The Role of Fermentable Carbohydrates on Appetite Regulation in Humans

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ABSTRACT

This thesis investigates the ability of fermentable carbohydrates to stimulate anorectic gut hormones, which in turn reduce appetite and body weight.

Fermentable carbohydrates have been shown to suppress food intake and body weight in rodents via the release of satiety hormones, glucagon-like peptide 1 (GLP-1) and peptide-YY (PYY). However, evidence of the effect of fermentable carbohydrates on modulating body weight in humans is contradictory. In this thesis, supplementing oligofructose over eight weeks has been shown to significantly reduce hunger and increase PYY secretion in overweight volunteers. However, there was no suppression on energy intake or body weight when compared with cellulose supplementation.

Studies in rodents also suggested that reductions in food intake and body weight following intake of fermentable carbohydrates are associated with activation in the central nervous system. In contrast, oligofructose supplementation in this study had no significant effect on reducing activation in pre-selected brain reward regions in response to visual food cues, as measured by functional MRI. Surprisingly, the activation in these brain regions was reduced by cellulose intake.

The negative findings from the oligofructose study may be due to insufficient concentrations of short chain fatty acids (SCFAs) in the colon. SCFAs, the fermentation end-products of ingested fermentable carbohydrates are thought to play a significant role in modifying energy homeostasis by stimulating PYY and GLP-1 from enteroendocrine L-cells in the colon. By applying a novel method of delivering propionate to the colon using a propionate carrier molecule (PCM), it has been shown that supplementation with PCM in healthy lean volunteers reduced hunger and energy intake in pilot studies via elevated PYY secretion. PCM also has a dose-dependent appetite inhibiting effect. The preliminary results in this thesis open up an interesting possibility of the development of the PCM as a dietary supplement to aid weight loss.

DECLARATION OF CONTRIBUTORS

All the work in this thesis was performed by the author except for otherwise stated
All of the contributors are listed below.

Chapter 2

The appetite study and 'In house' radioimmunoassays were carried out in teamwork with Nurhafzan Ismail. Glucose and insulin assays were performed by The Biochemistry Department at Hammersmith Hospital. The test product, oligofructose (Beneo P95) and cellulose were provided by Orafti, Tienen, Belgium.

Chapter 3

MRI whole body composition scans were performed by the MRC imaging group; Prof. Jimmy Bell, Dr. Louise Thomas and Julie Fitzpatrick. Images analyses were performed by Dr. Louise Thomas. Glucose, Insulin and lipid profile assays were performed by The Biochemistry Department at Hammersmith Hospital.

Chapter 4

fMRI brain scans were performed by MRC imaging radiologist, Guliana Durighel and were assisted by Nurhafzan Ismail, Dr. Tony Goldstone, Dr. Navpreet Chinna and Dr. Samantha Scholtz. fMRI imaging acquisition was performed with guidance from Dr. Tony Goldstone and Dr. Samantha Scholtz. 'In house' radioimmunoassays were performed in teamwork with Nurhafzan Ismail, Michelle Sleeth and Dr. Alexander Miras.

Chapter 5

Human studies 1 and 2 were carried out in collaboration with Dr. Sagen Zac-Varghese whilst study 3 and study 4 was performed in partnership with Dr. Alexander Viardot and Dr. Edward Chambers. Propionate carrier molecule was synthesised and kindly provided by Dr. Douglas Morrison, SUERC, Glasgow, Scotland and the control, inulin was provided by Orafti, Tienen, Belgium. The bread rolls used in study 1 and 2 were provided by Premier Foods (Lichfield, Staffordshire, UK). 'In house' radioimmunoassays were performed in teamwork with Sagen Zac-

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All the clinical studies were performed in the Sir John McMichael Centre. MRI and fMRI scans were carried out in Robert Stainer Unit, Hammersmith Hospital.

Dr. Michael Patterson, Dr. Paul Bech and Mr. Andrew Hogben were kindly provided advices and guidance in all ‘in-house’ gut hormones radioimmunoassay experiments and Professor Mohammad Ghatei established and maintained all the radioimmunoassay analyses.

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Untuk Ayahanda, Bonda

& keluarga tercinta

ABBREVIATIONS

ACC	Anterior cingulate cortex
AgRP	Agouti-related peptide
ANOVA	Analysis of variance
AP	Area postrema
ARC	Arcuate nucleus
ASAT	Abdominal subcutaneous adipose tissue
AT	Adipose tissue
BBB	Blood brain barrier
BMI	Body mass index
BOLD	Blood-oxygenation-level-dependent
CCK	Cholecystokinin
CNS	Central nervous system
DE	Degree of esterification
DEBQ	Dutch eating behaviour questionnaire
DMN	Dorsomedial nucleus
DPP-IV	Dipeptidyl-peptidase- 4
DEXA	Dual energy x-ray absorptiometry
DLPFC	Dorsolateral prefrontal cortex
DP	Degree of polymerization
FDR	False Discovery Rate
FFAR2 / FFAR3	Free fatty acid 2 / Free fatty acid 3
fMRI	functional magnetic resonance imaging
g	grams
GLP-1 / GLP-2	Glucagon like peptide-1 / Glucagon like-peptide 2
IAAT	Intra-abdominal adipose tissue
ICV	Intracerebroventricular
IHCL	Intrahepatocellular lipid
HOMA-IR	Homeostatic model assessment – Insulin resistance
LHA	Lateral hypothalamic area

MDM	Magnetic dipole moment
MEMRI	Manganese-enhanced MRI
Mins	Minutes
MRC	Medical research council
MRI	Magnetic resonance imaging
α-MSH	Alpha melanocortin-stimulating hormone
NAc	Nucleus accumbens
NTS	Nucleus tractus solitaries
NPY	Neuropeptide-Y
ObR	Leptin specific receptors
OFC	Orbifrontal cortex
OXM	Oxyntomodulin
PCM	Propionate carrier molecule
PET	Positron emission tomography
PFC	Prefrontal cortex
POMC	Pro-opiomelanocortin
ppm	Part per million
PVN	Paraventricular nucleus
PYY	Peptide tyrosine-tyrosine
RF	Radio frequency
ROI	Regions of interest
RYGB	Roux-en-Y gastric bypass
SAT	Subcutaneous adipose tissue
SCFA	Short chain fatty acid
SCOFF	Sick Control One Fat Food
SEM	Standard error of mean
SPECT	Single photon emission tomography
TAT	Total adipose tissue
tAUC	total Area Under the Curve
TR	Repetition time
vACC	ventral anterior cingulate cortex

VAS	Visual analogue scores
VMN	Ventromedial nucleus
VTA	Ventral tegmental area
WC	Waist circumference

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Chapter 1

General Introduction

1 INTRODUCTION

1.1 OBESITY

Obesity is a condition in which accumulation of excessive fat in the body has reached a certain level that causes various health problems, leading to increased morbidity and mortality. Obesity is a major global health problem with more than one billion adults now classified as overweight (Body mass index [BMI] $\geq$ 25 kg/m^2) and over 300 million people reported as clinically obese (BMI $\geq$ 30 kg/m^2) (WHO, 2002). In England, it was reported that 24.1% of males and 24.9% of females were classified as obese in 2008, an increase of 10.9% for males and 8.5% for females since 1993 (Boyle, 2011).

The cause of obesity is multifactorial, however increased consumption of palatable and energy-dense foods containing excessive amount of sugars and saturated lipids and lack of physical activity are suggested the main contributors to obesity. Genetic disturbances, medications and physiological illness also potentially lead to obesity but to a lesser extent compared to diet and behavioural factors. The consequences of obesity can be severe as obesity is associated with devastating co-morbidities such as hypertension, diabetes, cardiovascular diseases, musculoskeletal disorders and certain types of cancer (Eckel et al., 2005), which increase the risk of morbidity and mortality. Furthermore, in countries with a high percentage of obesity cases, there is a significant increase in healthcare costs for treating obesity-related diseases and also a financial burden on the employment system due to decreased productivity. Therefore, obesity is not just an individual health concern, but it affects the whole community.

To date, there are no known medications to cure obesity. However, reducing caloric intake and physical activity are the key elements in reducing body weight. Lifestyle modification programmes have been shown to induce body weight loss by utilising low energy diets. However, their effectiveness in the long term (between one to five years) is not sustained (Norris et al., 2004). Pharmaceutical therapies for long term treatment are limited to orlistat (Xenical®, Roche) after sibutramine (Reductil®, Abbott laboratories) was withdrawn as an anti-obesity treatment due to association with cardiovascular risk (Williams, 2010). Orlistat is suggested to

modestly reduce body weight by acting as a gastric and pancreatic lipase inhibitor in the gastrointestinal tract, which then subsequently reduces fat absorption. Although its efficacy in reducing body weight has been demonstrated in several studies (Hill et al., 1999; Rossner et al., 2000; Sjostrom et al., 2007), it can cause gastrointestinal side effects such as diarrhoea, flatulence (Padwal et al., 2004) and malabsorption of dietary fat (Filippatos et al., 2008). Furthermore, in 2009, United States Food and Drug Administration reported that orlistat maybe closely correlated to liver injury (U.S.Food and Drug Administration, 2011). To date, bariatric surgery is the most effective treatment for obesity and appears the only long term solution to promote sustained weight loss (Sjostrom et al., 2007). However, because the procedure has high complication rates and involves significant financial cost, these treatments are only limited to individuals with minimum BMI of 40 kg/m^2 or >35 kg/m^2 with co-morbidities (Fajnwaks et al., 2008). Several types of bariatric surgery have been developed since it was introduced in 1950s (O'Brien, 2010) including gastric banding, sleeve gastrectomy, Roux-en-Y gastric bypass (RYGB) and biliopancreatic diversion. However, the most common surgical operations performed in the UK and worldwide are gastric banding and RYGB (Buchwald and Oien, 2009; Flum et al., 2009).

Besides triggering weight loss, bariatric surgery has also been shown to stimulate the release of anorectic gut hormones, therefore resulting in reduced appetite and energy intake. The anorectic gut hormone, peptide tyrosine-tyosine (PYY) was shown to significantly increase in post-operative RYGB patients compared with the normal weight and obese volunteers three hours after the intake of a 420 kcal meal (Korner et al., 2005; Le Roux et al., 2006a). Interestingly, RYGB also preserved the levels of postprandial PYY in a long term period. A study showed that postprandial PYY levels in post-surgeries RYGB patients after 12, 18 and 24 months were significantly increased compared to the pre-operative patients whilst a tendency towards an increase glucagon-like peptide 1 (GLP-1) was also demonstrated at 18 and 24 months (Pournaras et al., 2010). In addition, the increased release of PYY and GLP-1 was followed with increased satiety and significantly decreased leptin, insulin and postprandial blood glucose levels (Le Roux et al., 2006a; Pournaras et al., 2010). In contrast, the role of RYGB on ghrelin secretion is still unclear as some studies reported a decrease in fasting and postprandial ghrelin (Leonetti et al., 2003; Morinigo et al., 2004) but others showed no significant effect in postoperative ghrelin

levels (Karamanakos et al., 2008; Le Roux et al., 2007). The discrepancies of the effects of bariatric surgery on ghrelin secretion are needed to be further investigated.

Even though gastric bypass surgery has been shown to maintain long term body weight management potentially by improving gut hormone release, the procedures have been related to various life-threatening complications such as pulmonary embolism (Podnos et al., 2003), leakage (Marshall et al., 2003), stomal obstruction, bleeding and nutrient malabsorption (Malinowski, 2006). However, it is agreed that naturally occurring anorectic gut hormones released following gastric bypass surgery are the potential candidates for weight loss treatment (Mitchell et al., 2001; Powers et al., 1999). Therefore, alternative therapies that stimulate gut hormone secretion are strongly needed for obesity treatment. Recently, certain dietary components have been suggested as potential sources for inducing the release of naturally occurring gut hormones and therefore receive considerable attentions to be developed as a treatment for obesity.

1.2 APPETITE REGULATION

Appetite is an eagerness feeling towards eating. It drives the consumption of food intake to provide adequate energy intake to maintain metabolic needs. Appetite is regulated by close networks between the gastrointestinal tract, adipose tissue and the brain. A loss of appetite regulation to balance energy intake and energy expenditure will lead to either weight loss or weight gain. Therefore, an energy balance is required in maintaining body weight.

Appetite regulation is controlled by three linked factors creating a network of interactions. These factors are; 1) physiological behaviours including hunger, appetite sensations 2) signals from the periphery and 3) neuronal activities in the brain (Blundell, 1999). In addition to individuals' internal cues, appetite regulation is also influenced by environmental factors which are social, economic, behavioural and cultural. Although the whole system is complex and the mechanisms linking these factors are currently not fully understood, it is now known that the close interaction between the gut signals and the brain influences human eating behaviour. Following food intake,

the gastrointestinal tract senses the presence of nutrients via receptors which results in the release of gut hormones and peptides from the periphery. This information is then signalled to the brain via the vagus nerve or the circulation. In the brain, these signals work together with the brain's receptors and neurotransmitters to translate the information received from the gut and to decide either to continue or stop the eating process. Cessation of eating process is described in two conditions; 1-satiation (a process that brings meal intake to the end following increase of fullness) and 2-satiety (a process that suppresses the eagerness to eat postprandially and maintain the satiated feeling for a certain period of time) (Blundell, 1999).

1.3 CENTRAL REGULATION OF APPETITE

1.3.1 Homeostatic Pathway

In the homeostatic system, there are four main sites involved, namely the hypothalamus, brainstem, gastrointestinal tract and abdominal viscera. These sites are linked to each other by a complex neuronal network with the hypothalamus and the brainstem as the centres where all the information is conveyed. The signals from the gastrointestinal tract and abdominal viscera are circulated in the blood or alternatively, conveyed to the hypothalamus and the brainstem through vagal nerve afferent. The information is processed by the hypothalamus and brainstem and then transmitted via efferent signals to influence satiety and food intake.

1.3.1.1 The Brainstem

The brainstem is located in the hindbrain and consists of the midbrain, pons and medulla oblongata. The dorsal vagal complex, which is situated in the medulla oblongata has been proposed to play an important role in the interpretation of peripheral signals via vagal afferent from the gut to the hypothalamus (Bailey, 2008). The dorsal vagal complex consists of the nucleus tractus solitaries (NTS), area postrema (AP) and dorsal motor nucleus of vagus. It is postulated that the presence of an incomplete blood brain barrier (BBB) in the AP facilitates the passage of peripheral satiety signals to the brainstem structures. Lesions in the AP have been demonstrated to cause hypophagia and

suppressed body weight gain, hence suggesting the role of AP in feeding behaviour (Hyde and Miselis, 1983). The AP has been identified as one of the circumventricular organ, a group of organs which lack a complete blood brain barrier (BBB) and located at various sites in the ventricular system in the brain. Therefore, nuclei residing in the circumventricular organ are accessible to the peripheral signals especially gut hormones without the need for a specific carrier to deliver the signals across the BBB. Peripheral signals can also use an alternative pathway via the brainstem and hypothalamus (Ricardo and Koh, 1978). In this pathway, the NTS acts as a receiver for vagal afferent fibres to integrate satiety signals from the periphery, which are then projected to the arcuate nucleus (ARC), paraventricular nucleus (PVN), lateral hypothalamic area (LHA) and dorsomedial nucleus (DMN) in the hypothalamus (Ter Horst et al., 1984; Ter Horst et al., 1989; Thompson and Swanson, 1998).

1.3.1.2 The Hypothalamus

The hypothalamus, a small region situated in the forebrain adjacent to the pituitary gland, plays an important role in regulating appetite and metabolism. It exerts its role by detecting signals from the periphery, such as hormones secreted from large intestine and adipose tissue and nutrients circulating in the blood. Furthermore, the hypothalamus also acts as an intermediary between the nervous and endocrine system, which are linked via the pituitary gland. Early investigation by Hetherington and Ranson suggested that the LHA was the ‘hunger centre’ whilst the ventromedial hypothalamic nucleus (VMN) functioned as the ‘satiety centre’ (Hetherington and Ranson, 1940). However, recent findings discovered that other hypothalamic nuclei in the hypothalamus are also involved in appetite regulation by having close interactions with the brainstem and higher cortical centres. A BBB, a barrier that separates cerebrospinal fluid from blood, can also be found in the hypothalamus. The existence of this barrier prevents circulating gut derived hormones from entering the brain, hence the delivery of gut hormones to the brain can only be performed using a specific carrier or in certain locations where the BBB is incomplete. The median eminence in the hypothalamus as well as the AP in the brainstem have been identified as the locations of an incomplete BBB, therefore permitting the integration of peripheral signals directly to the central nervous system (CNS) (Gotow and Hashimoto, 1979; Gross, 1992).

1.3.1.2.1 Arcuate Nucleus

The ARC, which is located at the base of the hypothalamus, has been suggested to play an important role in appetite regulation. It detects peripheral signals due to its close proximity to the median eminence. The ARC is modulated by two individual sets of neuronal populations that are working together in appetite regulation. The anorexigenic neurons expressing pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript, which can be found in the lateral ARC and orexigenic neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurones in the medial ARC (Broberger et al., 1998; Hahn et al., 1998). Both populations of neurons project to various hypothalamic areas that are involved in the central appetite centres such as the PVN, VMN, DMN and LHA.

1.3.1.2.2 Paraventricular Nucleus

The PVN is located parallel to the third ventricle in the anterior hypothalamus. The PVN plays a major role in controlling eating behaviour and energy expenditure. An ablation in the PVN performed in rat experiments resulted in obesity and overeating syndrome (Aravich and Sclafani, 1983; Leibowitz et al., 1981). It also has been shown to be stimulated by peripheral and central peptides. Intracerebroventricular (Dakin et al., 2002) injection of NPY has been shown to stimulate food intake (Stanley and Leibowitz, 1984) whereas ICV infusion of GLP-1 (McMahon and Wellman, 1998) and leptin (Satoh et al., 1997) suppressed food intake. The peripheral signals are transmitted to the PVN either by hypothalamic ARC and LHA or through a reciprocal linkage with brainstem nuclei such as the NTS.

1.3.1.2.3 Ventromedial Nucleus

Previously known as the ‘satiety centre’, the VMN is located on the top of the ARC in the hypothalamus from which it receives the periphery signals. Recently, the VMN has been shown to be influenced by both anorexigenic and orexigenic neurons. Administration of NPY into the VMN by ICV injection or microinjection increased food intake in rats (Clark et al., 1984; Kalra et al., 1991) while Jacob *et al*. showed that injection of leptin in rats resulted in a decrease of food intake (Jacob et al., 1997).

1.3.1.2.4 Dorsomedial Nucleus

The DMN is located above the VMN and has been suggested to play an important role as a satiety centre. It receives neuronal projections from the ARC and projects to the PVN. An abrasion of the DMN has been demonstrated to cause hyperphagia and obesity (Bellinger and Bernardis, 2002). ICV injection of galanin (Kuramochi et al., 2006) and orexin (Dube et al., 1999) in this region has been shown to increase food intake. The DMN is also reported to contain high levels of NPY/AgRP neurons (Broberger et al., 1998), which are suggested to be related with the development of obesity (Guan et al., 1998).

1.3.1.2.5 Lateral Hypothalamic Area

The LHA is a small region adjacent to the DMN. Early investigation by Anard and Brobeck in 1951 showed that lesions in this area resulted in hypophagia and weight loss (Anard and Brobeck, 1951), which indicated the LHA as a feeding centre. The LHA is comprised of α-melanocyte stimulating hormone (α-MSH), orexins and orexigenic hormones and NPY/AgRP neuropeptides. The hormones and neuropeptides are conveyed to the LHA from the ARC.

The summary of the complex neuronal network of appetite regulation is illustrated in figure 1.1.

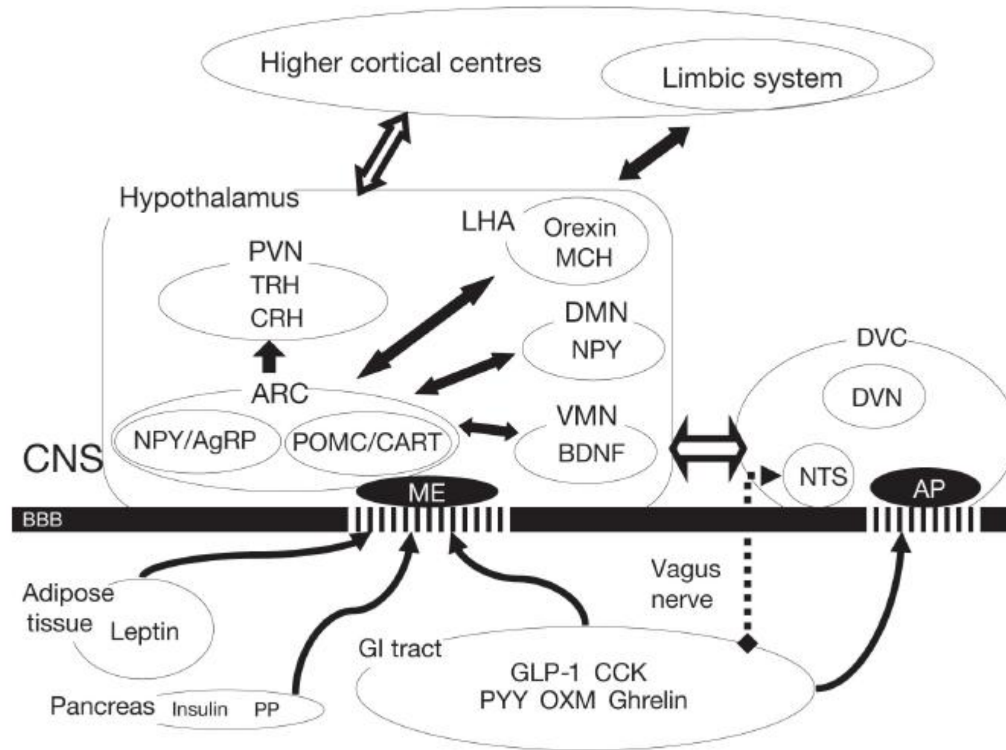


Figure 1.1 The hypothalamic and brainstem regions and its interaction with peripheral signals to regulate appetite regulation. From (Suzuki et al., 2010).

Abbreviations: CNS: central nervous system; ARC: arcuate nucleus; NPY/AgRP: neuropeptide Y and agouti related peptide; POMC/CART: pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript; PVN: paraventricular nucleus; LHA: lateral hypothalamic area; DMN: dorsomedial nucleus; VMN: ventromedial hypothalamic nucleus; ME: median eminence; DVC: dorsal vagal complex; DVN: the dorsal motor nucleus of vagus; NTS: the nucleus of the tractus solitarius; AP: area postrema; GI tract: gastrointestinal tract; TRH: thyrotropin-releasing hormone; CRH: corticotrophin-releasing hormone; MCH: melanocortin-concentrating hormone; BDNF: brain-derivedneurotrophic factor; GLP-1: glucagon-like peptide-1; CCK: cholecystokinin; PP: pancreatic polypeptide; PYY: peptide YY; OXM: oxyntomodulin; BBB: blood-brain barrier.

1.3.2 Non-Homeostatic Pathway

It is now widely accepted that besides a homeostatic pathway, appetite regulation is also controlled by a hedonic or also known as ‘non-homeostatic’ pathway. However, in contrast to the homeostatic pathway, the role of the non-homestatic pathway has not been fully explored. Hedonic is characterised as a pleasant (or unpleasant) sensations that could motivate people to

continue or stop the provided stimulation. It seems that taste, smell and visual cues from palatable foods are able to influence people to override homeostatic signals. Besides sensory cues, cognition, emotional and reward are among the factors that modulate the hedonic mechanism. These factors are established in the corticolimbic and higher cortical brain regions (Berthoud, 2004). The brain reward regions that are studied in this work are described below (figure 1.2).

1.3.2.1 Nucleus Accumbens

The nucleuc accumbens (NAc) is a structure which forms part of the ventral striatum. It can be classified into two components, the core and the shell, which have a distinct input and output projections (Heimer et al., 1991; Zahm and Brog, 1992). The NAc has a close interaction with other brain regions by which it receives dopaminergic projection from the ventral tegmental area (VTA) and glutamatergic projection from the prefrontal cortex, amygdala and hippocampus (Carlezon, Jr. and Thomas, 2009; Kelley, 2004). It has long been known that the NAc plays a significant role in the reward system, notably motivational drive in seeking food (Kelley, 2004). Numerous human studies have shown an increase activity in the ventral striatum of fasted volunteers when viewing food-related images (Fuhrer et al., 2008; Goldstone et al., 2009; Schur et al., 2009). In rodents, lesions of the NAc decreased the reward response to drugs of addiction (Kelsey et al., 1989). Similar results were shown when mice-null dopamine D2-receptor was unable to stimulate the rewarding effect of cocaine in the NAc (Welter et al., 2007). Evidence from these studies showed that the NAc and neuron-related factors play a significant role in modulating the reward system.

1.3.2.2 Amygdala

The amygdala is an almond-sized structure located in the anterior part of the temporal lobe and a part of the limbic system (Murray, 2007). It is widely known to be involved in emotion, reward, memory processing, motivation and attention (Murray, 2007). Bilateral ablation of the amygdala markedly diminishes emotional processing (Kalin et al., 2001; Meunier et al., 1999). In monkeys, lesions of the amygdala markedly reduce fear, impair emotional learning and decrease emotion awareness (Cardinal et al., 2002). The amygdala is also implicated in the

reward response to food. Studies show that the amygdala is highly activated when fasted volunteers are exposed to food-related sensory experiments (i.e. viewing food images or tasting food) in functional magnetic imaging resonance (fMRI) studies (Killgore et al., 2003; LaBar et al., 2001; Siep et al., 2009). The amygdala has a close connection with other brain nuclei. It receives afferent signals from the insula, fusiforum and the VTA and sends projections to the orbifrontal cortex (OFC), prefrontal cortex, NAc and anterior cingulate cortex (ACC) (Amaral and Price, 1984; Morgane et al., 2005).

1.3.2.3 Cingulate Cortex

The cingulate cortex can be divided into the anterior cingulate cortex (ACC) and the posterior cingulate cortex, which have a distinct function and pattern of projection. Whilst the anterior area is described as ‘executive’, the posterior area is evaluated as ‘evaluative’ (Vogt et al., 1992). The ACC mainly functions in cognition and emotional processing, which are regulated separately in the dorsal and ventral zones (Bush et al., 2000). The dorsal ACC exerts its effect on cognitive processing by regulating attention, motivation and conflict (Bush et al., 2000; Devinsky et al., 1995). It is connected to various nuclei including the prefrontal cortex, parietal cortex and motor system (Devinsky et al., 1995). On the other hand, the ventral ACC mainly regulates emotional responses and motivational information (Bush et al., 2000; Devinsky et al., 1995; Passamonti et al., 2008). It is closely connected with the amygdala, NAc, hypothalamus and anterior insula (Devinsky et al., 1995). The ACC has also been implicated to involve in eating process as this area was shown to be activated when volunteers were viewing food images in fMRI studies (Passamonti et al., 2009).

1.3.2.4 Orbifrontal Cortex

The orbifrontal cortex (OFC), which is a part of the limbic system has long been known to be involved in decision making and translational behavioural process. The OFC has been suggested to play a role in encoding sensory and food reward as well as in learning, decision making and expectation to translate emotional and reward drive into action (Gottfried et al., 2003; O'Doherty et al., 2000; Small et al., 2007). Numerous human neuroimaging studies showed that the OFC has pronounced roles in reward processing as it was shown to be activated when viewing food

images during fasting (Gottfried et al., 2003; Hinton et al., 2004; O'Doherty et al., 2000; Siep et al., 2009; Small et al., 2007; Wang et al., 2004). The OFC also has a close connection with other brain reward regions including the hypothalamus, amygdala, insula, dopaminergic midbrain as well as the ventral and dorsal striatum (Cardinal et al., 2002; Verhagen and Engelen, 2006).

1.3.2.5 Insula

The insula cortex is situated deep within the anterior part of the sylvian fissure between the temporal lobe and frontal lobe. The insula cortex can be divided into the smaller posterior insula and the large anterior insula. The insula is a primary gustatory cortex, which mainly involved in regulating chemical senses including taste aversion, sensory and reward-related food processing (James et al., 2009; Small, 2010; Small, 2012; Yaxley et al., 1990). The significant role of the insula on taste processing has been shown following viewing or tasting meal in several human fMRI studies (Siep et al., 2009; Wang et al., 2004). Nevertheless, the insula is also involved in regulating other sensations including pain, hunger and temperature (Craig, 2009; Damasio et al., 2000; Paulus and Stein, 2006). The insula projects efferent signals to the anterior cingulate cortex, NAc and also to various striatum regions and amygdala (Chikama et al., 1997).

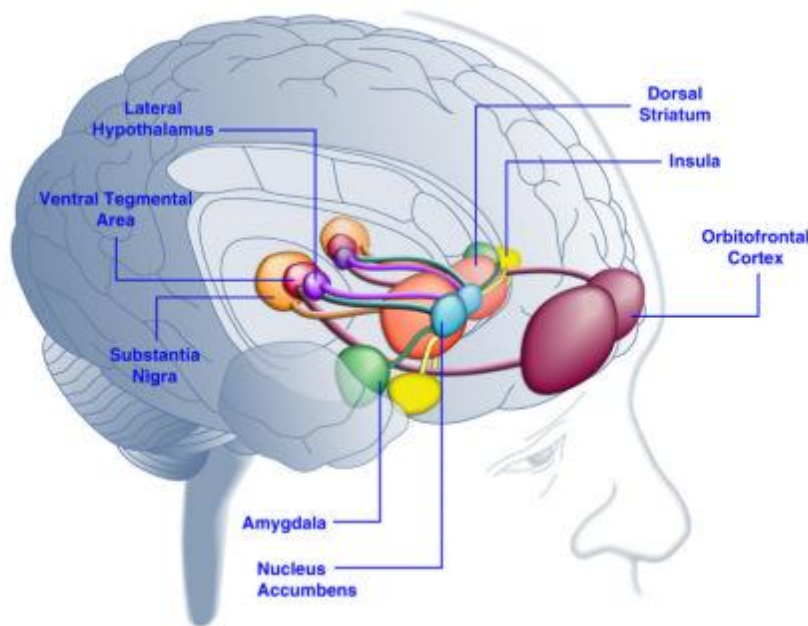


Figure 1.2 Key areas in the human brain involved in the hedonic system, from (Kenny, 2011).

1.4 THE ROLE OF PERIPHERAL SIGNALS IN APPETITE REGULATION

1.4.1 Gut Hormones

A large group of hormones are produced and secreted from numerous part of the enteroendocrine cells in the gut. These hormones transmit information of nutritional and metabolic activities from the intestinal system to the CNS via the bloodstream or vagal afferent to various hypothalamic nuclei and brainstem as discussed in section 1.3. Only selected gut hormones will be discussed in this section.

1.4.1.1 Glucagon- Like Peptide-1

Glucagon-like peptide-1 (GLP-1), a 30 amino acid peptide is mainly produced in the L-cells located in the distal gut where it is co-secreted with PYY (Eissele et al., 1992). GLP-1 is a product of post-translational cleavage of preproglucagon from which oxyntomodulin and glucagon are also produced. GLP-1 exists as two biological active forms, GLP-1_{7-37} and GLP-1_{7-36} amide. However, the most common circulating form is GLP-1_{7-36} amide (Orskov et al., 1994). Besides being secreted in the gut, a considerable amount of GLP-1 can also be found in the hypothalamus and brainstem, with the highest level of GLP-1 immunoreactive neurons found in the DMN, PVN and NTS (Kreymann et al., 1989; Larsen et al., 1997). GLP-1 exerts its effects via its receptor, GLP-1R which is widely distributed throughout the CNS including the hypothalamic ARC, PVN and DMN and the AP and NTS in the brainstem (Merchenthaler et al., 1999; Shughrue et al., 1996). GLP-1 is transmitted to the CNS via vagal afferent. Abbott *et al*. demonstrated that the anorectic effect of GLP-1 is abolished following bilateral vagotomy in rats, therefore confirming the involvement of vagal afferent in conveying circulating GLP-1 to the CNS (Abbott et al., 2005a).

GLP-1 is secreted into the circulation in response to calorie intake (Ghatei et al., 1983; Herrmann et al., 1995). The anorectic effect of GLP-1 has been demonstrated in both rodents and humans. In rats, administration of GLP-1 either by acute intravenous infusion (Chelikani et al., 2005) or chronic ICV injection (Meeran et al., 1999) reduced food intake and body weight. However, administration of a supraphysiological dose resulted in aversive side effects which highlighted its

effect as an anorectic gut hormone (Thiele et al., 1997). Therefore, the potential application of exogenous GLP-1 as a therapeutic agent needs to be carefully interpreted. In humans, GLP-1 has been demonstrated to reduce food intake in both lean (Flint et al., 1998; Gutzwiller et al., 1999) and overweight volunteers (Verdich et al., 2001a). Overweight volunteers are reported to have lower GLP-1 circulation levels compared to lean volunteers. Interestingly, the levels were reported to increase following weight loss (Verdich et al., 2001b). De Silva *et al*. demonstrated that infusion of GLP-$1_{7\text{-}36}$ in healthy normal weight volunteers reduced *ad libitum* meal intake but the effects were more robust when combined with $PYY_{3\text{-}36}$ (De Silva et al., 2011).

Although GLP-1 has been shown to have potential effects as a therapeutic anti-obesity agent, rapid degradation of circulating GLP-1 by dipeptidyl-peptidase-4 (DPP-IV) resulted in GLP-1 being inactivated with a short half-life of 1-2 minutes (Deacon, 2004; Kieffer et al., 1995; Mentlein et al., 1993b), thus making it unsuitable for an obesity treatment. Nevertheless, in 1991, Exendin-4 or exenatide, a GLP-1R agonist was developed with a longer biological half-life (Eng et al., 1992). Exendin-4 is synthesized from the saliva of the Gila monster lizard, *Heloderma suspectum*, and has been showed to improve glycemic control in patients with type 2 diabetes (Buse et al., 2004; DeFronzo et al., 2005; Kendall et al., 2005). Acute and chronic administration of exendin-4 in type 2 diabetic mice, rats and monkeys showed to have a glucose lowering effect, stimulated insulin secretion and weight loss (Young et al., 1999), hence highlighting exendin-4 role as an anti-diabetic treatment. In humans, administration of exendin-4 has been shown to improve glucose response, suppress energy intake in healthy volunteers (Edwards et al., 2001) and reduce body weight in diabetic patients (Kim et al., 2007; Zander et al., 2002).

1.4.1.2 Peptide Tyrosine-Tyrosine

Peptide tyrosine-tyrosine (PYY) is a 36 amino acid which was formerly isolated from porcine small intestine (Tatemoto and Mutt, 1980). It is a member of the polypeptide-fold protein family together with pancreatic polypeptide and NPY. PYY is synthesised and secreted together with GLP-1 in the intestinal L-cells, which can be found in high numbers in the colon and rectum

(Adrian et al., 1985). There are two circulating forms of PYY, which are PYY_{1-36} and PYY_{3-36}. PYY_{3-36} is the cleavage product of the Tyr-Pro amino terminal residues of PYY_{1-36} by enzymatic activities of DPP-IV and is distributed in the circulation (Grandt et al., 1994; Mentlein et al., 1993a).

PYY is released after ingestion of a meal and its release is proportional to calorie intake (Adrian et al., 1985; Batterham et al., 2003). Plasma PYY levels begin to rise in the circulation after 30 minutes, peak at one to two hours after meal intake and remain elevated for several hours (Adrian et al., 1985). Meals containing high fat and protein have been recognised as the most potent stimulator for PYY_{3-36} secretion (Batterham et al., 2006; Essah et al., 2007; Helou et al., 2008). PYY_{3-36} exerts its anorectic effects by reducing food intake in animals and humans. In rats, acute peripheral administration of PYY_{3-36} suppresses food intake and inhibits body weight gain (Batterham et al., 2002). Unexpectedly, Tschop *et al*. questioned this study as they were unable to reproduce the findings following acute and chronic PYY_{3-36} administration, either peripherally or centrally in 8 different strains of rodents (Tschop et al., 2004). This discrepancy is possibly due to the effect of stress in the rodents (Halatchev et al., 2004) and lack of acclimatization. Indeed, Abbott *et al*. showed that acclimatization, habituation and careful handling are important for rodents which are exposed to a new environment. A failure to account for these factors prior to the investigation may result in an attenuation of PYY's effect on food intake (Abbott et al., 2006). Clarification on this issue has helped the other groups in demonstrating the effect of PYY_{3-36} in reducing food intake as demonstrated in mice (Challis et al., 2004; Halatchev et al., 2004), rats (Chelikani et al., 2005; Chelikani et al., 2006) and rhesus monkey (Moran et al., 2005). Like PYY_{3-36}, PYY_{1-36} also suppressed food intake in rats, but the effect is less potent compared to PYY_{3-36} (Chelikani et al., 2004).

In humans, peripheral PYY_{3-36} reduces cumulative 24 hours food intake and decreases *ad libitum* meal intake in both normal and overweight volunteers (Batterham et al., 2003). Interestingly, the authors found that overweight volunteers have lower postprandial PYY levels compared to the normal weight volunteers, suggesting that a lack of PYY_{3-36} levels in obese volunteers results in reduced satiety and may therefore increase the risk of obesity (Batterham et al., 2003; Le Roux et

al., 2006b). The anorectic effect of PYY in humans has also been demonstrated in other studies (Batterham et al., 2006; Sloth et al., 2007).

PYY exerts its effect via the Y family of G coupled protein receptors. There are five subtypes of the receptors Y1, Y2, Y4, Y5 and Y6 (Blomqvist and Herzog, 1997). PYY has been shown to have a high affinity for the Y2 receptor (Keire et al., 2000), which is highly expressed on NPY neurons in the ARC (Broberger et al., 1997). The involvement of the Y2R in exerting the anorectic effect of PYY has been demonstrated using the Y2 receptor antagonist. In this study, the inhibitory effect of PYY on food intake and body weight is attenuated due to a blockade of the ARC Y2 receptor by BIIE0246, a specific antagonist of Y2 receptor. Administration of this antagonist possibly suppressed the NPY neurons thus resulting in increased food intake in rats (Abbott et al., 2005b). In addition, the effect of PYY is abolished in Y2 receptor null mice (Batterham et al., 2002), suggesting that the Y2 receptor plays a major role in the anorectic effect of $PYY_{3\text{-}36}$.

The satiety signal of PYY is transmitted to the brain using two mechanisms; firstly, by crossing the BBB that is located closely to the ARC (Nonaka et al., 2003) and secondly via the vagus nerve to convey information to the brainstem (Koda et al., 2005). In the ARC, PYY has been reported to activate POMC neurons, therefore POMC neurons may be involved in regulating food intake (Batterham et al., 2002). Deficiency of POMC-neurons either in humans or mice is related to hyperphagia and obesity (Krude et al., 1998; Yaswen et al., 1999). Unexpectedly, acute administration of $PYY_{3\text{-}36}$ in mice lacking POMC neurons significantly reduced food intake compared to wild type mice. However, the effect was abolished when $PYY_{3\text{-}36}$ was administered chronically. These observations suggest that POMC neurons are required to maintain energy balance towards obesogenic diet in the long term period (Challis et al., 2004). α-MSH neurons, a group of POMC derivative neurons have been shown to increase their activity by 20% following exogenous injection of $PYY_{3\text{-}36}$ in the ARC of the control rats but not in the vagotomised rats (Koda et al., 2005). This suggesting that $PYY_{3\text{-}36}$ exerts its anorectic effect via melanocortin-4 receptor system (Halatchev et al., 2004; Koda et al., 2005). Meanwhile, the involvement of the vagus nerve in exerting $PYY_{3\text{-}36}$ effect was demonstrated by Koda *et al*. In this study, bilateral subdiaphragmatic vagotomy or bilateral midbrain transection experiments

performed in rats inhibited the ability of PYY_{3-36} to reduce food intake, highlighting the important role of the vagus nerve in conveying information to the hypothalamus via the NTS (Koda et al., 2005).

1.4.1.3 Ghrelin

Ghrelin is the only identified orexigenic gut hormone at the present time. This 28 amino acid peptide is produced mainly in the stomach (Kojima et al., 1999) and elsewhere in the gastrointestinal tract (Date et al., 2000). In the CNS, the hypothalamus is the main source of ghrelin with the highest levels found in the NPY/AgRP neurons in the ARC. ICV injection of ghrelin has been shown to increase c-fos expression (neuronal activation marker) in NPY/AgRP neurons (Cowley et al., 2003; Nakazato et al., 2001), whilst ghrelin effect was abolished without NPY/AgRP neurons (Chen et al., 2004). Other brain regions that expressed higher levels of c-fos following ICV injection of ghrelin were the PVN, DMN, LHA of the hypothalamus and the NTS and AP of the brainstem (Lawrence et al., 2002). Ghrelin levels rise prior to nutrient ingestion and fall postprandially upon meal intake (Tschop et al., 2001) suggesting its role as a meal initiator (Cummings et al., 2001). Ghrelin modulates all three macronutrients with carbohydrate rich- meals showing the greatest suppression of ghrelin, followed by protein and lipid rich-meals (Al et al., 2005; Foster-Schubert et al., 2008).

ICV and intraperitoneal administration of ghrelin in rats has been shown to stimulate food intake, promoting weight gain and adiposity (Wren et al., 2001b) whilst in humans, intravenous infusion of ghrelin increased hunger and energy intake in healthy lean (Wren et al., 2001a) and in obese volunteers (Druce et al., 2005). High plasma ghrelin levels were demonstrated in anorexia nervosa patients, but the levels returned to normal after weight gain (Otto et al., 2001). Meanwhile, the low levels of plasma ghrelin in overweight volunteers were reported to be increased after weight loss (Cummings et al., 2002; Hansen et al., 2002). However, the ghrelin levels in patients underwent bariatric surgery are yet to be confirmed as some studies reported large falls following the surgery (Leonetti et al., 2003; Morinigo et al., 2004), but others showed no significant difference in RYGB patients compared to lean and obese volunteers (Karamanakos et al., 2008; Le Roux et al., 2006a).

1.4.1.4 Cholecystokinin

Cholecyctokinin (CCK) is a 115-amino acid cleavage product of pro-CCK and is expressed by the I-cells in the duodenum and jejunum (Buffa et al., 1976). Besides the gut, it can also be found in the CNS, in particular in the VMN and median eminence of the hypothalamus (Beinfeld et al., 1981). CCK is released postprandially as a response to nutrient ingestion (Liddle et al., 1985) and highly influenced by the presence of fat and protein (Lewis and Williams, 1990; Liddle et al., 1985). It exerts its anorectic effects by binding to its receptor, CCK_1R and CCK_2R which can be found in the brain, particularly in the LHA, median pons and lateral medulla (Schick et al., 1994). CCK starts to rise in the plasma within 15 minutes after meal ingestion and remains elevated up to 5 hours (Liddle et al., 1985). CCK exerts its effects in the gut by stimulating gallbladder contraction and pancreatic enzyme secretion (Liddle et al., 1985) as well as inhibiting gastric emptying (Moran and McHugh, 1982). Although CCK has been shown to reduce food intake in both rats and humans (Gibbs et al., 1973; Kissileff et al., 1981), its short-term effect resulted in failing to reduce food intake when administered more than 30 minutes prior to a meal (Gibbs et al., 1973). Furthermore, it has also been shown to increase feeding frequency by more than 162% as a compensation of reduced meal size by 44% in rats study (West et al., 1984). High dose of CCK cause nausea and taste aversion in rodents outweighing the potential use of CCK as an anti-obesity treatment (Ervin et al., 1995; Swerdlow et al., 1983).

1.4.2 Adipose Tissue Related Hormones

1.4.2.1 Leptin

Leptin, a 16 KDa peptide is an adipocyte-derived hormone and the product of the *ob gene* (Zhang et al., 1994). Leptin is released proportionally to the amount of body fat (Considine et al., 1996). Other sites that also produce leptin are the stomach, placenta, mammary gland, ovarian follicles, brain and fetal organs such as heart, bone and cartilage (Masuzaki et al., 1997; Trayhurn et al., 1999; Trayhurn and Beattie, 2001). Leptin mediates its anorexigenic effect by binding to leptin specific receptors which can be found in the ARC, PVN, DMH and LHA of the hypothalamus. The ObRa, the short leptin receptor isoform exerts its role as a leptin transporter

through the BBB (Bjorbaek et al., 1998) whilst the ObRb, the long leptin receptor isoform is mainly produced in the hypothalamus (Fei et al., 1997). Lack of leptin in mice (Zhang et al., 1994) and humans (Montague et al., 1997) leads to severe obesity. Administration of leptin in *ob/ob* mice (Halaas et al., 1995) and leptin deficient humans (Farooqi et al., 1999) has been shown to reduce food intake and prevent body weight gain. Interestingly, obese patients have been shown to have high circulating levels of leptin (Considine et al., 1996; Schwartz et al., 1996), but the levels were not related to a reduction in food intake (Considine et al., 1996). Meanwhile, a study showed that not all obese volunteers who underwent leptin treatment had a reduction in body weight (Heymsfield et al., 1999). These results suggest that the current use of leptin as a treatment for obesity is only applicable and effective in humans who are deficient in leptin. It is suggested that this could be due to the deterioration of transporting signals of leptin to the central of appetite regulation (Van et al., 1997).

1.4.2.2 Insulin

Insulin is secreted from the β-cells of the pancreatic islets of Langerhans. It is also known as an adiposity signal and involved in both short term and long term energy balance. Insulin is released in high levels after meal intake (Polonsky et al., 1988) and its sensitivity is highly correlated with the total adiposity (Kahn et al., 1993) and body fat distribution, in particularly visceral fat (Porte et al., 2002). Insulin levels are elevated during weight gain and reduced during weight loss. Insulin has been shown to cross the BBB by a saturable receptor mediated mechanism which is dependent on the levels of circulating insulin (Baura et al., 1993). Administration of insulin by ICV has been demonstrated to reduce food intake in sheep (Foster et al., 1991). Insulin exerts its anorectic effect by binding to its receptor which can be found in numerous sites of the hypothalamic regions including the ARC, VMN and PVN (Havrankova et al., 1981; Marks et al., 1990). In the ARC, insulin has been found to co-localise with NPY and α-MSH neurons (Pardini et al., 2006). In addition, POMC neurons have also been reported to co-express insulin receptor (Benoit et al., 2002). ICV injection of an insulin mimetic compound into the third ventricle of fasting rats decreased POMC mRNA expression (Air et al., 2002). Besides the hypothalamus, insulin receptors can also be found widely in the AP, NTS and the DMV of the brainstem (Pardini et al., 2006). Insulin regulates energy balance via the insulin receptor substrate 2 (Burks

et al., 2000). An experiment using insulin receptor substrate 2 knockout mice demonstrated that the absence of the receptor cause an increase in food intake, body weight, fat mass (Burks et al., 2000) and hyperphagia (Masaki et al., 2004).

1.5 INFLUENCES OF CARBOHYDRATES ON APPETITE REGULATION

Carbohydrate contributed the largest proportion in daily energy intake, therefore the quantity and the type of carbohydrate consumed are the most concern factors especially in maintaining weight management and controlling food intake. Carbohydrates can be divided into four classes which are monosaccharides, disaccharides, oligosaccharides and polysaccharides. This classification is based on the chemical class and molecular size such as type of individual monomers, linkage bonds and degree of polymerization. The monosaccharides and disaccharides are commonly known as 'sugars' and it is basically absorbed in the small intestine for energy production. Oligosaccharides are characterised by having a polymerization of small numbers of simple sugars typically from two to ten. Unlike monosaccharides, oligosaccharides are partially digested in the small intestine and the undigested portion will be transported to the large intestine for bacterial fermentation. This is because undigestible oligosaccharides are linked by β (2-1) glycosidic bond which can only be digested by gut microbiota. Polysaccharides are the longest and complex structures of carbohydrates which contain polymerization of monomer units between 200 to 2500. Similar with olisaccharides, some polysaccharides (such as starches and glycogen) are also absorbed in the small intestine due to binding to α (1-6) linkage. Interestingly, there are some polysaccharides that are able to escape digestion in the small intestine and fermented by gut bacteria in the large intestine (such as cellulose and β-glucan) (Figure 1.3 ilustrates the classification of the carbohydrates).

