**Please quote this number on all correspondence**

Yours sincerely
pp

Mr Ian Campbell
Vice Chair

Email: nrescommittee.northeast-tyneandwearsouth@nhs.net

Enclosures: List of names and professions of members who took part in the review

'After ethical review – guidance for researchers' SL-AR2

Copy to: Dr Keith Fildes – Research Dept, Sheffield Hallam University

Ms Philippa Collins – R&D Dept, The Rotherham NHS Foundation Trust

Ms Hanan Abdella /Ms Hameid El Farssi - Biomolecular Sciences Research Centre, Sheffield Hallam University

North East - Tyne & Wear South Research Ethics Committee

Attendance at PRS Sub-Committee of the REC meeting on 20 January 2017

Committee Members:

Name	*Profession*	*Present*	*Notes*
Ms Sam Barron	Clinical Lead, Speech and Language Therapist	Yes	
Mr Ian Campbell (Vice Chair)	Pharmacy	Yes	
Mrs Louise Jones	Faculty Research Administrator	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Ms Gillian Mayer	REC Manager

9.15 Appendix 15

NHS
Health Research Authority

Dr Caroline Dalton
Biomolecular Sciences Research Centre
Sheffield Hallam University
Sheffield
S1 1WB

Email: hra.approval@nhs.net

02 February 2017

Dear Dr Dalton,

Letter of HRA Approval

Study title:	**Study to investigate the psychological, hormonal and genetic factors influencing the success of a weight management plan**
IRAS project ID:	**208879**
REC reference:	**17/NE/0018**
Sponsor	**Sheffield Hallam University**

I am pleased to confirm that HRA Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application

IRAS project ID	208879

procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **208879**. Please quote this on all correspondence.

Yours sincerely

Thomas Fairman
HRA Assessor

Email: hra.approval@nhs.net

Copy to: *Dr Keith Fildes, Sheffield Hallan University, (Sponsor Contact)*
Ms Philippa Collins, The Rotherham NHS Foundation Trust,
(Lead NHS R&D Contact)