The following section will discuss on the effect of carbohydrates that escape digestion in the small intestine and transported to the large intestine for bacterial fermentation.

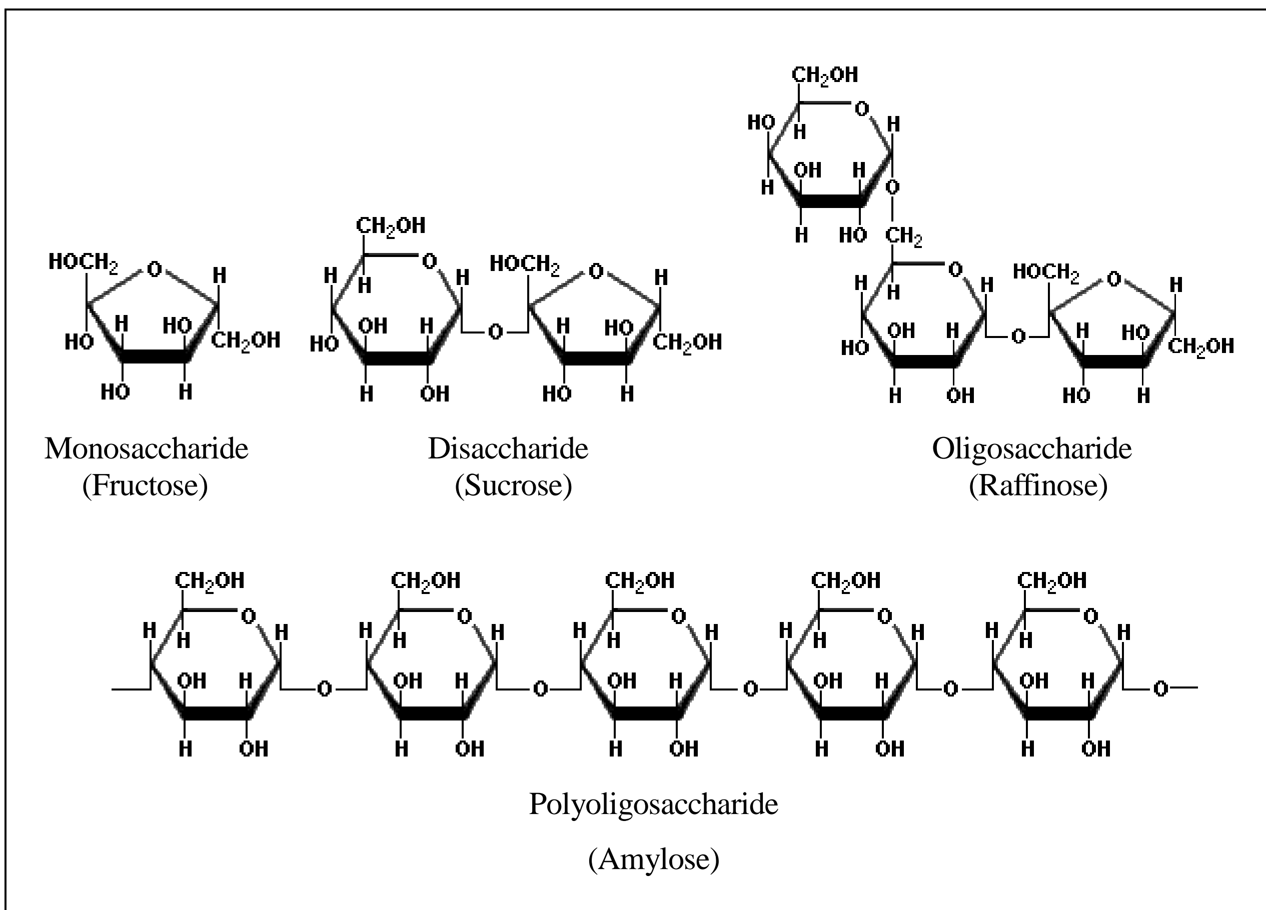


Figure 1.3 Molecular structure of carbohydrates

1.5.1 Dietary Fibre

Dietary fibres are defined as edible constituents of plant food that are not digested in the small intestine and therefore reach the large bowel relatively unchanged (Trowell, 1976). It is an essential nutrient and has been recommended as one of the key dietary components in a healthy diet. Dietary fibre can be classified into four classes (EFSA, 2010), which are:

1. Non-starch polysaccharides – cellulose, hemicelluloses, pectins, hydrocolloids (gums, mucilages and β-glucan).
2. Resistant oligosaccharides – fructo-oligosaccarides, galacto-oligosaccharides and other resistant oligosaccharides
3. Resistant starch – consisting of physically enclosed starch (RS1), some types of raw starch granules (RS2), retrograded amylose (RS3) and chemically and/or physically modified starches (RS4).
4. Lignin

It has been reported that intake of dietary fibre has changed markedly in the recent centuries. The ancestral human diet has been reported to contain a greater amount of dietary fibre compared to the modern westernised diet (Cordain et al., 2005). During the hunter-gatherers time, fruits and vegetables were the main source of dietary fibre with estimation of approximately 100 g of dietary fibre was taken per day (Eaton, 2006). In contrast, the current westernized diet comprised of fibre-poor added sugars, refined grains and processed foods. Current intake of fibre in America and Britain was reported to be in between 12-15 g per day, less than half of the dietary recommendation in both countries (US Department of Agriculture recommended 28 g/day for women and 36 g/day for men; British Nutrition Foundation suggested intake of 24 g/day for adults) (United States Department of Agriculture, 2005). The change of dietary intake between the ancentral diet and western diet are suggested due to the agricultural and animal husbandries, introduction of food processing, industrialized and advance technology (Cordain et al., 2005). A high intake of fibre has been suggested to be beneficial in reducing many risk factors of chronic diseases such as cardiovascular diseases (McKeown et al., 2002; Sahyoun et al., 2006), type 2 diabetes (Fung et al., 2002; Montonen et al., 2003; Schulze et al., 2007), certain type of cancers (Jacobs, Jr. et al., 1998) and mortality (Jacobs, Jr. et al., 2007).

Dietary fibre have recently received much attention due to their potential effect in reducing appetite and energy intake, which could be use to suppress body weight gain over the long term (Rozan et al., 2008). In humans, a large epidemiological study that involved 159 683 volunteers from a multiethnic population in a period of 25 years suggested that adding one gram of fibre in 200 kcal meals leads to 18 % and 23 % lower risk of being overweight in men and women respectively (Maskarinec et al., 2006). It is suggested that dietary fibres regulate body weight via several mechanisms. However, it is postulated that their ability to stimulate endogenous gut hormone secretion is the main mechanism in regulating long term body weight. The effects of dietary fibres however, vary from one fibre to another, possibly due to the differences in physicochemical properties (solubility, viscosity, fermentability and the site of fermentation in the gut) (Blackwood et al., 2000; Potty, 1996; Slavin and Green, 2007). The classification of dietary fibre based on their physicochemical properties, the source of fibres and their molecular structures are demonstrated in the Table 1.1 and Figure 1.4.

Table 1.1 Classification of dietary fibres. Adapted from (Bindelle et al., 2008; Meier, 2009; Nugent, 2005).

Type of Dietary Fibre	Solubility	Fermentability	Source
Structural NSP			
Cellulose	No	50	Whole-wheat flour, bran, vegetables
Hemicellulose A	Good	70	Bran, whole grains
Hemicellulose B	No	30	Bran, whole grains
Beta-glucan	Good		Barley, oats, rye
Structural non-NSP			
Lignin	No	5	Mature vegetables, wheat, fruits with edible seed
Non-structural NSP			
Pectins	Very good	100	Fruits (apples, citrus)
Gums	Very good	100	Oats, barley, legumes, guar
Mucilages	Good	100	Legumes
Oligosaccharides			
Inulin	Good	100	Chicory, onion, artichoke, asparagus,
Fructooligosaccharides (FOS)	Good	100	Cereals, jerusalem artichoke
Resistant Starch			
Physically inaccessibly starch (RS1)	Good	? – 100*	Whole or partly milled grains and seeds,
Crystalline resistant granules (RS2)			Raw potato, green bananas, legumes
Retrograded starch (RS3)			Bread, cornflakes, food products treated with repeated moist heat treatment

*** Depend to the type of resistant starch**

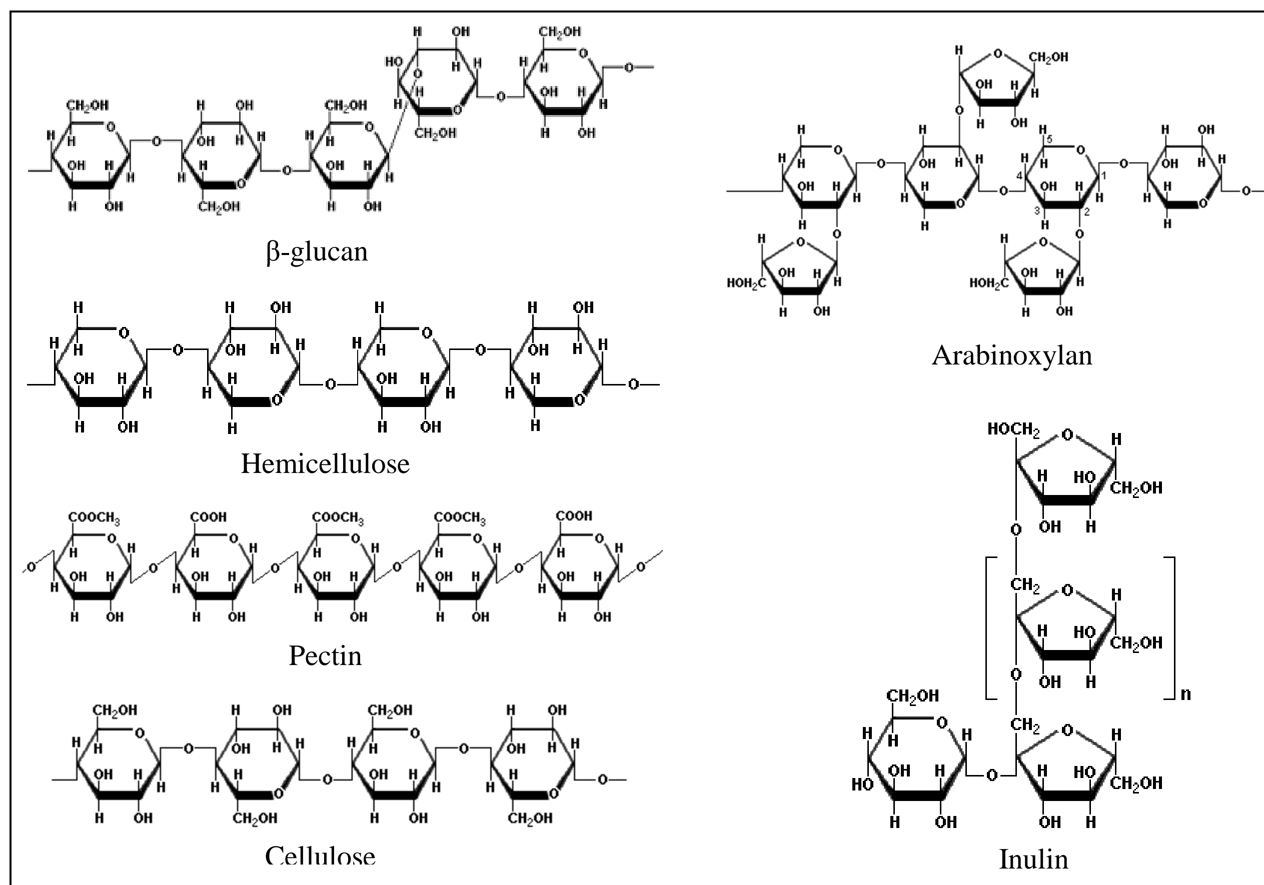


Figure 1.4 Molecular structures of dietary fibres

1.5.2 Dietary Fibre and Its Mechanism of Action

Dietary fibre is proposed to influence body weight by several mechanisms involving physiological actions in the gastrointestinal tract including mouth, stomach, upper and lower intestines. Figure 1.3 summarizes the proposed mechanisms of dietary fibre on modulating energy intake, satiety and ultimately affecting body weight.

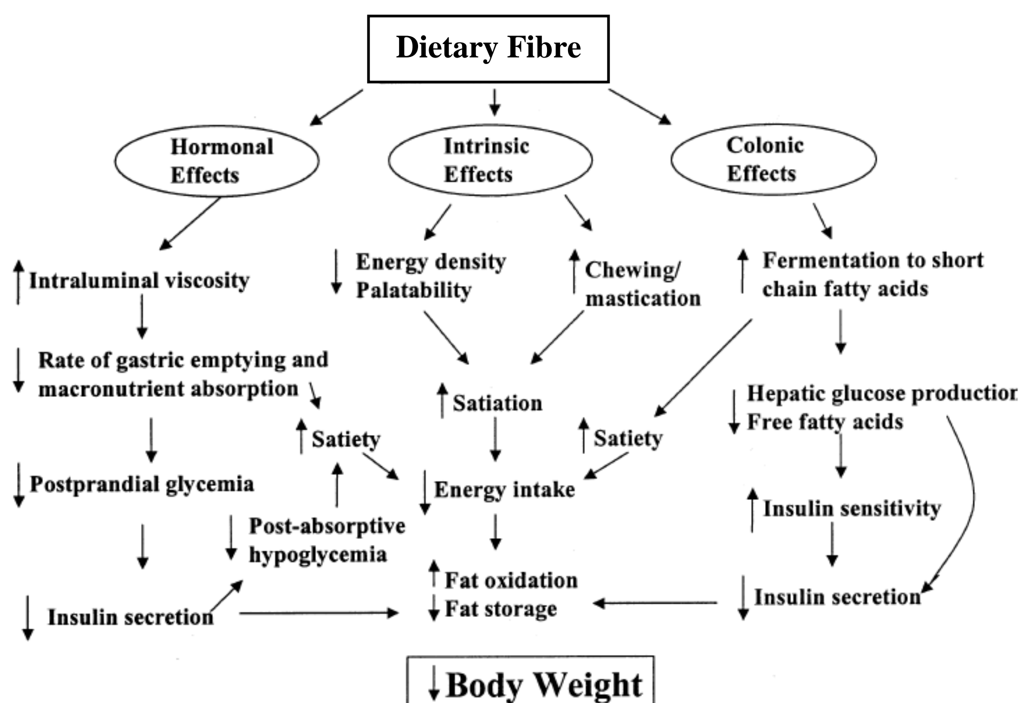


Figure 1.5 Potential mechanisms of dietary fibre in body weight regulation (Slavin, 2005).

1.5.2.1 The Effect of Dietary Fibre on Energy Density

Energy density is one of the major determinants in regulating food intake (Bell et al., 1998; Prentice and Poppitt, 1996; Rolls and Bell, 1999). In general, diet/food containing high energy density leads to overconsumption and ultimately, increase body weight in a long term period. Diet containing high fats are highly correlated with energy density (37.6 kJ/g) compared with CHO and protein (16.7 kJ/g) (Rolls and Bell, 1999). In contrast, diets-containing high fibres have a low energy density and commonly presented as a large amount of food. Adding fibre to the diet leads to an increased weight

of the diet (bulk effect), dilute energy density, induce satiation and subsequently leading to a reduction in energy intake (Drewnowski, 1998; Haber et al., 1977).

1.5.2.2 The Effect of Dietary Fibre on Chewing

Eating dietary fibre generally requires more time and efforts in mastication as high fibre diets commonly present in large quantities. It is hypothesised that this could lead to increased satiety because of a reduction in rate of ingestion (Howarth et al., 2001). Prolonging the time of chewing fibrous foods may also allow more satiety signals to be released from the gut and conveyed to the central appetite regulation, thus resulting in early satiety feeling and reduced amount of food intake. In a modified sham feeding study (a study in which specific nutrients are investigated mainly by oral technique such as smelling, tasting and chewing without involved swallowing process), chewing and tasting have been shown to increase secretion of several gut hormones such as ghrelin, CCK, pancreatic polypeptide, insulin and glucagon (Arosio et al., 2004; Simonian et al., 2005; Teff, 2000). In addition, a reduction in eating rate also has been demonstrated to influence the release GLP-1 and PYY (Kokkinos et al., 2010) and reduce food intake (Andrade et al., 2008; Duncan et al., 1983; Zijlstra et al., 2008). Moreover, eating rate can also be influenced by viscosity. A study investigated the effect of viscosity on energy intake demonstrated that semi-solid foods, but not liquid or semi-liquid food have the greatest effect on reducing *ad libitum* meal. The authors suggested that prolonging viscous foods in the mouth led to increased exposure to sensory receptors located in the oral cavity, increased subjective satiety and subsequently reduced energy intake (Zijlstra et al., 2008; Zijlstra et al., 2009).

1.5.2.3 The Effect of Dietary Fibre in the Stomach

The stomach is the second site that is suggested to be influenced by dietary fibres. In the stomach, dietary fibres such as β-glucan, alginates, pectins, psyllium and gums exert their effects by distending and delaying emptying of nutrients to the small intestine as a result of increased viscosity. These fibres influence gastric distension and gastric emptying by absorbing high amount of water and/or forming gels in the stomach, therefore delaying the transport of nutrients to the small intestine (Cameron-Smith et al., 1994; Dikeman et al., 2006; Juvonen et al., 2009). The effect of gastric distension in stimulating satiety has been shown in several human studies (Bergmann et al., 1992;

Geliebter et al., 1988; Oesch et al., 2006; Sturm et al., 2004). To date, it is still not clear which part of the stomach is actively involved in stimulating subjective appetite in humans. Some studies reported that the proximal stomach is mainly involved in inducing fullness (Feinle et al., 1997; Hebbard et al., 1996) while others suggested that fullness is induced from the antral site of the stomach (Hoad et al., 2004; Hveem et al., 1996; Sturm et al., 2004). However, the effects on appetite is not only contributed to the gastric distension alone, but also related to the type of nutrients that are present in the stomach (Oesch et al., 2006).

In general, high fibre foods are emptied more slowly from the stomach due to bulkiness effect. High viscous foods such as soluble fibres have also been suggested to influence gastric emptying (Benini et al., 1995; Bergmann et al., 1992; Marciani et al., 2000) due to their interaction with enzymes and mucosal absorption (Isaksson et al., 1982). In contrast, insoluble dietary fibres are less efficient as they have minimal ability to create a viscous environment in the stomach (Bianchi and Capurso, 2002; Vincent et al., 1995). Delay in gastric emptying has been shown to be related with increased release of CCK and PYY as well as decreased ghrelin levels in both rodents and humans (Li et al., 2011; Nguyen et al., 2007).

1.5.2.4 The Effect of Dietary Fibre on Nutrient Absorption

Whilst the effect of fibre in the stomach relates to the mechanical action of gastric distension and gastric emptying, the effect of fibre in the small intestine mainly depends on the type of nutrient supply (Kristensen and Jensen, 2011). In the small intestine, viscous fibres are suggested to increase digesta viscosity (Dikeman et al., 2006; Kristensen and Jensen, 2011; Vuksan et al., 2009), which in turns results in prolonged absorption of nutrients and increased release of satiety-related gut hormone (Howarth et al., 2001). This process is also suggested to induce a phenomenon known as 'ileal break'. The satiety-signals involved in relation with ileal break are CCK, GLP-1 and PYY (Blundell et al., 1994). Viscous fibres such as alginate, β-glucan, psyllium and guar gum have been demonstrated to increase satiety-related gut hormones following their effect in the small intestine (Beck et al., 2009a; Beck et al., 2009b; Juvonen et al., 2009).

1.5.2.5 The Effect of Dietary Fibre on Colonic Fermentation

Fermentable fibres, such as inulin-type fructans or resistant starch have been shown to exert their effect on energy homeostasis by stimulating colonic fermentation (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2007b; Daubioul et al., 2002; Delzenne et al., 2005; Delzenne et al., 2007). Fermentation processes of fibre include the breakdown and hydrolysis of complex carbohydrate to their basic sugars by gut microbiota which can be found in the caecum and ascending colon.

1.5.2.5.1 Gut Microbiota and its Effect on Energy Homeostasis

The humans' adult intestine contains between 10 trillion to 100 trillion microorganisms. These microorganisms can be categorised into 3 populations, bacteria, archaea and eukarya with the largest population belongs to bacteria. The bacterial population comprises a minimum of 10^{14} anaerobic bacteria from 500 to 1000 identified species (Xu and Gordon, 2003), which can be divided into the beneficial and the pathogenic communities. However, the most populated genera found in the human adults' intestine are *Bacteroidetes* (gram-negative bacteria) and *Firmicutes* (gram-positive bacteria). Both of the bacteria are mainly involved in digesting carbohydrates that escaped the absorption process in the small intestine (Gill et al., 2006).

Recently, the alteration of population levels of *Bacteroidetes* and *Firmicutes* in both rodents and obese people have been related to obesity. In rodents, *ob/ob* mice have been shown to contain lower population of *Bacteroidetes* but higher levels of *Firmicutes* compared to lean mice (Ley et al., 2005). This proportion levels of microflora have been suggested to have high ability for energy harvesting compared to lean mice (Turnbaugh et al., 2006). Moreover, the population of *Bacteroidetes* in human (twin volunteers) were also found in a lower amount compared to *Actinobacteria* populations (Turnbaugh et al., 2009). Interestingly, an intervention study demonstrated that obese volunteers with significantly less *Bacteroidetes* and increased *Firmicutes* composition at the beginning of the study were shown to reverse the bacteria composition pattern by increased *Bacteriodetes* and decreased *Firmicutes* after one year of low-fat or low-carbohydrate diets compared to normal weight volunteers. In addition, alteration in the bacterial population ratio was correlated with a reduction in body fat (Ley et al., 2006). Indeed, Santacruz *et al*. demonstrated that the reduction of body weight following lifestyle modifications seems to be influenced by the volunteers' gut microbiota

proportion. No correlation between the gut microbiota and total energy intake was reported, suggesting that there is possibly a link between diet, gut microbiota and the host (Santacruz et al., 2009).

Despite several studies showing that high *Firmicutes* / low *Bacteroidetes* are related to obesity, Duncan *et al*. suggested that an alteration of *Bacteroidetes* population is possibly not related to energy homeostasis. This suggestion has been made as the bacteria population was found to be in similar pattern in both overweight and lean volunteers. However, the authors agreed that *Firmicutes* populations were reduced after volunteers underwent a low-carbohydrate diet (Duncan et al., 2008). Therefore, the modification of gut microflora composition could possibly be involved in the regulation of energy homeostasis and could reduce the development of obesity. A number of dietary fibres have been suggested to alter the composition of gut microflora population. These dietary fibres are hypothesized to alter the gut microflora composition by selectively stimulating the growth of beneficial bacteria and suppressing the number of pathogenic bacteria composition. Dietary fibres with these physiological role are known as prebiotics (Gibson and Roberfroid, 1995). To be known as prebiotic, food ingredients must not be digested in the small intestine, fermented by at least one or more bacteria that symbiotically beneficial for gut health (e.g. bifidobacteria and lactobacilli), increase the composition of 'good' bacteria and reduce the pathogenic bacteria communities such as *Escherichia coli* and *Clostridium perfringens* (Kolida et al., 2002).

Inulin-type fructans have been suggested to fulfil these criteria and their prebiotic role has been demonstrated in a few studies. Adding inulin-type fructans (i.e. fructooligosaccharides, inulin and oligofructose) in the human diet have been shown to stimulate the number of *Bifidobacterium* spp. and *Lactobacillus* spp. in humans (Bouhnik et al., 2006; Kolida et al., 2007; Kruse et al., 1999; Sghir et al., 1998). In addition, including oligofructose and inulin in the diet for 45 days significantly increased *Bifidobacteria* composition and at the same time reduced the number of *Bacteroides, clostridia, Fusobacteria* and gram positive cocci (Gibson et al., 1995). The bifidogenic effect of inulin was also demonstrated in Kleessen *et al*. (Kleessen et al., 1997). Recently, supplementing oligofructose in high fat diet has also been exhibited to reduce the pathogenic effect of endotoxemia. The effect was negatively correlated with an increase number of *Bifidobacterium* spp. compared with the high fat diet alone. Interestingly, the bifidogenic effect of oligofructose also results in improved

glucose tolerance, stimulates insulin release and suppresses endotoxemia following increased bifidobacteria (Cani et al., 2007c). Endotoxemia is a condition in which plasma lipopolysaccharide (an endotoxin produced following intake of high fat diets) is increased in high levels in the periphery and its abundance has been associated with increased body weight as well as a reduction in the number of gram positive bacteria, bifidobacteria (Cani et al., 2007a). In general, intestinal microflora are important to digest non-digestible fibres and resistant starch to yield short chain fatty acid (SCFA), gasses, heat and energy as the products of fermentation (Cummings, 1981). The main products of the fermentation, SCFAs have been hypothesized to play an important role in energy homeostasis (Delzenne et al., 2005; Reimer and McBurney, 1996). The roles of SCFAs are explained further in the section 1.5.3.

Recently, there has been a controversy regarding the secretion of SCFA-derived intestinal fermentation by the gut microbiota. Evidence demonstrated that obesity is related to high energy harvesting of intestinal microbiota (Turnbaugh et al., 2006). In addition, SCFAs were also demonstrated to be significantly increased in the caecum of genetically obese mice compared to lean mice (Murphy et al., 2010). Schwiertz *et al*. showed that a high production of propionate and total production of SCFA were released in obese volunteers compared to lean volunteers. However, this observation could also possibly be due to differences in the microbe, shift in transit time or decreased rate of mucosal absorption (Schwiertz et al., 2010). At the same time, SCFAs-derived intestinal fermentation have extensively been shown to affect hosts' health by reducing hunger, improving glucose tolerance and modulating body weight (Cani et al., 2005b; Cani et al., 2006b). The contradict effects of gut-derived SCFAs on energy homeostasis following dietary factors especially in humans are still unclear. Although dietary factors seem to play a role in the contrasting effect of SCFA, there is possibly an unknown mechanism by which colonic gut-microbiota interacts with the host in regulating energy homeostasis. This remains to be further clarified.

1.5.3 Short Chain Fatty Acids

SCFAs that arise from fibre degradation by colonic gut bacteria have been proposed to beneficially influence human health by modifying energy homeostasis. There are many suggestions of how their

physiological role can influence energy homeostasis, however their main role as anorectic gut hormone stimulators has attracted much attention as this could be developed as an obesity treatment.

SCFAs are organic fatty acids which contain 1 - 7 carbons. Acetate (2 carbons), propionate (3 carbons) and butyrate (4 carbons) are the main products of the bacterial degradation of dietary fibre apart from gasses (CO_2, CH_4 and H_2), heat and energy. The chemical structures of acetate, propionate and butyrate are illustrated in figure 1.4.

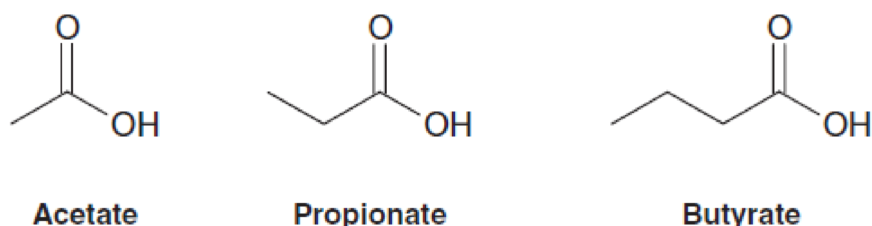


Figure 1.6 Chemical structure of acetate, propionate and butyrate produced from fermentation of fibre in the large intestine. Adapted from (Darzi et al., 2011).

SCFAs can be found in herbivorous and omnivorous animals, but the highest production of SCFAs can be found in herbivors as SCFAs are produced from the fermentation of plant-type materials. Ruminants have the highest SCFA production which provides approximately 60 – 80 % of metabolised energy (Bergman, 1990). In contrast to ruminants, SCFAs in humans are produced typically from western diet which contains less plant-based materials. Therefore, this produces comparably low amounts of metabolised energy (5 – 10%) (Mortensen and Clausen, 1996).

There are several factors that influence the production of SCFAs, such as the population and amount of bacteria present in the gut, the bowel transit time and the type of substrate or fibre ingested (Cook and Sellin, 1998). Evidence suggested that the fermentation of dietary fibre and malabsorbed carbohydrate contributed between 220 – 760 mmol SCFAs daily (Freeland and Wolever, 2010). Protein is also a potential fermentation substrate. It is digested into branched amino acid such as valine, leucine and isoleucine by a group of bacteria called proteolytic.

However, the amount of SCFAs produced by protein digestion is much lower than carbohydrate digestion (Macfarlane and Macfarlane, 1995; Macfarlane and Macfarlane, 2003).

The overall SCFAs intestinal production is in the order of acetate > propionate > butyrate, which comprise 90-95 % of the total colonic SCFA production (Cummings, 1981). The SCFAs production and the type of SCFAs are determined by the location and availability of the substrate. The caecum and proximal colon contain the highest concentration of SCFAs because of higher bacterial fermentation activities and high amounts of substrate availability compared to other parts of the colon. The SCFAs concentrations are slowly reduced towards the distal colon as the levels of available substrate declined (Bach Knudsen et al., 1991; Govers et al., 1999; Marsono et al., 1993; Topping et al., 1993). It is estimated that the caecum and proximal colon contain between 100 – 140 mmol/l of total colonic SCFAs whilst the distal colon contains 40 – 80 mmol/l (Bach Knudsen et al., 1991). From the colon, SCFAs are rapidly absorbed and transported to the liver and the periphery via portal blood. Investigation of SCFA concentrations in sudden death victims revealed that the portal blood contained the highest amount of the SCFAs (375 ± 70 µmol/l) compared to the hepatic circulation (148 ± 42 µmol/l) and the peripheral (79 ± 22 µmol/l). Nevertheless, the SCFAs levels reported in the portal blood was 1/1000th less than the amount produced in the colon (Cummings and Englyst, 1987). SCFAs play their role at different sites in the body. The three most important sites where SCFAs exert their roles are the colon for butyrate, the liver for acetate and propionate, whilst muscle cells are predominantly fuelled by acetate. The individual SCFA productions in the large intestine and blood are summarised in Table 1.2.

Table 1.2 Individual SCFA levels in the large intestine and blood circulations in humans. Adapted from (Cummings et al., 1987).

SCFA	Blood (µmol/l)			Large intestine (mmol/kg)			
	Portal	Hepatic	Peripheral	Ascending	Transverse	Descending	Sigmoid
Acetate	258 ± 40	115 ± 28	70 ± 18	63.4 ± 6.8	57.9 ± 5.4	43.5 ± 11.1	50.1 ± 16.2
Propionate	88 ± 28	21 ± 10	5 ± 2	26.7 ± 4.0	23.1 ± 2.8	14.2 ± 3.1	19.5 ± 6.7
Butyrate	29 ± 8	12 ± 4	4 ± 2	24.5 ± 4.2	24.4 ± 2.2	14.7 ± 2.9	17.9 ± 5.6
Total	375 ± 70	148 ± 42	79 ± 22	114.6 ± 15.0	105.4 ± 10.4	72.4 ± 17.1	87.5 ± 28.5

Results are expressed as mean ± SEM (n=6)

1.5.3.1 Acetate

Acetate is the most abundant SCFA produced in the gut. Once produced, it is absorbed and transported to the liver. In the liver, acetate is the main substrate for cholesterol synthesis. Rectal infusion of 90 mmol of acetate (+ 30 mmol propionate) and 180 mmol of acetate (+ 60 mmol propionate) positively correlated with elevated levels of cholesterol and triglycerides, which strongly suggested the role of acetate in lipogenesis (Wolever et al., 1989; Wolever et al., 1989). Theoretically, acetate stimulates lipid synthesis by converting into acetyl-CoA with the help from acetyl-CoA synthetase, an enzyme that can be found mainly in the adipocytes and mammary glands (Wong et al., 2006).

Lactulose has been suggested as a good source of acetate as it has been shown to increase acetate levels compared to a control group (Fernandes et al., 2000; Pomare et al., 1985; Pouteau et al., 1998). Intake of lactulose has been shown to increase fasting serum cholesterol in humans. The authors suggested that this effect could potentially be due to increased acetate, which is a substrate for cholesterol synthesis (Jenkins et al., 1991). In addition, lactulose might have produced insufficient levels of propionate (Fernandes et al., 2000) to suppress cholesterol synthesis (Wolever et al., 1991; Wolever et al., 1995). Indeed, Wolever *et al.* also highlighted the role of propionate in suppressing cholesterol synthesis after they demonstrated that adding propionate into acetate infusion leads to a

decrease in serum cholesterol but the effect was diminished when infusing acetate alone. In addition, these observations also suggested that the effect of acetate and propionate on lipogenesis is not solely depend on individual SCFAs production, but also depends on acetate and propionate ratio (Wolever et al., 1991).

1.5.3.2 Propionate

The role of propionate on human health will be discussed in Chapter 5

1.5.3.3 Butyrate

Butyrate is the main energy regulator for colonocytes (Roediger, 1980). It is reported that about 70 – 90 % of butyrate is metabolised by the colonocytes (Cook and Sellin, 1998). Indeed, the importance of butyrate as the energy provider in the colonocytes when compared to propionate and acetate is estimated in the ratio of 90:30:50 (Cook and Sellin, 1998). As a regulator in the colon, butyrate has been shown to influence colonic cell proliferation and differentiation (Li and Elsasser, 2005; Topping and Clifton, 2001) and inflammatory response (Topping and Clifton, 2001). Nilsson & Nyman demonstrated that butyrate concentration is higher in the distal colon compared to the caecum, whereas butyrate proportion was found to be elevated both in the caecum and the distal colon (Nilsson and Nyman, 2005). Oligofructose, *Plantago ovate*, guar gum, butyrylated starch, lactulose, cornstarch and barley kernels are the plants and food components that produce high amount of butyrate (Bajka et al., 2006; Fernandes et al., 2000; Morrison et al., 2006; Nordgaard et al., 1996; Stewart and Slavin, 2006).

1.5.3.4 Short Chain Fatty Acids Receptor

Although many studies suggest that SCFAs may have numerous physiological effects on intestinal health and improving the host health, the exact mechanisms of how they regulated these effects remains unclear. It was not until 2003, Brown *et al.,* Nilsson *et al.* and Le Poul *et al.* identified the presence of two receptors, free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3) were activated by SCFAs (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). The discoveries of these two SCFAs receptors have opened a new perspective in appetite research and

hopefully this could lead to a better understanding of how SCFAs mediate their effect on energy homeostasis.

Interestingly, FFAR2 and FFAR3 have a different affinity towards the SCFAs. In FFAR3, the potential orders of SCFA binding are propionate ≥ butyrate > acetate whilst in FFAR2, each SCFA has equal affinity for the receptor (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003). FFAR2 and FFAR3 are expressed in various types of tissues. FFAR3 is highly expressed in the adipose tissue, pancreas, spleen and bone marrow (Brown et al., 2003; Le Poul et al., 2003; Xiong et al., 2004) and FFAR2 has been reported to be expressed in the immune cells, particularly monocytes and neutrophils (Brown et al., 2003; Le Poul et al., 2003), adipocytes, spleen, skeletal muscle and heart (Ge et al., 2008; Hong et al., 2005; Nilsson et al., 2003; Xiong et al., 2004; Zaibi et al., 2010). In the intestine, mRNA and protein of both receptors can be found in the whole wall and mucosa extracts (Karaki et al., 2008; Tazoe et al., 2008). It has also been demonstrated that FFAR2 and FFAR3 were bound to Gi/o-proteins that can be found in the adipose tissue (Brown et al., 2003; Le Poul et al., 2003). G-proteins is a group of signalling protein that exert their effects by binding to the cytoplasmic of the receptors and help in sensing and aid in their coupling downstream process. Binding of G-protein with SCFAs receptors in the adipose tissue highlighted the important of adipose tissue as the target for SCFA. The localization of both of the receptors in various types of tissues have suggested that the receptors functions may not just be limited to the stimulation of gut hormones secretion but possibly involved in the regulating of various immune and inflammatory responses (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003).

1.5.3.4.1 The Role of Short Chain Fatty Acids Receptors

Recently, studies demonstrated that PYY containing enteroendocrine cells, the L-cells also expressed FFAR2 and FFAR3 which suggests that the cells possibly sense SCFAs directly via both receptors in rats and human (Karaki et al., 2006; Karaki et al., 2008; Tazoe et al., 2008) to release PYY. However, it is still not fully understood how the cells respond to this stimulus. In addition to this finding, activation of SCFA on FFAR2 may also stimulate the expression of 5-hydroxytryptophan, also known as serotonin. This hypothesis has been made following the recent finding of co-localisation of FFAR2-IR cell with serotinin in the mucosal mast cells (Karaki et al., 2006). However, this observation has only been demonstrated in rat, but not in human colonic mucosa (Karaki et al.,

2008). Serotinin is a neurotransmitter, which is secreted primarily in the gastrointestinal tract and also in the CNS. It is mainly involved in regulating energy homeostasis and food intake, mood and gastrointestinal motility (Berger et al., 2009). The importance of FFAR3 in releasing PYY levels has been investigated in a mice null FFAR3 study. In this study, Samuel *et al*. reported that the lack of FFAR3 in mice resulted in reduced PYY levels and body weight compared to the wild type mice, potentially due to the lack of PYY expression (Samuel et al., 2008). The L-cell, the enteroendocrine cell located in the intestine has been postulated as an important site in regulating appetite. This is due to its physiological role in the synthesis and secretion of anorectic gut hormones such as GLP-1 and PYY, which have been shown as an important inhibitor of food intake and body weight in both rodents and human (Batterham et al., 2002; Batterham et al., 2003; Cani et al., 2005b; Cani et al., 2007b).

Besides stimulating gut hormone secretion, SCFAs receptors are also postulated to play significant roles in the adipose tissue. Hong *et al*. and Zaibi *et al*. demonstrated that acetate and propionate stimulated adipogenesis and reduced expression of leptin, which was activated via FFAR2 (Hong et al., 2005; Zaibi et al., 2010). Activation of FFAR2 by both of the SCFAs, acetate and propionate in the adipocytes resulted in reducing lipolysis while acetate has been shown to suppress plasma free fatty acid levels *in vivo* in both male and female mice (Ge et al., 2008). Surprisingly, it seems that FFAR3 is not involved in this process (Hong et al., 2005).

1.5.3.5 Short Chain Fatty Acids and Gut Hormones Release

Numerous evidences demonstrated that SCFAs-derived from colonic fermentation potentially modulate energy homeostasis by stimulating the release of anorectic gut hormones such as PYY, and GLP-1, GLP-2 and reducing the amounts of orexigenic hormone, ghrelin (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2009; Parnell and Reimer, 2009) possibly via their receptors, FFAR2 and FFAR3. Nevertheless, although all three main SCFAs can be generated endogenously via fat oxidation and gluconeogenesis metabolism, the amounts of SCFAs produced seem insufficient to stimulate the production of gut hormones. It was reported that the average colonic production was 100 mM whilst the stimulation of PYY occurred at 300 mM (Cherbut et al., 1998). Therefore, increased intake of other source of SCFAs such as dietary fibre are highly encouraged in order to

increase colonic pools of SCFAs needed to stimulate gut hormone release. Based on this objective, appetite research has now focusing on finding ways to increase endogenous production of SCFAs either by finding new plant source or approaches that could induce SCFAs production.

1.5.4 The Effect of Dietary Fibre on Appetite Regulation

1.5.4.1 The Effect of Dietary Fibres in Rodents

Dietary fibres with viscous and fermentable properties have been shown to reduce food intake and body weight compared to low fermentable non-viscous fibres. Adding β-glucan in the obese mice for six weeks reduced food intake and suppressed weight gain possibly due to increased postprandial plasma $PYY_{3\text{-}36}$ concentration (Huang et al., 2011). In other study, Hara *et al.* suggested that fermentable viscous fibre was more effective in reducing food intake and weight gain compared to other tested fibres. In this study, the effect of 10 % guar gum, psyllium, sugar-beet fibre, cellulose or diet without fibre were investigated in three weeks. Guar gum, a high fermentable viscous fibre showed the highest effect in attenuate weight gain compared to psyllium, sugar-beet fibre, cellulose and fibre-free diet (138 ± 8.9 g, 142 ± 3.7 g, 143 ± 5.2 g, 148 ± 7.0 g and 149 ± 5.2 g respectively) (Hara et al., 1996). The effect of guar gum on food intake and body weight is also reported elsewhere (Henningsson et al., 2002; Johnson and Gee, 1986; Lu et al., 2000; Vachon et al., 1988). A study showed that although guar gum significantly reduced food intake compared to cellulose, bran or fibre-free diet in a short term (six weeks), the effect was not sustained to reduce weight gain in the long term (67 weeks) (Track et al., 1985). The same observation was also reported in other studies (Davies et al., 1991; Isken et al., 2010). Isken *et al.* suggested that the energy produced from high SCFA levels of fermented soluble fibre in the gut may have influenced the overall endogenous energy production therefore outweighed the known acute effect of fermentable carbohydrates on food intake and body weight (Isken et al., 2010).

Diet supplementing with 5 % pectin, 5 % cellulose and combination of 2.5 % pectin and 2.5 % cellulose for six weeks showed no significant effect on food intake or body weight although there was a significant modulation on plasma cholesterol and HDL-cholestrol levels in rats fed pectin-

enriched diet (Krzysik et al., 2011). It is possible that 5 % of fibres used in this study were too low to attenuate food intake and body weight. This is because the effect of dietary fibre on reducing food intake and body weight in rodents were usually reported following intake of 10 % fibre per total dietary intake (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2006b). Surprisingly, adding 500 g mixed fibre diet of oat based products consists of resistant starch, β-glucan and dietary fibre in Wistar rats for six weeks also showed no significant effect on food intake and body weight although the treatment did increased caecal and colon walls weight, increased the genus *bifidobacterium* and reduced coliform numbers upon the treatment (Drzikova et al., 2005). The pronounced effects of the treatment on gut architecture could possibly be due to the presence of resistant starch, which mainly modulated its effect on appetite and body weight via colonic fermentation (Keenan et al., 2006; Zhou et al., 2008; Zhou et al., 2009). The effect however, diminished in this study.

Resistant starch, via its colonic fermentation effect has been shown to suppress body weight gain, deposition of fat mass and increased release PYY and GLP-1 gene expression (Charrier et al., 2011; McCutcheon et al., 2009; Shen et al., 2009; So et al., 2007; Zhou et al., 2008). However, Keenan *et al*. did not find the same observation in their study. They demonstrated that supplementing the same amount of either resistant starch or methylcellulose diet to rats resulted in no significant effect on food intake and body weight. The reason behind this observation is possibly due to increased food intake in order to compensate for low energy density from the fibre-enriched diet. Oligofructose, another fermentable carbohydrate also has been suggested to alter food intake and body weight through colonic fermentation (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2007b; Daubioul et al., 2002; Delzenne et al., 2005). The effect of oligofructose treatment on body weight is potentially due to the increased release of GLP-1 and PYY (Cani et al., 2004; Cani et al., 2005b; Delzenne et al., 2005). The role of oligofructose is discussed in more detail in Chapter 2.

1.5.4.2 The Effect of Dietary Fibres in Suppressing Appetite in Humans

A systematic review comparing the effect of different physico-chemical properties of dietary fibres on subjective appetite, energy intake and body weight suggested that fibres with viscous property suppressed appetite and acute energy intake to a greater extent compared with other type of fibres (Wanders et al., 2011). β-glucan, one of the viscous fibres, has been demonstrated to modulate

subjective appetite and gut hormones when added to different type of foods such as beverages (Barone et al., 2012; Lyly et al., 2009; Lyly et al., 2010), breakfast cereals (Beck et al., 2009a) and bread (Vitaglione et al., 2009). Surprisingly, although it has been shown to affect subjective appetite, it does not always reduce food intake (Juvonen et al., 2009; Vitaglione et al., 2010). Peters *et al*. also demonstrated that β-glucan when taken as cereal bars had no significant effect on both appetite and energy intake, although it increased *in vitro* digesta viscosity (Peters et al., 2009).

Nevertheless, a study comparing the effect of different viscosities on satiety demonstrated that guar gum, the highest viscous fibre investigated in this study, increased subjective satiety and decreased desire to eat compared to other low viscous fibre and wheat bran (Lyly et al., 2009). French and Read demonstrated that supplementing 3 % of guar gam to low and high fat soup hindered the return of hunger sensations in young men, due to delayed gastric emptying (French and Read, 1994). In contrast, Lavin *et al*. suggested that reduced glycaemic and insulinemic response following three hours intake of 2% of guar gum added in a 250ml glucose beverage was not related to delayed gastric emptying, but possibly due to increased circulating plasma GLP-1, insulin and gastric inhibitory polypeptide levels (Lavin and Read, 1995).

The role of alginate in modulating subjective appetite and food intake has been demonstrated in healthy normal weight and obese volunteers (Georg et al., 2011; Hoad et al., 2004; Peters et al., 2011; Solah et al., 2010) either in controlled clinical studies (Georg et al., 2011; Pelkman et al., 2007) or in free living volunteers (Paxman et al., 2008). It is suggested that alginate exerts its effect by forming gelling particles either in the acidic environment (pH less than 3.5) in the stomach or by the interaction with divalent ions such as Ca^{+} (Hoad et al., 2004). However, some studies cannot replicated the same effect (Mattes, 2007; Odunsi et al., 2010). Inconsistencies in showing the effect of alginate on subjective appetite and food intake may be due to several factors; such as differences in study design and the levels of alginate investigated, the carriers (whether solid or liquid) and type of biopolymer used in alginate (guluronate or mannuronate). Different types of biopolymer can influence the viscosity and strength of gel mass production during the reaction in the stomach, due to different gelling abilities and molecular weight (Georg et al., 2011).

Adding psyllium in the diet has been shown to reduce hunger and increase satiety in twelve healthy lean volunteers following six hours intake of the test meal. It was suggested that psyllium exerts it effects by delaying gastric emptying, which was demonstrated to be started three hours after meal intake in this study (Bergmann et al., 1992). In contrast, Rigaud *et al*. reported that the effect of 7.4 g psyllium on suppressing hunger and energy intake was not related to a delay in gastric emptying, but primarily due to high viscous environment, which subsequently leads to slow intestinal absorption of nutrients. The effect of psyllium in reducing energy intake was also reported by other studies (Cybulski et al., 1992; Rigaud et al., 1998; Turnbull and Thomas, 1995). Surprisingly, adding psyllium in breads has resulted in no significant effect on appetite although postprandial glucose and insulin, plasma ghrelin, GLP-1 and PYY were modulated by psyllium treatment (Karhunen et al., 2010). The authors suggested that the unaffected subjective appetite found in this study was related to solid foods (bread) as the carriers. Furthermore, because subjective appetite has a large inter-individual variation, the number of volunteers involved in the study (sixteen volunteers) maybe insufficient to detect small differences on subjective appetite (Karhunen et al., 2010).

A non-viscous, fermentable carbohydrate is another group of fibre which has been postulated to have a major impact on appetite regulation. Fermentable carbohydrates control appetite and energy intake by acting in the distal gut and therefore little or no modulation of its effect on the small intestine is expected. There are a few type of fibres categorised in this group, which are methylcellulose, resistant starch, fructans, xylans and mannans families. The role of non-viscous fermentable carbohydrates on the short term effect of appetite has been investigated in several studies (Archer et al., 2004; Bodinham et al., 2010; Cani et al., 2006a; Cani et al., 2009; Delargy et al., 1997; Isaksson et al., 2009; Peters et al., 2009; Raben et al., 1994; Weickert et al., 2006; Willis et al., 2009). A study comparing the effect of different fibres supplemented in muffins; β-glucan (viscous fibre), corn bran (insoluble fibre), resistant starch (fermentable carbohydrate) and low fibre on appetite sensations exhibited that resistant starch consistently affects appetite by reducing hunger-related scores and increased satiety-related ratings (Raben et al., 1994; Willis et al., 2009). However, assessment on energy intake was not performed in this study. Meanwhile, inclusion of 30 g resistant starch, high-amylose corn starch (RS2) and retrograded high-amylose corn starch (RS3) had little effect on subjective appetite and energy intake (de Roos et al., 1995)

Interestingly, although increased resistant starch to 48 g taken twice per day during breakfast and lunch in 20 healthy BMI volunteers had no significant effect on subjective appetite, the supplement reduced energy intake 4 hours after meals and in 24 hours thereafter (Bodinham et al., 2010). Late effect on energy intake was also demonstrated in Archer *et al*. In their study, inclusion of inulin (24 g) in a fat-reduced sausage patty reduced energy intake compared to the full fat sausage patty during breakfast, although no significant effect on subjective appetite was observed. This observation suggests that the effects of resistant starch on appetite did not necessarily appear at the meal time but could be affected later in the day (Archer et al., 2004). Besides resistant starch, inulin has also been suggested to modulated satiety and energy intake following intake of 16 g oligofructose for two weeks (Cani et al., 2009). The effect of oligofructose on appetite and energy intake potentially modulated via increased circulating plasma gut hormones after fibre fermentation by gut bacteria. The effect of inulin-type fructans on appetite will be discussed in detail in Chapter 2.

1.5.4.3 The Effect of Dietary Fibres on Reducing Body Weight in Humans

Based on the evidence discussed in the previous paragraphs, dietary fibres have a great potential to affect acute subjective appetite and energy intake. However, whether the modulations of subjective appetite and energy intake in the short term could be translated to weight loss or prevention of weight gain are remain controversial. Nevertheless, until now, large epidemiological, cross-sectional and intervention studies consistently showed that high fibre diet or fibre-related products in a long term have a negative correlation with body weight, weight gain, WC and adiposity (Du et al., 2010; Koh-Banerjee et al., 2004; Liu et al., 2003; Ludwig et al., 1999; Maskarinec et al., 2006; Miller et al., 1994; Nelson and Tucker, 1996; van, V et al., 2009).

Alginate, a viscous fibre is proposed to reduce energy intake (Georg et al., 2011; Paxman et al., 2008; Pelkman et al., 2007) by forming gels with the presence of ionic cations (Ohta et al., 1997). The gelling property of alginate is the main feature that differentiates its mechanism of action with other viscous fibres such as guar gum and psyllium. A study showed that intake of hypocaloric diet containing 15 g alginate three times per day for two weeks reduced body weight (-1.42 ± 0.38 kg). However, the effect was less pronounced than the effect showed by the control group (-1.56 ± 0.21 kg) (Georg et al., 2011). It was postulated that two weeks was insufficient to affect body weight. In

addition, the authors suggest that body weight reduction can be induced if the supplement is continuously taken in a long term period. Indeed, recent findings showed that a 15 g alginate-containing hypocaloric diet significantly suppressed body weight by 6.78 ± 3.67 kg after 12 weeks treatment in 80 obese volunteers compared to 5.04 ± 3.40 kg in the placebo group. However, no significant difference was demonstrated in intention-to-treat analysis (Georg et al., 2012).

Guar gum has been suggested to exert its effect on appetite and energy intake by delaying gastric emptying (Todd et al., 1990). Investigations into the effects of guar gum on controlling body weight has been studied in several populations, hypercholesterolemic patients (Blake et al., 1997; Tuomilehto et al., 1980; Uusitupa et al., 1984), dyslipidemic patients (Tuomilehto et al., 1983), overweight or obese volunteers (Heini et al., 1998; Kovacs et al., 2001; Kovacs et al., 2002) and post-menopausal women (Makkonen et al., 1993; Pasman et al., 1997b). Supplementing 2.5 g guar gum to a semi-solid meal of a low energy diet showed a tendency to alter subjective appetite compared to meals without guar gum and solid meals in 28 overweight healthy volunteers. However, no significant effect on weight loss was demonstrated (Kovacs et al., 2001). In contrast, adding 20 g guar gum in orange juice twice daily for a week in 17 obese women three months after weight loss significantly reduced energy intake by 1.3 ± 0.2 MJ compared with the control group (Pasman et al., 1997a). Meanwhile, supplementing 20 g partially hydrolysed guar gum in a low calorie diet in 25 obese women increased postprandial CCK levels, but showed no significant effect in reducing appetite or weight loss during the treatment course (Heini et al., 1998). Similarly, no significant body weight loss was also reported in 20 weight-reduced women who received 20 g/day of guar gum supplementation for 14 months (Pasman et al., 1997b) and in 15 menopausal women supplemented with 15 g/day guar gum for six months (Makkonen et al., 1993). Indeed, a meta-analysis on eleven randomized guar gum studies concluded that guar gum is not effective as a weight loss treatment. Furthermore, a high number of side effects (constipation, diarrhoea, flatulence) reported by volunteers may have masked the beneficial effect of this fibre (Pittler and Ernst, 2001).

Intake of two capsules of 500 mg glucomannan three times per day in 20 obese women induced weight loss by 2.5 kg and reduced serum related-cholesterol after eight weeks supplementation period (Walsh et al., 1984). Furthermore, addition of 4 g/day of glucomannan in a low caloric diet in 25 severely obese volunteers for three months significantly reduced body weight compared to a

hypocaloric diet without the supplement (Vita et al., 1992). Meanwhile, combination of glucomannan and other fibres (inulin, psyllium and pectin) in a dose of 7 g/day suppressed an increase in BMI (26.3 ± 1.7 to 25.0 ± 1.41 kg/m^2) in ten volunteers over four weeks (Bortolotti et al., 2008). Indeed, Birketvedt *et al*. also showed that adding glucomannan alone or in a combination with other fibres (guar gum or alginate) in hypocaloric diet significantly increased weight loss (3.8 – 4.4 kg) compared to a low caloric diet alone or placebo after five weeks treatment in 176 volunteers (Birketvedt et al., 2005). A similar observation was demonstrated when Salas-Salvado *et al*. investigated the effect of delivering mixed fibre (3 g *Plantago ovata* and 1 g glucomannan) in a large scale, parallel, double-blinded, randomised and placebo-controlled study. In this study, 4 g of mixed fibres taken either twice or three times per day induced weight loss (-4.52 ± 0.55 and -4.60 ± 0.56 kg respectively) compared with the control group (-0.79 ± 0.59 kg) in 200 overweight or obese volunteers for 16 weeks, but no significant difference was found between the treatments (Salas-Salvado et al., 2008). However, no effect on body weight loss was also demonstrated by Hylander *et al*. and Rodriguez-Moran *et al*. (Hylander and Rossner, 1983; Rodriguez-Moran et al., 1998).

β-glucan has been proposed to alter subjective appetite and energy intake in the short term by increased viscosity in the stomach (Beck et al., 2009a; Lyly et al., 2009; Lyly et al., 2010; Vitaglione et al., 2009). However, there is currently a controversy whether continuous consumption of β-glucan for a longer period can aid weight loss. This is either because the data on the role of β-glucan on weight loss is insufficient or the findings from the previous studies are not supporting its role in weight management. Saltzman *et al*. demonstrated that consumption of a hypocaloric diet containing oats (45g/1000 kcal) in 41 adults did not affect body weight compared to the control, although there was a tendency towards a reduction of hunger. The authors suggested that this observation was possibly due to the lower amount of supplementation or the number of volunteers in this study were insufficient to effect body weight change (Saltzman et al., 2001). No significant effect on reducing body weight was also observed when Maki *et al*. supplemented 3 g/day oat β-glucan-enriched hypocaloric diet in 144 overweight women for 12 weeks (Maki et al., 2010).

Although non-viscous fermentable carbohydrates have been shown to reduce subjective appetite and acute energy intake, evidence on the effect of long term studies on maintaining body weight

is inconsistent. Numerous evidence have demonstrated that resistant starch reduced energy intake and body weight in rodents (de Deckere et al., 1995; Keenan et al., 2006; Shen et al., 2009; Zhou et al., 2009). However, evidence in humans is currently insufficient to support the role of resistant starch in reducing body weight. Supplementing resistant starch (40 g/day) for 12 weeks in 20 insulin resistance volunteers did not affect body weight and fat deposition although it significantly improved insulin response. These results suggest that modulation on insulin is not directly mediated via change in body fat but possibly other mechanisms that need further investigation (Johnston et al., 2010). Supplementing 24 g/day resistant starch in healthy overweight volunteers for 21 days also had no significant effect on body weight although it reduced postprandial glucose and cholesterol levels (Park et al., 2004). However, none of the effect was demonstrated by Heijnen *et al.* following supplementing 30 g of either high-amylose cornstarch (RS2) or retrograded high-amylose cornstarch (RS3) for three weeks. It is thought that a lack of statistical study power, low doses and short study period had caused no clear effects of resistant starch on glycaemic and lipidemic responses (Heijnen et al., 1996). Interestingly, de Roos *et al*. managed to show a little effect in reducing body weight in 24 healthy male volunteers after intake of 30 g resistant starch for four weeks (de Roos et al., 1995). Based on this study, resistant starch might have a potential to be developed as a weight suppressant if the study is extended to a longer period. Investigations are needed to support this hypothesis.

Administration of a non-viscous fibre in a conjunction with a low calorie diet (1200 kJ) showed mixed findings. Supplementing 6 g/day of non-viscous fibre (cereal, citrus, insoluble fibre) for eight weeks and followed with intake of 4 g/day for 16 weeks in 53 healthy overweight females showed to reduce body weight by 8.0 kg compared to only 5.8 kg in the placebo group (Birketvedt et al., 2000). However, Astrup *et al*. showed that after adding 30 g/day cellulose in a very low calorie diet (women: 388 kcal/day, men: 466 kcal/day) in 21 obese volunteers had no significant effect in reducing body weight although the treatment reduced subjective hunger (Astrup et al., 1990). Interestingly, Fragala *et al*. demonstrated that supplementing cellulose and cetylated fatty acids in combination with a caloric restricted diet and exercise significantly reduced body weight and body fat. Moreover, the authors also showed that the treatment

significantly decreased adipose-related hormones, leptin, insulin as well as increased adiponectin levels after the treatment (Fragala et al., 2009).

In contrast, a randomised crossover study comparing the effect of 27 ± 0.6 g/day fermentable carbohydrates (pectin, β-glucan) and non-fermentable carbohydrate (methylcellulose) for three weeks in healthy adults showed no significant effect on energy intake and body weight. Nevertheless, the non-fermentable carbohydrate group showed an increased satiety compared to the fermentable carbohydrate group (Howarth et al., 2003). Inulin-type fructans have been shown to consistently reduce body weight in rodents through its colonic fermentation effect (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2006b). However, data in humans is currently insufficient to support this observation. However, Parnell and Reimer have shown a promising result as supplementing 21 g/d oligofructose for 12 weeks significantly reduced body weight in healthy overweight volunteers (Parnell and Reimer, 2009). The role of inulin-type fructans in weight maintenance will be discussed in more detail in Chapter 3.

In conclusion, there is evidence that dietary fibres have the potential to be developed as a weight loss agent. Beneficial effects of fermentable carbohydrates in rodents have been used as guidance in human studies, however, to date, data regarding dietary fibre in human studies is inconsistent and needs further investigation. The contradict effects of dietary fibre on weight loss may be due to different methodologies used, type and dose of fibres investigated as well as different type of carriers. Whilst this warrants further investigations, it is worthwhile to explore a new approach to increase the efficacy of dietary fibre on suppressing appetite and body weight.

1.6 THE EVALUATION OF BODY FAT AND WEIGHT MANAGEMENT

Since 1972, BMI has been used as a classification system to determine human body size. The BMI is determined by dividing an individual's body weight (in kg) by the squared height (m^2). The WHO has classified BMI into categories, which are 18.5 to 25 kg/m^2 as healthy individuals, 25 to 30 kg/m^2 as overweight individuals and BMI from 30 kg/m^2 or more are known as obese people. As BMI has been used to describe body size, the same index also can be used to predict body fat (Deurenberg and

Yap, 1999). Individuals with a high BMI are predicted to have high body fat compared to individuals with lower index numbers. However, because BMI estimates body size and fat composition by correcting weight for height, it can be unsuitable to use as an assessment tool for some specific populations such as pregnant women and body builders.

Furthermore, BMI is not a precise method to assess adiposity as it cannot differentiate between fat mass or fat-free mass (Kontogianni et al., 2005; Wellens et al., 1996). Anthropometric measurements such as skinfold thickness and body circumferences seem to be more efficient in estimating body fat composition. In this method, total body adiposity is estimated based on the thickness of the subcutaneous fat measured by a special calliper designed to assess skinfold thickness. This is based on the assumption that total adiposity has a constant relationship with subcutaneous fat (Deurenberg and Yap, 1999). The skinfold thickness can be measured in any part of the body, but the most common areas are the upper arm (biceps and triceps), scapula (subscapular) and upper area of iliac crest (suprailiac). Although anthropometric measurements are not able to provide a precise estimation of total body fat, it is a common method used in estimating body adiposity due to its practicality, availability, non-invasive and relatively inexpensive method. Therefore, it is suitable to use in large scale and field studies. However, the reliability of these studies relies on the skills of the observers (Wang et al., 2000) as large errors in body fat estimations could arise from untrained observers. In addition, skinfold measurements are also influenced by age, sex, race and body fat contents (Wang et al., 2000).

Recently, many studies have shown that the metabolic consequences of obesity are not determined by the total amount of adiposity, but the effects are highly associated with body fat specific regions (Kelley et al., 2000; Pi-Sunyer, 2004). Therefore, in contrast to skinfold thickness which estimates general total adiposity, WC and waist to hip ratio (WHR) are suggested to be more accurate in predicting abdominal fat (Prentice and Jebb, 2001). Indeed, WC has been shown to have a strong correlation with abdominal fat compared to WHR or BMI (Han et al., 1995; Lean et al., 1995; Pouliot et al., 1994). However, all of these techniques (skinfold thickness, WC and WHR) are based on assumption and therefore inaccurate in quantifying the actual *in vivo* levels of abdominal fat and other non-adipose tissue depot such as ectopic areas. This is important as numerous studies have shown that these regions are associated with

metabolic consequences such as insulin resistance, hyperinsulinemia, hypertension and cardiovascular diseases (Boyko et al., 2000; Mathieu et al., 2008; Patel et al., 1999; Vega et al., 2005; Consitt et al., 2009; Thomas et al., 2005). Therefore, precise and effective assessment tools which can measure and distinguish body fat compartments are needed in order to identify individuals that are at high risk of metabolic diseases. Advances in the development of modern equipment have lead to improve body fat measurement techniques. In these techniques, body fat is no more estimated based on assumption but is directly measured from individuals and conveyed to computerised devices for quantification. Interestingly, some of the devices are able to distinguish different tissues of body composition including regional body fat and visualized it as segmental of human body compositions.

Bioeletrical impedance (BIA) quantifies body composition by measuring the resistance or impedance of human tissues to an electric current. In this method, a small electric current is emitted by the machine through the body and the impedance measurement is performed as the current travels via the body’s water pool. BIA is used to measure many types of human body composition including total body water, fat-free mass, body cell mass and body fat. Although it is among the attractive methods used in clinical and research studies (due to its practicality, portability and low cost), the accuracy of BIA depends on many variables such as body fluid, hydration, food intake, skin temperature water in the tissues. In addition, the precision of BIA also can be influences by technician skills and instrumentation (Deurenberg and Yap, 1999). Other technique, dual-energy X-ray absorptiometry (DEXA) uses X-ray beams to measure fat storage and body composition. In measuring fat content, DEXA can also be used to determine both total and regional (e.g. trunk, arms or legs) fat and fat-free tissues. However, DEXA measures adiposity as a total amount as it is unable to distinguish between subcutaneous and visceral adipose tissue. Moreover, DEXA measures fat mass through tissue thickness, which could led to increase errors in measurement as the tissues thickness increased. Apart from these limitations, DEXA is a preferable method to use in assessing body composition studies due to its practical, non-invasive approach, its availability and its low-radiation exposure as well as suitable to use in all ages.

Computed tomoghraphy (CT) measures body fat or muscles by creating cross-sectional images or ‘slices’ based on its different attenuation towards the X-ray source. This X-ray beam rotates around the volunteers by producing a pencil or fan-shaped beam that penetrates the body. The rotation of the X-ray produces three-dimensional images which are known as tomograms. These images are then conveyed to a computer for image reconstruction. Although CT seems able to give accurate information of specific and regional muscles, adipose tissue and organs compared to the other aforementioned body composition methods (Thibault et al., 2012), CT uses ionizing radiation which limited its application for repeated measurements especially in vulnerable populations such as children and pregnant women.

1.6.1 Magnetic Resonance Imaging

Among of all the methods used to measure body composition, MRI has been recognised as a gold standard mainly because it is a non-invasive technique, without exposure to ionizing radiation, allowing for repeated measures in longitudinal studies (Fowler et al., 1991; Ross et al., 1993) in all age-groups and clinical settings. Most importantly, MRI produces good contrast images of body fat tissues, and provides great structural detail about complex organs such as the brain, heart and muscle. This feature is particularly important in assessing total, regional and ectopic fat distribution which cannot be performed with other techniques (Hu et al., 2011; Thomas et al., 1998; Thomas et al., 2012). The accuracy of structural MRI for body fat assessment has been validated against dissection in human cadavers (Abate et al., 1994; Mitsiopoulos et al., 1998).

Since the 1990s, another application of functional MRI – fMRI – has been introduced in the field of neuro-obesity research. fMRI is able to provide functional data about regional neuronal activation within the brain, and has effectively been used to identify and map specific brain regions that involved in modulating appetite regulation. In fMRI neuroimaging studies, brain activity has been measured following stimulation with food images (Goldstone et al., 2009), infusion of gut hormones (De Silva et al., 2011; Rosenbaum et al., 2008) which have been performed in obese and lean volunteers (Martin et al., 2010) in both the fed and fasted state (Batterham et al., 2007; De Silva et al.,

2011), to provide information about which brain regions play a significant role in mediating satiety and hunger. The application of fMRI in neuro-appetite studies will be discussed in detail in Chapter 4.

1.6.1.1 Principles of Magnetic Resonance Imaging

Clinical MRI machines primarily image protons (or hydrogen nuclei) within the body. Each proton has its own magnetic property known as a magnetic dipole moment (MDM), by virtue of the fact that it is essentially a spinning positive charge, and each proton can therefore be thought of as a tiny bar magnet. In the absence of an external magnetic field, the protons have randomly oriented magnetic moments, but when an external magnetic field (B_0) is applied, they try to align with it. The combined effect of the aligned MDMs is known as the magnetization vector (M_z). MRI signal is produced by the application of another magnetic field, in the form of a short electromagnetic wave, which is the radio frequency (RF) pulse. This second magnetic field flips the M_z by 90^0. The longitudinal magnetization is now known as transversal magnetization (M_{xy}). Alignment of M_z to M_{xy} is important as this step produces signal needed for generating MRI images (Huettel et al., 2009) (see figure 1.6).

Once the RF pulse is finished, M_{xy} always tries to return to the stable ground, *z* axis. This happens because the *MR signals* losses its strength due to two independent phenomenons known as spin-lattice relaxation and spin-spin relaxation. Spin-lattice relaxation occurs when the transverse (xy) magnetization is slowly attenuated by releasing the absorbed energy obtained from the RF pulse to the surroundings and at the same time slowly increasing the longitudinal magnetization in the *z* axis. This phenomenon is called T1 longitudinal relaxation. At the same time, spin-spin relaxation or T2 transverse relaxation process occurs when the orbicular rotations of transverse magnetization slowly losses its spin affinity and subsequently decays after some period, but, unlike the spin-lattice relaxation that gives away its energy to the surrounding, the spin-spin relaxation exchanges its energy with other nuclei. Both T1 and T2 are measured in milliseconds. Body tissues have longer T1 compared to T2; Fluid has 1500-2000 ms T1 compared to 700 – 1200 ms T2, water-based tissues have 400 – 1200 ms T1 compared to 40 – 200 ms T2 and fat tissues have 100 – 150 ms T1 compared to 40 – 200 ms T2.

However, an RF pulse is not a single process. It is a serial process that occurs in several time periods. In between repetitions of the RF pulses, there are two periods occur; repetition time (TR) and echo time (TE). These periods are important factors in creating the image contrast of MRI. TR is the relaxation time from one RF pulse to the next RF of the same slice and mainly influences T1 as it quantifies the magnitude of longitudinal relaxation after the end of one RF pulse to the next. In comparison to TR, TE is a time length from the RF pulse to the signal peak stimulated in the coil and it is used to determine T2 images.

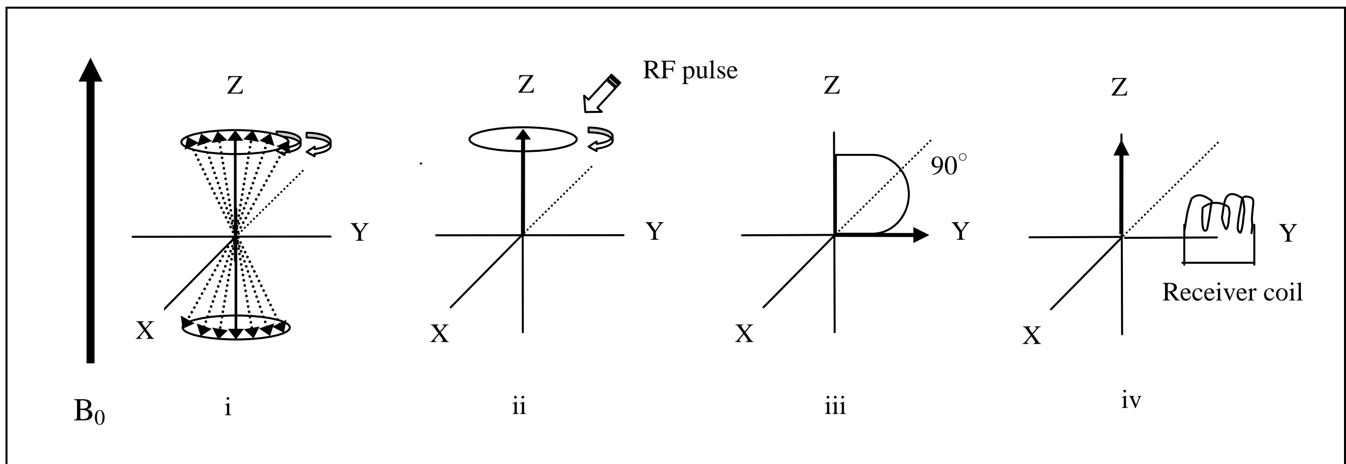


Figure 1.7 The concept of magnetic resonance imaging.

1.6.1.2 Image contrast

In generating image contrast, body tissues are discriminated from each other by using signal intensities. A typical MRI image appears as bright for tissue with high signal property whilst a tissue with low signal property is viewed as dark. The signal intensities of human tissue are influenced by the combination of the intrinsic (proton density, T1 and T2) and extrinsic factors (TR and TE). Intrinsic factors describe the features of body tissues and therefore cannot be changed but extrinsic factors can be altered as T1-weighted images or T2-weighted images. In T1-weighted images, a bright tissue image is produced due to high longitudinal magnetization. Meanwhile, in T2-weighted image, a bright tissue image is created for a tissue containing large transverse magnetization. The brightness is due to the increased amount of MR signal transmitted to the receiver. In contrast, a dark

image is exhibited for a tissue with low transverse magnetization which therefore results in low signal transmission to the receiver coil (Westbrook et al., 2011).

1.6.1.3 Limitations

MRI is costly, since both the use and maintenance of the machinery is expensive. It is estimated that a one hour scanning session requires approximately £300. Subsequently, the images produced need to be analysed with specific software by trained staff. MRI studies typically produce several hundred images which are time intensive to analyse. Furthermore, this method is also limited in claustrophobic and severely obese volunteers with BMI of > 40 kg/m^2. This disadvantage becomes extremely important especially in assessing body fat distribution in patients who will undergo gastric bypass, which usually involves the morbidly obese. Despite of these limitations, MRI is the only applicable method to quantify regional and ectopic body fat distribution.

1.7 SUMMARY

Gut hormone secretion has been suggested as a potential mechanism to modulate appetite regulation in rodents and humans. Although it can be stimulated via gastric bypass surgery, this approach is associated with life-threatening complications, adverse side effects and high costs which make this approach the last option in obesity treatment. Furthermore, gastric bypass is only limited to severely obese patients, thus limiting treatment options at a population level. Interestingly, some dietary sources are able to increase the production of naturally occurring gut hormones. One of the suggested dietary sources is dietary fibre. Fermentable carbohydrate, a dietary fibre-derivative, via its end products, SCFAs, has been suggested to modulate appetite regulation via gut hormone secretion. This thesis investigates the role of dietary fibres on stimulating gut hormone production and its effect on appetite, body weight and change in brain activation following food image stimulation in healthy individuals.

1.8 AIMS

To investigate my hypotheses, It is aimed to:

1) Investigate the effects of supplementing oligofructose on plasma PYY and GLP-1 levels, subjective appetite and energy intake in overweight/obese volunteers.

2) Investigate the effects of supplementing oligofructose on body weight, total, regional, ectopic body fat and insulin sensitivity in healthy overweight/obese volunteers following an eight week supplementation period.

3) Assess the effect of oligofructose intake on reducing brain activation in NAc, amygdala, OFC, hippocampus, insula and vACC.

4) Investigate the effects of supplementing propionate carrier molecule directly to the colon on plasma PYY and GLP-1 levels, subjective appetite and energy intake in healthy normal and overweight volunteers.

1.9 Hypothesis

Fermentable carbohydrate, through increase of PYY and GLP-1 may have an important role in regulating appetite and maintenance body weight following release from entero-endocrine L-cells in the gut. In this thesis, two types of fibre components are investigated, which are oligofructose and propionate carrier molecule.

General hypothesis

It is hypothesised that intake of fermentable carbohydrate and its product, propionate increases plasma PYY and GLP-1 levels, decreases subjective appetite and energy intake thus leads to a reduction of body weight in humans.

Oligofructose study

It is hypothesised that oligofructose supplementation increases circulating PYY and GLP-1 levels, decreases subjective appetite, energy intake and decreases neuronal activation and leads to a reduction of body weight and body adiposity in overweight volunteers following eight weeks supplementation period.

Propionate carrier molecule study

It is hypothesised that intake of propionate carrier molecule, a novel dietary substance, delivers propionate directly to the large intestine, increases plasma PYY and GLP-1 secretion, reduces hunger and therefore decreases energy intake in healthy normal and overweight volunteers.

Chapter 2

The Effect of Oligofructose on Appetite and Gut Hormones in Healthy Overweight Volunteers: A Randomised, Controlled, Single-blind Study

2 BACKGROUND

2.1 INTRODUCTION

2.1.1 Inulin-type fructans

Inulin-type fructans are a group of linear fructose polymers or oligomers bound β (2-1) linkages and its first monomer of the structure is either β-D-glucopyranosyl or β-D-fructofuranosyl residue (figure 2.1). Inulin is a plant storage carbohydrate which can be found in wheat, bananas, onion, garlic and leek. Besides inulin, also categorized in this group are oligofructose and fructooligosaccharides, which are produced from partial enzymatic hydrolysis of inulin. In the food industry, the main source for inulin and oligofructose are either chicory roots or Jerusalem artichoke (Bach Knudsen and Hessov, 1995). Inulin and oligofructose are estimated to have a caloric value of 1.5 kcal / g (6.3 kJ/g) (Roberfroid, 1999). Although containing similar caloric contents, inulin and oligofructose have different amounts of monomeric sugars, or also known as the degree of polymerization. Inulin has been reported to contain a degree of polymerization between 2 – 60 units, whilst oligofructose contains a degree of polymerization between 3 – 8 units, which also determined their physical characteristics. Inulin, which is a longer chain of fructan has lower solubility level than oligofructose and capable to crystallise when dissolved in a liquid, whilst oligofructose, a shorter form of inulin is a soluble component and completely dissolves in a liquid. Because of their natural sweet taste, oligofructose has been used as a sugar replacer while inulin is used for fat replacement in the food manufacturing industry (Niness, 1999).

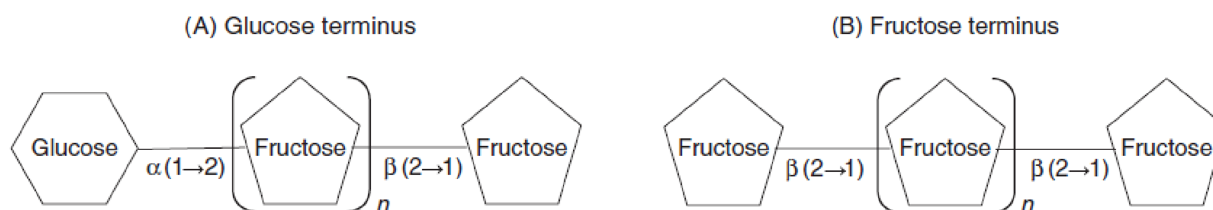


Figure 2.1 The chemical structure of inulin-type fructans. Inulin-type fructans are series of fructose units linked to each other by β-(2-1) linkage. The first monomer of the structure is either β-D-glucopyranoside or β-D-fructofuranoside. From (Darzi et al., 2011).

2.1.1.1 Consumption of Inulin-Type Fructans

It is reported that Americans consume between 1 - 4 g inulin-type fructans daily, with wheat as the most common food source (Moshfegh et al., 1999). In Europe, it was reported that daily intake of inulin-type fructans is between 3 – 11 g/day (van et al., 1995d). Recently, Dunn *et al*. studied the reliability of food frequency questionnaires in quantifying intake of inulin and oligofructose in comparison with seven day food diaries in the UK population and reported intakes of 3.8 to 4.0 g/day of oligofructose and inulin. However, it is most likely that this level is not applicable to describe the actual intake of the UK population, as the majority of the volunteers involved in this study were young female (49 females out of 66 subjects in total) with limited age group and high academic background (Dunn et al., 2011).

2.1.1.2 Fermentation of Inulin-Type Fructans

Inulin-type fructans are classified as fermentable carbohydrate due to its capability to escape enzymatic digestion in the small intestine (Bach Knudsen and Hessov, 1995) and therefore, delivers intact to the large intestine. In the large intestine, inulin-type fructans undergo bacterial fermentation (Roberfroid et al., 1998) in which the process will produce short chain fatty acids (SCFAs) (acetate, propionate and butyrate), gasses (H_2, CH_2 and CO_2) and energy. Inulin-type fructans are fermented at different location depending on the degree of polymerization of each substrate. Oligofructose, which has an average degree of polymerization of 4.5 is fermented predominantly in the caecum and in the upper part of the colon, whereas inulin with an average degree of polymerization of 25, is fermented in the lower part of the colon (van et al., 1995c). Inulin-type fructans are fermentation agents. It has been reported that no residues of fructans were found in the stools or urine upon fructo-oligosaccharides supplementation in healthy men. Indeed, fructo-oligosaccharides also increased breath hydrogen release, a surrogate marker of colonic fermentation (Alles et al., 1996). High breath hydrogen excretion following intake of inulin-type fructans has also been demonstrated in other studies (Cani et al., 2009; Gibson et al., 1995; Hess et al., 2011; Oku and Nakamura, 2003; Piche et al., 2003).

2.1.1.3 The Role of Inulin-Type Fructans on Colonic Fermentation

Recently, inulin-type fructans have attracted much attention because of their role in reducing food intake, body weight and fat mass in rodent. It is postulated that inulin-type fructans modulated these effects by stimulating anorectic gut hormones into the circulation following colonic fermentation (Cani et al., 2005b; Cani et al., 2007b; Cani et al., 2009; Delzenne et al., 2005; Parnell and Reimer, 2012; Verhoef et al., 2011). Supplementing fructans with different degree of polymerization: oligofructose, inulin and oligofructose-enriched inulin in the diet of rodents reduced energy intake in all groups. However, the shorter chain of fructans, oligofructose and oligofructose-enriched inulin were more efficient in reducing epidydimal fat mass, inducing caecum enlargement, increasing levels of GLP-$1_{7\text{-}36}$ amide and proglucagon mRNA in the proximal colon as well as GLP-$1_{7\text{-}36}$ amide levels in the portal vein and suppressing active ghrelin levels (Cani et al., 2004).

In addition, supplementing 10 % (w/w) oligofructose with a high fat diet has been shown to suppress weight gain and accumulation of body fat in Wistar rats (Cani et al., 2004; Cani et al., 2007b). In this study, the effect of oligofructose on body weight was correlated with two fold increases in release of GLP-$1_{7\text{-}36}$ in the proximal and medial colon, increased portal GLP-$1_{7\text{-}36}$ amide and GLP-2 levels (Cani et al., 2004). The latter effect of oligofructose has been related to the increase of proglucagon mRNA in the proximal colon, caecal and colonic pools of GLP-$1_{7\text{-}36}$ amide due to the enlargement of caecal and colonic tissues (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2007b; Delzenne et al., 2005; Kok et al., 1998). Furthermore, oligofructose also reduced DPP-IV activity by 30% which could have contributed to the increase in portal vein GLP-$1_{7\text{-}36}$ amide levels (Cani et al., 2005b). The increased in GLP-1 secretion might be related to oligofructose's capability to stimulate differentiation of the L-cells in the proximal colon and therefore increase the number of GLP-1-expressing cells (Cani et al., 2007b). In addition, Lippl *et al*. showed that increased portal GLP-1 levels might also be related to the reduced plasma ghrelin levels (Lippl et al., 2004). However, the effect of oligofructose on reducing plasma ghrelin levels can only be seen when it was added to normal chow as no effect was demonstrated after consumption of high fat-enriched oligofructose diet (Cani et al., 2004; Cani et al., 2005b; Delzenne et al., 2005; Parnell and Reimer, 2012). Besides GLP-1, plasma PYY levels were also found in the high levels in the caecum and portal vein after intake of oligofructose, possibly due to caecal tissue hypertrophy and gene expression in the L-cells (Cani et al., 2005b; Delzenne et al., 2005).

The effects of fructans on gut hormone secretion and peptide gene expression are suggested to be mediated via a release of SCFAs. Addition of oligofructose / inulin to the diet of rats led to an increase of caecal butyrate production and butyrate pools (Campbell et al., 1997; Le et al., 1999) which subsequently modulated proglucagon gene expression in L-cells (Tappenden et al., 1998). Besides butyrate, treatment with oligofructose also increased caecal propionate and acetate pools (Campbell et al., 1997; Le et al., 1999). It was reported that oligofructose yielded 65:20:15 (Gibson et al., 1995), whilst inulin provided 60:26:14 (Kleessen et al., 1997) (acetate:propionate:butyrate) of SCFA profile detected in the human faeces. However, faecal SCFA ratios may not be a reliable indicator to evaluate colonic fermentation activity, as small amount of SCFAs has been found to be excreted in the faeces. However, as human colon is inaccessible for direct SCFAs measurement, human SCFAs colonic production can only be evaluated using stool sampling (Campbell et al., 1997). Meanwhile, it was reported that several studies experienced difficulties in developing suitable methods for measuring SCFA in humans' faecal sample (Brighenti et al., 1999; Costabile et al., 2010; Kleessen et al., 1997; Kruse et al., 1999; Nyman, 2002; Ramnani et al., 2010).

Interestingly, Tarini *et al*. clearly demonstrated the effect of inulin in postprandial SCFA plasma profiles four hours after ingestion of inulin (Tarini and Wolever, 2010). To date, this is the only study which has shown an increase in all of the three major SCFAs after intake of fermentable carbohydrate. Other studies reported that β-glucan, resistant starch and L-rhamnose increased serum propionate (Nilsson et al., 2008; Robertson et al., 2005; Vogt et al., 2004b; Vogt et al., 2004a) whilst wheat and low fibre increased acetate and butyrate levels in a long term study (Freeland and Wolever, 2010; Wolever et al., 2002). The discrepancies in SCFA patterns in different type of fibres depended on the type of basic monomeric content of the carbohydrates, the complexity and combination of substrates, type of linkages that binds the carbohydrate monomers as well as the degree of polymerisation and the site for carbohydrate fermentation (Henningsson et al., 2002; Nilsson and Nyman, 2005).

2.1.1.4 The Role of Inulin-Type Fructans on Appetite Regulation

Recently, Rozan *et al*. showed that adding oligofructose-enriched inulin in the diet has increased the longevity of rats by 33.3% compared to a control group. This is the first study to demonstrate the

lifelong effect of inulin and the authors suggested that the prolonged life of rats fed oligofructose-enriched inulin is potentially due to its effects on maintaining body weight, thus preventing the deposition of fat mass and obesity as well as reduced obesity-related diseases such as cardiovascular disease and diabetes (Rozan et al., 2008). Whilst fructans have shown promising effects on food intake and fat mass in rodents, the effects in humans are still inconclusive. Initial investigation of fructans' role in human appetite regulation began when Piche *et al*. investigated the effect of fructo-oligosaccharides in patients with gastroesophageal reflux disease. Although appetite assessment was not one of their outcome measures, they showed that including 19.8 g fructo-oligosaccharides (6.6 g taken three times per day) in volunteers' diet for seven days increased postprandial plasma GLP-1. In addition, they also found an increase in breath hydrogen levels (fermentation marker) which suggested that increase in plasma GLP-1 levels is closely related to a modulation on colonic fermentation (Piche et al., 2003). Following that, Cani *et al*. showed that supplementing 16 g/day oligofructose for two weeks increased satiety, reduced hunger and energy intake in normal BMI subjects (Cani et al., 2006a).

In contrast, Hess *et al*. showed that intake of five grams and eight grams of fructo-oligosaccharides increased breath hydrogen levels in 240 minutes but no significant effect on reducing appetite was found (Hess et al., 2011). However, the lack of effects of fructo-oligosaccharides on appetite in this study could be due to acute study period (240 minutes). Furthermore, in comparison to Cani *et al*., Hess *et al*. provide volunteers with half of the amount of oligofructose used by Cani *et al*. Therefore, the dose might be insufficient to suppress appetite (Cani et al., 2006a; Hess et al., 2011). Interestingly, Archer *et al*. showed that although inulin-enriched sausages had no significant effect on appetite in 33 overweight volunteers, the dose reduced fat intake and 24 hours energy intake compared to a full-fat sausage patty (Archer et al., 2004). Similarly, Hess *et al*. also demonstrated that addition of fructo-oligosaccharides in the diet had no significant effect on energy intake during *ad libitum* meal, but the dose reduced 24 hour energy intake. Surprisingly, this observation was only demonstrated in female volunteers as no change in energy intake was found in male volunteers, suggesting that there might be gender variations in satiety response to oligofructose (Hess et al., 2011).

Recently, Cani *et al.* investigated the effect of supplementing the diet with 16 g oligofructose-enriched inulin for two weeks. They showed that intake of oligofructose-enriched inulin reduced subjective hunger and total energy intake assessed at the end of the supplementation period. Interestingly, these effects were associated with a significant decrease in area under the curve (AUC) of postprandial plasma glucose levels and increase in circulating PYY and GLP-1 levels. Therefore, it is possible that modulation of gut hormones and glucose response were among the factors that contributed to the alteration in hunger and energy intake demonstrated in this study (Cani et al., 2009). Findings from this study have lead to a speculation whether 16 g/ day is the minimum dosage of oligofructose to alter appetite profiles. Verhoef *et al*. compared the effect of supplementing 10 g/day and 16 g/day for 13 days and found that 16 g/day is more efficient than 10 g/day in increasing circulating plasma GLP-1 and PYY levels as well as reducing energy intake. This study suggests that 16 g/d is the least amount of oligofructose to induce an effect on energy intake (Verhoef et al., 2011). Based on these studies, it might be possible that inulin-type fructans have a beneficial role to modulate body weight following long term supplementation. This will be investigated in more detail in Chapter 3.

2.1.1.5 The Role of Inulin-Type Fructans on Lipid, Glucose and Insulin Levels

Effects of inulin-type fructans on modulating appetite and energy intake might also associate with improve postprandial lipid, glucose and insulin levels. Inulin-type fructans have been suggested to modulate lipid metabolism by lowering triacylglycerol levels (Delzenne and Kok, 1999; Fiordaliso et al., 1995; Kok et al., 1996) possibly due to increased levels of propionate released in the portal vein. During the process, propionate exerts its effect by preventing the conversion of acetate into lipid (Brighenti et al., 1999; Daubioul et al., 2000; Grysman et al., 2008; Tarini and Wolever, 2010; Fiordaliso et al., 1995; Kok et al., 1996; Rozan et al., 2008). In humans, a meta-analysis of 15 randomised, controlled studies showed that inulin-type fructans also significantly reduced serum triacylglycerol (Brighenti, 2007). In contrast, the role of inulin-type fructans on modulating human blood glucose levels is equivocal. Whilst some studies demonstrated the hypoglycaemic effect of inulin-type fructans (Daubioul et al., 2005; Jackson et al., 1999; van Dokkum et al., 1999; Yamashita et al., 1984), others found that inulin-type fructans had no role in reducing blood glucose levels (Alles et al., 1996; Causey et al., 2000; Giacco et al., 2004).

However, rodent studies showed contrast effect from human studies. Supplementing inulin-type fructans in rodents' diet were shown to reduce glycaemic response potentially due to increased release of GLP-1 (Cani et al., 2006b; Cani et al., 2009; Kok et al., 1998) and associated with an increase release of insulin (Daubioul et al., 2000). In diabetic rats, supplementing oligofructose in combination with streptozocin has increased pancreatic and portal insulin levels (Cani et al., 2005a). Kok *et al*. showed that there was a significant decrease in serum insulin after oligofructose treatment, which potentially related to improved glucose disposal following increased of circulating serum GLP-1 from L-cells (Kok et al., 1998).

2.1.2 Rationale of Supplementing 30 g Oligofructose in This Study

This study is established from a preliminary work performed by Pedersen and Frost (Pedersen, 2010). In this study, a dose escalation study was developed in order to characterise the optimum dose needed to modulate appetite, gut hormone and energy intake. This is because there is no consensus of the oligofructose dose to modulate appetite sensations and energy intake in the literature. In this study, a dose of 15 g, 25 g, 35 g, 45 g and 55 g were supplemented in 12 healthy volunteers (4 men and 7 women), aged 25.0 ± 3.9 years and BMI 21.6 ± 2.2 kg/m^2 for five weeks. The outcome parameters were assessed on the appetite study days (subjective appetite ratings, energy intake, gut hormones, glucose and insulin) and also during free-living supplementation (appetite assessment, energy intake, BMI and body fat).

The result from this study suggested that high doses of oligofructose (van et al., 1995b) induced the most pronounce effects on energy intake and plasma PYY release. However, intake of doses more than 30 g/day may potentially caused gastrointestinal discomforts, therefore it is not encouraged to be used in humans (Kaur and Gupta, 2002). Due to these reasons, a dose of 30 g/day oligofructose was chosen as the potential dose to be developed as an appetite suppressor in this study. Furthermore, USDA 2005 recommended that 28 g dietary fibre in 2000 kcal/day for women and 36 g in 2600 kcal/day for men should be consumed to achieve adequate intake requirement. In UK, British Nutrition Foundation recommended to take 24 g fibre daily in both men and women. Therefore,

supplementing 30 g/day oligofructose in this study would fulfil the dietary guidance recommendation suggested in both countries. To date, 30 g oligofructose supplemented in this study is the highest dose that has been supplemented in human appetite studies. Apart from my study, Kleessen *et al*. also have supplemented 40 g/day inulin in their study (Kleessen et al., 1997). However, this dose was used to investigate the role of inulin on constipation in older volunteers (mean age 76.4 years old). Other human studies; Antal *et al*., Cani *et al*., Verhoef *et al*. and Parnell and Reimer used 14g, 16g and 21g respectively (Antal et al., 2008; Cani et al., 2006a; Cani et al., 2009; Parnell and Reimer, 2009; Verhoef et al., 2011).

2.2 AIMS AND HYPOTHESIS

2.2.1 Aims

The main aim for this study was to investigate the change in gut hormone response, appetite and energy intake following intake of 30 g/day oligofructose for six weeks (following two weeks run-in period) in contrast to cellulose supplementation in healthy overweight and obese volunteers. To achieve this objective, the below parameters were measured:

- Plasma glucose and insulin levels and gut hormones, GLP-1 and PYY levels
- Breath hydrogen levels as a marker for colonic fermentation
- Visual analogue scales to access subjective appetite
- Energy intake during supplementation period and *ad libitum* meal assessment.

2.2.2 Hypothesis

It was hypothesized that supplementing 30 g/day oligofructose for six weeks (following two weeks run-in period) would suppress subjective appetite, reduce food intake and body weight to a greater extent compared with a non-fermentable carbohydrate, cellulose.

2.3 MATERIALS AND METHODS

2.3.1 Materials

2.3.1.1 Supplements

Oligofructose, Beneo™ P95 and cellulose were kindly provided by ORAFTI, (van et al., 1995a). Both of these supplements were provided in sachets. Each sachet contained 10 g of white, powdery form and has a similar appearance. Oligofructose has a slight sweet taste whilst cellulose is a supplement without any taste. However, volunteers were not aware of the taste differences as they only took one treatment during the study. The caloric content of oligofructose is approximately 15.8 kcal/ 66.2 kJ (per sachet or 10 g). As cellulose does not contain any calories, 13 g of maltodextrin was added to the 30 g cellulose in order to provide the same amount of energy as the oligofructose supplement. Therefore, both of the supplements provided approximately 47.4 kcal/ 198.6 kJ per 30 g supplement.

2.3.1.2 Randomization

Volunteers were randomly allocated to the treatment prior the start of the study using enveloped system based on gender by a member of the laboratory who was not directly involved in the day-to-day execution of this study.

2.3.1.3 Power Calculation

The study sample size was based on power calculations that used plasma PYY results from a pilot dose finding study as a primary outcome (Pedersen, 2010). With the estimation of AUC_{480min} of 5234 ± 3638 ρmol*min based on 0.8 power to detect a significant difference of P = 0.05, two sided, a minimum of 18 participants were needed. To allow for dropouts, 22 volunteers were recruited for this study.

2.3.1.4 Volunteers

Healthy overweight male and female volunteers were recruited to take part in this randomised, controlled, single blinded and parallel study (subject characteristics are described in Section 2.4.1). Volunteers were recruited by advertisement either from posters at the Imperial College London

campus sites (Hammersmith Hospital, St. Mary's and Charing Cross), Metro and Evening Standard newspapers or by search engine website, Gumtree. They were also recruited from Sir John McMichael Centre's Healthy Panel clinic and by word of mouth. Volunteers were excluded if they had any of serious illnesses including gastrointestinal diseases, physiological problems such as depression and anorexia nervosa, gastrointestinal surgeries, metallic or electronic implants such as pacemaker and fixed dental braces, claustrophobic, regular user of prebiotics, probiotics or laxatives, lactose-intolerance, vegan or vegetarians, were pregnant or breastfeeding and excessive exercise more than five hours per week, donated blood, used antibiotics or had unstable body weight within three months before the study started. Before they could participate in the study, volunteers had to attend the screening study day. During the screening visit, they were screened for abnormal eating behaviour using Sick Control One Fat Food (SCOFF) (Morgan et al., 1999) (appendix 4), Dutch Eating Behaviour Questionnaire (DEBQ) (van Strien et al., 1986) (appendix 5) and Eating Attitude Test (Garner et al., 1982) (appendix 6). They also had a meal testing for a meal which will be served during the buffet meal on the three appetite study days. A three day food diary (appendix 8) was also assessed for abnormal food and fibre intake prior the study. Written informed consent was obtained from each volunteer during the screening visit. Ethical approval was obtained from the Hounslow and Hillingdon Research Ethics Committee (Project registration number: 09/H0709/18) and the study was performed in accordance with the Declaration of Helsinki.

2.3.2 Methods

2.3.2.1 Study design

Volunteers were required to attend seven baseline and post-supplementation visit for appetite, whole body MRI and brain fMRI scanning days. Supplementation period was started on day 1 until day 56, which comprised of two weeks run-in period and six weeks full supplementation period. This chapter will discuss the appetite study days and the supplementation period. MRI body composition and fMRI brain scanning will be discussed in the Chapter 3 and Chapter 4 respectively. The protocol design is summarized in Figure 2.2.

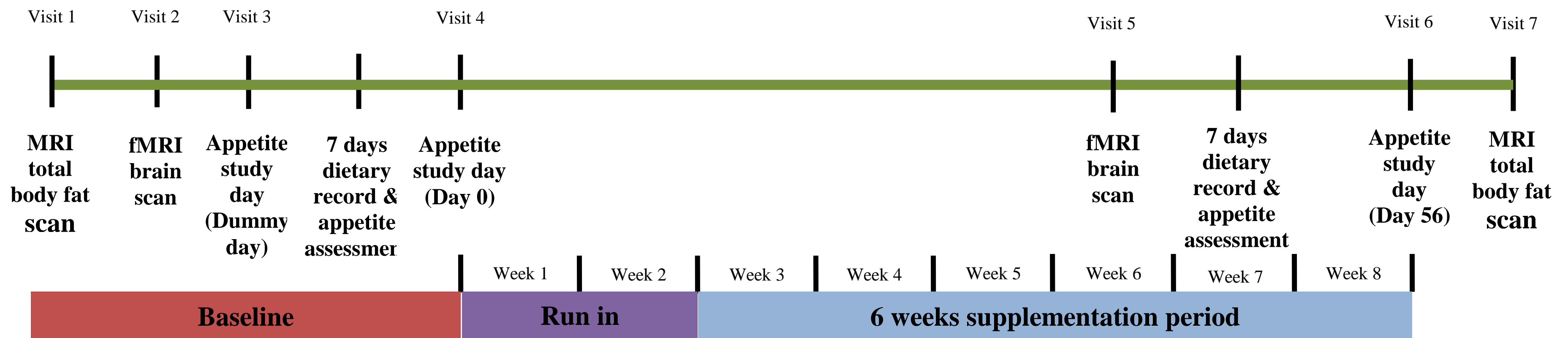


Figure 2.2 A schematic diagram of the study design. Volunteers are instructed to attend 7 visits in the period of 10 weeks. Before volunteers started their supplementation, they have to attend a baseline study visits which are MRI fat scanning study day which they had a full body fat scan, fMRI brain scanning study day on visit 2 and an acclimatization appetite study day on visit 3. A week before they started their study, they were asked to fill in 7 days food dietary records and appetite assessment. On day 0, they attended an appetite study day (visit 4) which they were assessed for subjective appetite ratings, meal intake, breath hydrogen production and blood were withdrawn for gut hormones measurement. On the next day (day 1), they started their oligofructose or cellulose supplementation depended to the randomisation procedure which had been done prior of the study. The first and the second weeks of the supplementation were the run-in supplementation period which 10 g/d need to be taken in the first week and followed with 20 g/d in the second week. They were instructed to start their actual dose (30 g/d) of supplementation on day 15 and the dosage is remained to be taken until week 8. In week 6 (between day 32-42), they were asked to come to the hospital for post-supplementation fMRI brain scan study day. 7 days post-supplementation dietary record and appetite assessment were obtained at week 7 which was a week before the end of the supplementation period. On day 56, subjects attended a visit 6, a post-supplementation appetite study day assessment. In this visit, the same observation as visit 4 has been done. Lastly, subjects attended their final visit on visit 7 which they had a post-supplementation MRI total body fat.

2.3.2.2 Appetite study day

The appetite study protocol is summarized in Figure 2.3. The study was performed in the Sir John McMichael Centre, Hammersmith Hospital. Volunteers were instructed to arrive at 0845 on each study day. There were three appetite study days, acclimatization day (Visit 3), pre supplementation (Visit 4), which was performed on day 0 and post supplementation visit (visit 6) performed on day 56. The study days started at 0900 and finished at 1700. Participants arrived fasted overnight for at least 12 hours. During the fasting, only water was allowed. During the appetite study day, visual analogue scales (VAS), breath hydrogen and blood were obtained from the volunteers. Blood pressure was also measured once at the start of the study. Breakfast was served at 0 minute and lunch was provided at 240 minutes. At this meal time, they were instructed to finish their meal in 20 minutes. For energy intake assessment, an *ad libitum* meal based on their choice determined at the screening visit was served at 420 minutes. Volunteers were given 30 minutes to eat the meal. During this time, no reading materials, TV or electronic devices were allowed. Before they had the meal, they were instructed to eat until comfortably full. The final assessment was performed at 450 minutes.

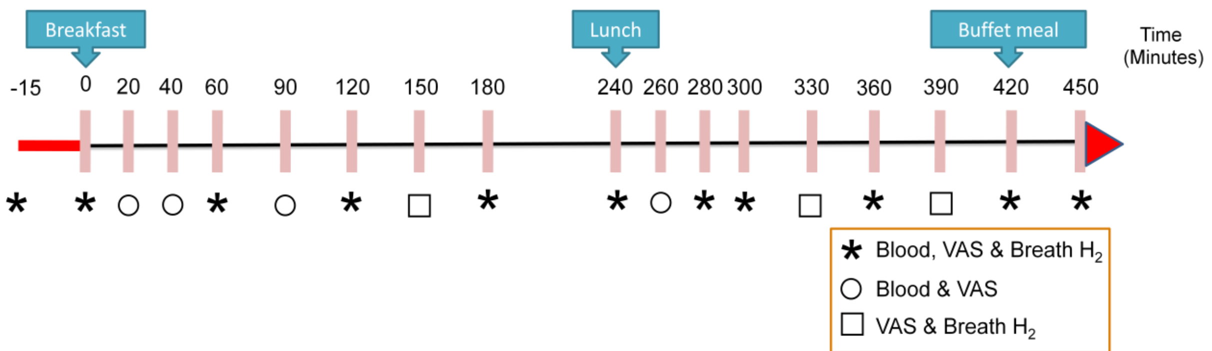


Figure 2.3 Oligofructose appetite study day protocol. Subjects attended appetite study days on visit 3 (acclimatization visit), visit 4 and visit 6. VAS, breath hydrogen levels and blood for gut hormones analyses were assessed on these study days. Breakfast, lunch and *ad libitum* meal were provided at 0, 240 and 420 minutes. 30g oligofructose / cellulose supplementation was provided on post-supplementation visit (day 56).

2.3.2.2.1 Meals

Volunteers were provided 500 kcal breakfast which comprised of a cereal (a choice of cornflakes or rice crispies), semi-skimmed milk, orange juice and croissant or bread roll. At lunch, volunteers had sandwiches (a choice of a piece of ham, chicken and/or a piece of cheese), water, orange juice, a choice to have two foods of either: a bag of crisp, a pot of yogurt or mini kit kat, which containing 550 kcal. On visit 6 only (the last appetite study), supplements were provided by mixing it with orange juice during breakfast and lunch. At 420 minutes, a preweighted *ad libitum* meal was served to the volunteers. Volunteers were given a choice between macaroni cheese, bolognaise bake or chicken tikka masala which was served in excess. A jug of water was also served with the meal. The weight of both the meal and water were recorded at the end of the study session.

2.3.2.2.2 Subjective Appetite Scores

Subjective appetite scores were measured using 100 mm VAS (Appendix 7) at the same time breath hydrogen was accessed. The VAS questionnaires assessed motivation to eat, palatability, fullness, hunger and satiety sensation using questions such as "how hungry do you feel now?", "how full do you feel now?" which were assessed between the extremes of "not at all" to "extremely" at either end of the scale (Flint et al., 2000). Volunteers were asked to mark a small vertical line on the VAS to indicate their feelings at every 20 - 30 minutes during the study period. Besides of subjective appetite, we also used VAS to monitor gastrointestinal side effects; stomach discomfort, bloating, nausea, flatulence, diarrhoea and flatulence which were accessed at hourly until the end of the study day.

2.3.2.2.3 Breath Hydrogen Assessment

Hydrogen gas is one of the end products produced by bacterial fermentation of dietary fibre in lower intestine and was therefore use in the study as an indicator for bacterial fermentation activity. The assessment was performed using a portable hand-held breath hydrogen monitor (Gastrolyzer, Bedfont Scientific Ltd. Kent, UK). Breath samples were taken twice before breakfast (-10 and 0 minutes) and at 60, 120, 150, 180, 240, 280, 300, 330, 360, 390, 420 and 450 minutes post supplementation during breakfast. The hydrogen levels was measured in parts

per million (ppm). Monitors were calibrated before use with the calibration gas (200 ppm hydrogen). Volunteers were asked to inhale as deeply as possible, hold their breath for 15 seconds before exhaled it directly into a mouthpiece attached to the apparatus. The peak H_2 levels in the expired air were then recorded.

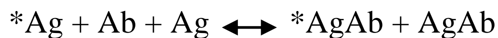
2.3.2.2.4 Blood Sampling

A cannula was inserted in the forearm for blood sampling throughout the day. Blood for gut hormones, glucose and insulin were withdrawn at -15, 0, 20, 40, 60, 90, 120, 180, 240, 260, 280, 300, 360, 420 and 450 minutes. 160 ml of blood were collected during visit 4 and visit 6 whilst only 15 ml were withdrawn on the acclimatization study day. Collecting blood on the acclimatization study day is a necessary step so that volunteers could get the idea of the real situation on the study day. Blood for gut hormones was transferred to EDTA containing tubes (Teklab) containing aprotinin (Bayer trasylol 500.000 KIU; 200 μL/7.5 ml blood), a protease inhibitor to prevent the degradation of gut hormones. Blood for glucose analysis was transferred to fluoride oxalate tubes. Blood for insulin analysis was transferred to yellow gel separator tubes and allowed the blood to clot before centrifugation. Blood was centrifuged at 3000 g at 4°C for 10 minutes. The separated plasma was then aliquoted into eppendorf tubes and was stored in the -20°C freezer until assaying process.

2.3.2.2.5 Gut Hormones Analysis (Radioimmunoassay)

'In house' radioimmunoassay using iodine-125 experiment has been used to analyse the gut hormones PYY and GLP-1. The objective of this analysis was to measure the levels of unlabelled antigen bound to the antibody. In this analysis, antibody site of binding was competed between a radioactively labelled and unlabelled radioactive ligand. A standard curve was plotted from a known level of labelled ligand and the antibody used. This could be achieved by setting up a series of standards of known levels. This allows determination of known levels of unlabelled peptide from the samples by interpolation from the standard curve. As the level is increased, the binding percentage will decrease. The antibody-bound and free-ligands are able to

separate from each other before the radioactively-labelled was measured by counting the radioactivity.



*Ag = Radiolabelled antigen
Ag = Unlabelled antigen
Ab = Antibody

2.3.2.2.5.1 Methodology

Prior the radioimmunoassay experiment, gut hormones plasma samples, which had been stored at -20°C, were thawed and vortexed for 1 minute to homogenise the sample. All samples were analysed in one batch to prevent inter-assay variation and were run in duplicate after being thawed once. In this experiment, there were a series of reagents were involved; buffer, label, antibody, standards and samples *per se*. A few days before the assay experiment, tubes were numbered and arranged in duplicate in racks. The first 30 tubes have been designated with the assay specific order. The first 2 tubes were designated for non specific binding and contained no antibody. Tubes 3-6 were used for iodinated peptide containing 50 µl for 1/2x label and 200 µl for 2x label. This step is needed in order to measure specific activity of the label. “Zero” tubes were followed in quadruplicate after that, which start from tubes 7 to 10. These tubes were used to detect any intra-assay drift and usually included approximately in every 100 tubes in an assay. Tubes 11 to 30 were designated for standards which arranged in gradual increased from 1, 2, 3, 5, 10, 15, 20, 30, 50 and 100 µl. In this assay, three sets of standards were included and were located at the beginning of the assay (usually rack 1), middle and the end of the assay. Similar to the ‘zero’ function, a number of standards were needed to detect any intra-assay drift. These standard curves were then used to determine the levels of the antigen contained in the samples. The next tubes were allocated for the samples. 100 µl was added in each assay tube for PYY and GLP-1 gut hormones.

The assay was performed in 0.06 M phosphate buffer containing 0.3% bovine serum albumin. Then, standards or samples were added to the allocated tubes as described in the previous paragraph. After that, each assay was added 100 µl of label with inclusion of 1/2x label which contained 50 µl (tube 3 and 4) and 2x which contained 200 µl (tube 5 and 6). Finally, 100 µl of antibody was added in each tube with the exception of non-specific binding tubes. Total volume in each tube was therefore 700 µl. At the end of the assay, excess antibody was added in order to determine the maximal binding of the antibody to labelled peptide. At the end of day 1, the assay racks were covered in aluminium foil and incubated at 4ºC for 3-5 days before separation of antibody-antigen complex from the free antigen were performed later.

There are various methods of separating the antigen-antibody complexes from the supernatant including charcoal adsorption and secondary antibody separation. For the GLP-1 assay, 250 µl dextran-coated charcoal in 0.06 M phosphate buffer with gelatine was added to each tube and the tubes were subsequently vortexed gently to mix the contents and centrifuged at 4ºC at 2500 rpm or 748 g for 20 minutes. In GLP-1 assay, the free antigen is trapped in the porous charcoal whereas the antigen-antibody complex was left in the supernatant. For the PYY assay, a secondary antibody (sheep anti-rabbit antibody) separation technique was used. Upon addition of secondary antibody, the assay was then left at room temperature for one hour before 500 µl of 0.01% Triton-X-100 (Sigma, Poole, UK) was added to each tube. Each assay was then centrifuged at 4ºC at 2500 rpm or 748 g for 20 minutes. Addition of second antibody into the PYY assay induced the antigen-antibody complex to form a pellet whilst the free antigen remained in the supernatant. Aspiration of the supernatant into empty tube was performed using pipetting. Tubes were the sealed using heated paraffin wax.

The radioactivity of each PYY and GLP-1 supernatant and pellet were counted in a gamma counter (NE 1600, Thermo Electron Corporation). Counting time was set at 179 seconds. The peptide levels were then determined using a non-linear plot (Radioimmunoassay software, Thermo Eletron Corporation) and results calculated in terms of the standard. The results for the peptide levels in the unknown samples were interpolated from the standard curve.

2.3.2.2.5.2 PYY Immunoassay

A specific and sensitive radioimmunoassay was used to measure plasma PYY, as previously described (Adrian et al., 1985). This technique allowed for total PYY to be measured, which comprised of the truncated fragment PYY_{3-36} and the full-length hormone PYY_{1-36} which are both biologically active. The antiserum Y21 used in the assay was produced in a rabbit against synthetic porcine PYY (Bachem, St. Helen's, UK) coupled to bovine serum albumin by gluteraldehyde and used at a final dilution of 1:50000. The antibody also has full cross reactivity with the biological active circulating forms of PYY but not with pancreatic polypeptide, neuropeptide Y or other known gut hormones. Label was prepared on the assay day with the required volume of buffer so the specific activity of the iodine-125 labelled PYY was 25-30 Bq/100 µl. The ^{125}I PYY was prepared by the iodogen method and purified by HPLC. The detection limit of PYY assay was 2.5 pmol/l with an intra-assay coefficient of variation of 5.8% and interassay variation below 10%.

2.3.2.2.5.3 GLP-1 Immunoassay

Plasma GLP-1 like immunoreactivity was measured with a specific and sensitive radioimmunoassay as described in Kreymann *et al*. (Kreymann et al., 1987). The antibody was produced in rabbits against GLP-1 coupled to bovine serum albumin. Antibody was used at a final dilution of 1:190 000. The antibody cross-reacted 100% with all amidated forms of GLP-1 but did not cross react with glycine extended form ($GLP\text{-}1_{1-37}$ and $GLP\text{-}1_{7-37}$) or any other known pancreatic or gastrointestinal peptide. Like ^{125}I PYY, ^{125}I-labelled GLP-1 was also prepared by iodogen method and purified by HPLC. The specific activity of the ^{125}I-labelled GLP-1 was 48 Bq/fmol. The detection limit of GLP-1 assay was 7.5 pmol/l with an intra-assay variation of 5.4% and interassay variation below 10%.

2.3.2.2.6 Insulin Assay

Insulin levels at -15, 0, 20, 40, 60, 90, 120, 180, 240, 260, 280, 300, 360, 420 and 450 minutes were performed by using iodine-125 RIA kits (Millipore, Missouri, USA). In the beginning of

the experiment, samples were thawed and vortexed for 1 minute to remove any fibrin clots. To avoid any inter-assay variation, samples were analysed in one assay and in duplicate.

Each tube in the assay needs to be numbered and arranged according to the assay protocol. Tube 1 and 2 were allocated for total count, tube 3-4 were for non-specific binding, tube 5-6 were for 'zero', tube 7-20 were allocated for standards, tube 21-24 was specified for quality controls the rest of the assay were continued with study samples. Tube 1 and 2 only contained the ^{125}I-insulin therefore this would allow 100 counts. The blank tube 3-4 contained all assay reagents except of antibody and it serves to assess non-specific binding of the label to other plasma fractions. The principle of Millipore RIA kit and 'in house' assay is similar except of the kits provided all the reagents and solution whilst in 'in house' RIA, the reagents and solutions such as buffer, label and antibody need to be prepared before performing the assay experiment.

The assay was performed in a total volume of 50 µl of 0.05 M phosphosaline buffer pH 7.4 containing 0.025 M EDTA, 0.08% sodium azide and 1% RIA grade bovine serum albumin with the exception of total count tube (tube 1-2) which only contained ^{125}I-insulin. The next step was the addition of 50 µl of standards or sample to the allocated tubes (tube 7-20). In this assay, the standard levels were set in the gradual increase. Standards contained 3.125 µU/mL, 6.25 µU/mL, 12.5 µU/mL, 25 µU/mL, 50 µU/mL, 100 µU/mL and 200 µU/mL purified recombinant human insulin. Followed after that was 50 µl of the ^{125}I-insulin. The tracer was prepared by the iodogen method and purified by HPLC. Next, 50 µl of antibody was added after that. The antibody was produced in guinea pig against purified human insulin. The antibody was cross reacted with human proinsulin but did not cross react with any other known gastrointestinal hormones. As the step 5 (addition of antibody) completed, each tube was vortexed and covered with foil and incubated for 20-24 hours in the room temperature.

The next day, the experiment was continued with the addition of 500 µl precipitating reagent. The precipitate reagent is raised in goat against guinea pig lgG and contained 3% PEG and 0.05% triton X-100 in 0.05M phosphosaline, 0.025 M EDTA and 0.08% sodium azide. The assay was then vortexed and incubated for 20 minutes at 4°C and centrifuged for 20 minutes at

3000 g at 4°C. The limit of detection was 1.3575 μU/mL for 50 μl sample size with an intra-assay variation of 4.4%.

2.3.2.2.7 Glucose Assay

Plasma glucose levels collected at -15, 0, 20, 40, 60, 90, 120, 180, 240, 260, 280, 300, 360, 420 and 450 minutes were analysed in the Department of Clinical Biochemistry, Hammersmith Hospital. Analyses of the glucose were performed using an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK). Glucose assay sensitivity was 0.3 mmol/L with an intra-assay coefficients of variation of 1.0% .

2.3.2.3 Free-Living Supplementation Period

On the day following the baseline appetite study day (Visit 4), volunteers were instructed to start their supplementation for 8 weeks. During this period, they lived at their own accommodation and prepared their own meals but supplemented oligofructose or cellulose at the same time. The first and second week was the gut adaptation weeks. 10 g/day need to be taken in the first week and increased to 20 g/day in the latter week. This is important for gut to adapt with the fibre and also to reduce potential gastrointestinal side effects (Parnell and Reimer, 2009). On day 15, the actual supplementation period (30 g/day) began and continued until day 55. They were asked to take three sachets per day. Each sachet provided 10 g fibre. Volunteers were asked to take the supplement with main meals. They were given the choices of whether to mix it with drinks, sprinkle or mix it into food. The supplement must not be cooked or exposed to high temperatures. During these 6 weeks supplementation period, volunteers were contacted by investigators by phone to ensure that instructions were followed and for gastrointestinal side effect assessment.

2.3.2.3.1 Compliance

Compliance of supplementation was monitored by counting of unused sachets returned by volunteers after the supplementation period.

2.3.2.3.2 Energy intake Assessment

Assessment of energy intake was performed using the seven day food diaries obtained at baseline (day -8 until day -1), whilst the post supplementation energy intake assessment was measured on day 49 until day 55. Subjects were advised to keep their intake of prebiotic or probiotic products to a minimum (they were given a list of food that have high prebiotic, probiotic and synbiotic food products to avoid) (appendix 11), not to start any diet regime and not to gain or lose weight consciously during the supplementation period. The seven day food diaries were analysed using a standard dietary calculator, Dietplan6 (Forestfield Software Ltd, West Sussex, UK). Whenever portion sizes were not provided by the volunteers, standard portion sizes provided with this programme were used.

2.3.2.4 Statistical Analysis

Data are presented as mean ± standard error means (SEM). Prior the analyses, data were checked for Gaussian distribution using D'Agostino & Pearson omnibus normality test. An analysis of covariance (ANCOVA) with baseline data (day 0), age, gender and BMI as covariates were used to determine the effect between treatments for the hydrogen breath test, subjective appetite, gastrointestinal side effects assessment, gut hormones GLP-1, PYY, insulin and glucose levels. In addition, a two-tailed paired *t-test* was also used to compare the mean total area under the curve (tAUC) value within group analysis (baseline visit [day 0] vs. post-supplementation [day 56] group effect). Gastrointestinal side effect data were not normally distributed, thus data analysis for this parameter only, was performed on log10-transformed variables prior the ANCOVA and t-test analyses. Statistical significance is defined by a P value of 0.05 or less and all statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego CA, USA). ANCOVA was performed using SPSS 20.0 (SPSS Inc. Chicago, IL, USA).

2.4 RESULTS

2.4.1 Appetite Study day

Appetite study days were performed on baseline study day (day 0) (visit 2) and post-supplementation study day (day 56) (visit 6). On appetite study day, breath hydrogen test, visual analogue scales (VAS) and blood for gut hormones, PYY and GLP-1 were obtained from each volunteer. The result of the appetite study days are described below.

2.4.1.1 Volunteers Characteristics

Twenty two, [oligofructose = 12 (male=4, female=8) and cellulose = 10 (male=2, female=8)] healthy overweight and obese volunteers were participated in this study. The mean ages were 36.5 ± 2.2 years (range 21 – 49 years) in the oligofructose group and 28.7 ± 2.3 years (range 20 – 47 years) in the cellulose group. The mean BMI were 29.7 ± 1.0 kg/m^2 (range 25.0 – 34.6 kg/m^2) for oligofructose group and 31.1 ± 1.1 kg/m^2 (range 26.0 – 35.0 kg/m^2) for cellulose group.

2.4.1.2 Compliance

Adherence to the supplementation was calculated based on the return of unused sachets at the end of the supplementation period (day 55). Four of 22 volunteers involved in this study were reported to experience mild side effects such as bloated, flatulence or stomach discomfort between week three and week four and therefore they were allowed to continue taking two sachets per day (20 g) during this time. They were on 30 g of oligofructose or cellulose treatment on week five and week six. The reduced intake of supplement resulted in reduced compliance in the range of 60 – 77 % whilst the rest of the volunteers had high compliance rates between 86 – 100 %. Nevertheless, the reduced compliance rate demonstrated in four volunteers in the oligofructose group did not affect mean group compliance rate, oligofrucose (89.8 ± 13.1 %) (n=12). Cellulose group had a compliance rate of (89.7 ± 12.8 %) (n=10).

2.4.1.3 Energy Intake Assessment

Figure 2.4 demonstrates energy intake assessed during *ad libitum* meal assessment. There was no significant difference between oligofructose and cellulose group (P=0.739). Nevertheless, intake of oligofructose significantly reduced *ad libitum* energy intake (P=0.007) (760.2 ± 51.6 kcal) compared to baseline visit (873.2 ± 54.1 kcal) whilst cellulose supplementation has a tendency to reduce energy intake (P=0.066) (725.3 ± 117.4 kcal [day 56] and 867.6 ± 159.5 kcal [day 0]).

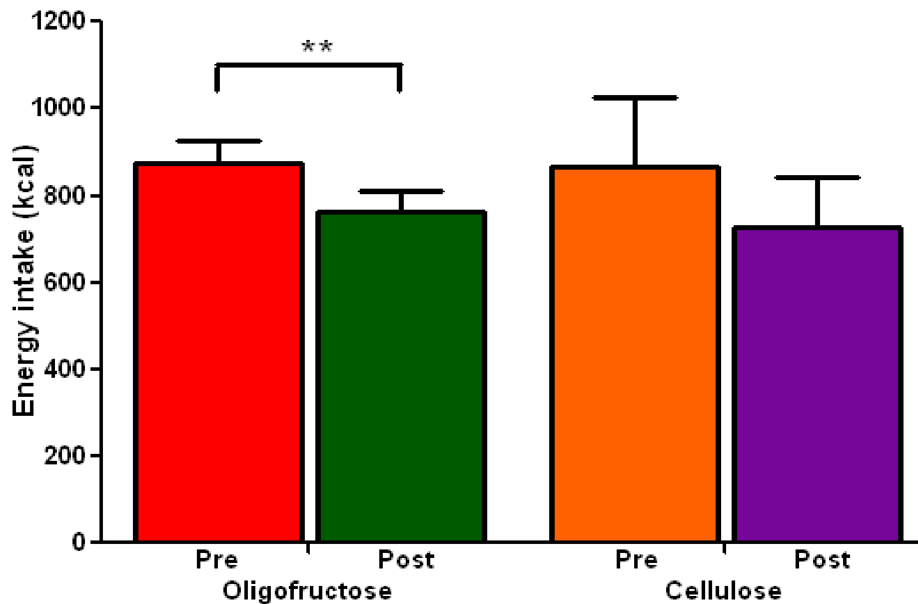


Figure 2.4 Postprandial energy intake assessment (kcal) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10). **P<0.01 oligofructose *vs.* baseline

2.4.1.4 Subjective Appetite Ratings

Subjective appetite ratings for hunger, fullness, motivation to eat and side effects were assessed at 0, 20, 40, 60, 90, 120, 180, 240, 260, 280, 300, 330, 360, 390, 420 and 450 minutes on both pre-supplementation (day 0) and post-supplementation study day (day 56). The results are shown in Figures 2.5 – 2.11.

2.4.1.4.1 Appetite Assessment

2.4.1.4.1.1 Hunger

Figure 2.5 shows subjective hunger scores (i) and $tAUC_{450min}$ hunger scores (ii). Intake of oligofructose significantly reduced $tAUC_{450min}$ hunger scores (P=0.034) compared to cellulose treatment. In addition, oligofructose also significantly reduced $tAUC_{450min}$ within the group (P=0.001) (1387.3 ± 263.2 cm*min [day 56] and 2053.7 ± 308.7 cm*min [day 0]). No significant effect on $tAUC_{450min}$ hunger scores was demonstrated in the cellulose group (P=0.964) (1838.6 ± 315.9 cm*min [day 56] and 1850.6 ± 395.3 cm*min [day 0]).

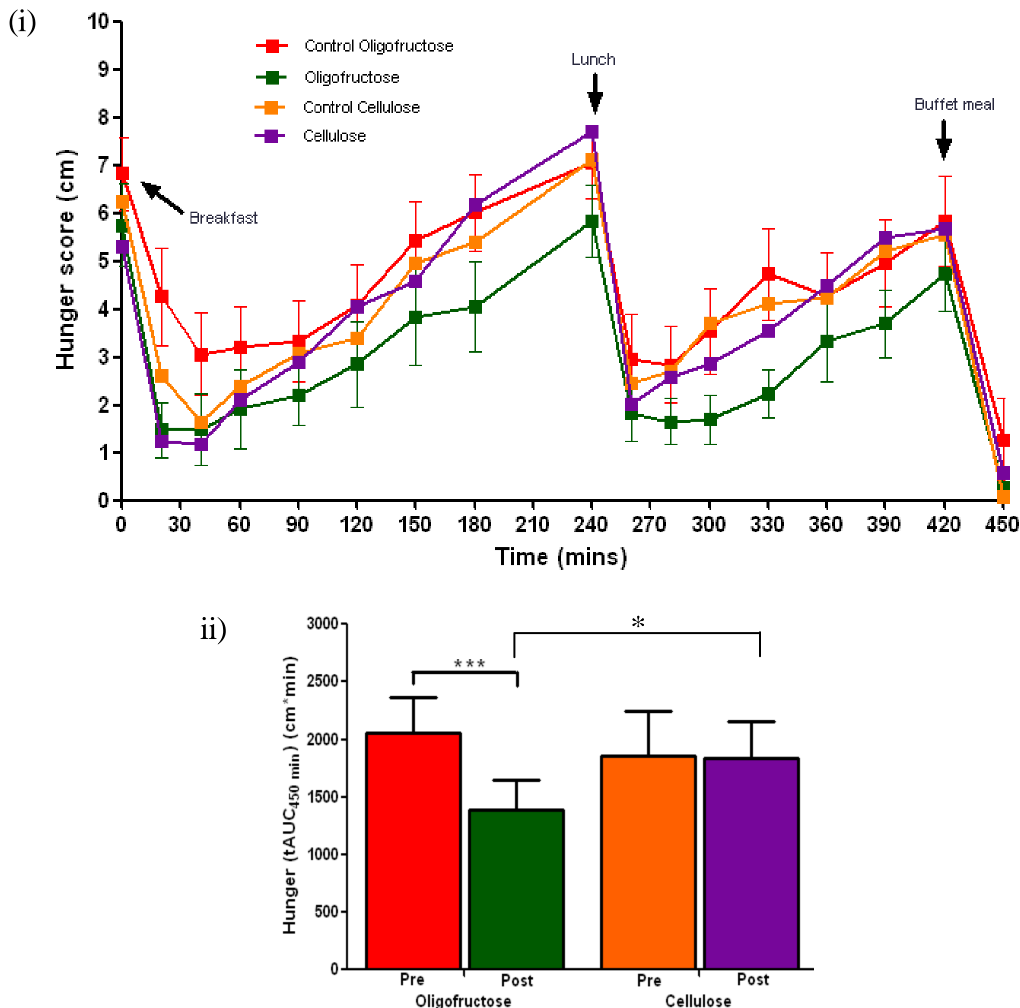


Figure 2.5 Subjective hunger scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). *P<0.05 oligofructose *vs.* other treatments, ***P<0.001 oligofructose *vs.* baseline,

2.4.1.4.1.2 Fullness

Figure 2.6 exhibits subjective fullness scores (i) and $tAUC_{450min}$ fullness scores (ii). Oligofructose supplementation had no significant effect on $tAUC_{450min}$ fullness scores (P=0.493) compared with cellulose treatment. Nevertheless, oligofructose was shown to significantly increase (P=0.029) $tAUC_{450min}$ fullness scores (2095.3 ± 249.3 cm*min [day 56] compared to baseline 1790.8 ± 243.7 cm*min [day 0]). No significant effect (P=0.431) was demonstrated in the cellulose group (1805.3 ± 254.7 cm*min [day 56] and 1615.1 ± 314.5 cm*min [day 0]).

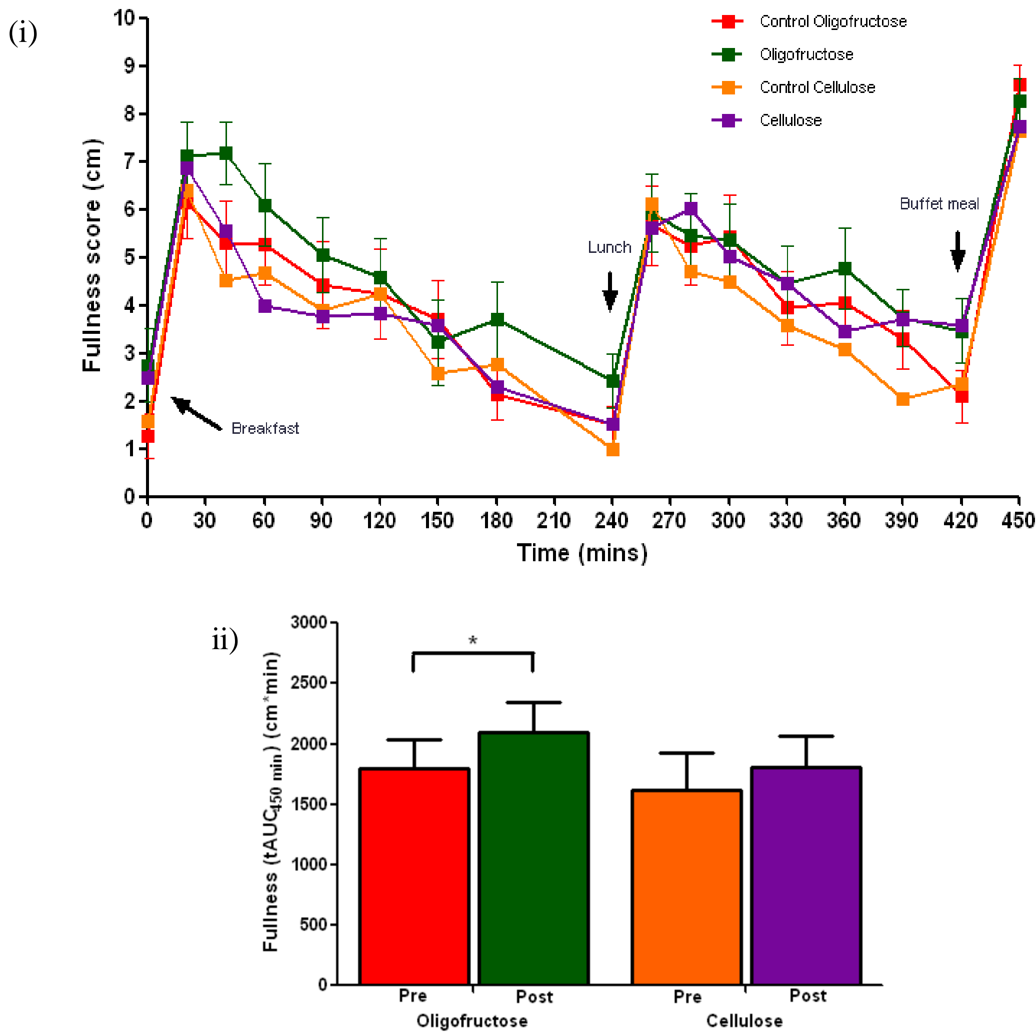


Figure 2.6 Subjective fullness scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). *P<0.05 oligofructose *vs.* baseline.

2.4.1.4.1.3 Motivation to Eat

Figure 2.7 shows subjective motivation to eat scores (i) and $tAUC_{450mins}$ motivation to eat scores (ii). Oligofructose supplementation significantly reduced $tAUC_{450min}$ motivation to eat scores (P=0.013) compared with cellulose treatment. In addition, oligofructose also significantly reduced $tAUC_{450min}$ motivation to eat scores within the group (P=0.004) (1412.5 ± 229.2 cm*min [day 56] and 2059.6 ± 294.5 cm*min [day 0]) whereas no significant modulation on $tAUC_{450min}$ scores was demonstrated in the cellulose group (P=0.635) (1847.4 ± 313.4 cm*min [day 56] and 1946.4 ± 367.6 cm*min [day 0]).

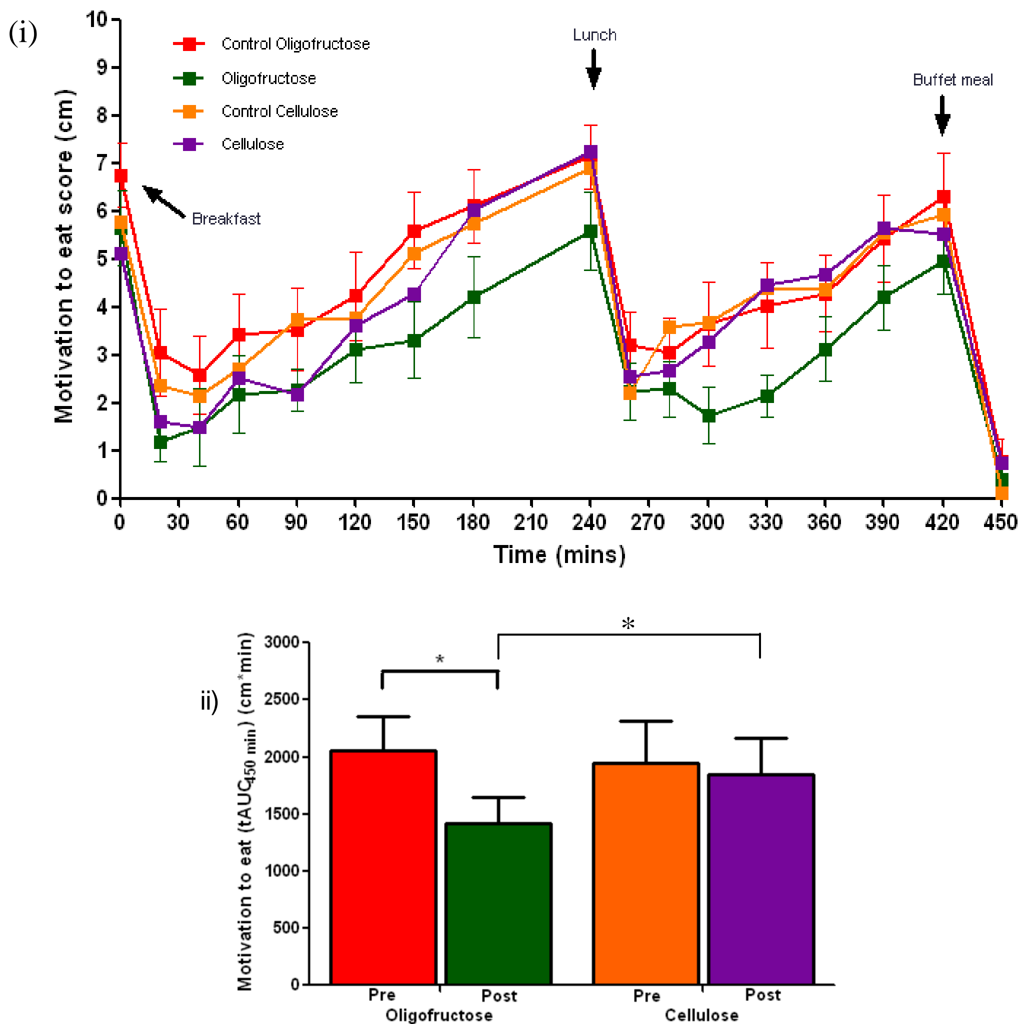


Figure 2.7 Subjective motivation to eat scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). *P<0.05 oligofructose *vs.* baseline.

2.4.1.4.1.4 Desire to Eat Sweet Food

Figure 2.8 demonstrates subjective desire to eat sweet food scores (i) and $tAUC_{450min}$ desire to eat sweet food scores (ii). Oligofructose supplementation had no significant effect on $tAUC_{450min}$ desire to eat scores compared with cellulose (P=0.342). Nevertheless, oligofructose significantly reduced the $tAUC_{450min}$ scores (1232.8 ± 361.3 cm*min) compared to baseline (day 0) (1759.5 ± 372.2 cm*min) (P=0.022), but no significant effect was demonstrated in the cellulose treatment (P=0.353) (1904.3 ± 364.1 cm*min [day 56] and 2022.8 ± 444.9 cm*min [day 0]).

i)

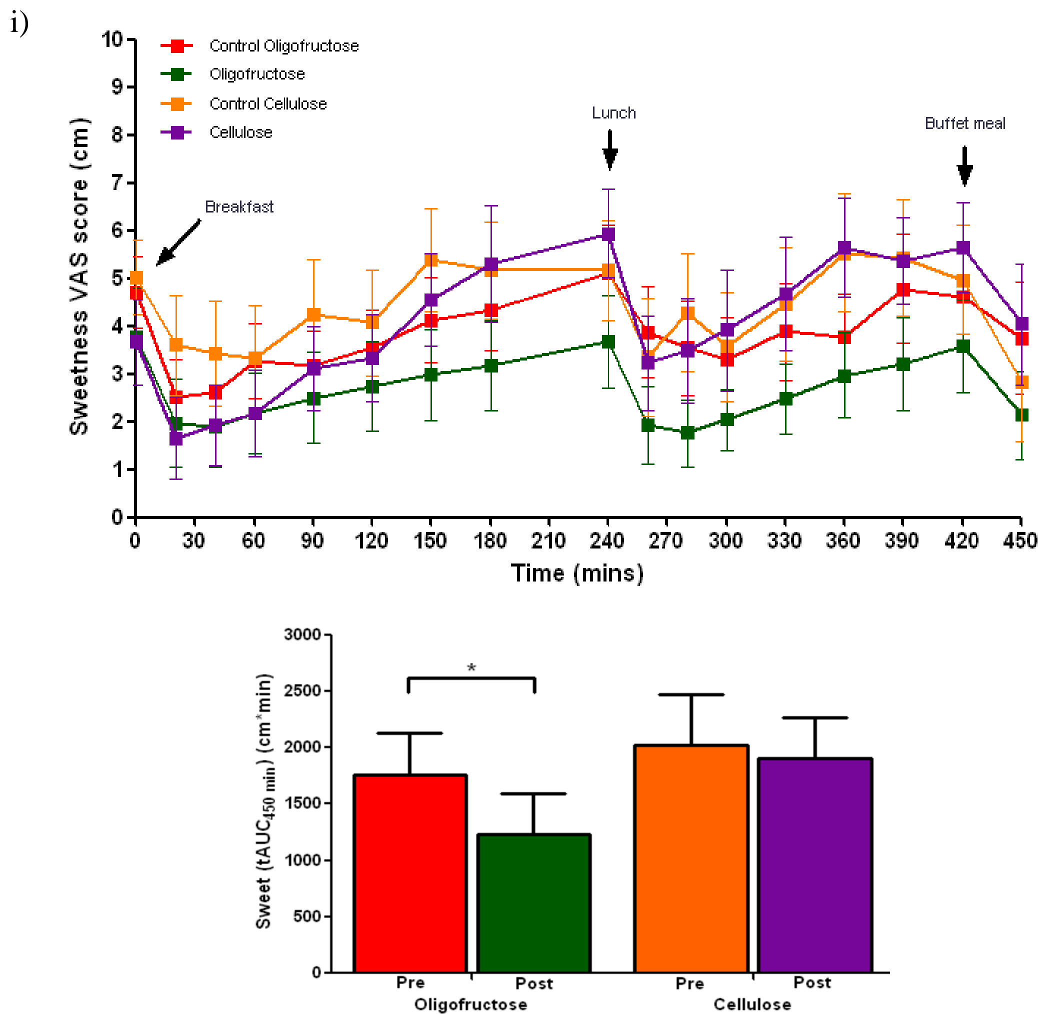


Figure 2.8 Subjective desire for sweet food scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). *P<0.05 oligofructose *vs.* baseline.

2.4.1.4.1.5 Desire to Eat Savoury Food

Figure 2.9 depicts subjective desire towards savoury food scores (i) and $tAUC_{450min}$ desire towards savoury food scores (ii). Oligofructose supplementation significantly reduced $tAUC_{450min}$ desire for savoury food scores (P=0.003) compared to other treatments. In addition, oligofructose also significantly reduced within group $tAUC_{450min}$ scores (P=0.004) (1090.6 ± 251.1 cm*min [day 56] and 1658.0 ± 365.3 [day 0]). However, no significant effect on savoury $tAUC_{450min}$ scores in the cellulose group (P=0.203) (cellulose: 1757.7 ± 420.0 cm*min [day 56] and baseline: 1534.7 ± 445.3 cm*min [day 0]).

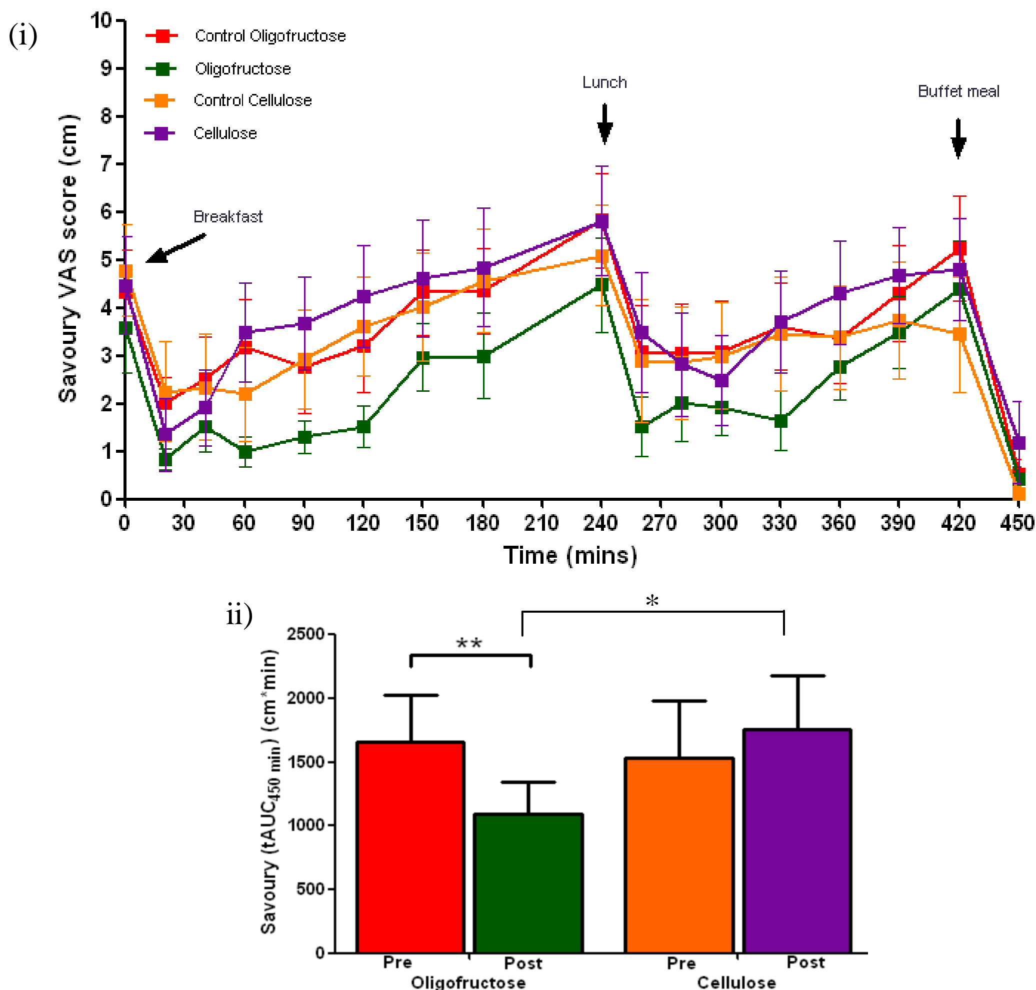


Figure 2.9 Subjective desire for savoury food scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). **P<0.01 oligofructose *vs.* baseline, *P<0.05 oligofructose *vs.* other treatments.

2.4.1.4.1.6 Desire to Eat Fatty Food

Figure 2.10 exhibits subjective desire to eat fatty food scores (i) and $tAUC_{450min}$ desire to eat fatty food scores (ii). Inclusion of oligofructose in the diet significantly reduced $tAUC_{450min}$ fatty scores compared to other treatments (P=0.013) and $tAUC_{450min}$ within oligofructose group (P=0.001) (566.3 ± 191.2 cm*min [day 56] and 1401.9 ± 324.8 cm*min [day 0]). No significant effect was found in $tAUC_{450min}$ desire to eat fatty scores in the cellulose group (P=0.408) (1095.5 ± 320.9 cm*min [day 56] and 1022.4 ± 380.8 cm*min [day 0]).

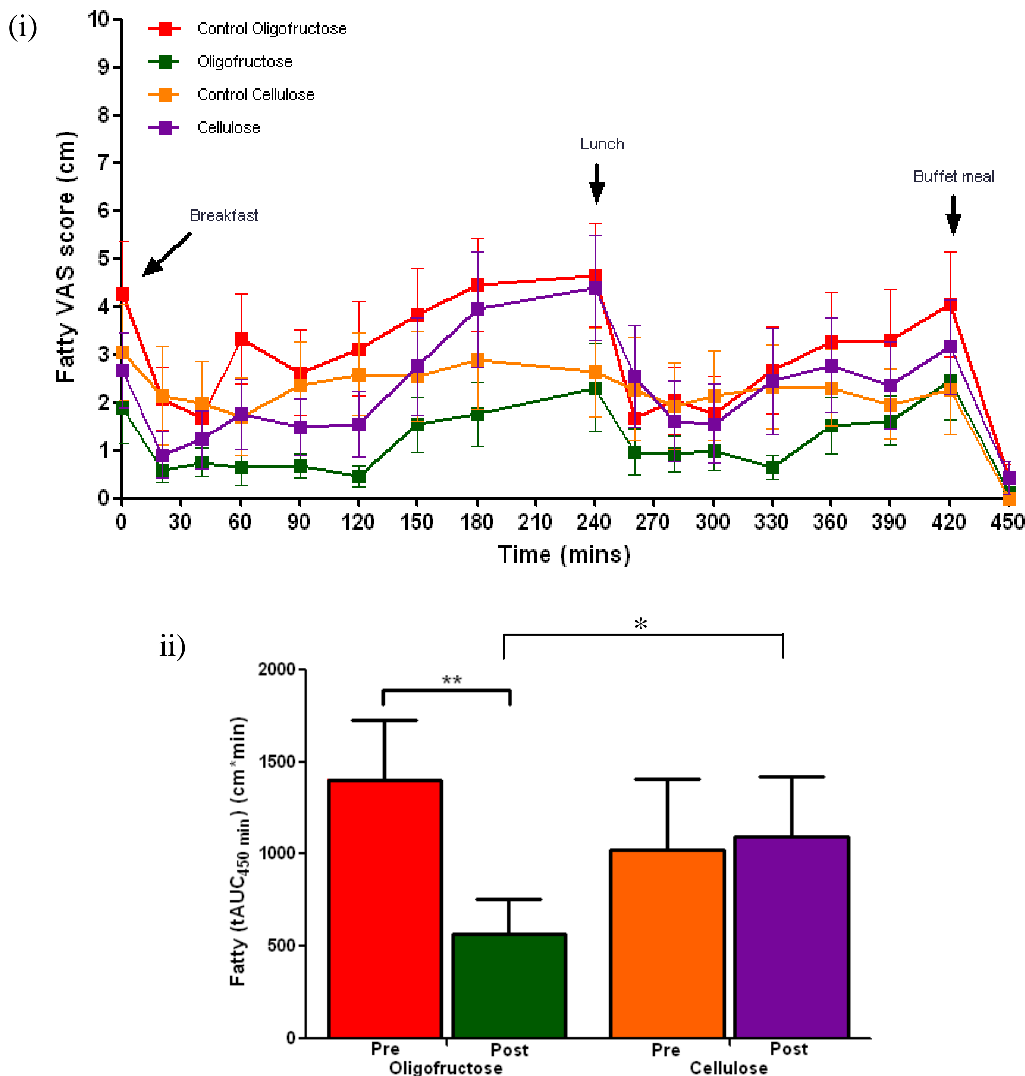


Figure 2.10 Subjective desire for fatty food scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). **P<0.01 oligofructose *vs.* baseline, *P<0.05 oligofructose *vs.* cellulose.

2.4.1.4.1.7 Desire for Salty Food

Figure 2.11 depicts subjective desire for salty food scores (i) and $tAUC_{450mins}$ desire for salty food scores (ii). There was a significant reduction in reducing $tAUC_{450mins}$ desire for salty food in the oligofructose group compared to other groups (P=0.009). $tAUC_{450mins}$ for desire salty food at 450 minutes in the oligofructose group (880.8 ± 238.8 cm*min) was significantly reduced compared to baseline (day 0) (1617.4 ± 369.6 cm*min) (P=0.002). No significant effect was demonstrated in the cellulose group (1322.0 ± 341.4 cm*min) compared to baseline (day 0) (P=0.190) 1035.7 ± 350.6 cm*min [day 0]).

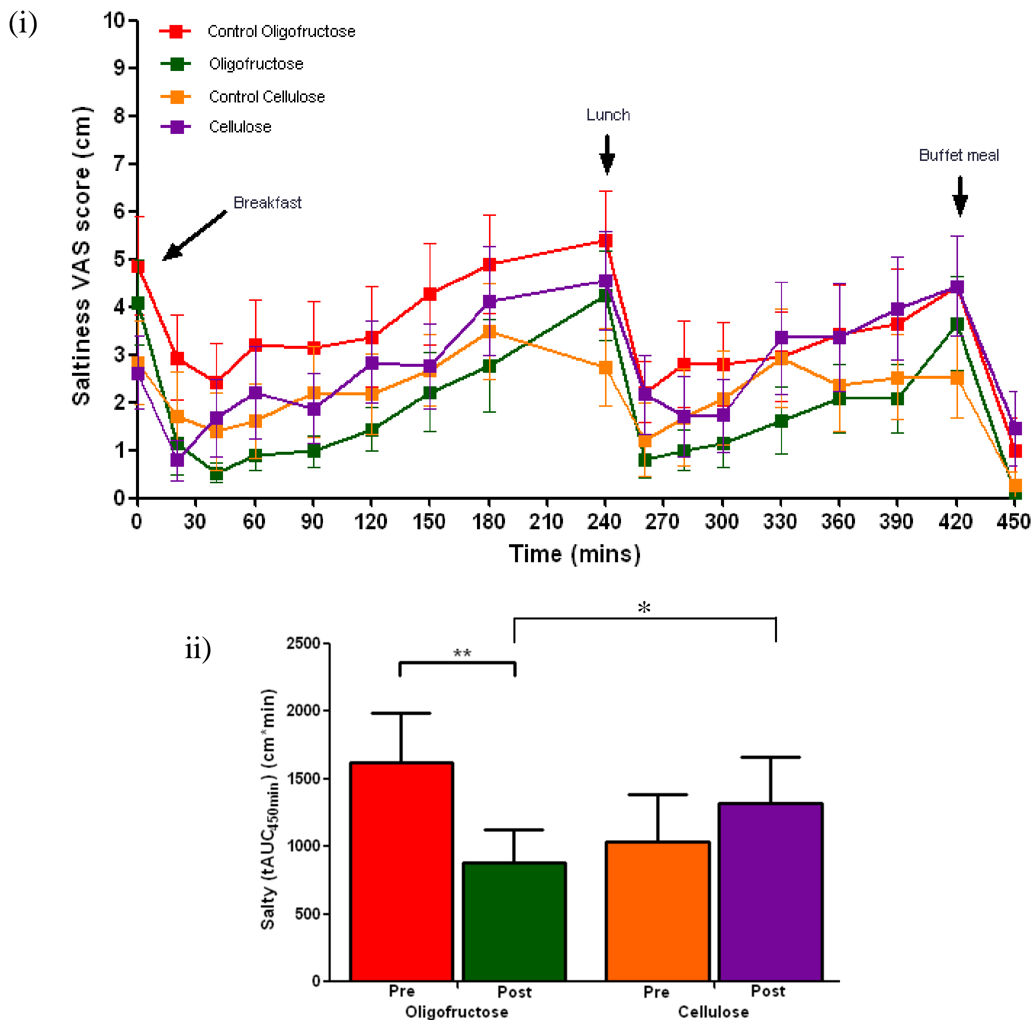


Figure 2.11 Subjective desire for salty food scores (cm) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10).**P<0.01 oligofructose *vs.* baseline, **P<0.01 oligofructose *vs.* cellulose

2.4.1.4.2 Gastrointestinal Side Effect Assessment

Gastrointestinal side effect data were not normally distributed, thus data analysis was performed on log10-transformed variables. The geometric means (95% confident interval) are presented in Table 2.1. Oligofructose supplementation significantly increased $tAUC_{450mins}$ bloating and flatulence scores compared to cellulose supplementation (P=0.007 and P=0.005 respectively) and a tendency to increase both of bloating and flatulence when compared to baseline study day (day 0) (P=0.097 and P=0.077 respectively). No significant effect was demonstrated in the cellulose group (P=0.629 and P=0.496 respectively).

Table 2.1 Gastrointestinal side effects at baseline (day 0) and following intake of 30 g/day oligofructose and cellulose supplementation on post-supplementation (day 56) of appetite study day.

Appetite sensation	Oligofructose (n=12)		Cellulose (n=10)		P[c] Value
	Day 0	Day 56	Day 0	Day 56	
Bloated (cm*min)[b]	15.1 (1.0 – 218.6)	51.6 (2.7 – 985.8)[a]	105.2 (21.6 – 512.8)	14.1 (0.6 – 363.4)	0.007
Stomach discomfort (cm*min)[b]	72.6 (19.4 – 271.5)	4.0 (0.2 – 75.4)	43.3 (11.6 – 161.0)	10.6 (1.1 – 106.1)	0.011
Flatulence (cm*min)[b]	11.1 (0.9 – 132.4)	57.6 (7.0 – 470.5)[a]	32.8 (3.8 – 280.5)	5.9 (0.4 – 99.1)	0.005
Diarrhoea (cm*min)[b]	1.2 (0.1 – 9.8)	0.5 (0.1 – 3.9)	5.1 (1.1 – 24.0)	0.5 (0.1 – 5.1)[a]	0.896
Sick (cm*min) [b]	14.4 (2.5 – 81.8)	1.9 (0.2 – 20.9)	19.4 (5.6 – 67.8)	2.4 (0.2 – 37.9)	0.613

[a]significantly different compared to baseline (day 0) study day.

[b]Data are presented as geometric mean (95% confidence interval)

[c]Statistical analysis was performed by ANCOVA

2.4.1.5 Breath Hydrogen Analysis

Figure 2.12 demonstrates breath hydrogen levels (i) and $tAUC_{450mins}$ breath hydrogen levels (inset). Both of the supplementation showed no significant effect on fasting breath hydrogen levels when compared to baseline (oligofructose: P=0.431 and cellulose: P=0.871). Intake of oligofructose significantly increased breath hydrogen levels and $tAUC_{450min}$ (P< 0.0001 and P=0.001 respectively) compared to cellulose treatment. Breath hydrogen levels following ingestion of oligofructose supplementation started to increase from 180 minutes and peaked around 260 to 300 minutes. One participant showed a late peak at 420 minutes with hydrogen levels of 88 ppm. After 300 minutes, hydrogen levels remained stable at 15 – 20 ppm until 450 minutes. This means that fermentation was still increased when the *ad libitum* meal intake was administered at 420 minutes. In contrast, no significant effect was demonstrated in the cellulose group, with the levels being consistently below than 10 ppm throughout the study day. Please refer to Section 2.4.1.3 for *ad libitum* meal assessment.

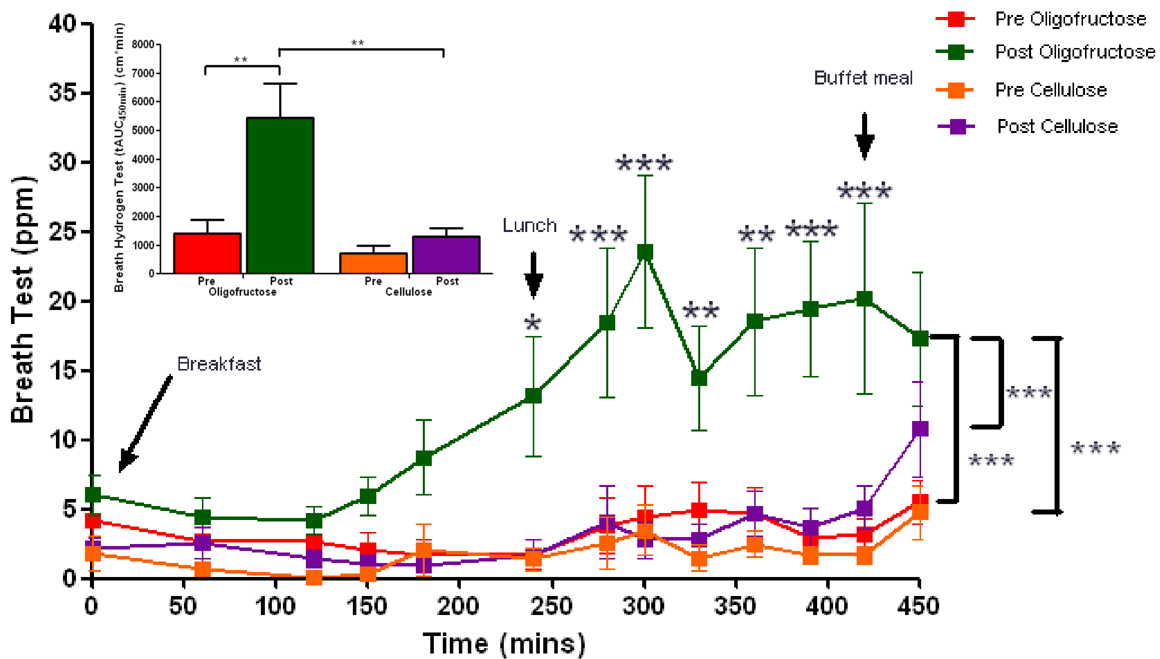


Figure 2.12 Breath hydrogen levels (ppm) and $tAUC_{450min}$ (inset) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10). **P<0.01 oligofructose *vs.* cellulose and **P<0.01 oligofructose *vs.* control oligofructose $tAUC_{450mins}$). ***P<0.001 oligofructose vs. other treatments (time course).

2.4.1.6 Gut Hormones, Glucose and Insulin Analyses

2.4.1.6.1 Peptide Tyrosine-Tyrosine

Figure 2.13 illustrates plasma PYY levels (i) and $tAUC_{420min}$ plasma PYY levels (ii). Supplementing oligofructose in volunteers' diets had no significant difference compared with cellulose (P=0.219) No significant effect on fasting plasma PYY levels was also demonstrated (P=0.941). However, PYY levels were shown to be increased after two hour ingestion of oligofructose supplement and remained elevated throughout the study day compared to cellulose treatment. In addition, the plasma PYY levels peaked at 260 minutes and significantly increased $tAUC_{420min}$ plasma PYY levels (13876.2 ± 1078.3 pmol/L*min) compared to baseline (P=0.037) (12377.5 ± 794.5 pmol/L*min) whilst no effect was shown in the cellulose group (P=0.432).

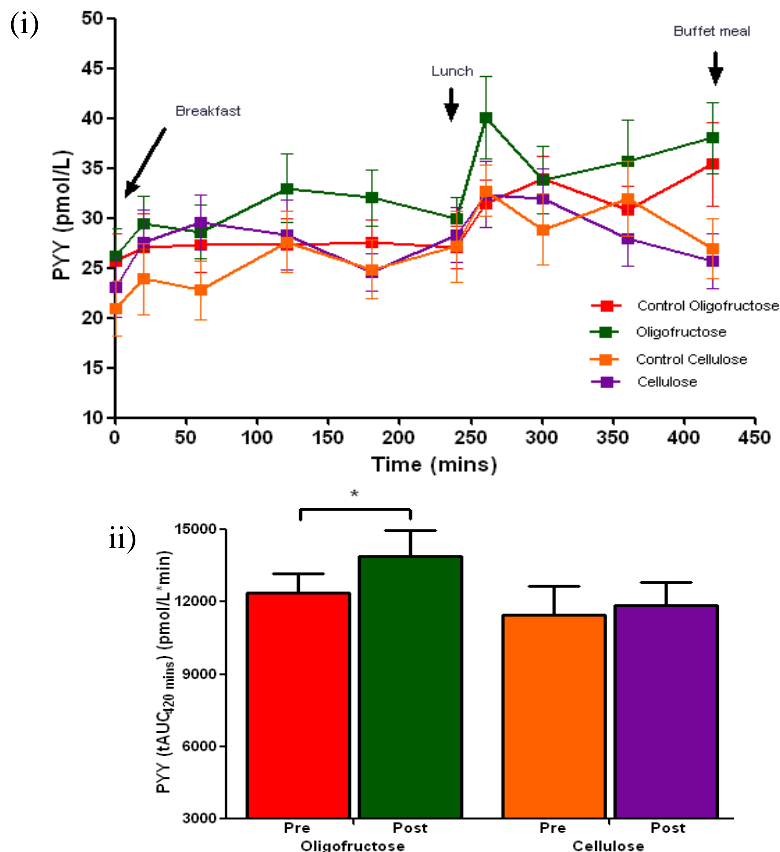


Figure 2.13 Postprandial plasma PYY levels (pmol/L) (i) and $tAUC_{420min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10).*P<0.05 oligofructose *vs.* baseline.

2.4.1.6.2 Glucagon-like Peptide 1

Figure 2.14 exhibits plasma levels of GLP-1 (i) and $tAUC_{420min}$ of plasma GLP-1 (ii). Cellulose supplementation significantly increased (P=0.006) $tAUC_{420min}$ postprandial plasma GLP-1 compared to baseline but no significant effect when compared to oligofructose group (P=0.327). No significant effect was also demonstrated in the oligofructose group (P=0.412).

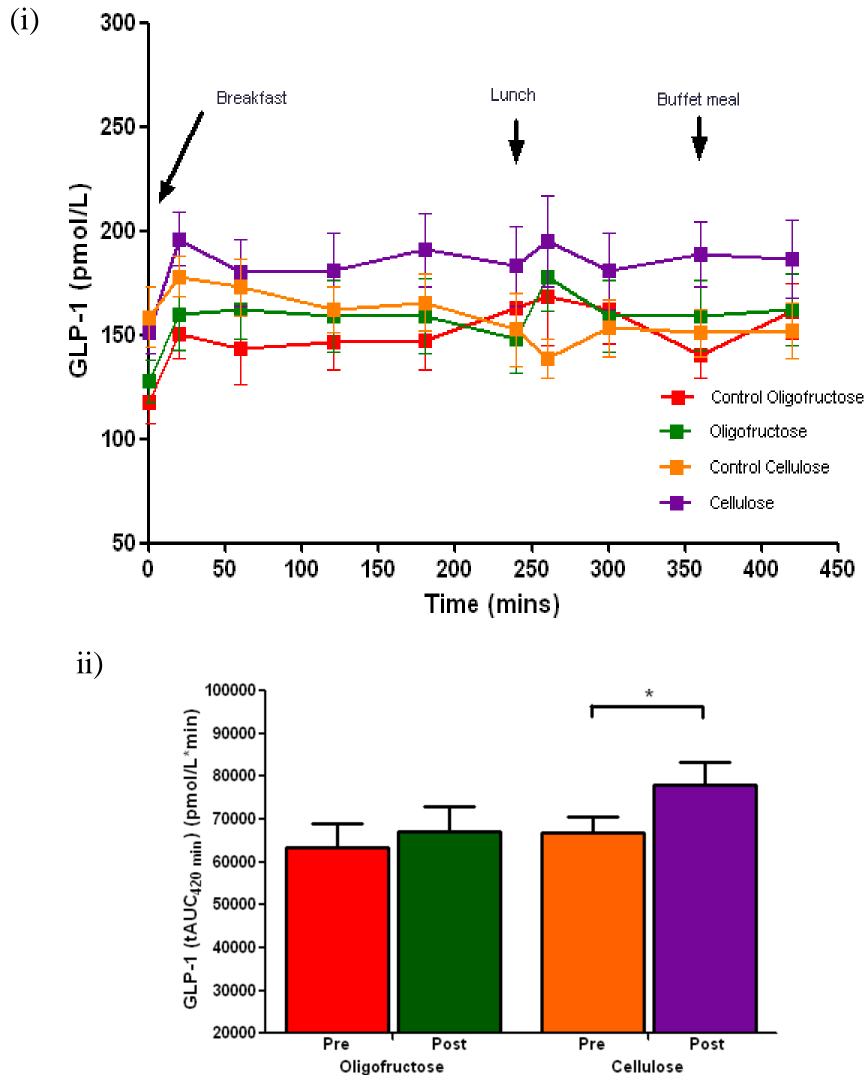


Figure 2.14 Postprandial plasma GLP-1 levels (pmol/L) (i) and $tAUC_{420min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10).*P<0.05 cellulose *vs.* baseline

2.4.1.6.3 Insulin

Figure 2.15 depicts plasma levels of insulin (i) and $tAUC_{450min}$ of insulin levels (ii). Oligofructose had no significant effect when compared to cellulose treatment (P=0.750). No significant effect also demonstrated in $tAUC_{450min}$ plasma insulin in both oligofructose and cellulose group (P=0.413 and P=0.895).

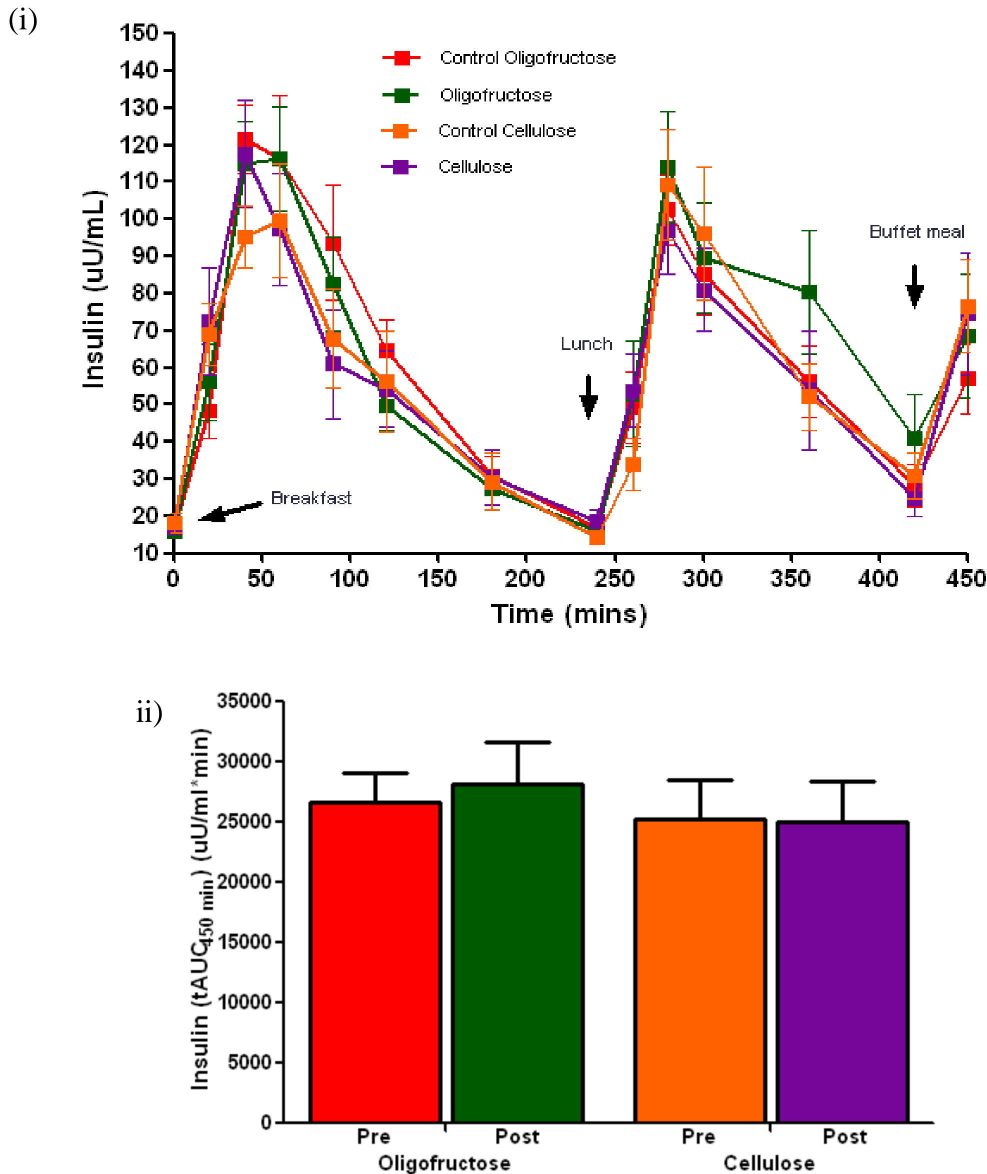


Figure 2.15 Postprandial plasma insulin levels (uU/mL) (i) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10).

2.4.1.6.4 Glucose

Figure 2.16 demonstrates plasma levels of glucose (i) and $tAUC_{450min}$ (ii). Inclusion of oligofructose in meals significantly increased glucose response when compared to the baseline (P=0.040) whilst cellulose had no significant effect on plasma glucose levels (P=0.846) compared to the baseline. No significant difference between the oligofructose and cellulose treatments was also demonstrated (P=0.643).

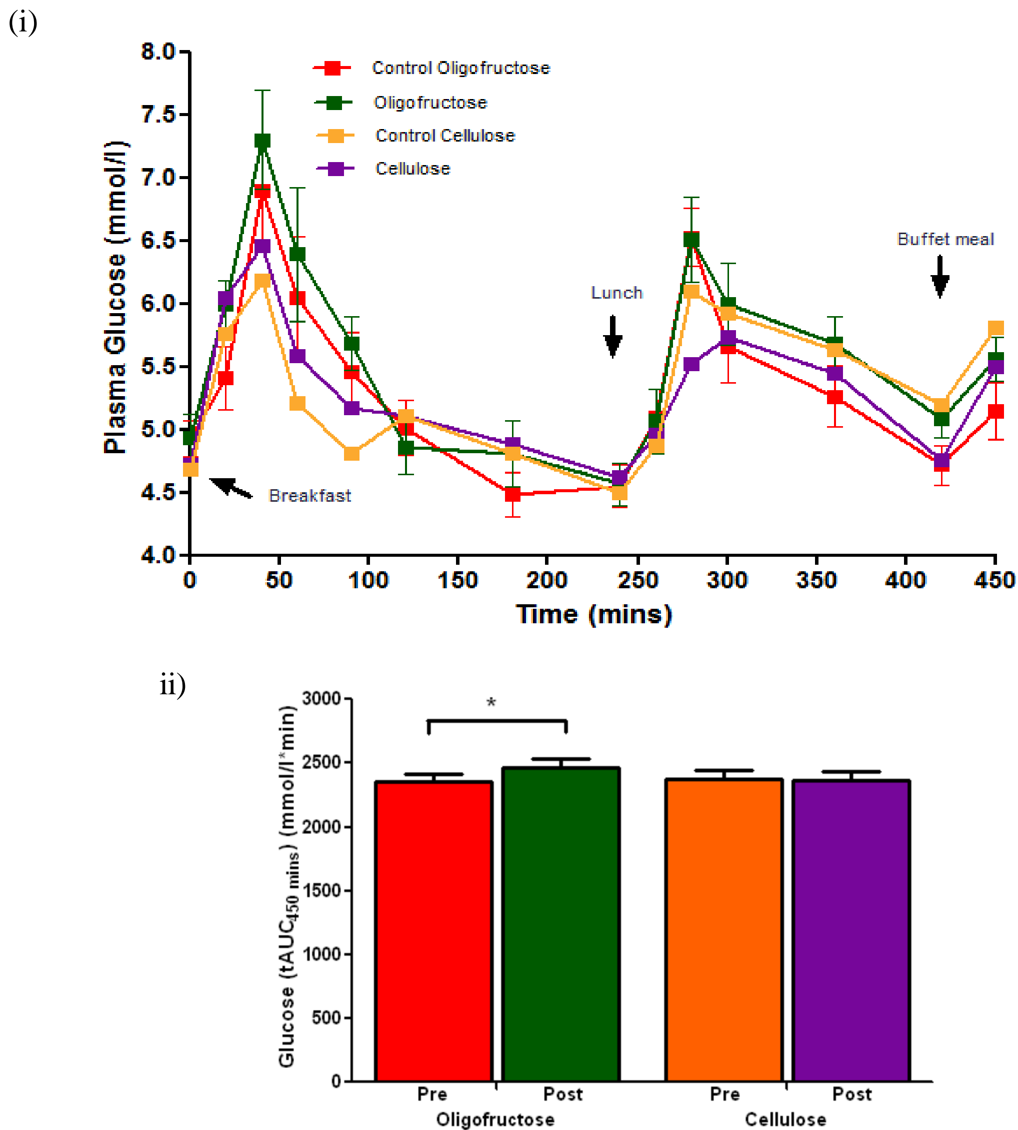


Figure 2.16 Postprandial plasma glucose levels (mmol/L) and $tAUC_{450min}$ (ii) in oligofructose and cellulose groups. The doses were supplemented into the meal during breakfast (0 min) and lunch (240 minutes) in the post-supplementation visit (day 56). The *ad libitum* meal was assessed at 420 minutes. Data is expressed as mean ± SEM. Oligofructose (n=12), cellulose (n=10).*P<0.05 oligofructose *vs.* baseline.

2.4.2 Free-living Supplementation Period

Assessment was performed at prior to the supplementation period (Day -8 to -1) (baseline) and during the last week of supplementation (day 49 to 55) (post-supplementation), VAS and energy intake were assessed.

2.4.2.1 Energy intake Assessment

Figure 2.17 shows energy intake assessment. There was no effect of treatment in reducing energy intake at home in the oligofructose and cellulose group when compared to the baseline; oligofructose (P=0.522) and cellulose (P=0.652). Oligofructose group also showed no significant difference when compared to cellulose supplementation (P=0.821). For this analysis, the result represents the mean value for 21 volunteers as one volunteer in the cellulose group lost the food diary (oligofructose = 12 and cellulose = 9 volunteers).

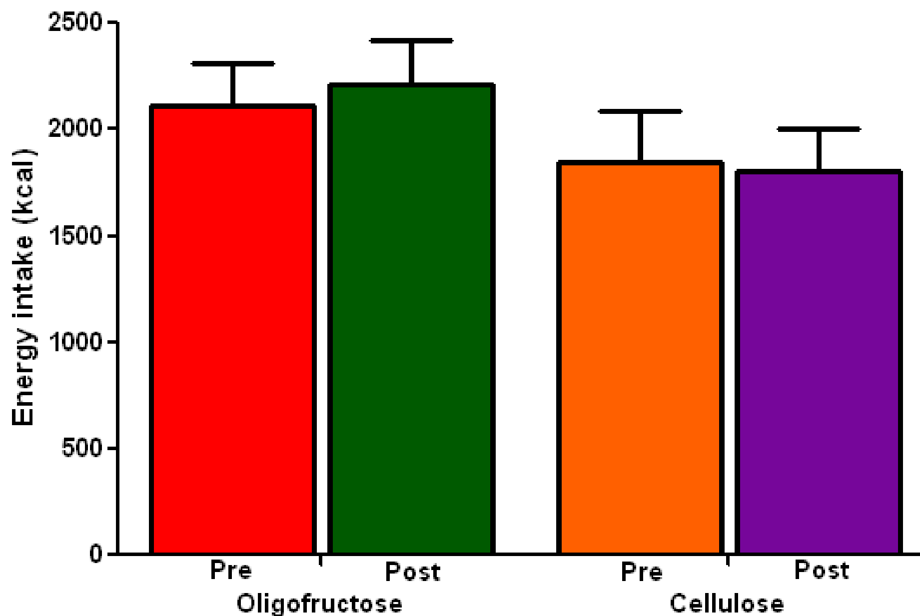


Figure 2.17 Seven days energy intake assessment (kcal) in oligofructose and cellulose groups. The supplementation were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline (day -8 to day -1) and post-supplementation period (day 49 to day 55). Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=9).

2.4.2.2 Subjective appetite ratings

2.4.2.2.1 Hunger

Figure 2.18 shows subjective hunger scores. There was a tendency in the oligofructose group to reduce hunger scores (3.9 ± 0.6 cm) compared to baseline (day 0) (P=0.054) (5.4 ± 0.7 cm) whilst no significant effect was demonstrated within cellulose group (P=0.711) (5.1 ± 0.3 cm [day 56] and 4.7 ± 0.9 cm [day 0]). No significant effect was also demonstrated between the supplementations (P=0.219).

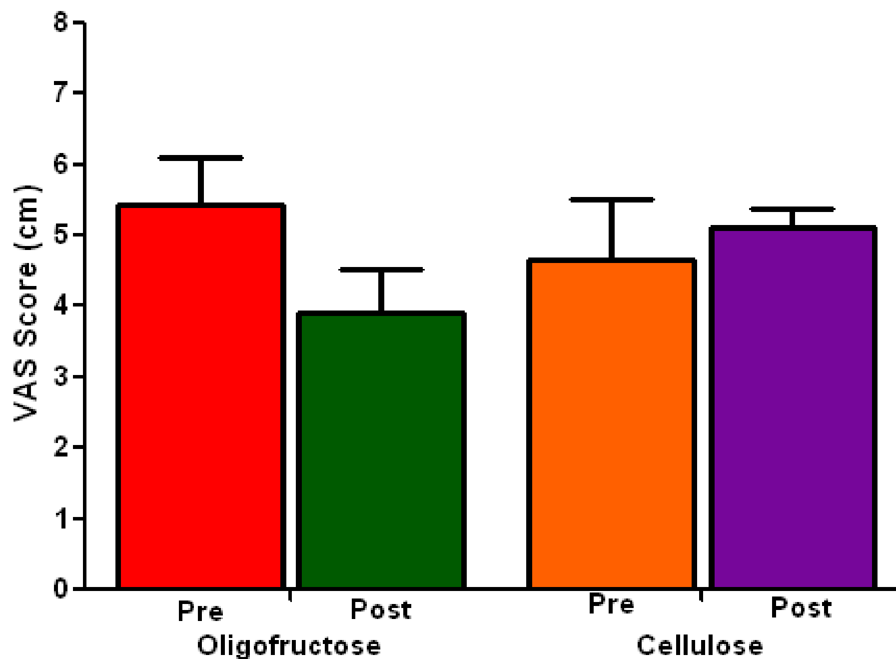


Figure 2.18 Subjective hunger scores (cm) in oligofructose and cellulose groups. The supplementation were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline (day -8 to day -1) and post-supplementation period (day 49 to day 55). Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

2.4.2.2.2 Fullness

Figure 2.19 demonstrates the subjective fullness scores. There was no significant effect of treatment on increasing fullness score (4.7 ± 0.4 cm) compared to baseline (5.1 ± 0.4 cm) in both oligofructose group (P=0.210) and cellulose group (P=0.688) (4.3 ± 0.6 cm [day 56] and 4.4 ± 0.8 cm [day 0]). No significant effect was also seen when comparing between oligofructose and cellulose treatment (P=0.634).

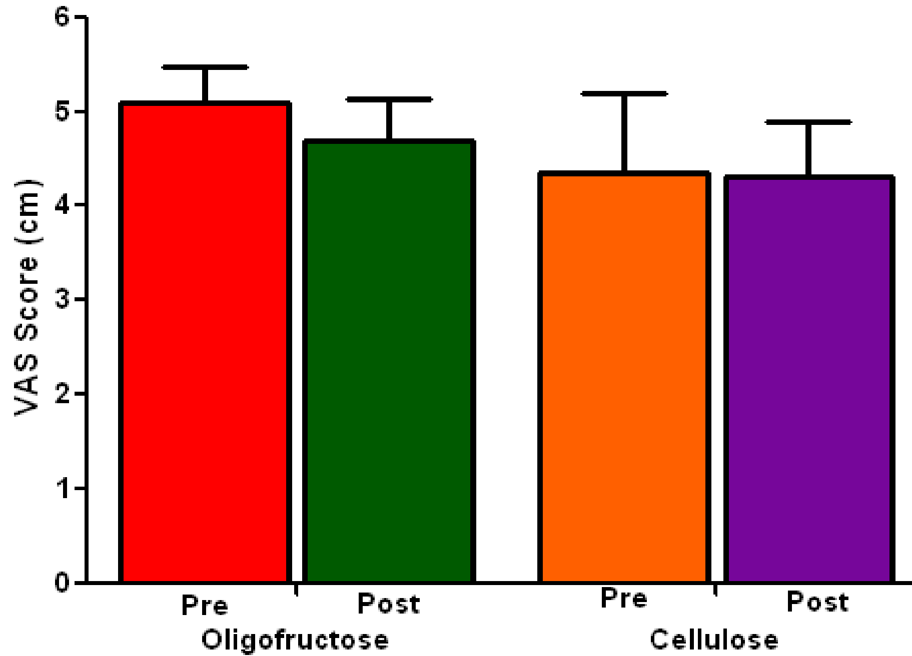


Figure 2.19 Subjective fullness scores (cm) in oligofructose and cellulose groups. The supplementation were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline (day -8 to day -1) and post-supplementation period (day 49 to day 55). Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

2.4.2.2.3 Gastrointestinal Side effects

Table 2.2 demonstrates the geometric mean and 95% confidence interval of gastrointestinal side effect assessed during free-living supplementation period. No significant effect was showed between oligofructose and cellulose supplementation.

Table 2.2 Gastrointestinal Side effects following intake of 30 g/day oligofructose or cellulose during free-living supplementation period, baseline (day -8 to -1) and post-supplementation (day 49 to 55) appetite study day.

Side effects (cm)	**Oligofructose (n=12)**		**Cellulose (n=10)**		**P Value**
	Baseline	Post-supplementation	Baseline	Post-supplementation	
Bloating	0.9 (0.2 – 1.5)	2.3 (0.6 – 3.9)	0.4 (0.1 – 1.2)	0.4 (0.1 – 1.2)	0.9737
Stomach discomfort	0.5 (0.2 – 1.2)	0.4 (0.1 – 1.2)	0.2 (0.1 – 0.8)	0.2 (0.1 – 0.8)	0.8682
Flatulence	0.9 (0.4 – 1.5)	1.7 (0.3 – 3.2)	0.3 (0.1 – 1.1)	0.3 (0.1 – 1.3)	0.224
Diarrhoea	0.1 (0.04 – 0.2)	0.1 (0.04 – 0.5)	0.01 (0.03 – 0.3)	0.1 (0.04 – 0.4)	0.837
Sickness	0.1 (0.05 – 0.3)	0.1 (0.05 – 0.4)	0.1 (0.04 – 0.4)	0.2 (0.04 – 0.6)	0.760

*Data are presented as geometric mean (95% confidence interval)

2.5 Discussion

2.5.1 Appetite Study Day Assessment

The result of this study is consistent with the hypothesis that intake of oligofructose for eight weeks significantly increased breath hydrogen secretion and reduced several subjective appetite scores (hunger, motivation to eat, desire to eat savoury, fatty and salty) compared to cellulose group. However, oligofructose supplementation had no significant effect on energy intake, fullness scores and plasma PYY levels compared to cellulose although the effects were significant within the group.

Data in the literature suggests that intake of fermentable carbohydrates, oligofructose (Cani et al., 2009), inulin (Fernandes et al., 2011) and fructo-oligosaccharides (Piche et al., 2003) increased colonic fermentation (Cani et al., 2004; Delzenne et al., 2005). Indeed, the present study showed that colonic fermentation activity assessed by breath hydrogen test was significantly increased two hours after oligofructose intake and the levels were maintained throughout the study day. Similar to our study, Gee *et al*. reported that intake of 10 g lactitol also resulted in peaked breath hydrogen levels after 3-5 hours ingestion (Gee and Johnson, 2005). Nevertheless, it is surprising that oligofructose has no significant effect on fasting breath hydrogen levels as demonstrated in Cani *et al*. and Piche *et al*. (Cani et al., 2009; Piche et al., 2003). Hydrogen breath test is a non invasive, simple and common test used in clinical studies to test for the malabsorption of carbohydrates (Simren and Stotzer, 2006), therefore it is also suitable to be used as a surrogate marker for fermentability of fibre. However, there are some limitations to hydrogen breath test analysis. Some people do not have bacteria that produce bydrogen and this make the breath hydrogen measurement impossible in these people. Therefore, it is important to identify volunteers that able to produce breath hydrogen before the start of the study. In addition, hydrogen can also be metabolised to other metabolites such as ethanol and methane. Instead of hydrogen, some people have bacteria that produce a different gas, such as methane or produce both methane and hydrogen concurrently. Although methane can also be measured in the breath just like hydrogen, the production of methane is more complex and its analysis is less experience in carbohydrate malabsorption studies.

It is widely known that stimulation of colonic fermentation by fermentable carbohydrates produced SCFAs. Although SCFAs were not measured in this study, other studies have suggested that intake of

fermentable carbohydrates elevated SCFAs, which is associated with increase expression of proglucagon genes in the gut (Delzenne et al., 2005; Tappenden et al., 1998). In addition, the relationship between SCFAs and L-cells derived hormones has been demonstrated in both human and rodent studies. In humans, rectal infusion of acetate has been shown to significantly increase plasma PYY and GLP-1 levels (Freeland and Wolever, 2010). In rodents, intracolonic infusion of SCFA has been shown to trigger the release of PYY in the circulation (Cherbut et al., 1998). Interestingly, Zhou *et al*. showed that SCFAs directly induced release of proglucagon and PYY mRNA expression *in vitro* study (Zhou et al., 2008). In the present study, the peak levels of hydrogen release following oligofructose intake was detected between 260 to 300 minutes and this is well-correlated with the peak levels of plasma PYY showed at 260 minutes. Moreover, oligofructose significantly increased $tAUC_{420mins}$ plasma PYY secretion compared to the baseline study day (day 0). To date, the mechanism of how SCFAs modulated the release of PYY is still unclear. However, SCFAs might modulate their effects on stimulating gut hormone release by binding to their receptors, FFAR2 and FFAR3. FFAR2 and FFAR3 are co-localised with cell producing GLP-1 and PYY in the colonic L-cells, therefore activation of these receptors by SCFAs could potentially triggers GLP-1 and PYY secretion (Brown et al., 2003; Karaki et al., 2008; Le Poul et al., 2003; Xiong et al., 2004; Zaibi et al., 2010).

Despite of a significant increase $tAUC_{420mins}$ PYY levels in the oligofructose group, the levels were not significantly different when compared with the cellulose group. The result of this study is in agreement with Parnell and Reimer's result. In Parnell and Reimer study, oligofructose was shown to significantly increase $tAUC_{360mins}$ plasma PYY levels after 12 weeks oligofructose intake, but the effect was not different with the control, maltodextrin (Parnell and Reimer, 2009). Contrary to our observation, Cani *et al.* and Verhoef *et al.* showed that intake of 16 g oligofructose for 14 and 13 days significantly increased plasma PYY levels compared to the control treatment. However, the results of these studies are debateable. In Cani *et al*., a significant increase in plasma PYY levels in the oligofructose group was demonstrated only at 10 minutes after ingestion whilst no clear effects were demonstrated at other timepoints. Furthermore, this effect was compared with the control at individual timepoints whilst no measurement of tAUC was performed (Cani et al., 2009). On the other hand, Verhoef *et al*. showed a significant increase of AUC 0 – 230 mins (morning until lunch)

following intake of oligofructose, but the effect was not maintained until the end of the study day, thus resulting in no significant different in the $tAUC_{420mins}$ (Verhoef et al., 2011).

Evidence suggests that fermentable carbohydrates suppress food intake via increased in plasma PYY secretion (Delzenne et al., 2005). However, the present study showed that the $tAUC_{420mins}$ plasma PYY levels were not related to subjective hunger scores. The lack of relationship between hormonal changes and subjective appetite was reported to be common in human studies (Cani et al., 2009; Parnell and Reimer, 2009; Weickert et al., 2006; Weickert and Pfeiffer, 2008). Nonetheless, intake of oligofructose in this study significantly reduced $tAUC_{450mins}$ subjective appetite scores. Similarly, associations between oligofructose supplementation and modulation of subjective appetite scores have been previously demonstrated (Cani et al., 2006a; Cani et al., 2009). In contrast, Archer *et al*. explained in their study that appetite suppression following intake of fermentable carbohydrates does not necessarily happen during the meal testing time but the effect can be delayed until later in the day. This is due to the extra time needed for colonic fermentation and subsequently increases SCFAs secretion (Archer et al., 2004). The same observation was found by Verhoef *et al*. (Verhoef et al., 2011). On the other hand, other studies showed that neither reduction of energy intake nor modulation of subjective appetite scores were demonstrated after addition of oligofructose in meal replacement bars and beverages (Hess et al., 2011; Karalus et al., 2012; Peters et al., 2009). However, it is suggested that this might be due to the lower oligofructose dose provided in the study (10 g or less per day) compared to 30 g of oligofructose provided in this study.

Consistent with the change in appetite scores, oligofructose was also showed to significantly reduce energy intake at *ad libitum* meal and the effect is possibly resulted from an increase postprandial $tAUC_{420mins}$ plasma PYY levels. The same result was observed in Parnell and Reimer study (Parnell and Reimer, 2009). Unexpectedly, intake of cellulose in the current study also reduced *ad libitum* meal intake (cellulose: 142.27 ± 67.89 kcal; oligofructose: 112.98 ± 33.80 kcal). However, no different between the treatments was found. In contrast, Howarth *et al*. suggested that intake of 27 ± 0.6 g/day cellulose in 11 healthy volunteers with BMI 20.0-34.4 kg/m^2 for 3 weeks had no significant effect on appetite and energy intake (Howarth et al., 2003). The mechanisms of how cellulose reduced energy intake are unclear, but it might be related to an increase in plasma GLP-1 levels. In this study, plasma GLP-1 levels in the cellulose group were significantly increased compared to after

intake of oligofructose. However, this observation was not really unexpected as cellulose is an insoluble fibre, thus it could have its own effect on stimulating GLP-1 secretion. Insoluble fibres in humans act as a bulking agent and help to slow down the transit time (Jenkins et al., 1978), which leads to a decrease in starch absorption (Lewis and Heaton, 1997). In addition, polongation of time for carbohydrate to reach the distal small intestine also resulted in secretion of GLP-1 (Qualmann et al., 1995; Seifarth et al., 1998). Moreover, results from other fermentable carbohydrates human trials also showed conflicting results. Whilst some studies demonstrated a high release of GLP-1 after oligofructose supplementation (Cani et al., 2009; Piche et al., 2003; Verhoef et al., 2011), some oligofructose (Parnell and Reimer, 2009; Pedersen, 2010), inulin (Tarini and Wolever, 2010) and resistant starch (Gee and Johnson, 2005; Weickert and Pfeiffer, 2008) studies showed no significant effect on plasma GLP-1 levels.

In contrast, evidence from rodents' studies showed that the levels of plasma GLP-1 and PYY were significantly increased following fermentable carbohydrates intake (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2006b; Cani et al., 2007b; Gee and Johnson, 2005; Kok et al., 1998). The possible explanation of the discrepancy in findings between human and rodents' plasma GLP-1 and PYY levels might be due to several reasons. Firstly, humans have a low tolerability towards high fibre intake as this could leads to gastrointestinal adverse effects (Kaur and Gupta, 2002). In contrast, rodents are able to tolerate fibre doses up to 10 % of their body weight without any gastrointestinal side effects (Cani et al., 2005b; Peters, 2010). Secondly, plasma GLP-1 and PYY levels in rodents were obtained mostly from portal blood, where these gut hormones are expected to be released in high amounts. However, in humans, this location is not accessible for direct measurement; hence both of the plasma satiety hormones can only be quantified from the periphery, where the lowest amount of gut hormone levels is found. Thirdly, having a large caecum might have been an advantage to rodents to increase fermentation activity. The caecum is an organ in the gastrointestinal tract that involved in microbial fermentation activities in mammals (DeSesso and Jacobson, 2001).

In this study, oligofructose supplementation significantly increased postprandial glucose, but had no significant effect on insulin levels compared to cellulose. This observation is contrary to rodent studies which have consistently shown to reduce postprandial glucose and insulin levels (Cani et al., 2005a; Cani et al., 2006b; Kok et al., 1998; Urias-Silvas et al., 2008). Although there were evidence

to show that inulin-type fructans significantly reduced glucose and insulin levels in humans (Cani et al., 2009; Daubioul et al., 2005; Jackson et al., 1999; Russo et al., 2008), several groups showed no significant effect of inulin-type fructans on both postprandial glucose and insulin levels (Giacco et al., 2004; Parnell and Reimer, 2009; Tarini and Wolever, 2010; van Dokkum et al., 1999). The role of inulin-type fructans on modulating blood glucose levels has been disscused in more details in a systematic review. The authors demonstrated that out of the thirteen studies they have reviewed, only four studies have shown a decrease in serum glucose levels after inulin-type fructans intake and only one of these studies were statistically significant. The nine other studies showed no significant effect on serum glucose levels. This review reveals that the effect of inulin-type fructans on reducing blood glucose in humans remains unclear and needs further investigation (Bonsu et al., 2011).

2.5.2 Self-living Supplementation Period

During the 8-week supplementation period, four volunteers in the oligofructose group showed a mean compliance of 72.9%. However, this does not affected the overall group compliance as oligofructose group was shown to have compliance rate of 89.8 ± 13.1 % whilst 89.7 ± 12.8 % was demonstrated in the cellulose group. Furthermore, assessment of gastrointestinal side effects using VAS questionnaires suggested that both oligofructose and cellulose were well-tolerated. No significant effect was reported with the dosage except for a significant increase in delta change of nausea scores in the oligofructose group when compared to cellulose group. However, the mean scores was less than 1.0 cm (0.45 ± 0.22 cm) and that the increase scores were actually obtained from one volunteer who scored 3.1 cm in the baseline study day and 5.4 cm in the post-supplementation study day. The rest of the volunteers scored nausea below 1.5 cm on both of the study days. Some volunteers also complained of the occurrence of flatulence and bloating, however these were reported as mild and no significant effect was reported.

Generally, dosage of less than 30 g of fibre per day is related to fewer gastrointestinal side effect (Kaur and Gupta, 2002) whilst dosage of 40-50 g/day has been shown to correlate with rumbling stomach and diarrhoea (Briet et al., 1995; Carabin and Flamm, 1999; Marteau and Flourie, 2001). Serious gastrointestinal side effects could discourage the consumption of fibre, which therefore could affect the study's compliance. As a precaution, studies using a dosage of more

than 20 g/day may need to introduce a gut adaptation period, including a lower starting dose of fibre and a stepwise increase to the actual fibre dose investigated. Thus, this would help to reduce gastrointestinal symptoms during the investigation and allow for higher dosages of fibre to be consumed.

Home-supplementation VAS scores showed that hunger was decreased in the oligofructose group compared with cellulose group, but this was not significant. Fullness scores were also not affected by the treatment in both groups. During the supplementation period, volunteers were asked to rate VAS once a day for seven days at baseline and during the supplementation period as a summary of their subjective appetite feeling. Using VAS for measuring subjective appetite has been related to increased variability (Flint et al., 2000; Stubbs et al., 2000) which possibly resulted from methodological and biological day-to-day differences in individuals (Raben et al., 1995). Moreover, differences in the setting of the study might also influence the VAS scores. Appetite scores which were rated during study days in a clinical setting are possibly more robust as volunteers were supervised and in controlled conditions compared to during free-living. Therefore, performing studies in clinical settings is more sensitive to detect and reduce any confounding variables that may influence the result (Livingstone et al., 2000) (e.g. during the appetite study day, meals were provided at a fixed time whilst volunteers might have their meals at their convenience during free-living supplementation period).

Although subjective hunger score was reduced in the oligofructose group, no significant effect on energy intake was demonstrated when assessed using the food diaries. The results showed that energy intake in the oligofructose group was slightly increased (101.92 ± 154.04 kcal) from the baseline whilst there was a reduction in the cellulose group (-46.82 ± 172.57 kcal), but this was not significant. However, as mean energy intake was low for both groups, this indicated that subjects underreported their energy intake. Evidence from the literature suggests that under-reporting is very common in overweight/obese individuals (Briefel et al., 1997; Johansson et al., 1998; Kretsch et al., 1999; Lafay et al., 1997). So it is not a surprise that volunteers in this study underreported their energy intake. Moreover, diet diary data are also not that reliable / accurate in reporting energy intake (Hoidrup et al., 2002; Trabulsi and Schoeller, 2001; Westerterp and Goris, 2002). However, in this study, the lack of change in energy intake is supported by the fact

that body weight did not change either (the effect of oligofructose on body weight is discussed in chapter 3). Adding oligofructose in the diet also might have increased the caloric content on the post-supplementation assessment compared to the baseline assessment. However, cellulose also contained the same amount of calories as oligofructose. Therefore, an increase in calorie intake following supplementation if any, can be found in both groups.

As a conclusion, supplementation of oligofructose in the volunteers' diet significantly increased colonic fermentation which subsequently elevated PYY, but not GLP-1 secretion. It is suggested that PYY suppressed appetite scores and energy intake in the oligofructose group. However, the amount of postprandial plasma PYY levels was insufficient to suppress energy intake when compared to cellulose. Oligofructose also had no significant effect on postprandial glucose and insulin levels. Based on the result of this study, it can be concluded that oligofructose when compared to cellulose has temporal effect on appetite during acute clinical investigation, but the dose levels were insufficient to alter appetite and energy intake over eight weeks free-living supplementation period.

Chapter 3

The Effect Of Oligofructose On Adiposity & Insulin Sensitivity

3 Background

3.1 Introduction

3.1.1 Magnetic Resonance Imaging and Metabolic Disorders

Over the past 10 years, large numbers of studies have used MRI to investigate the relationship between regional body fat distribution and its related metabolic disorders. Indeed, via MRI, it is now known that metabolic disturbances are primarily related to regional body fat, particularly intra-abdominal adipose tissue (IAAT) or visceral fat. IAAT has been shown as the major contributor in the development of insulin resistance, hyperinsulinemia, hypertension, cardiovascular disease and mortality (Carey et al., 1996; Goodpaster et al., 2005; Hayashi et al., 2004; Nicklas et al., 2003; Pouliot et al., 1992; Yamashita et al., 1996). Another related region, abdominal subcutaneous adipose tissue (ASAT) also associates with these diseases, but to a lesser extent compared to IAAT (Demerath et al., 2008; Fox et al., 2007; Goldstone et al., 2001; Kelley et al., 2000; Kuk et al., 2006). This is because IAAT is more metabolically active in secreting hormones and inflammation markers compared to ASAT (Bjorntop, 1996). IAAT is hypothesised to influence hepatic insulin resistance (Cnop et al., 2002; Kelley et al., 2000) by releasing its lipolysis product, free fatty acids. These free-fatty acids are transported to the portal circulation which results in increasing hepatic glucose production, reducing insulin secretion and leads to the development of insulin resistance. In contrast to IAAT, free fatty acids from subcutaneous fat are delivered to the systemic circulation (Gastaldelli et al., 2007; Kershaw and Flier, 2004).

Besides IAAT and ASAT, evidence reveals that saturation of adipose tissue (AT) due to excessive accumulation of fat from energy intake causes the fat to accumulate in other non-adipose tissue organs. This group of fat is known as ectopic fat and it can affect important organs such as liver, pancreas, skeletal muscle, heart and kidneys (Heilbronn et al., 2004). As a consequence, ectopic fat also associated with pathogenesis of various metabolic diseases such as insulin resistance, type 2 diabetes and cardiovascular disease (Lettner and Roden, 2008; Montani et al., 2004; Rasouli et al., 2007; Szendroedi and Roden, 2009).

Looking at the patho-physiological effects of regional and ectopic fat on generating metabolic disturbances, a reduction of body fat distribution is now considered as the main focus in obesity research. Recent evidence demonstrated that a reduction of IAAT repository either via surgical removal (Barzilai et al., 1999; Gabriely et al., 2002) or lifestyle modifications; including exercise (Johnson et al., 2009; Thomas et al., 2000), dietary changes (Gasteyger et al., 2009) or combination of exercise and dietary changes (Idoate et al., 2011) have led to improved insulin sensitivity (Larson-Meyer et al., 2006; Ross et al., 2004). Therefore, increase insulin sensitivity may be used as a predictive tool for regional and ectopic fat reduction following weight loss-related treatments.

Although surgical removal is an effective approach in reducing body fat, it can relate to many adverse side effects. Besides, it is also an expensive procedure compared to a lifestyle modification approach. Therefore, lifestyle modifications are potentially a better option in reducing body fat distribution (Carr et al., 2005; Laaksonen et al., 2003; Slentz et al., 2004; Slentz et al., 2011). A 5 year intervention Insulin Resistance and Atherosclerosis study (IRAS) among 1114 African and Hispanic Americans showed that lifestyle modifications of consistent physical activity and consumption of soluble fibre led to a reduction of visceral fat accumulation. Interestingly, this study also highlights the role of fibres in controlling body fat by suggesting that every 10 g increase of soluble fibres resulted in a 3.7 % reduction of visceral AT (Hairston et al., 2012). This observation is also supported by Davis *et al.* which have shown that increased intake of insoluble fibres in 85 overweight Latino adolescents in a 2-year longitudinal study significantly decreased visceral AT. In addition, they also reported that a slight reduction of 3 g insoluble fibre / 1000 kcal per day resulted in a 21 % increase of visceral fat (Davis et al., 2009).

These studies suggest that dietary fibres have a potential role in modulating body fat distribution and in stimulating insulin sensitivity. On the other hand, the role of isolated dietary fibres on AT metabolism has not been fully explored yet. Interestingly, recent evidence suggests that fermentable carbohydrates may have a significant role in modulating insulin sensitivity (Robertson et al., 2003; Robertson et al., 2005; Robertson et al., 2012) and therefore raising a possibility to be developed as anti-obesity treatment.

3.1.2 Fermentable Carbohydrates, Adipose Tissue Metabolism and Insulin Sensitivity

Evidence suggests that the role of fermentable carbohydrates in regulating adiposity maybe related to its end fermentation products, SCFAs. SCFAs' receptors, FFAR2 and FFAR3 have been shown to be expressed in the AT (Ge et al., 2008; Hong et al., 2005; Le Poul et al., 2003). In addition, acetate and propionate have also been demonstrated to inhibit lipolysis of adipocytes *in vitro* which potentially leads to suppression of plasma non-esterified fatty acid levels in the circulation (Ge et al., 2008). Indeed, intraperitoneal infusion of sodium acetate into wild-type mice significantly decreased non-esterified fatty acid levels as a result of increased circulating plasma acetate levels. This effect has been shown to be abolished in FFAR2-null mice, suggesting that acetate exerts this effect via activation of FFAR2 (Ge et al., 2008). Besides lipolysis, acetate and propionate also have been shown to mediate its effect on adipogenesis via FFAR2. This result highlights the role of FFAR2 in adipocytes development and differentiation (Hong et al., 2005).

Evidence reported that adipocyte size has a negative association with insulin sensitivity, in which the larger the adipocytes, the more they become less-insulin sensitive (Salans et al., 1968; Unger, 2003). Obese / overweight individuals have been shown to have large adipocytes (Salans et al., 1973). Therefore, adipocytes of obese/overweight individuals are more likely to become less-insulin sensitive. It has also been shown that increased size of abdominal adipocyte is strongly associated with whole body insulin resistance and tissue inflammation (Lundgren et al., 2007; Maffeis et al., 2007; Weisberg et al., 2003). Nevertheless, the cell will restore its sensitivity following weight loss (Lofgren et al., 2005; Shadid and Jensen, 2003) via a reduction in adipocyte size.

Therefore, it is proposed that increased circulating SCFAs following intake of high amount of fermentable carbohydrates in the diet could influence AT metabolism by improving insulin sensitivity. Evidence from a mouse study showed that eight weeks of high resistant starch diet lead to significant reduction of total body fat, subcutaneous, visceral and intrahepatocellular. It is suggested that the effect of resistant starch on body adiposity is due to significant reduction of adipocyte size as no change in body weight was demonstrated compared to the control group. Moreover, insulin secretion was also reduced in the treatment group compared to the control group, suggesting that mice-fed high resistant starch are insulin-sensitive compared to the control group (So et al., 2007).

In humans, supplementing 30 g/day resistant starch in ten obese volunteers for four weeks significantly increased insulin sensitivity compared to the control group. This could be explained by reduced non-esterified fatty acid secretion from visceral adipocytes and increased levels of circulating plasma acetate and propionate (Robertson et al., 2005). In another study, intake of 40 g/day resistant starch for twelve weeks in 20 insulin-resistance volunteers led to improved insulin sensitivity, which was positively correlated with a reduction of WC and tibialis fat depot (Johnston et al., 2010). Robertson *et al.* suggested that resistant starch improved insulin sensitivity via peripheral muscle and adipocytes, but not via hepatic metabolism (Robertson et al., 2012).

In contrast to resistant starch, the role of inulin-type fructans on AT metabolism, specifically in humans, is not fully explored. Nevertheless, as inulin-type fructans are also fermentable carbohydrates, it might be possible that both of these fibres have similar mechanisms in regulating body adiposity. Moreover, recent evident showed that adding oligofructose in mice-fed high fat diet for four weeks has been shown to suppress overexpression of FFAR2, derived from accumulation of adipocyte cells following obesogenic diet (Dewulf et al., 2011). This finding highlighted the role of inulin-type fructans in modulating AT metabolism. Studies in rodents showed that supplementing 5% fructooligosaccharides for five weeks significantly reduced visceral fat mass although no significant difference on body weight or food intake were demonstrated when compared to control group. In addition, the beneficial effect of fructooligosaccharides on suppression abdominal fat also lead to improved insulin sensitivity (Shinoki and Hara, 2011). The effect of inulin-type fructans on AT suppression has been demonstrated by other group. Anastasovska *et al.* showed that supplementing oligofructose-enriched inulin in high fat diet in mice led to significantly reduce total, subcutaneous, internal and intrahepatocellular fat depot (Anastasovska et al., 2012).

In humans, Abrams *et al.* showed that 8 g of oligofructose-enriched inulin supplemented for a year as an adjunct supplementation with calcium for pubertal growth in young adolescents reduced BMI, BMI z-score and fat mass compared to individuals who were on calcium treatment alone (Abrams et al., 2007). However, as the body weight change was not the main study outcome, no plasma-related AT and gut hormones were investigated in this study. In another study, the authors showed that the effect of 14 g/day oligofructose on reducing body fat and body

weight in 16 obese school children and 17 obese women for 12 weeks possibly related to significant decreased resistin levels (an adipokine) (Antal et al., 2008). However, no investigation on gut hormone release was performed. Interestingly, Parnell and Reimer showed that the effect of 21 g/day oligofructose for 12 weeks on reducing body weight was related to significant increased AUC plasma PYY, lowered insulin and ghrelin levels. The treatment however, did not affect plasma GLP-1 levels and subjective appetite (Parnell and Reimer, 2009).

To our knowledge, Parnell and Reimer is the only study which closely investigated the effect of supplementing high dose of fructans on body composition in adults over a long term period (Parnell and Reimer, 2009). Moreover, no studies have investigated the role of oligofructose or inulin-type fructans on regional and ectopic body fat distribution. Insufficient data on the effects of supplementing high dose of oligofructose in human adult has therefore encouraged us to investigate the effect of supplementing 30 g/day oligofructose in 22 overweight volunteers for eight weeks (including two weeks run-in supplementation period).

3.2 Aims and Hypothesis

3.2.1 Aims

The aims of this study were to investigate the role of oligofructose supplementation on body weight, total body fat, regional body fat, ectopic fat as well as insulin sensitivity and compare these effects with cellulose supplementation. To achieve these objectives, the following parameters were measured:

- Changes in body weight, waist circumference and waist hip ratio (WHR)
- Change in total and regional body fat distribution
- Change in intrahepatocellular and intramyocellular muscle (soleus and tibialis) fat
- Plasma glucose, insulin, lipid, Homeostatic model assessment – Insulin resistance (HOMA-IR) levels.

3.2.2 Hypothesis

It was hypothesized that supplementing 30 g per day oligofructose for six weeks (following two weeks run-in period) would reduce body weight, total, regional body fat as well as ectopic adiposity, leading to a reduction in insulin and glucose levels, and subsequently decreased insulin resistance.

3.3 Material and Methods

3.3.1 Materials

3.3.1.1 Volunteers

Healthy male and female overweight/obese volunteers that participated in this study were the same volunteers that involved in the appetite study days, described in Chapter 2. The inclusion and exclusion criteria for this study were described in Section 2.3.1.4.

3.3.2 Methods

3.3.2.1 Study Design

This study is a part of a randomised, single-blinded, placebo-controlled study on the effects of oligofructose on appetite, gut hormones, body composition and brain activity. The day before attending the study day, volunteers were asked to avoid any strenuous physical activity and alcohol. They were also required to eat standardized evening meal before the start of fasting at 9 pm for 12 hours until the study day. On the study day, volunteers attended MRC Clinical Sciences Imaging Unit, Hammersmith Hospital at 9 am for whole body composition MRI scans. These were acquired on the baseline (day 0) and post-supplementation study day. Each study visit lasted three hours. The scanning procedures and images analysis were performed by the Metabolic and Molecular Imaging Research group; Prof. Jimmy Bell, Dr. Louise Thomas and Mrs. Julie Filtzpatrick.

Upon arrival, volunteers were asked to complete a metal check form to ensure they were free from any metal prior to the scanning procedure. They were required to change into scrubs before anthropometric measurements were recorded. Volunteers then had their whole body MRI scanning for total and regional fat quantification whilst liver and muscle fat were measured using MR spectroscopy performed on a 1.5 Tesla Philips Achieva MR scanner (Phillips Medical Systems, Best, the Netherlands) in a period of one hour. All of these measurements are described in the next sections.

Upon scanning completion, 30 ml fasting blood samples for glucose, insulin and lipid profile, were withdrawn from volunteers. VAS and blood pressure were assessed at 20 minute before scanning and at 20 minutes post scanning. Volunteers were discharged from the unit at 11 am.

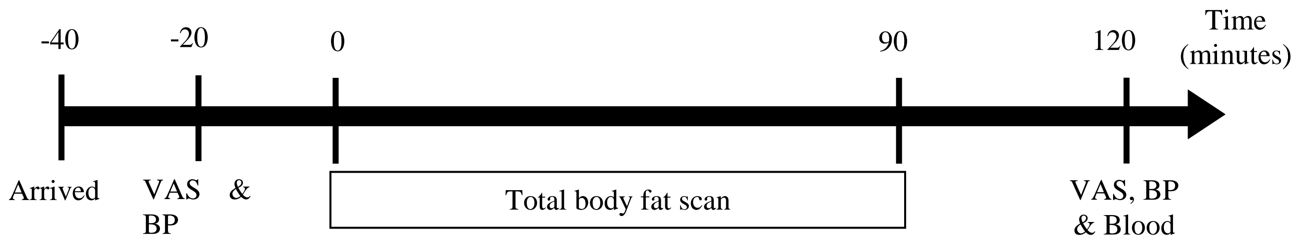


Figure 3.1 Schematic diagram for MRI total body fat scan study day.

3.3.2.2 Anthropometric Measurements

Body weight, waist and hip circumference were measured prior to the scanning. Body weight was measured in volunteers lightly clothed in scrubs and barefoot, to the nearest 0.1 kg whilst height was measured to the nearest 0.1 cm with a Harpenden stadiometer (Holtain Ltd, Crosswell, UK) only on the first visit. Waist and hip circumference were quantified using a tape measure based on the WHO recommendation in which the measurement was performed at midpoint between the distal border of the lower rib and the superior border of the iliac crest (Lean et al., 1996). Hip circumference was measured at the level of the largest circumference around the hip. From these measurements, BMI and WHR were calculated.

3.3.2.3 Body Fat Distribution Measurements

3.3.2.3.1 Magnetic Resonance Body Fat Measurements

Total body and regional fat masses were quantified using 1.5 Tesla Philips Achiva MRI scanner (Phillips, Best, The Netherlands) by using a rapid T1-weighted spin-echo sequence imaging as described elsewhere (Thomas et al., 2012). Volunteers were asked to lie in a prone position on the scanning table with arms straight above their head before they were moved into the magnet for the scanning. Volunteers were scanned from fingertips to toes by, acquiring 10-mm thick

transverse images with 10 mm gaps between slices. The total and regional fat masses were quantified in litres (Lean et al., 1996) and the images were analyzed using SliceOmatic (Tomovision, Montreal, Quebec, Canada).

In measuring body fat distribution (Thomas et al., 1998; Thomas et al., 2012), the following AT depots were quantified:

1- Total adipose tissue = Subcutaneous adipose tissue (SAT) + total internal adipose tissue (IAT)
2- Subcutaneous adipose tissue = Abdominal subcutaneous adipose tissue (ASAT) + non-abdominal subcutaneous adipose tissue (non-ASAT)
3- Total internal adipose tissue = intra-abdominal adipose tissue (IAAT) + non-intra-abdominal adipose tissue (non-IAAT)
4- Total trunk
5- Abdominal subcutaneous adipose tissue (ASAT)
6- Intra-abdominal adipose tissue (IAAT)

3.3.2.3.2 Magnetic Resonance Spectroscopy (MRS) of the Liver

Following whole body fat measurement, ^{1}H MR spectra for liver was obtained from the right lobe of the liver using a PRESS sequence without water saturation (repetition time 1,500 ms, echo time 135 ms) and with 128 signal averages. Transverse images were used in order to ensure accurate positioning of the (20 x 20 x 20 mm) voxel in an area in the liver avoiding blood vessels, the gall bladder and fatty tissue. Spectra were analysed using AMARES (Naressi et al., 2001; Vanhamme et al., 1997). Peak areas for all resonances were obtained and intra-hepatocellular lipid resonances measured relative to water resonance after correcting for T_1 and T_2 (Thomas et al., 2005).

3.3.2.3.3 Magnetic Resonance Spectroscopy (MRS) of the Muscle

Subsequently after intrahepatocellular lipid measurement, spectra of intramyocellular lipid were obtained from the soleus and tibialis muscles using ^{1}H MR as previously described (Thomas et al., 2005). Spectra were obtained from 20 x 20 x 20 mm voxels were placed in the soleus and tibialis muscles of the left calf and avoiding apparent streaky fat and main blood vessels using a

PRESS sequence (repetition time 1,500 ms, echo time 135 ms) and with 128 signal averages. Following the acquisition, intramyocellular were measured with reference to total muscle creatine signals after correcting for T1 and T2 as previously described (Rico-Sanz et al., 1999).

3.3.2.4 Biochemical Analysis

3.3.2.4.1 Fasting Glucose, Insulin and Lipid Profile Analysis

Blood samples for glucose, lipid profile and insulin levels were collected at 120 minutes after the scanning. The samples were analysed in the Department of Clinical Biochemistry, Hammersmith Hospital using either an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK) or an Axsym analyser (Abbott Diagnostics, Maidenhead, UK) respectively. The intra-assay coefficients of variation of these analyses were between 1.0 -5.0% .

3.3.2.5 HOMA-Insulin Resistance Assessment

The level of insulin resistance was calculated based on the HOMA model (Matthews et al., 1985):

$$\text{HOMA-IR} = \frac{\text{Fasting insulin levels } (\mu\text{U/L}) \times \text{ fasting glucose levels (mmol/L)}}{22.5}$$

3.3.2.6 HOMA-Pancreatic B-cell Function Assessment

$$\text{HOMA-B} = \frac{20 \times \text{ fasting insulin levels } (\mu\text{U/L})}{\text{Fasting glucose levels (mmol/L)} - 3.5}$$

3.3.2.7 Statistical Analysis

Data are presented as mean ± SEM. Data were checked for Gaussian distribution using the D'Agostino & Pearson omnibus normality test prior the analysis. Non-normally distributed variables were logarithmically transformed, and non parametric tests were used if normality was not obtained. An ANCOVA with baseline data (day 0), age and gender as covariates were used to determine the effect between oligofructose and cellulose treatments. Effects of treatment within the groups (baseline visit [day 0] vs. post-supplementation [day 56]) were compared using two-tailed paired *t-test* if normally distributed and Wilcoxon signed-ranks test if the data is not normally distributed. A P value of < 0.05 was considered significant. Statistical analysis was performed on Graph Prism 5 (GraphPad Software, Inc., La Jolla, USA) and ANCOVA analysis was performed using SPSS 20.0 (SPSS Inc. Chicago, IL, USA).

3.4 Results

3.4.1 Volunteers Characteristics

Twenty two, [oligofructose=12 (male=4, female=8) and cellulose=10 (male=2, female=8)] healthy overweight and obese volunteers participated in this study. The mean ages were 36.5 ± 2.2 years (range 21 – 49 years) in the oligofructose group and 28.7 ± 2.3 years (range 20 – 47 years) in the cellulose group. The mean BMI were 29.7 ± 1.0 kg/m^2 (range 25.0 – 34.6 kg/m^2) in the oligofructose group and 31.1 ± 1.1 kg/m^2 (range 26.0 – 35.0 kg/m^2) in the cellulose group.

3.4.2 Anthropometric Measurements

3.4.2.1 Body Weight and Body Mass Index

Supplementing oligofructose and cellulose into volunteers' diet for eight weeks had no significant difference on body weight (P=0.743) and BMI (P=0.637) between the groups. No significant difference was also found within the groups, body weight (oligofructose: P=0.392 and cellulose:P=0.474) [Figure 3.2 (i)] and BMI (oligofructose:P=0.321 and cellulose:P=0.505) [Figure 3.2 (ii)].

i) ii)

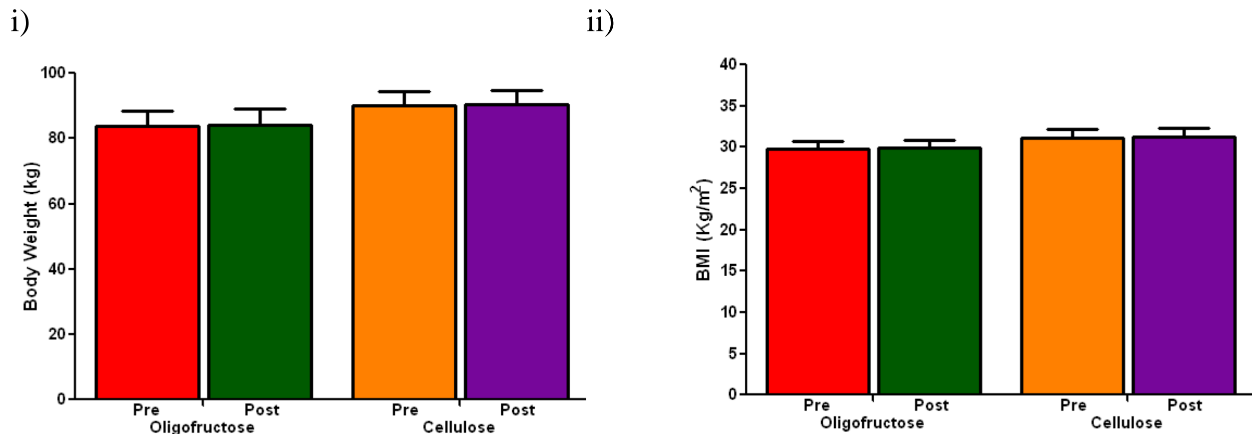


Figure 3.2 Body weight (i) and BMI (kg/m^2) (ii) in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

3.4.2.2 Waist Circumference (WC) and Waist Hip Ratio

Adding oligofructose and cellulose into volunteers' diet showed no significant difference on WC (P=0.920) and WHR (P=0.257) between the groups and also within each groups, WC (oligofructose:P=0.281 and cellulose:P=0.254) [Figure 3.3 (i)] and WHR (oligofructose:P=0.065 and cellulose:P=0.177) [Figure 3.3 (ii)].

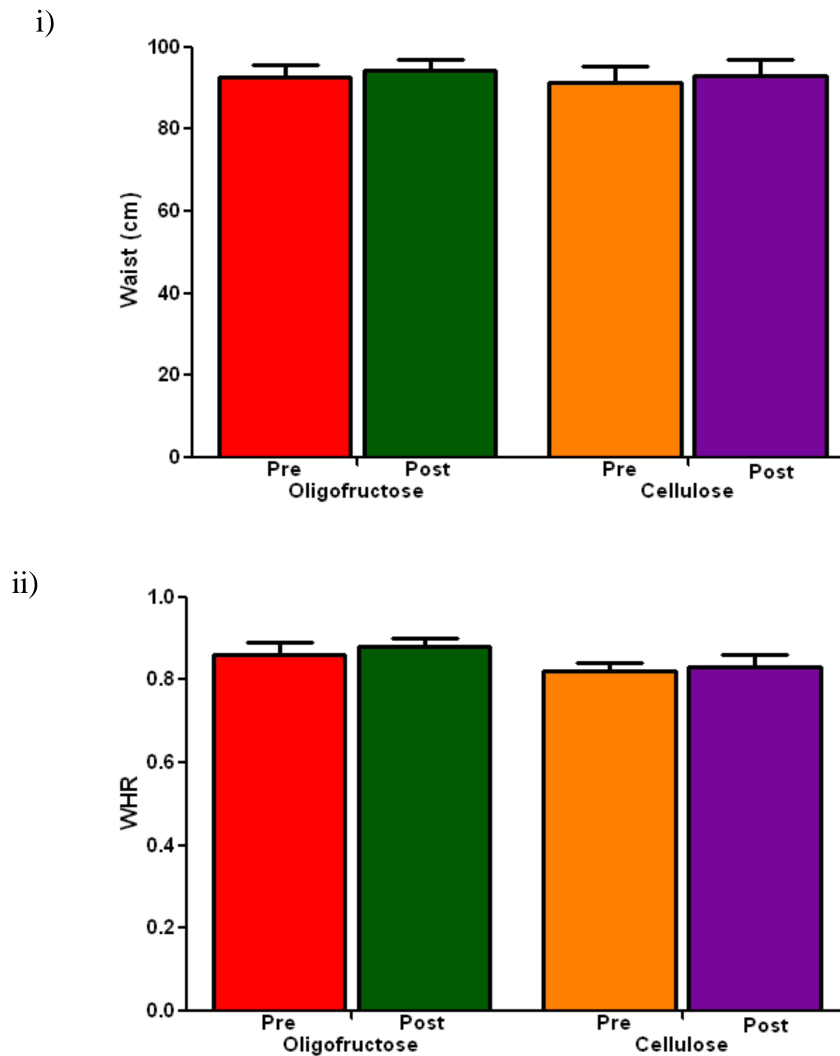
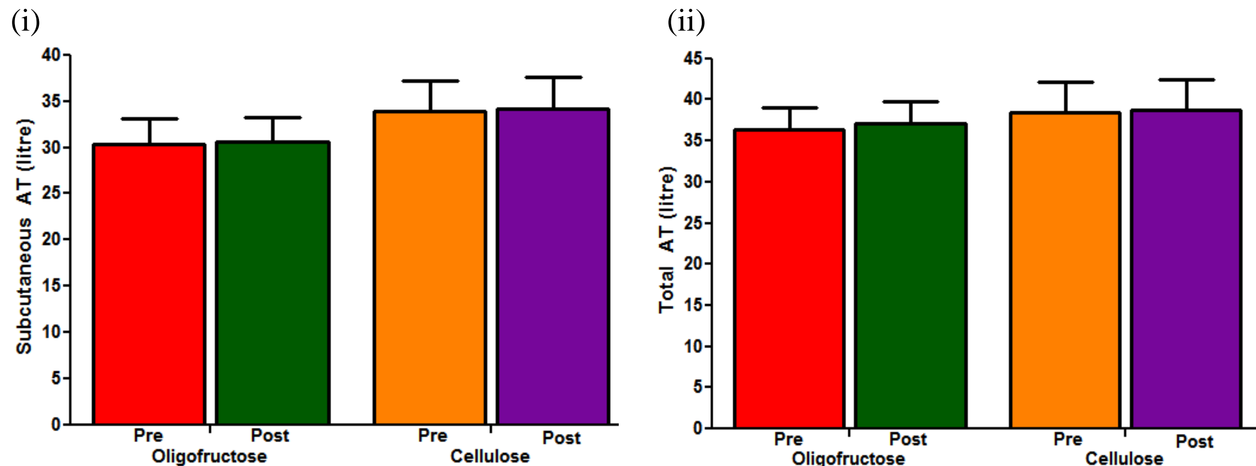


Figure 3.3 Waist circumference (cm) (i) and WHR (ii) in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

3.4.3 Body Composition Measurements

3.4.3.1 Total Body Fat Distribution

Supplementing the diet with oligofructose or cellulose for eight weeks showed no significant effect between oligofructose and cellulose group in reducing total AT (P=0.858) and subcutaneous AT (P=0.914) contents. Similarly, within group analysis demonstrated that both oligofructose and cellulose had no significant trend in reducing total AT (oligofructose:P=0.178 and cellulose:P=0.700) and subcutaneous AT (oligofructose:P=0.566 and cellulose:P=0.592) [Figure 3.4 (i) - (ii)]. Total AT distribution demonstrates that male volunteers in the oligofructose group have high internal AT levels compared with female volunteers (Figure 3.4 (iii) and (iv). Clear separation between male and female volunteers in both groups can also be seen in IAT:SAT ratio. Male volunteers were shown to have high IAT:SAT ratio compared with female volunteers (Figure 3.4 (v) and (vi)).



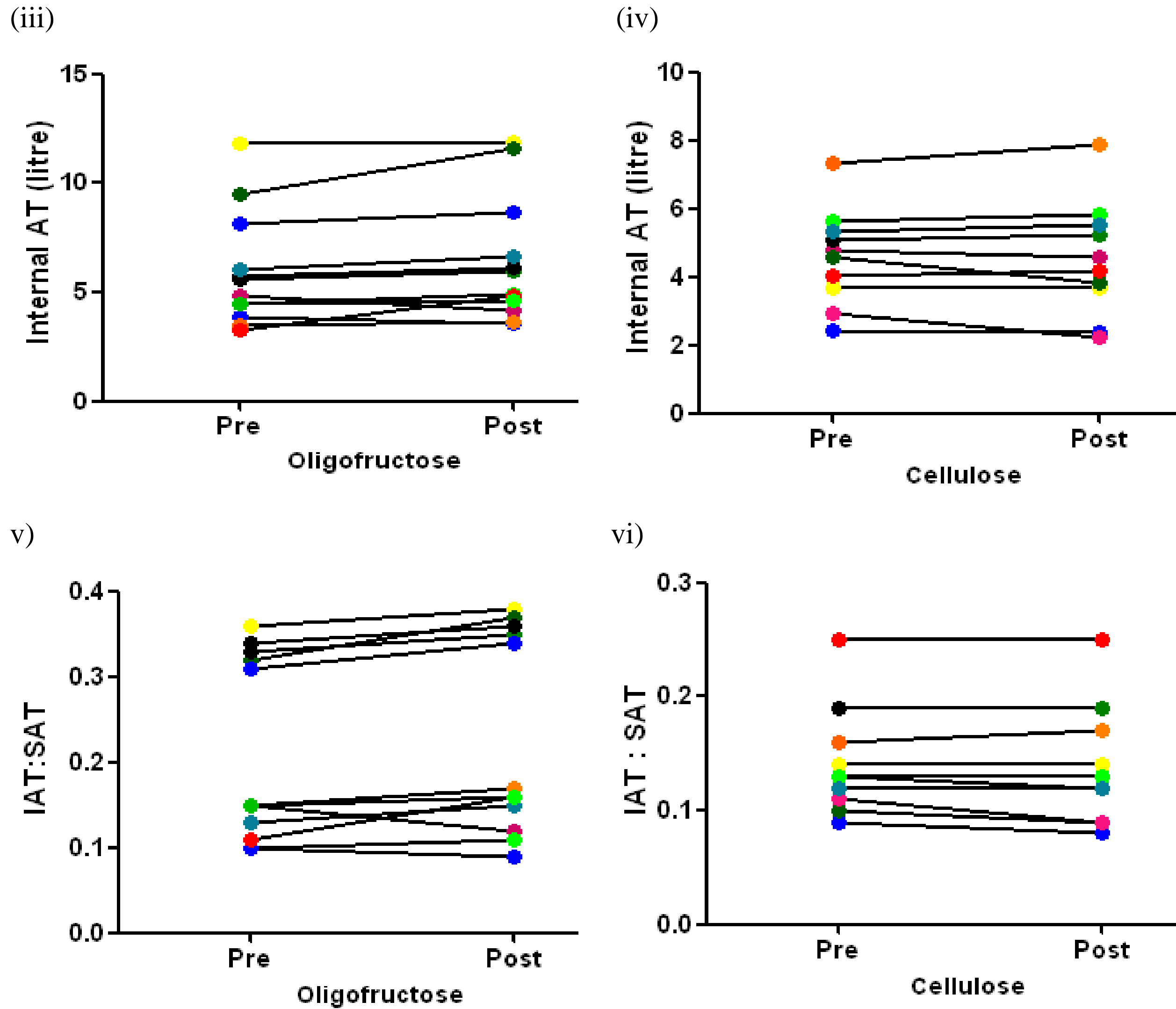
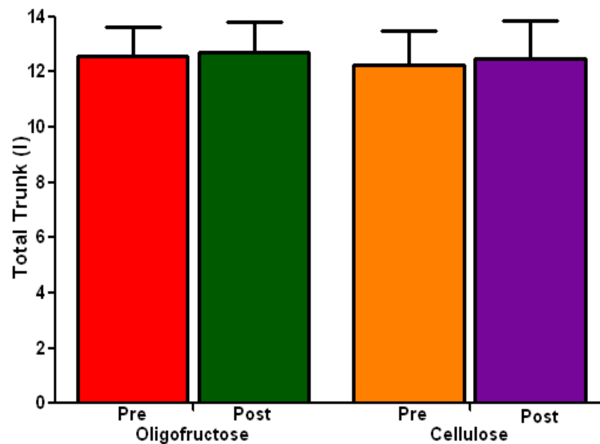


Figure 3.4 Subcutaneous AT (l) (i), total AT (l) (ii), internal AT of oligofructose (l) (iii), internal AT of cellulose (l) (iv), IAT:SAT oligofructose (v) and IAT:SAT cellulose (vi) in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10). Abbreviations: AT = adipose tissue, SAT = Subcutaneous adipose tissue, IAT = Internal adipose tissue.

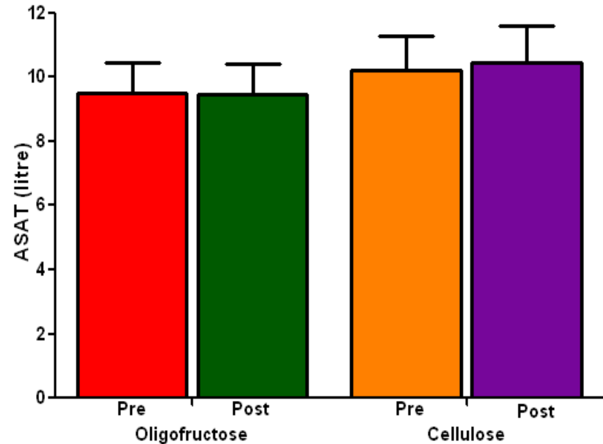
3.4.3.2 Abdominal Body Fat Distribution

Both of the supplements had no significant effect in reducing ASAT within the groups (oligofructose:P=0.923 and cellulose:P=0.114) or between the treatments (P=0.194). Similarly, no significant effect in total trunk was demonstrated (P=0.717) [Figure 3.5 (i) – (ii)]. Oligofructose was shown to significantly increase IAAT when compared to baseline (P=0.043). This significant effect might be influenced by increased IAAT in two male volunteers in this group. No significant effect was demonstrated in the cellulose group (P=0.869) (Figure (iii) and (iv)). Surprisingly, both groups showed that male volunteers (oligofructose = 4 and cellulose = 2) have high IAAT: ASAT ratio compared with female volunteers [Figure 3.5 (v) and (vi)].

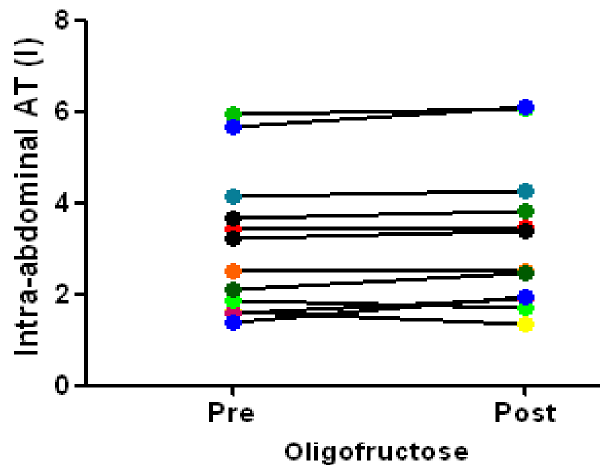
(i)



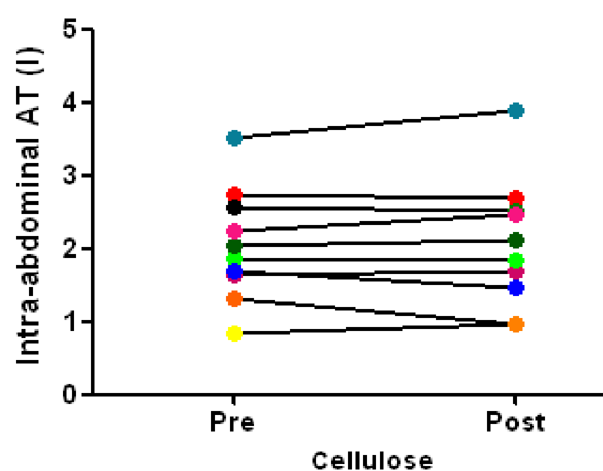
(ii)



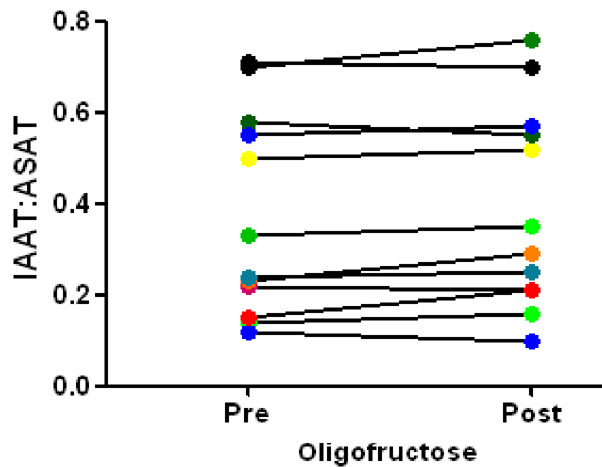
iii)



vi)



v)



vi)

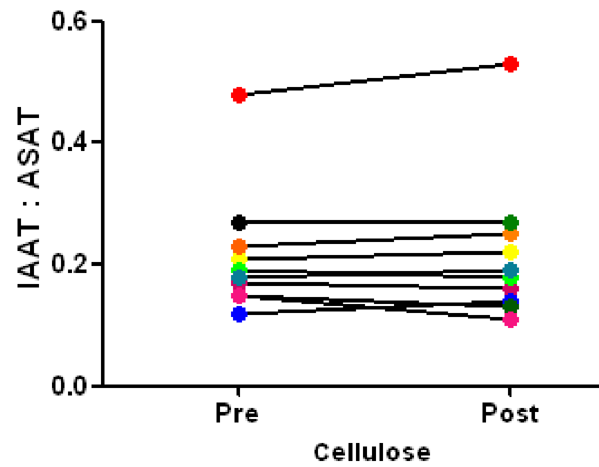


Figure 3.5 Total trunk (l) (i), ASAT (l) (ii), individual IAAT oligofructose (l) (iii) individual IAAT cellulose (iv) IAAT:SAAT oligofructose (v) and IAAT:SAAT cellulose (vi) in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10). *P=0.043 oligofructose *vs.* baseline. Abbreviations: SAAT = Abdominal subcutaneous adipose tissue, IAAT = intra-abdominal adipose tissue.

3.4.3.3 Intrahepatocellular Lipids

Intrahepatocellular levels between volunteers demonstrated a large inter-variation, ranging from 0 to 43.18 litres. Three volunteers in the oligofructose group had high intrahepatocellular lipid levels compared to other volunteers [Figure 3.6 (i) and (ii)]. This high inter-individual variation potentially may have influenced the mean value in the oligofructose group and resulted in no significant change in log transformed intrahepatocellular oligofructose compared to cellulose (P=0.814). There was no significant effect demonstrated in the oligofructose group (P=0.176) and cellulose treatment (P=0.343) although the mean levels of post supplementation in the oligofructose group were reduced compared to baseline, mean ± SEM (baseline:7.2 ± 3.4 and oligofructose:6.4 ± 3.9) and (baseline:0.9 ± 0.3 and cellulose:0.9 ± 0.3) [Figure 3.6 (i) and (ii)].

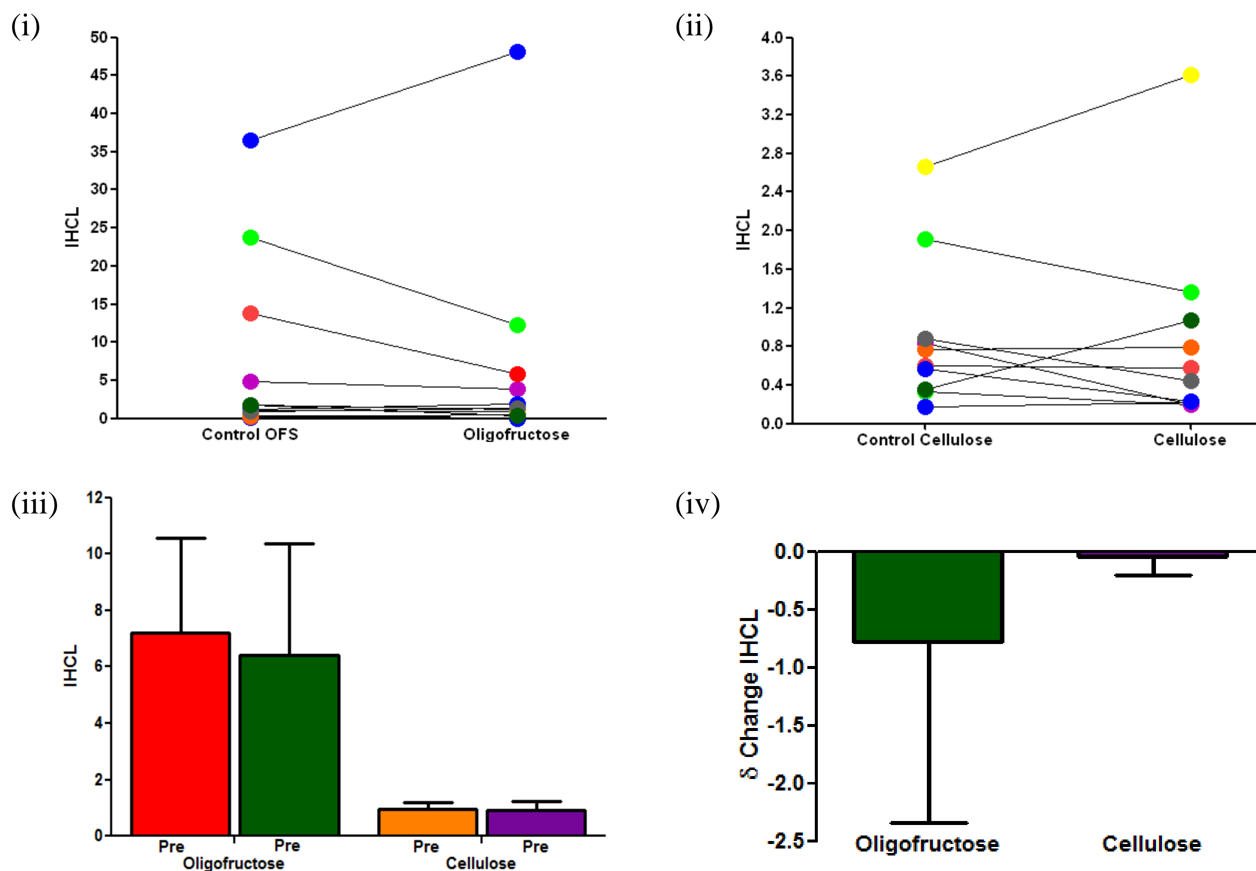


Figure 3.6 Intrahepatocellular fat oligofructose (i), cellulose (ii), mean (iii) and delta change intrahepatocellular (iv) in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

3.4.3.4 Soleus and Tibialis Intramyocellular lipids

Including oligofructose or cellulose supplementation in the diet for eight weeks showed no significant difference on log transformed soleus intramyocellular compared to baseline (oligofructose:P=0.340 and cellulose:P=0.667) [Figure 3.7 (i)]. No significant effect was also demonstrated between oligofructose and cellulose group (P=0.883). However, some volunteers in the cellulose group were demonstrated to have high variation of soleus lipid. Therefore, this may have influenced the lowering mean values of soleus intramyocellular in post-supplementation data of cellulose group, mean ± SEM; 19.3 ± 8.9 (baseline) and 15.5 ± 4.9 (post-supplementation) compared to mean values in the oligofructose group, mean ± SEM; 14.1 ± 3.6 (baseline) and 14. 9 ± 3.3 (post-supplementation) No significant difference was demonstrated in tibialis intramyocellular analysis between the groups (P=0.366) [Figure 3.7 (ii)].

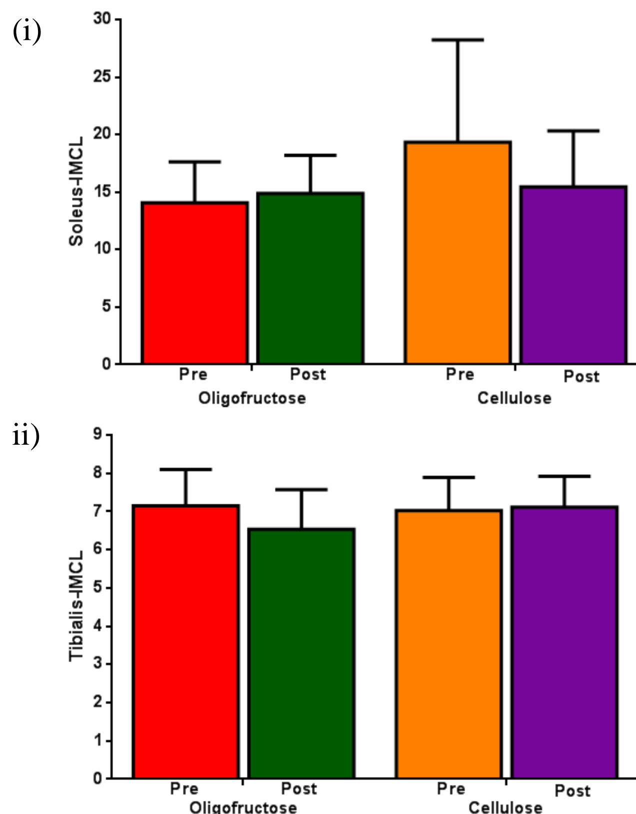


Figure 3.7 Soleus intramyocellular (i) and tibialis intramyocellular (ii) lipid levels in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

3.4.4 Biochemical Analysis

3.4.4.1 Fasting Plasma Glucose and Insulin Levels

Figure 3.8 demonstrates fasting plasma glucose (i) and plasma insulin (ii) levels. Intake of oligofructose supplementation showed no significant effect on both fasting plasma glucose levels (P=0.986) and fasting plasma insulin levels (P=0.770) between the groups. Only 11 volunteers from the oligofructose group were involved in this analysis as data from one volunteer in the oligofructose was excluded due to a haemolysed sample.

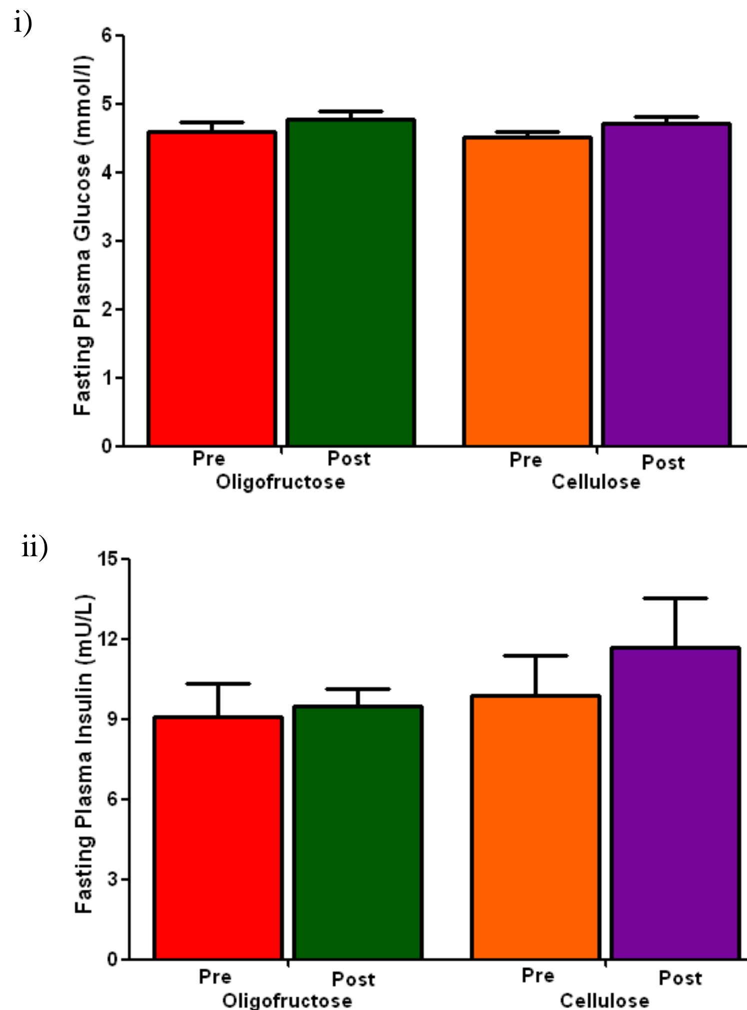


Figure 3.8 Plasma glucose (mmol/l) (i) and plasma insulin levels (mU/L) (ii) in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

3.4.4.2 HOMA-IR and HOMA-B

Figure 3.9 demonstrates HOMA-IR (i) and HOMA-B (ii) levels. Adding oligofructose into volunteers' diet showed a trend toward higher HOMA-IR (P=0.058) but no significant effect on HOMA-B (P=0.992) levels [Figure 3.10 (i) and (ii)] was found. However, cellulose supplementation significantly increased HOMA-IR levels compared to baseline (P=0.049) [Figure 3.10 (i)]. However, the effect was not significant between these groups, (P=0.880). No significant effect was found in HOMA-B levels, (P=0.861) [Figure 3.9 (ii)].

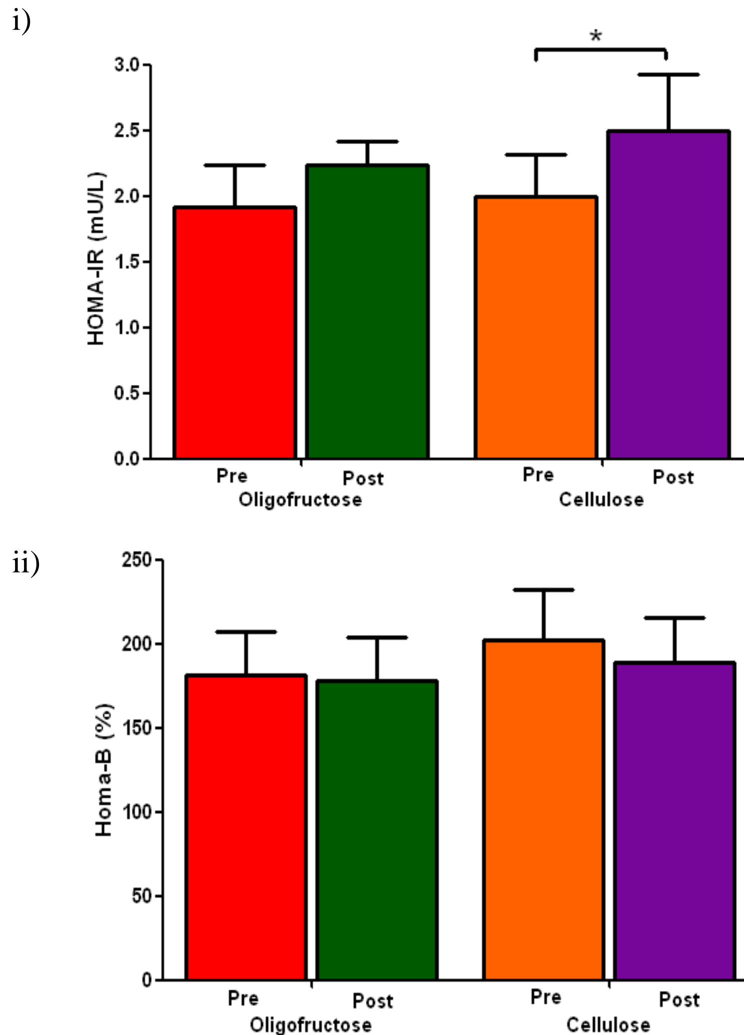


Figure 3.9 HOMA-IR (i) and HOMA-B (ii) levels in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10). *P=0.049 cellulose vs. baseline. *P<0.05 cellulose *vs.* control.

3.4.4.3 Lipid Profile

Figure 3.10 demonstrates plasma lipid profile levels in delta change. No significant difference was demonstrated in cholesterol levels between oligofructose and cellulose group (P=0.746) (0.02 ± 0.12 mmol/L and 4.65 ± 0.89 mmol/L respectively). In contrast, intake of oligofructose appeared to reduce triglycerides, HDL and LDL compared to the cellulose group. However, the effect was not significant (P=0.391, P=0.847 and P=0.671 respectively).

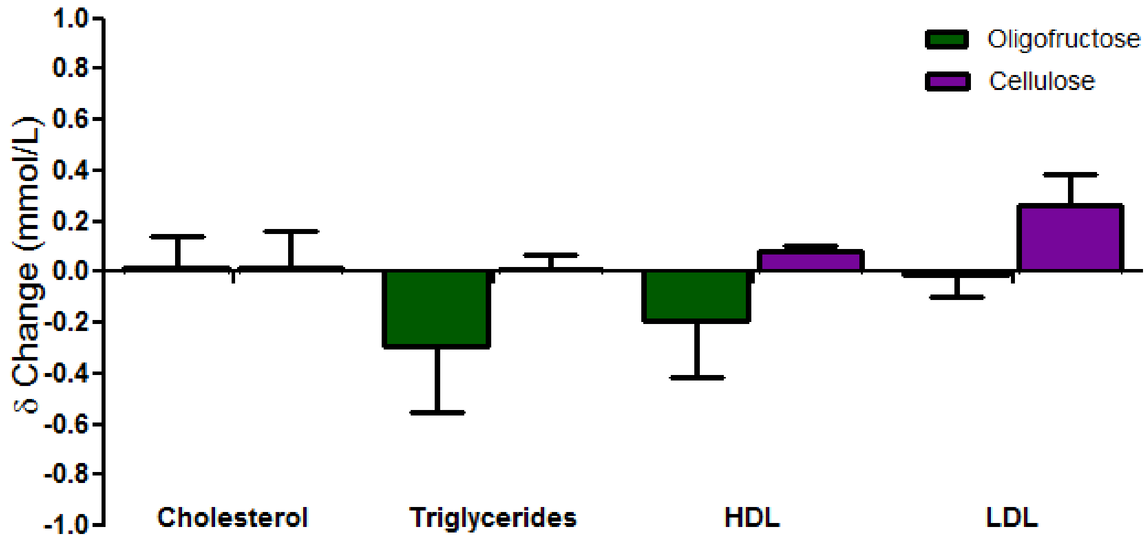


Figure 3.10 Delta change plasma lipid profile levels in oligofructose and cellulose groups. The supplementations were taken thrice daily during meal intake (10 g each time) for six weeks following two weeks run-in supplementation period. Assessment was performed at baseline and 8 weeks post-supplementation period. Data is expressed as mean ± SEM. oligofructose (n=12), cellulose (n=10).

3.5 Discussion

This chapter discusses the effect of supplementing 30 g/day oligofructose into 22 volunteers' diet for six weeks following two weeks run-in supplementation period on body fat distribution (total, abdominal and ectopic body fat distribution), insulin resistance and its related plasma metabolites compared to cellulose supplementation in a randomised, single-blinded, placebo-controlled study. The results of this study did not support the hypothesis that oligofructose would have beneficial effects on body weight, body adiposity and plasma metabolites. In this study, no significant effect on reducing body weight, BMI, WC, WHR, total body fat (total AT, subcutaneous AT, internal AT), regional body fat (ASAT and IAAT) and ectopic fat (intrahepatocellular, soleus and tibialis intramyocellular lipid) were demonstrated. This was surprising as evidence from rodent studies showed positive effects of fermentable carbohydrates on reducing total, intra-abdominal, subcutaneous, and intrahepatocellular AT (Shinoki and Hara, 2011; Anastasovska et al., 2012).

However, rodent studies typically used high dose that humans are unable to tolerate. Therefore, it is unlikely to replicate these findings in humans. The effect on suppressing body weight in rodents usually demonstrated following addition of 10% of their total dietary intake with fermentable carbohydrates (Cani et al., 2005b; Johnson and Gee, 1986; Peters et al., 2011). However, this effect was only demonstrated when fermentable carbohydrates are added to high fat diets (Anastasovska et al., 2012; Cani et al., 2005b; Delzenne et al., 2007) as the effect was abolished when fermentable carbohydrates are included in a normal diet (Anastasovska et al., 2012; Cani et al., 2005b). In contrast, 30 g/day oligofructose supplemented in the volunteers' diet in this study corresponds to ≈ 5% of rodents' total dietary intake (Jenkins et al., 1999), half the dose used in rodent studies. There is evidence to suggest that humans are only able to tolerate a maximum dose of 30 g/day. Beyond than that amount, intake of fermentable carbohydrates are highly correlated with gastrointestinal side effects (Briet et al., 1995; Carabin and Flamm, 1999; Marteau and Flourie, 2001). Therefore, it is not possible to implement the same dose from rodents study to humans' diet.

In humans, current evidence is insufficient to support the role of oligofructose on body weight maintenance. Until now, only a few publications have shown that oligofructose significantly

reduces body weight (Abrams et al., 2007; Antal et al., 2008; Parnell and Reimer, 2009). Parnell and Reimer showed that 21 g/day oligofructose for 12 weeks significantly reduced 1.03 ± 0.43 kg body weight in 48 healthy overweight and obese volunteers. In another study, Antal *et al*. demonstrated that 14 g/day oligofructose in combination with a hypocaloric diet of 33 obese young adolescents and women also significantly decreased BMI and percentage of body fat.

There are a number of factors that have resulted in discrepancy between the current study and these studies. Whilst these studies comparing the effect of oligofructose with maltodextrin as their study control, this study comparing intake of oligofructose with cellulose, a non-fermentable fibre. However, 13g maltodextrin was also added to 30 g cellulose in order to provide equal caloric content with oligofructose. Maltodextrin is a digestible carbohydrate with α-1-4 linkages and a glycaemic index of 110 (Foster-Powell et al., 2002). Meanwhile, oligofructose is a fermentable carbohydrate and not digested in the small intestine due to the lack of α- linkages, which subsequently transported to the large bowel for colonic fermentation. Unlike maltodextrin, cellulose has similar digestive properties with oligofructose. As a fibre, it is also able to escape small intestinal digestion and transported to the large intestine for stool bulking. Furthermore, cellulose is also fermented in the large intestine (Topping and Clifton, 2001), but to a lesser extent compared to oligofructose. Therefore, physiological properties of both treatments in the large intestine able to promote satiety signals at different part of the gut, thus this potentially the reason why there was no significant effect on body weight and body fat between the treatments. However, this hypothesis was not proven as the post-supplementation body weight and body fat were not significantly different compared to baseline (day 0) measurement.

Currently, studies comparing the effect of oligofructose with non-fermentable carbohydrates are lacking. Apart from this study, Howarth *et al.* was the only study in humans which has investigated the role of fermentable carbohydrate on body weight with a non-fermentable carbohydrate. Similarly, the authors also demonstrated no significant difference between fermentable carbohydrates, pectin and β-glucan with a non-fermentable carbohydrate, cellulose on appetite, energy intake and body weight in 11 healthy adults with BMI 20.0 – 34.4 kg/m^2 for three weeks. The lack of effect of fermentable carbohydrate in this study could possibly be due

to short term period (three weeks). Thus, the findings of this study cannot be used to evaluate the role of fermentable carbohydrate on promoting body weight loss (Howarth et al., 2001).

However, increasing the study period from three weeks to eight weeks in the present study also showed no significant effect on modulating body weight and body fat. It seemed that eight weeks supplementation period may not long enough for oligofructose to exert its effect on body weight loss. To date, there is still no consensus to suggest the minimum duration needed for fermentable carbohydrates to exert its effect on reducing body weight. Nevertheless, Parnell and Reimer and Antal *et al*. showed that body weight can be suppressed after 12 weeks supplementation (Antal et al., 2008; Parnell and Reimer, 2009). In contrast, 12 weeks supplementation of 40 g/day resistant starch showed no significant effect on reducing body weight, abdominal, liver and muscle fat after for in insulin resistance volunteers. However, the study found a correlation between reduced WC as well as tibialis muscle fat with insulin sensitivity following the intervention (Johnston et al., 2010). However, volunteers in Johnston *et al*. were patients with metabolic syndrome whilst the volunteers in this study were relatively young healthy overweight volunteers, thus this might have different effect between the groups. Interestingly, a year supplementation with 8 g/day oligofructose with calcium in 97 young school children was also showed to suppress body weight gain during their pubertal growth although the study was not initially designed to evaluate body weight change (Abrams et al., 2007). Based on the findings from these studies, it is postulated that fermentable carbohydrates require more than 12 weeks to modulate body weight and AT metabolism.

Additionally, it is also possible that the lack of effect of oligofructose on body weight in the present study is attributed to the small sample size. Whilst the effect of oligofructose on body weight in the current study were performed in 22 volunteers, the effect of oligofructose on body weight in the previous studies were demonstrated in 33 volunteers (Antal et al., 2008), 48 volunteers (Parnell and Reimer, 2009) and 97 volunteers (Abrams et al., 2007). Therefore, it is not impossible that the current study may have low statistical power to detect a significant difference between the treatments.

Despite of the study showed no significant change in body weight and body adiposity, the result reveals that using MRI for assessing body adiposity showed large inter-individual variations, particularly in male volunteers in the intrahepatocellular fat, internal AT, IAAT, IAT:SAT and IAAT:ASAT ratio although there were known as healthy volunteers. It is widely known that men and women have different body fat distribution, with men normally have fat deposition around mid and upper body (central adiposity) and women are more associated with high fat distribution in the lower body area, particularly in the hip and thigh areas. Based on the different fat composition between gender, therefore, it is wisely to separate the analysis. However, because of limited number of male volunteers in this study (oligofructose = 4 and cellulose = 2), it is impossible to show gender effects on body adiposity on separate analysis. Nevertheless, separation between the genders within each group could still be seen in the aforementioned regions, particularly in IAT:SAT and IAAT:ASAT ratio (Figure 3.4 (v) - (vi) and Figure 3.5 (v) – (vi)), i.e. all males in the oligofructose and cellulose group have relatively high body fat distribution compared to female volunteers. It might be possible that the gender effect could be the confounding factor in determining the effect of oligofructose on body adiposity. Perhaps the effect of oligofructose in reducing body fat would be better explained if the volunteers are divided into each gender. The role of gender in modulating intra-abdominal fat storage has been discussed more details in (Thomas et al., 2012).

In dietary intervention studies, low compliance also can reduce the efficacy of supplement/treatment. In the current study, although four volunteers in the oligofructose group reduced compliance to 60 – 77% due to mild gastrointestinal side effects in the first two weeks following increased supplementation from 20 g/day to 30 g/day, this does not affect group mean compliance (Section 2.4.1.2). Both treatment groups in the current study were demonstrated to have compliance with ≈ 90% (oligofructose = 89.8 ± 13.1% and cellulose 89.7 ± 12.8%). Therefore, the non-significant effect of oligofructose on body adiposity is not related to low compliance. Another possible explanation for no effect on body weight can be related to changes in energy expenditure. In this study, volunteers were advised to maintain their usual physical activity levels throughout the study period and avoided any extreme physical activities programme in order to prevent any bias outcome. As the physical activities were not measured during the intervention period, hence it is unconfirmed whether this has changed. However, as

body weight and adiposity were not changed during the intervention period, it is most likely that no significant modification on physical activities have been made.

Evidence from previous studies showed that intake of inulin-type fructans led to improved lipid profile, reduced glucose and insulin levels as well as insulin sensitivity in both rodent and human studies (Beylot, 2005; Daubioul et al., 2005; Robertson et al., 2003; Robertson et al., 2005). Interestingly, a recent investigation by Johnston *et al*. demonstrated improved insulin sensitivity following intake of 40 g/day resistant starch in 12 weeks of metabolic syndrome volunteers and associated with change in WC, tibialis fat depot as well as IAAT:ASAT ratio (Johnston et al., 2010). Contrary to these results, adding oligofructose into volunteers' diet in this study did not have any significant effect on lipid profile, plasma glucose and insulin levels as well as insulin resistance markers, HOMA-IR and HOMA-B. However, these results were predictable as no significant modulation on body weight and body adiposity were found in this study.

Although the exact mechanism is still unclear, it is suggested that fermentable carbohydrates improved insulin sensitivity via its end products, SCFAs (Robertson et al., 2003; Robertson et al., 2005). SCFAs, which have been delivered to the periphery following colonic fermentation have been shown to reduce lipolysis (Crouse et al., 1968; Robertson et al., 2005) in adipose tissue thus result in decrease circulating non-esterified fatty acid levels in the periphery (Venter and Vorster, 1989; Wolever et al., 1989). Interestingly, Robertson *et al*. in their very recent study demonstrated that intake of 40 g/day resistant starch for 8 weeks in insulin resistance volunteers significantly reduced HOMA-IR, which is a marker for insulin sensitivity. The authors suggested that resistant starch improved insulin sensitivity by modulating adipose tissue and muscle fat depot but not through hepatic metabolism. This suggestion is made due to the significant elevation of the genes expression of lipase, lipoprotein lipase and perilipin, enzymes that involved in regulating adipose tissue differentiation (Robertson et al., 2012).

In conclusion, the results of this study showed that supplementing 30 g/day oligofructose for six weeks following two weeks run-in supplementation period, had no significant effect on modulating AT metabolism, glucose, insulin and lipid profile levels in healthy, overweight and obese volunteers when compared with cellulose supplementation. Based on the promising result

demonstrated by other fermentable carbohydrates studies which showed that 12 weeks are needed to modulate AT metabolism, therefore it might be possible that duration of > 12 weeks are needed to alter adiposity. Further work is required to ascertain whether these findings can be achieved using oligofructose supplementation.

Chapter 4

The Effect of Oligofructose on Brain Reward Regions

4 Background

4.1 INTRODUCTION

It is generally accepted that appetite and feeding behaviour are controlled by two complex systems which are the homeostatic and hedonic networks. The homeostatic system is centred around the hypothalamus, where peripheral signals about the body's energy status are assembled and subsequently regulated appetite and energy expenditure. On the other hand, hedonic network is a higher network of mesolimbic structures, where the rewarding properties of food are encoded. Hedonic or 'non-homeostatic' pathways are influenced by external factors such as food cues (sensory properties and palatability), emotional, learning, cognitive and environmental influences (Berthoud, 2011). Although traditionally it has been thought that obesity is caused by the disruption of the homeostatic network *per se*, modern functional MRI has led to a re-evaluation of hedonic network that also plays a significant role in causing obesity. In addition, fMRI also has highlighted the importance of the mesolimbic reward system in humans. The latest evidence now suggests that these homeostatic and non-homeostatic processes do not work in isolation, but have a concerted role in the control of feeding (Taghva et al., 2012).

In functional neuroimaging studies, positron emission tomography (PET), single photon emission computed tomography (SPECT), fMRI and manganese-enhanced MRI (MEMRI) have been used to investigate changes in brain activation with factors that modulate feeding behaviour. However, PET, SPECT and MEMRI pose several disadvantages that limit its application as an assessment tool in humans. Firstly, PET and SPECT require the intravenous use of radioactive biological tracers. Secondly, these tracers have to be produced in a specific device known as a cyclotron, an expensive and high maintenance machine. MEMRI uses Mn^{2+} ions as a contrast medium (manganese ions are preferentially taken up into activated neurones), so its use is strictly limited in humans because of toxicity. fMRI has been known as the best technique for performing repeated measures of brain activity because of its lack of invasiveness and no exposure to ionising radiation.

4.1.1 functional Magnetic Resonance Imaging

fMRI is an adaptation of the structural MRI scanning technique, to create images of brain regions related to localised neuronal activities following stimulation of cognitive, sensory and motor tasks (Huettel et al., 2009) (principle of MRI is discussed in section 1.8.1.3). fMRI and MRI share the same technique, nuclear magnetic resonance to image nuclei of atoms in the body. However, although both of the techniques were built on the same principle, MRI is mainly used to image tissue structures whilst fMRI is used to evaluate the function of localised brain areas. The major fMRI paradigm in use today localises neuronal activity in response to a stimulus, by measuring the change in blood flow to that region (Huettel et al., 2009; Kristensen et al., 2009).

4.1.1.1 Blood-Oxygenation-Level-Dependent fMRI

In recent years, BOLD-fMRI has been widely used as an assessment tool in evaluating brain activity in appetite-related neuroimaging studies. BOLD fMRI applies an imaging paradigm which detects changes in localised blood flow. It is based on the fact that oxygenated and deoxygenated bloods have differential effects on the localised magnetic field. When brain tissue (collections of neurones) is activated or stimulated with sensory, motor or cognitive stimulation, it requires more oxygen supply than normal, which consequently increases blood flows to that area. However, the physiological response always provides a greater increase in blood flow than is actually required. As a result, there is an increase in the ratio between oxygenated and deoxygenated blood in the activated region. Deoxyhaemoglobin is paramagnetic. It creates local field inhomogeneities which result in a quicker decay of the T2 signal. Thus, in activated brain areas, where the level of deoxyhaemoglobin is relatively lower (because of the physiological overshoot in blood supply greater than the local metabolic demand), there is greater MRI signal on a T2-weighted protocol (Huettel et al., 2009).

4.1.2 fMRI Studies and Appetite Control

The main objective in fMRI appetite research is to investigate brain activity that is associated with cognition, motivation and behaviour to stimulate or terminate the eating process. This can

be achieved via several methods including whole brain exploration, resting state scans or investigation in pre-selected regions of interest (ROI) brain region analysis, which is the most common method used in fMRI appetite-related experiments. ROI analysis is an approach in which the signals are extracted from specific brain regions that is pre-determined prior the analysis. It is preferably chosen for fMRI studies potentially due to its high chances to detect statistically different result in comparison with whole-brain analysis. Nevertheless, choosing ROI analysis also can increase chances of missing significant signals from other excited brain areas that were excluded from view (De Silva et al., 2012).

In fMRI, stimulations including olfactory, gustatory or visual images of food are typically studied either via block designs or event-related designs. In block designs, two or more alternating sets of stimuli are presented to volunteers in separate runs whilst event-related designs presents stimuli as individual events in a mixed, pseudo-randomised order, hence individual evaluation on each single stimuli can be performed. In appetite studies, the most common paradigm used are visual food cues. It is usually presented in groups such as high calorie and low calorie, or high palatability and low palatability, and the effect/changes in brain activation are typically investigated in two different nutritional states (i.e. fasted vs. fed) or intervention (i.e. before vs. after intake of tested meals or gut hormone infusion).

By using visual food images in BOLD-fMRI experiments, it is now known that activation of brain regions within reward systems (i.e. NAc, amygdala, insula, OFC and etc.) is highly related to the nutritional state, most notably in the fasting state. LaBar *et al.* was among the earliest group to investigate the effect of viewing food pictures vs. non-food pictures on amygdala and corticolimbic system during fasted and fed conditions. They found that the amygdala, parahippocampal gyrus and fusiform gyrus were highly activated during the hunger state (LaBar et al., 2001). Since then, other brain areas have also been shown to be activated during hunger condition, such as the ACC, superior temporal gyrus, insula, OFC, striatum, and the dorsolateral prefrontal cortex (Schur et al., 2009; Goldstone et al., 2009; Fuhrer et al., 2008; St-Onge et al., 2005). Interestingly, increased activation in the amygdala, ventrial striatum, insula and medial OFC during the fasting state was shown to be blunted when the experiments were repeated in the fed state/satiety condition (Goldstone et al., 2009; Fuhrer et al., 2008; LaBar et al., 2001; Farooqi et al., 2007).

Most notably, viewing high calorie food images during fasted state was shown to induce greater activation in several brain regions including the insula, ventral striatum, amygdala, medial OFC, medial prefrontal cortex and the dorsolateral prefrontal cortex compared to when viewing low calorie food images (Goldstone et al., 2009; Killgore et al., 2003; Siep et al., 2009). In contrast, viewing low calorie foods has been shown to attenuate activation in the aforementioned ROI areas (even in the fasted state), but it is also suggested to induce greater activation in the somatosensory areas possibly due to lesser cephalic phase signals by classical conditioning to less appealing food images (Killgore et al., 2003).

However, these observations were performed in healthy lean volunteers. Therefore, it is also of interest to investigate the neuronal responses in obese/overweight subjects and whether these subjects respond differently to normal lean individuals in response to food cues. Rothemund *et al*. investigated the effect of exposing obese women (BMI>31) and lean women (BMI 19-24) to food images (high calorie and low calorie food pictures) and non-food images. They found that obese women had augmented activation in the caudate/putamen, anterior insula, claustrum, posterior cingulate, hippocampus and lateral OFC following viewing high calorie food pictures vs. non-food pictures (Rothemund et al., 2007) during the fasted state. In agreement with this, heightened activation of the NAc / ventral striatum, medial and lateral OFC, insula, amygdala and arterior cingulate cortex following viewing of high calorie foods vs. non-food images were also demonstrated in 12 fasted obese and 12 lean women (Stoeckel et al., 2008). Based on these studies, it has been suggested that a hyper-reactive reward system in response to high calorie food cues contributes to the pathogenesis of obesity.

Unexpectedly, evidence suggests that brain activation in obese volunteers' does not just increase during fasting/premeal condition, but also during satiety/postmeal. Martin *et al*. showed that when viewing food images, obese volunteers had augmented activation in the ACC (premeal) and medial prefrontal cortex before and after intake of 500 kcal meal compared with healthy lean volunteers (Martin et al., 2010). This observation was also supported by Dimitropoulos *et al*. In this study, increased activation was demonstrated in the anterior prefrontal area following viewing food stimuli when fasted. In contrast, eating has led to heightened activation in frontal, temporal and limbic areas (Dimitropoulos et al., 2012).

An interesting study of women who had increased body weight over a six month period showed an attenuate postprandial striatal BOLD fMRI signal when BMI had gone up. The authors suggested that weight gain potentially reduced striatal activation as a consequence of down-regulation of dopamine D2 receptors (Stice et al., 2010). Lack of dopamine expression D2 receptors in obese subjects also has been shown in PET studies (Wang et al., 2001). Therefore, decreased levels of dopamine D2 in obese subjects may be another mechanism that causes overeating syndrome in humans (De Silva et al., 2012).

Evidence suggests that gut and adipose tissue-derived hormones have a significant role in the initiation and termination of energy intake. Whilst there are many studies have shown their role in systemic regulation, the role of these hormones on regulating neuronal activity remains unclear. The orexigenic hormone, ghrelin and anorectic hormones, leptin, insulin, PYY and GLP-1 have been postulated to influence neuronal response towards appetite-related stimuli. Evidence suggests that intravenous infusion of ghrelin increases activation in the amygdala, OFC, insula, prefrontal cortex and striatum after viewing food images vs. non-food images, alongside an increase in subjective hunger scores (Malik et al., 2008). On the other hand, viewing food pictures following leptin treatment not just decreased activation in the accumbens-caudate and putamen-globus pallidus areas (Farooqi et al., 2007), it also reduced activation in the insula, parietal and temporal cortex in leptin-deficient patients (Baicy et al., 2007). In another study, administration of intranasal insulin in nine healthy lean volunteers has been shown to reduce activation in the fusiforum gyrus, hippocampus, temporal superior cortex and frontal middle cortex (Guthoff et al., 2010). Based on these studies, it is suggested that circulating leptin and insulin reduce the subjective reward value of food and also motivation to eat (Palmiter, 2007).

It is widely known that PYY and GLP-1 are co-secreted together from the gut's L-cells. Recently, infusion of PYY_{3-36} has been shown to significantly alter activation in the hypothalamus and OFC following resting BOLD-signals study. The authors showed that the positive correlation between energy intake and BOLD hypothalamic activation was transformed into negative correlation when PYY_{3-36} was administered. The result of this study suggested that the existence of PYY_{3-36} in the system has shifted the regulation of energy intake from a

homeostasis (hypothalamus) to a hedonic region (OFC) (Batterham et al., 2007). Very recently, De Silva *et al*. showed that viewing food-related stimuli following infusion of combination PYY_{3-36} and $GLP\text{-}1_{7-36\ amide}$ infusion in healthy normal weight volunteers reduced BOLD activation mainly in the insula and to a lesser extent in the OFC and NAc when compared to saline infusion. Interestingly, this effect was also associated with a reduction of *ad libitum* energy intake provided at the end of the study day (De Silva et al., 2011). These encouraging findings of the role of gut and adipose tissue-related hormones on neuronal responses may help to emphasis the regions that involved in controlling motivation and reward, thus regulating energy intake.

Fermentable carbohydrates are suggested to modulate food intake and body weight via stimulation of the release of PYY and GLP-1 as a result of increased release of SCFAs (Cani et al., 2004; Delzenne et al., 2005; Delzenne et al., 2007). Interestingly, in 2009, Shen *et al*. showed that resistant starch potentially exerts its effect on food intake and body weight by increasing the hypothalamic POMC expression (Shen et al., 2009). Starting from this finding, a few studies also investigated the effect of fermentable carbohydrates on hypothalamic regions. So *et al.* showed that adding resistant starch in the diet of mice not only resulted in reducing energy intake and body fat, but the effect was also related to increased uptake of Mn^{2+} in the hypothalamic VMH and PVN (So et al., 2007). Very recently, the effect of resistant starch in hypothalamic regions was also supported by another fermentable carbohydrate, oligofructose-enriched inulin (synergy 1). In this study, including synergy1 in high fat diet of mice decreased energy intake and body weight alongside of significant increased activation of ARC (Anastasovska et al., 2012). Moreover, evidence also demonstrated that acetate, a 2-carbon SCFA also has been shown to transport to the brain (Song et al., 2009). Therefore, these results highlighted the potential role of fermentable carbohydrates on central appetite regulation. In contrast to rodent studies, no known human studies have investigated the role of fermentable carbohydrates on neuronal response. This has therefore encouraged us to investigate the role of fermentable carbohydrates on neuronal responses and its effect on satiety in humans.

4.2 Aims and Hypothesis

4.2.1 Aims

The aim of this study was to investigate the effect of oligofructose supplementation on brain activation and subjective appetite when viewing food images. To achieve this objective, the below parameters were measured:

- Change in fMRI signals in pre-selected ROIs pertaining to mesolimbic reward structures.
- Food appeal ratings
- Subjective appetite scores
- Circulating levels of gut hormones, PYY and GLP-1.

4.2.2 Hypothesis

It was hypothesized that intake of 30 g per day oligofructose for six weeks (following two weeks run-in period) would reduce brain activation in six pre-selected ROI regions, reduce appetite ratings and food appeal scores following viewing of food images.

4.3 Materials and Methods

4.3.1 Study Visit Protocol

This study is a part of **The effect of oligofructose on appetite, gut hormones, body composition and brain activity in healthy overweight subjects.** Volunteers were asked to attend two fMRI brain scan study days (Visit 2 and Visit 5), the baseline and supplementation study day which were held at the Robert Steiner MRI unit, MRC Clinical Sciences Centre, Hammersmith Hospital after 10 – 12 hours overnight fasting. Upon arrival, they were asked to remove any metal from their body before changing into hospital scrubs and filling in a metal checklist form as a confirmation that no metal was attached to their body. A mood/depression assessment questionnaire was also obtained from volunteers. This questionnaire enabled confirmation of the volunteers' mental state and that they were free from depression on the scanning day (Appendix 7). Female volunteers were also confirmed not to be pregnant prior to scanning using a urine HCG dipstick testing. In addition, to avoid any hormone induced changes that influence food selection and brain activation that might have occurred due to variable stage of menstrual cycle during the scanning (Dreher et al., 2007; Frank et al., 2010), study visits for female volunteers were only arranged during the follicular phase of their menstrual cycle (preferably between 2-10 days). Due to technical problems with the scanner during the study period, only 10 out of 22 volunteers managed to complete the scanning section (pre and post brain scans).

Prior to scanning, a cannula was inserted into the antecubital vein preferably on their left arm as a hand keypad for picture rating will be placed on the right arm during the scanning. On the study day, the first blood withdrawal was performed at 35 minutes prior to the scanning, followed with second assessment at 0 minute and lastly at 1 hour and 30 minutes after the scanning session started. VAS and blood pressure were also obtained concurrently with the blood withdrawal. They were also required to fill in three-day food diaries started from the day before the scanning and ending a day after the scanning day. The food diary was performed to assess for differentiation of nutritional intake before and after taking the supplementation (the food diary data has not been analysed for this thesis).

The study visits took 3-4 hours to complete. On the supplementation visit only (Visit 5), volunteers took the supplement at 7 am prior attending the study visit in order to allow for colonic fermentation activities to be maximal when volunteers had their brain scans later in the afternoon. It is estimated that approximately five hours is needed for the supplement to be fully fermented by the gut bacteria, therefore, the scanning session was started at noon (please refer to Section 4.3.2 for fMRI scanning protocol). Volunteers were discharged at 3 pm after final blood and VAS assessment (Figure 4.1).

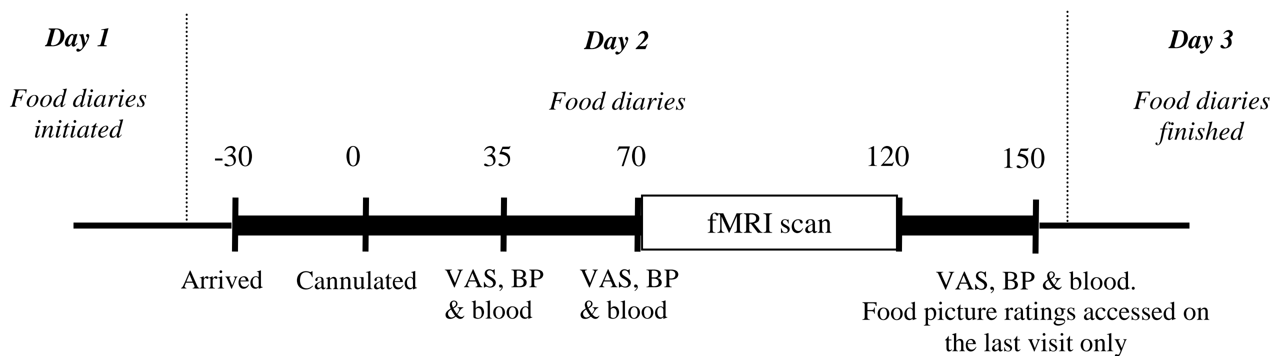


Figure 4.1 Schematic diagram for fMRI study day. Volunteers attended 2 fMRI study days at baseline (visit 2) and supplementation (visit 5). After cannulation, VAS, BP and blood were obtained from volunteers at 35 and 70 minutes. After that, volunteers had fMRI scan for 60 minutes and post-scanning VAS, BP and blood withdrawal were obtained at 150 minutes. On the supplementation study day only, volunteers took the supplementation at 7 am before attending the study session. In addition, they were also asked to rate the liking and frequency of consumption of the food pictures seen during the fMRI scanning. Three-day food diaries were completed from a day prior the study day and finished a day after each fMRI study day.

4.3.1.1 Randomization

Prior to the baseline fMRI study day, volunteers were randomised to view four different preudo-randomised food picture orders known as AB, CD, EF and GH using a computerised excel sheet by a member of the department which was not directly involved in the study. These picture orders were then randomly selected for the fMRI picture paradigm on the study days.

4.3.1.2 Visual Analogue Scales

VAS for hunger, fullness and motivation to eat were obtained from each volunteers at 35, 70 and 150 minutes in the same way as described in the previous Section 2.4.1.2. However, there are some additional subjective appetite questionnaires related to the scanning procedure such as anxiety, sleepiness and pleasantness to eat which were also assessed. Volunteers were also asked to rate hunger three times during the scanning (Figure 4.3).

4.3.1.3 Picture Ratings

On the fMRI supplementation visit only (visit 5), volunteers were asked to name each of the food pictures that they viewed during the scanning. This task was performed in order to monitor whether volunteers could recognised the pictures (expressed in percentage correct recognition). In addition, they were also asked to rate their liking and frequency of eating of each picture seen by rating questions *of how much do you usually like to eat this food:* hate a lot (score -3) to like a lot (score +3) and a question of; *On average how often do you actually eat this food*: never (score 0) to once per day or more (score 8) (Figure 4.2). The scores were summed separately based on high calorie and low calorie foods so that high calorie vs. low calorie intake can be accessed.

How much do you usually LIKE to eat this food (please circle)?

Hate a lot	Hate somewhat	Hate a little	Neither like nor hate	Like a little	Like somewhat	Like a lot

On average how OFTEN do you actually eat this food (please circle)?

Never	3 times or less per year	6 times or less per year	Once per month or less	2-3 times per month	Once per week	2-3 times per week	4-6 times per week	Once per day or more

Figure 4.2 Preference and frequency questionnaires asked during food ratings pictures.

4.3.1.4 Plasma Gut Hormones Analysis

Blood for total PYY and GLP-1 measurement were drawn at 35, 70 and 150 minutes and were collected into lithium heparin tubes containing aprotinin (40 µl/10 ml bloods). Each sample was then spun at 4000g at 4°C for 10 minutes and the plasma separated and divided into aliquots before immediate storage at -80°C. PYY and GLP-1 were analysed by radioimmunoassay using the in-house RIA as described in Section 2.3.2.2.5.

4.3.2 fMRI Scanning Protocol

Each fMRI scanning experiment took approximately an hour to complete. In the MRI scanner, volunteers were asked to lie in the supine position. They were provided with ear plugs to wear and headphone to reduce noise from the MRI scanner. Then, a padded head coil was placed around their head for supports. An alarm buzzer was also given to hold in their left arm, in case they needed to stop or were uncomfortable with the scanning. On the right hand, a keypad was placed for the picture appeal button scores task. Volunteers were advised to stay still and not to move their head as this would disturb the scanning process. Seven tasks were performed during the scan. These tasks were test run, resting scans, the visual images run 1 and run 2, field maps, an audio-motor-visual task (AMV) and DTI scans (Figure 4.3). During the scanning, volunteers were also asked to rate hunger ratings three times (after test run, resting scans and field maps) on a scale from 1 to 5 from not at all (little finger), not really (ring finger), neutral (middle finger), a little stimulation (index fingers) and a lot (thumbs) (Figure 4.4)

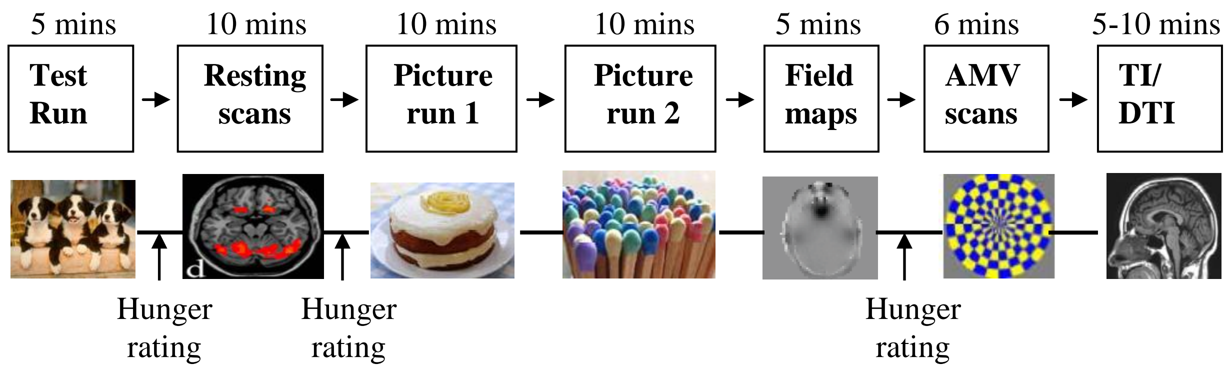


Figure 4.3 Schematic diagram of fMRI protocol. The scanning procedure was performed in a fixed order for 60 minutes, 1) Test run consisting of animal pictures as a practice and for volunteers to familiarize themselves with the keypad pressing to rate pictures, 2) Resting state fMRI scan to assess brain activity without the presence of any stimulation 3) fMRI picture run 1 and 4) run 2 using evaluation of food and non-food pictures 5) Field maps to correct for signal distortion, 6) Audio-Motor-Visual (AMV) control fMRI task, 7) TI and DTI anatomical scans to look for brain abnormalities and allow image registration. Each volunteer were also asked to rate hunger ratings three times during the scanning.

Figure 4.4 A hand-held keypad used for accessing hunger and picture appeal scores during the fMRI scanning.

4.3.2.1 Visual Stimuli Images

A total of 372 coloured photographs were presented during the brain scans. These photographs were presented in a block design and divided into 4 different groups: 1) 60 high-calorie foods, 2) 60 low-calorie foods, 3) 60 pictures of non-food-related household objects and 4) 192 Gaussian blurred images of high-calorie foods (e.g. chocolate and cakes), low-calorie foods (e.g. salads, fish-based dishes) and objects (e.g. keys, chairs, clothes) pictures (example of the stimuli are displays in figure 4.5). These photographs were acquired from the internet and the International Affective Picture System (IAPS, NIMH Centre for the study of Emotion and Attention, University of Florida, Gainesville, FL, USA). All of these images were arranged to be of similar luminosity and resolution. Food pictures, high and low calorie food images were the tested tasks whilst non-food and Gaussian blurred images were used as low level baseline images and to allow study of the effect of dietary intervention (before and after intake of supplementation) on control stimuli. Only foods that are consumed in the Western diet were used in the study. The nutritional contents of both high calorie and low calorie foods were analysed using DietPlan6 (Forestfield Software Ltd, West Sussex, UK) (Table 4.1).

Table 4.1 The total caloric load, caloric density and macronutrients composition of the high calorie and low calorie foods.

Type of foods	Total caloric (kCal)	Caloric density (kCal/100g)	Macronutrient composition (%)		
			CHO	Protein	Fat
High calorie	834 ± 100	321 ± 13	48 ± 1	10 ± 1	48 ± 1
Low calorie	157 ± 18	64 ± 5	35 ± 3	29 ± 3	35 ± 3
High calorie vs. low calorie	P<0.001	P<0.001	P<0.001	P<0.001	P=0.03

In each scanning session, two runs of stimuli images were shown to the volunteers for 18 minutes. Stimuli were presented in a block design experiment. In total, five blocks of pictures from the categories were showed in one of two pseudo-randomised block orders containing randomised pictures in each block. Each block has six different pictures either from the high-calorie food, low-calorie food or objects categories. Each of these pictures were presented for 2.5 seconds and followed by a 0.5 seconds fixation cross image in between the images. Therefore,

each block category was shown in 18 seconds. Each block of high-calorie foods contained two pictures of chocolate, non-chocolate sweet and savoury non-sweet food pictures. Each stimuli block was followed by a block of six blurred images with the same duration as food and object images in each block. This step was performed in order to allow the BOLD signal to return back to baseline after complete viewing each block of stimuli. Images were viewed via a mirror mounted above an 8 channel RF head coil which displayed images from a projector using the IFIS image presentation system (In Vivo, Wurzburg, Germany) using ePrime 2.0 software (Psychology Software Tools Inc, Pittsburgh,PA, USA).

In this task, volunteers were also asked to immediately rate each picture as soon as the images were shown in the scanner by pressing the keypad button on their right hand (1 = not at all, 2 = not really, 3 = neutral, 4 = a little, 5 = a lot).

4.3.2.2 Auditory-Motor-Visual (AMV) Task

An AMV task was used as a control task to rule out non-specific changes of fMRI BOLD activation between pre-supplementation and supplementation visits in both oligofructose and cellulose groups (the schematic diagram for this process is described in Figure 4.5). This six minute task was performed at the end of the scanning session. In this paradigm, two tasks were performed at the same time from:

1. Watching a 4Hz colour flashing checkerboard and listening to a story or
2. Watching a 4Hz colour flashing checkerboard and pressing the right index finger once every second, or
3. Listening to a story and pressing the right index finger once every second.

The single run consisted of nine 33 second blocks, with each task performed in six blocks, and instructions about whether to start or stop the motor task displayed for three seconds prior each block.

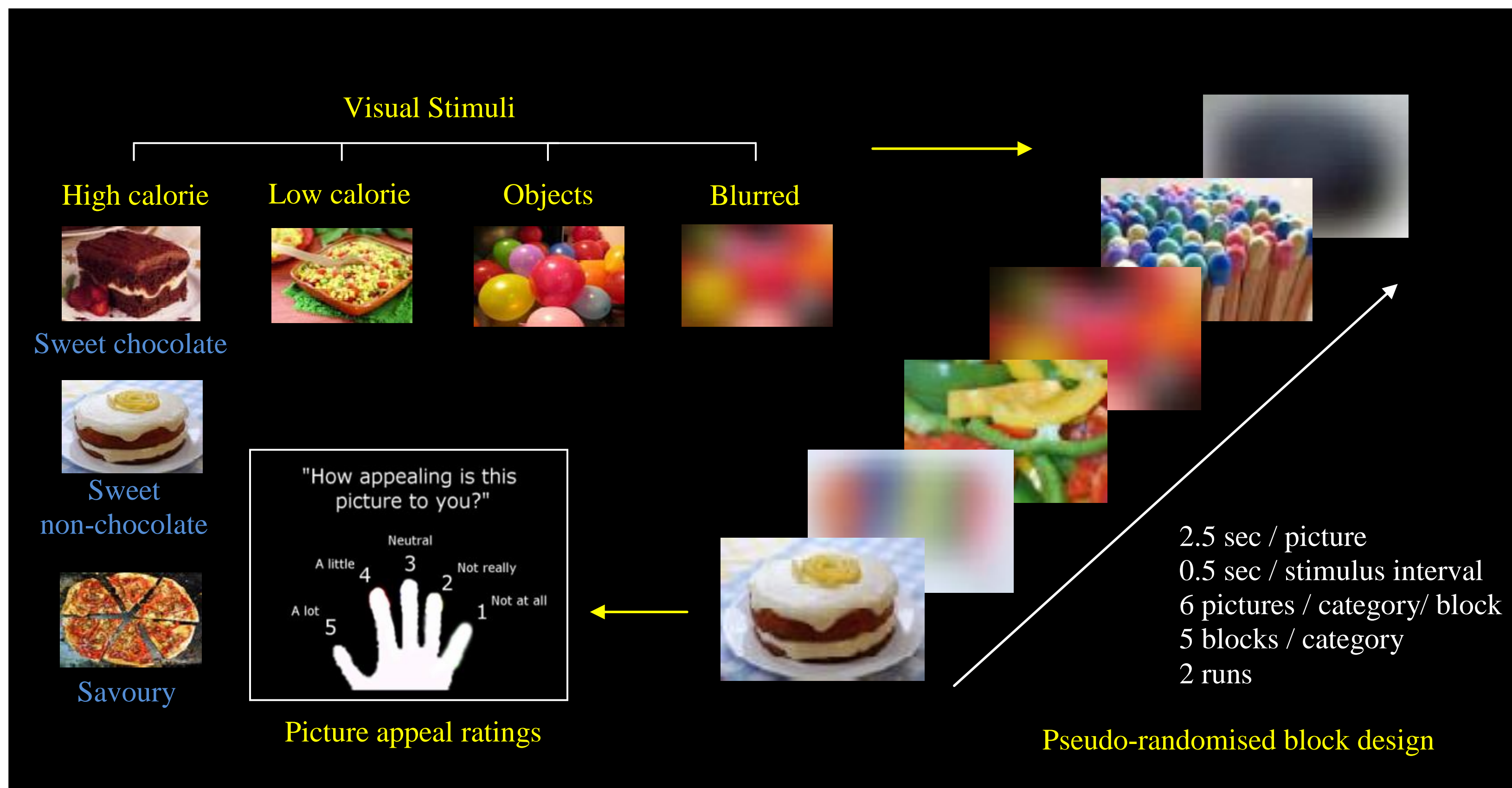


Figure 4.5 Schematic diagram for fMRI visual stimulation tasks. Visual stimuli are presented in blocks and comprised of four types of stimuli: high calorie foods (sweet chocolate, sweet non-chocolate and savoury), low calorie foods, objects and blurred images (i). During the fMRI scanning, these pictures are shown in a pseudo-randomised block design. Each picture was shown for 2.5 seconds and six pictures were presented in a block. Each category (high-calorie foood, low calorie food and objects) has 5 blocks per run which were shown in 2 pre-randomised picture runs (AB, CD, EF and GH). Volunteers were asked to rate the appeal of each pictures that they saw during the scanning on a 1-5 scale from not at all (little finger), not really (ring finger), neutral (middle finger), a little (index finger) and a lot (thumb).

4.3.2.3 fMRI Imaging Acquisition

T2*-weighted gradient-echo planar images were acquired with BOLD contrast using a 3T Philips Intera whole body scanner using a -30º tilted acquisition angle from anterior and posterior commissure plane to decrease signal dropout in orbifrontal regions resulting from air sinuses. Image parameters were echo time (TE) = 30 ms; repetition time (TR) = 3000 ms; flip angle = 90º, 44 slices ascending contiguous; slice thickness = 3.25mm, 2 x 2 mm voxels, matrix size =112x112, field of view 190 x 219 mm^2. B0 fieldmaps were collected to correct for geometric distortions caused by inhomogenities in the magnetic field as follows: TR 29 ms; TE 3.6 ms, 30º flip angle; field of view 190 x 219, 44 ascending contiguous 3.25mm thick slices, 2 x 2 mm voxels, ∂TE 0 and 2.5. A high resolution T1-weighted structural scan was also collected at each visit for fMRI image co-registration and mapping the ROIs (TE = 4.6 ms; TR = 9.7 ms; flip angle = 8°; field of view = 240 mm; voxel dimensions = 0.94 x 0.94 x 1.2 mm).

4.3.2.4 Pre-processing and Analysis of fMRI Images

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). No subjects had excess head movement (average relative motion across both food runs >0.5mm/TR). The following preprocessing was applied: motion correction using MCFLIRT (Jenkinson et al., 2002); fieldmap-based EPI unwrapping using PRELUDE+FUGUE (Jenkinson, 2003; Jenkinson, 2004); non-brain removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 6.0mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=100.0s).

Time-series statistical analysis was carried out using FILM with local autocorrelation correction including picture onsets (high-calorie and low-calorie food and objects) integrated with the gamma HRF, with temporal derivative and motion parameters as co-variates (Woolrich et al., 2001). Registration to high resolution T1 structural images was carried out using FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002). Registration from high resolution structural

to standard space was then further refined using FNIRT nonlinear registration (Andersson et al., 2007b; Andersson et al., 2007a). For the food pictures, higher level analysis was carried out using a fixed effect model to combine the 2 runs, by forcing the random effects variance to zero in FLAME (FMRIB's Local Analysis of Mixed Effects) (Beckmann et al., 2003; Woolrich et al., 2004) to determine activation for the following contrasts: high-calorie or low-calorie food > object, high-calorie food > object and low-calorie food > object. Similar time-series statistical analysis was performed for the single run AMV paradigm including the onsets of each task (auditory, motor and visual) to contrast activation during performance of each task with that when the other tasks were being performed. All higher-level analysis was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) stage 1 (Beckmann et al., 2003; Woolrich et al., 2004).

4.3.2.4.1 Generation of functional ROIs

The first stage in fMRI analysis was to create group activation maps in order to locate voxels of brain regions that were activated by the food image paradigm (any food [high-calorie or low-calorie] vs. object) in order to generate functional ROIs. This was performed using an identical paradigm and analysis with fMRI scans from another study. An activations were thresholded at false discovery rate (FDR) $P<0.05$ for correction of multiple comparisons. In this analysis, scans were used from an initial baseline fasted scanning visit from 22 healthy overweight volunteers before undergoing another dietary intervention, omega-3 polyunsaturated fatty acid (PUFA) study. This cohort had no-significant difference in BMI, age and weight with the volunteers involved in this study (Table 4.2). The group activation is illustrated in Figure 4.6. Similar localizers were made from this group analysis for the control auditory, motor and visual tasks (Figure 4.8).

Table 4.2 Demographic profiles for PUFA and oligofructose study

Profiles	PUFA	Oligofructose / cellulose study	P value
n	22	10	-
Age (years)	33.4 ± 1.7	34.8 ± 3.0	P = 0.719
Body weight (kg/m^2)	80.6 ± 3.3	85.4 ± 4.0	P = 0.366
BMI	28.7 ± 0.7	29.9 ± 1.0	P = 0.308
% fat	35.1 ± 1.5	36.2 ± 2.5	P = 0.455
Gender	18 females, 4 males	6 females, 4 males	-

The functional ROIs were obtained by masking these group activation maps with a priori anatomical ROI. For the food picture task these were the nucleus accumbens (NAc), amygdala, anterior OFC, hippocampus, insula and ventral ACC (Figure 4.7). For the AMV task these were bilateral primary visual cortex (lingual gyrus), bilateral secondary auditory cortex (superior temporal gyrus), and left supplementary motor area (Figure 4.8). These were defined by the relevant bilateral ROIs from the cortical and subcortical structural Harvard FSL atlas thresholded at 10% probability. The OFC functional ROI included regions in the OFC and frontal pole with $y > 22$ and $z < -6$, since analysis of functional activation in this region demonstrated distinct bilateral clusters overlapping the anatomical Harvard atlas regions.

4.3.2.4.2 Comparison of group Activation for Oligofructose and Cellulose

The average (median) magnitude of bilateral BOLD activation within each a priori fROI was then extracted for each individual subject for each of the visits (pre- and post-supplementation visits) in each oligofructose and cellulose group for the following contrasts: (i) any food (high-calorie or low-calorie food) > objects, (ii) high-calorie food > objects, (iii) low-calorie food > object and (iv) objects > blurred pictures, using featquery in FSL, to measure the differences in activation between visits and groups for the different picture categories. Similar analysis was performed to compare auditory, motor and visual activation in the relevant fROIs between visits and groups. Average change in BOLD activation for each of these contrasts within each ROI was then compared between visits and groups outside FSL.

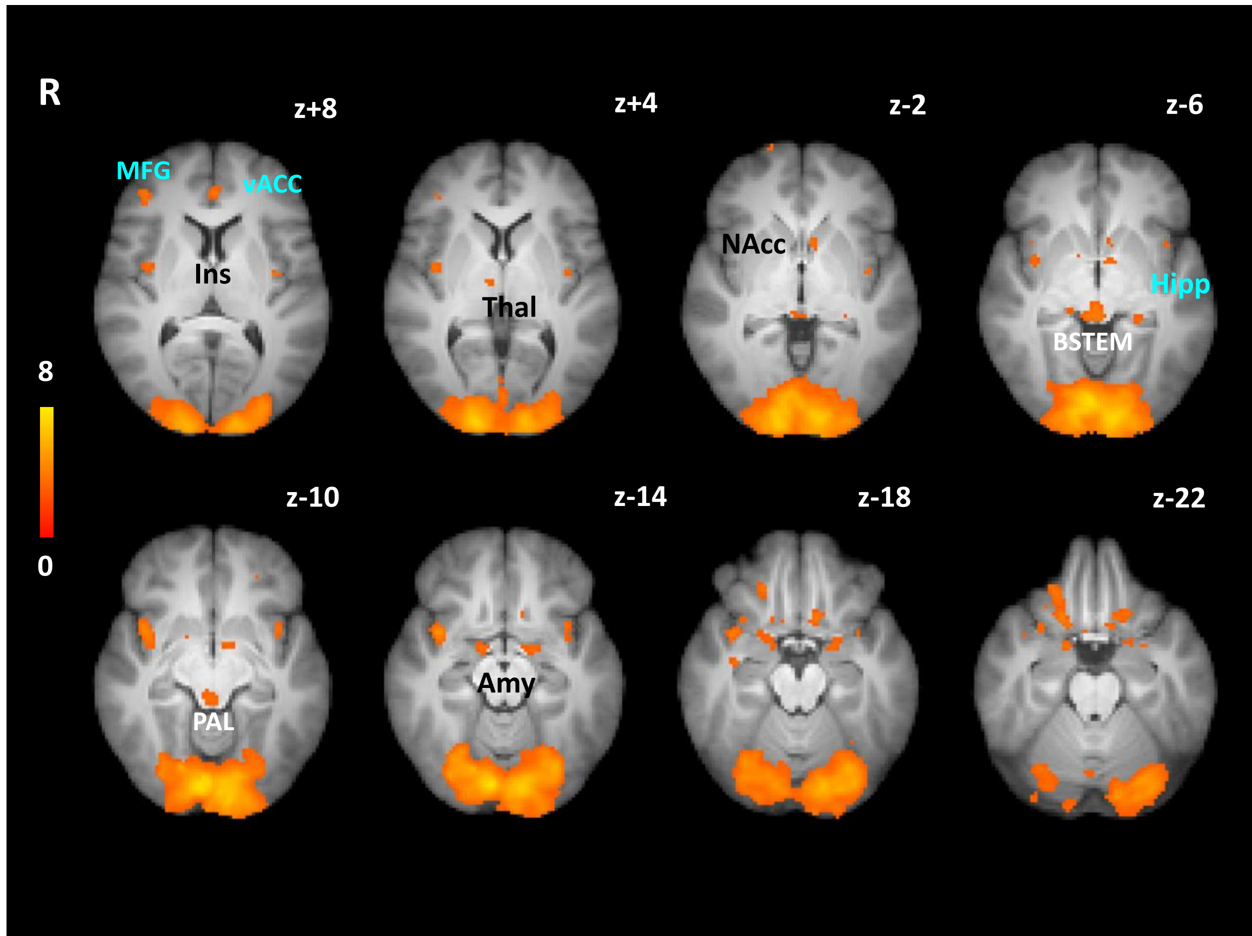


Figure 4.6 Group brain activation to food pictures used to generate functional ROIs. Group activation map for any food (high-calorie or low-calorie) minus object picture contrast at the pre-supplementation visit (red yellow). Colour bar indicates z value. Activations are thresholded at FDR $P<0.05$, overlaid onto the average T1 scan for all subjects (n=22 subjects from PUFA study). Co-ordinates are given in standard MNI space. Abbreviations; Amy: amygdala, BSTEM: brainstem, Hipp: hippocampus, Ins: insula, MFG: medial frontal gyrus, NAc: nucleus accumbens, PAL: pallidum, Thal: Thalamus, vACC: ventral anterior cingulate cortex, Colour bar gives Z statistic.

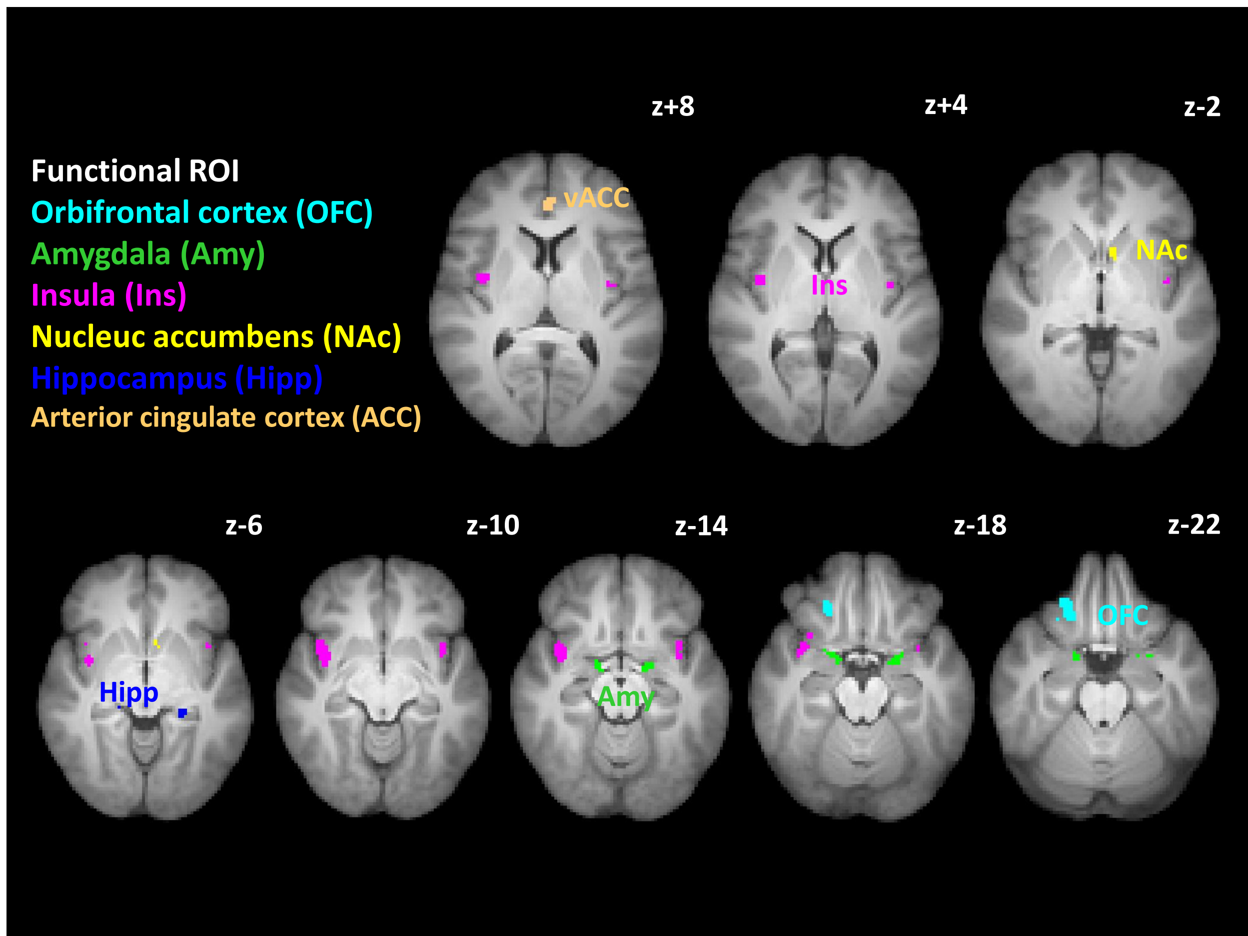


Figure 4.7 Funtional ROIs used in analysis for food picture task. Activation is thresholded at FDR P<0.05, overlaid onto the average T1 scan for all subjects (n=21). *A priori* functional regions of interest (ROI) are indicated: OFC (OFC, light blue), amygdala (Amy, green), NAc (NAc, yellow), insula (Ins, magenta), hippocampus (Hipp, dark blue) and ventral anterior cingulate cortex (vACC, light brown). Coordinates are given in standard MNI space.

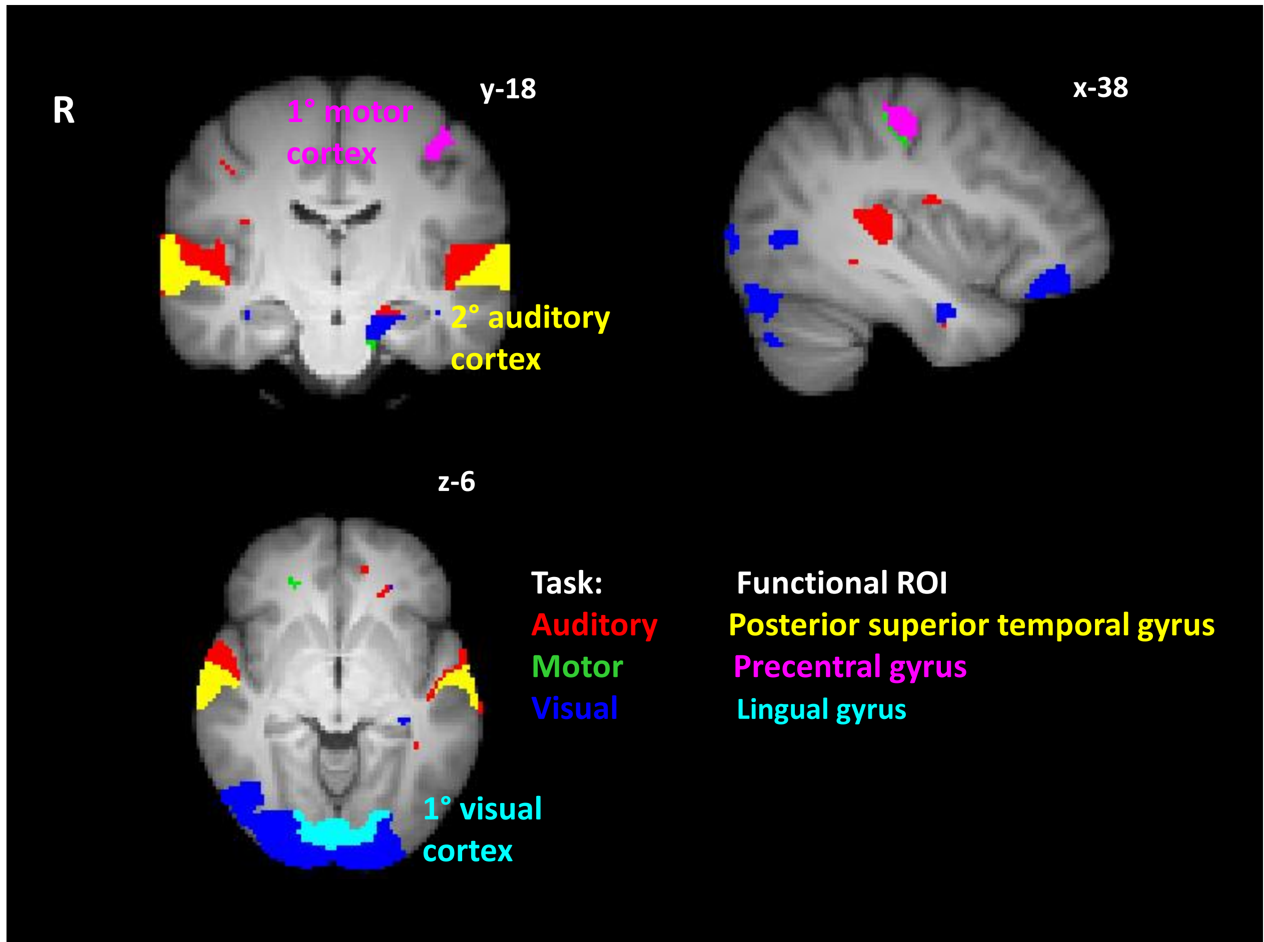


Figure 4.8 Functional regions of interest for control Auditory-Motor-Visual task: Group activation maps thresholded at FDR P<0.05 are overlaid with functional ROIs on background image of average T1 scan for all subjects (n=22 subjects from PUFA study): auditory (red:listening to story) with bilateral posterior division of superior temporal gyrus (overlaid in yellow), motor task (green: button press) with left pre-central gyrus (overlaid in magenta), visual (dark blue: flashing checkerboard) with lingual gyrus (overlaid in light blue). Co-ordinates are given in standard MNI space.

4.3.2.5 Statistical Analysis

Results are presented as mean ± SEM. As this study has a small sample size (oligofructose=4, cellulose=6), Gaussian distribution cannot be used to assess normality of the data distribution. Therefore, statistical analyses were performed using parametric tests. Changes in ROI activation on viewing and rating food vs. objects, high calorie foods vs. objects, low calorie foods vs. objects and objects vs. blurred pictures, and during auditory, motor and visual control tasks between oligofructose and cellulose groups were calculated by substracting BOLD activation at post-supplementation fMRI scans from baseline visit and the result was compared using two-tailed unpaired t-test. Within groups effects were examined using two-tailed paired t-test (oligofructose and cellulose vs. baseline) to compare effect on picture appeal rating scores. The change in activation was then compared between oligofructose and cellulose using two-tailed unpaired t-test. An ANCOVA analysis with baseline value, BMI and gender as co-variates was used to compare the differences between the treatments for subjective appetite VAS (tAUC), picture appeal ratings, plasma GLP-1 and plasma PYY levels and the end of study food picture ratings. Statistical significance is defined by a P value of 0.05 or less. Statistical analysis for within group student t-test was performed using GraphPad Prism 5 (GraphPad Software, San Diego CA, USA) whilst ANCOVA analysis was performed using SPSS version 20.0 (SPSS Inc, Chicago, IL, USA).

4.4 Results

4.4.1 Volunteers Characteristics

10 healthy overweight/obese right-handed volunteers participated in this study. Four volunteers were from the oligofructose group (2 male, 2 female, mean age 39.3 ± 4.1 years, range 31 – 49 years, mean BMI 27.5 ± 0.9 kg/m2, range 25.4 kg/m^2 – 29.7 kg/m^2) and 6 volunteers were from the cellulose group (2 male, 4 female, mean age 27.3 ± 1.6 years, range 22 – 47 years, mean BMI 31.7 ± 0.5 kg/m^2, range 27.0 – 35.7 kg/m^2).

4.4.2 Activation to Food Pictures in ROI analysis

Figure 4.9 – Figure 4.11 demonstrates changes in BOLD signal in the NAc, amygdala, OFC, insula, hippocampus and vACC on viewing food vs. objects (i), high calorie vs. objects (ii) and low calorie vs. objects (iii) after intake of oligofructose and cellulose supplementations.

4.4.2.1 Food vs. Object Pictures

There was no significant difference in the change in activation on viewing food vs. object pictures after oligofructose vs. cellulose supplementation though there was a suggestion that OFC and insula activation was reduced more by cellulose than oligofructose (NAc: P=0.479, amygdala: P=0.633, OFC: 0.273, insula: P=0.261, hippocampus: P=0.441 and vACC: P=0.733) (Figure 4.9)

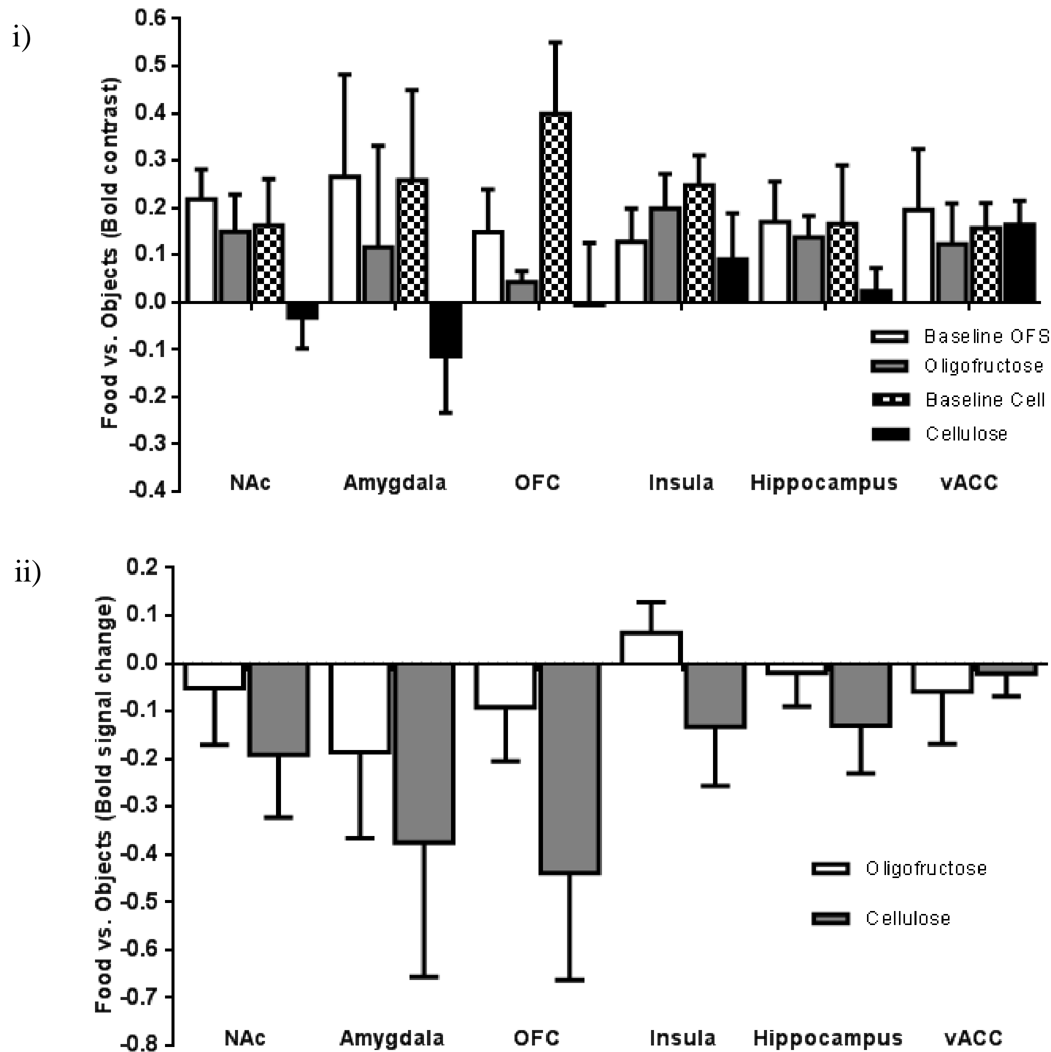


Figure 4.9 BOLD signal (i) and change in BOLD signal (ii) in NAc, amygdala, OFC, insula, hippocampus and vACC on viewing of food vs. objects pictures in oligofructose and cellulose groups. Change in BOLD signal is expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.2.2 High-calorie Food vs. Object Pictures

Figure 4.10 shows the change in ROI activation on viewing high calorie food vs. object pictures in the oligofructose and cellulose group. Intake of cellulose reduced activation significantly more than oligofructose in the OFC (P=0.018) with a trend for a reduction in NAc and insula, but not in other regions (NAc: P=0.146, amygdala: P=0.599, OFC: P=0.273, insula: P=0.111, hippocampus: P=0.396 and vACC: P=0.985).

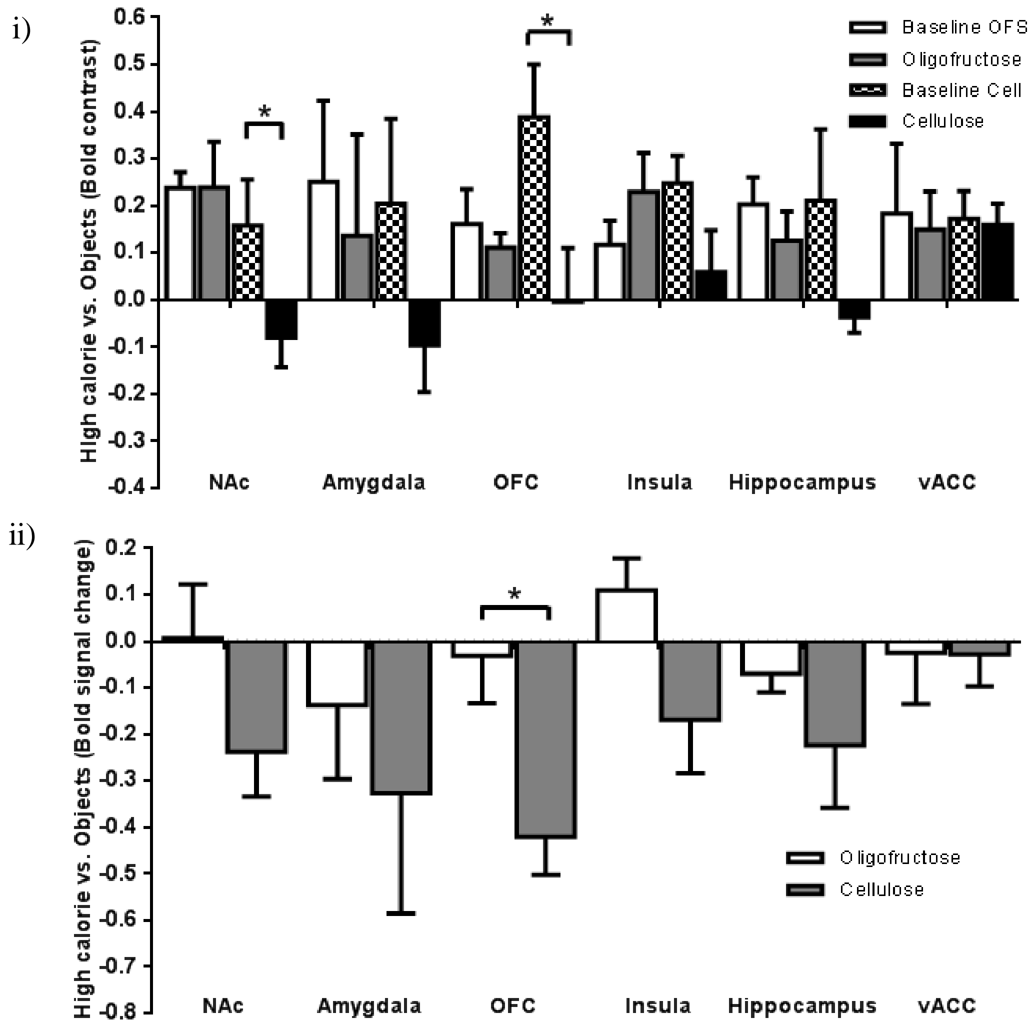


Figure 4.10 BOLD signal (i) and change in BOLD signal (ii) in NAc, amygdala, OFC, insula, hippocampus and vACC on viewing of high calorie food vs. object pictures in oligofructose and cellulose groups. Change in BOLD signal is expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6). P<0.05 cellulose vs. oligofructose group.

4.4.2.3 Low-calorie Food vs. Object Pictures

No significant difference or trend in change of activation on viewing low-calorie food vs. object pictures was demonstrated between supplementation with oligofructose and cellulose in any ROI, NAc: P=0.872, amygdala: P=0.646, OFC: P=0.663, insula: P=0.573, hippocampus: P=0.801 and vACC: P=0.624 (Figure 4.11).

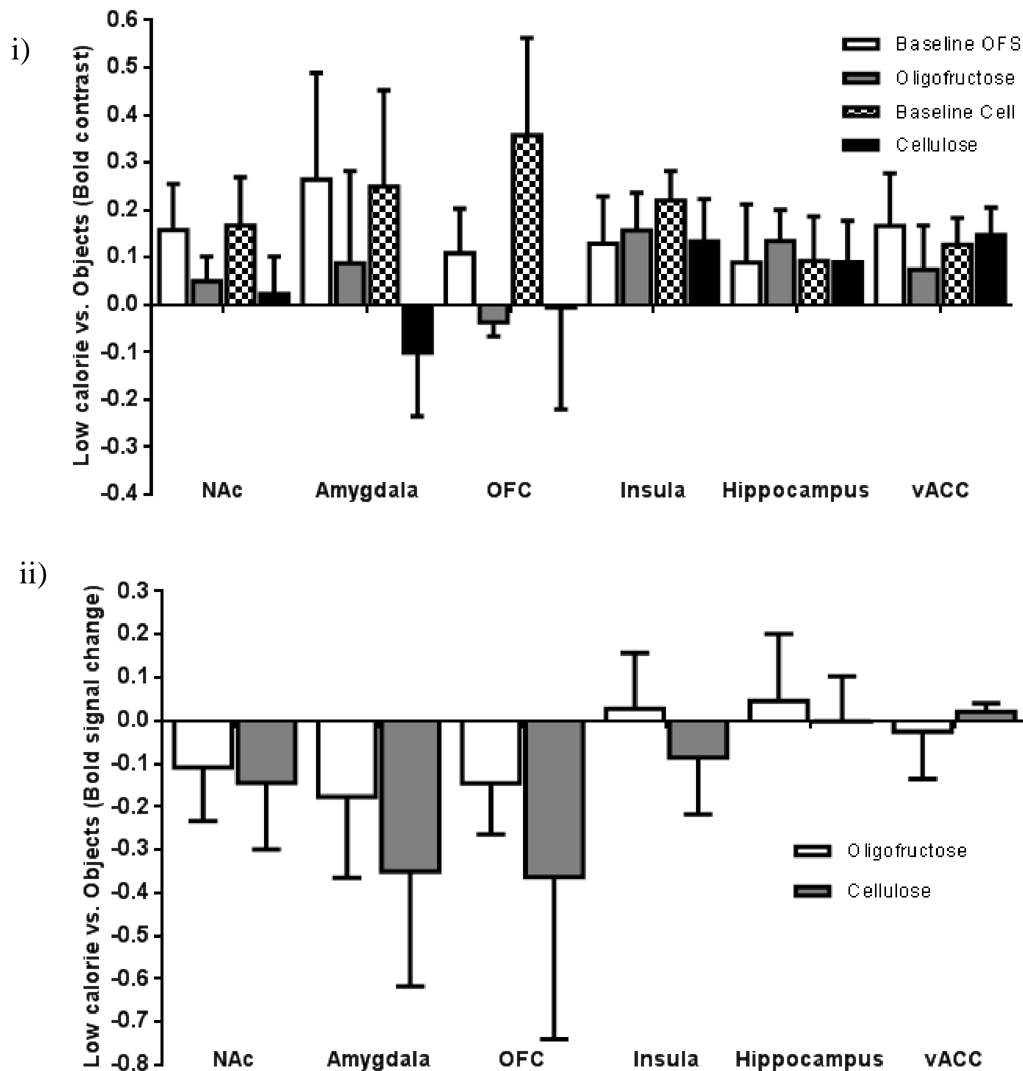


Figure 4.11 BOLD signal (i) and change in BOLD signal (ii) in NAc, amygdala, OFC, insula, hippocampus and vACC on viewing of low calorie food vs. object pictures in oligofructose and cellulose groups. Change in BOLD signal is expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.2.4 Control Activation

In this study, blurred vs. objects picture contrast and auditory, motor, visual task activation were used as control tests for any non-specific changes in BOLD activation following intake of oligofructose or cellulose.

4.4.2.4.1 Object vs. Blurred Pictures

There was no significant difference in the effects of oligofructose vs.cellulose supplementation on ROIs activation to viewing object vs. blurred pictures though there was a trend for insula activation to increase more with cellulose, NAc: P=0.437, amygdala: P=0.659, OFC: P=0.969, insula: P=0.123, hippocampus: P=0.538 and vACC: P=0.847 (Figure 4.12).

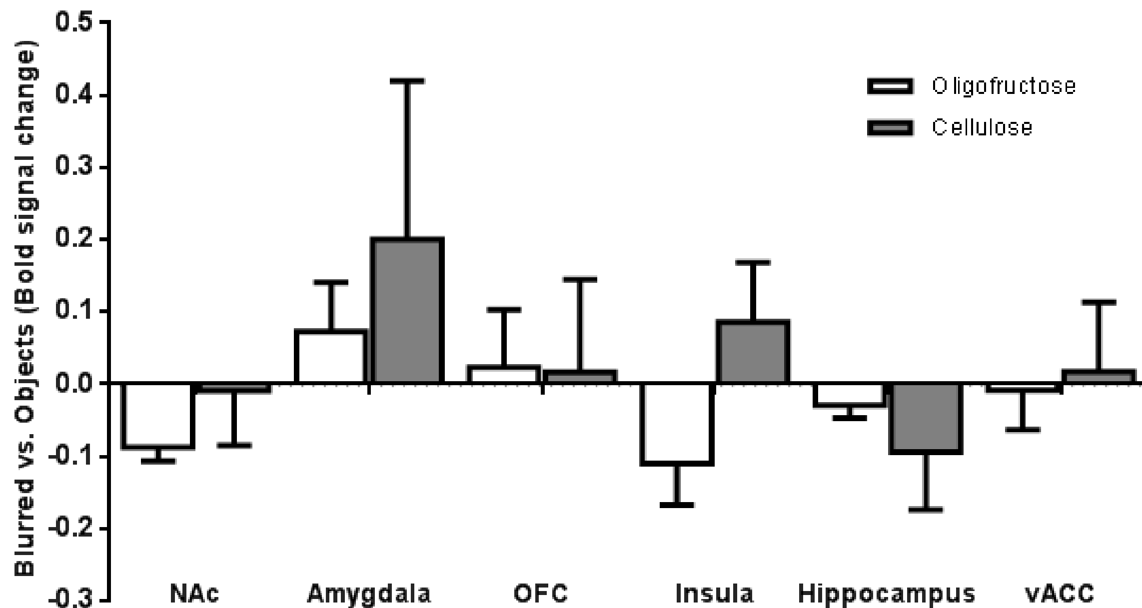


Figure 4.12 Change in BOLD signal change in NAc, amygdala, OFC, insula, hippocampus and vACC on viewing of objects vs. blurred pictures in oligofructose or cellulose groups. Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.2.4.2 Visual Checkerboard

There was no significant difference or trend in change of activation in superior temporal gyrus, precentral gyrus and lingual gyrus during the control auditory-motor-visual task between oligofructose and cellulose supplementation: superior temporal gyrus (P=0.917), precentral gyrus (P=0.347) and lingual gyrus (P=0.523) (Figure 4.13).

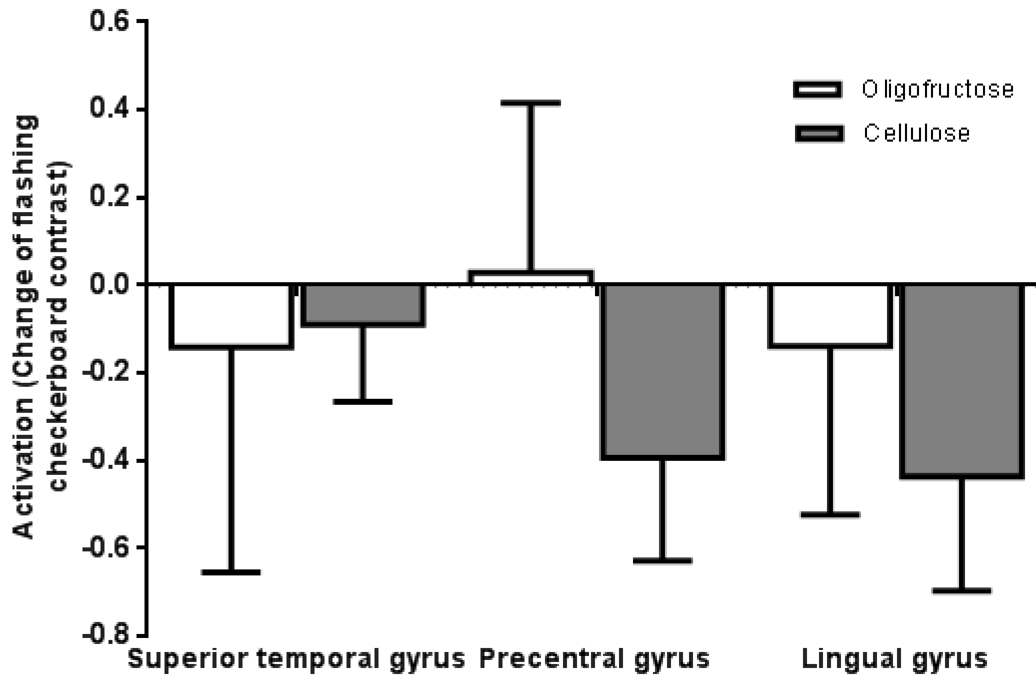


Figure 4.13 Change in BOLD signal change in superior temporal gyrus, precentral gyrus and lingual gyrus during auditory-motor-visual control task in oligofructose or cellulose groups. Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.3 Picture Appeal Rating

Table 4.3 demonstrates the appeal rating scores in mean ± SEM. High calorie food pictures were rated as more appealing compared to other visual stimuli in both groups. However, no significant difference was found between before and after supplementation in both oligofructose and cellulose groups, oligofructose [blurred (P=0.639), high calorie foods (P=0.396), low calorie foods (P=0.598) and objects (P=0.260) pictures] and cellulose [blurred (P=0.994), high calorie foods (P=0.336), low calorie foods (P=1.00) and objects (P=0.708) pictures] and also between the treatment, blurred (P=0.396), high calorie foods (P=0.056), low calorie foods (P=0.766) and objects (P=0.284).

Table 4.3 Picture appeal rating scores

Tasks	Oligofructose (n=4)			Cellulose (n=6)		
	Pre	Post	Change	Pre	Post	Change
Blurred	1.42 ± 0.27	1.25 ± 0.20	-0.17 ± 0.32	1.43 ± 0.13	1.38 ± 0.15	-0.05 ± 0.13
High calorie foods	3.74 ± 0.16	3.89 ± 0.19	0.15 ± 0.16	4.08 ± 0.29	3.98 ± 0.49	-0.10 ± 0.21
Low calorie foods	3.30 ± 0.40	3.24 ± 0.49	-0.07 ± 0.11	3.70 ± 0.25	3.71 ± 0.18	0.02 ± 0.15
Objects	2.28 ± 0.49	1.91 ± 0.47	-0.36 ± 0.26	2.21 ± 0.37	2.28 ± 0.32	0.07 ± 0.21

4.4.4 End of Study Food Picture Ratings

Figure 4.14 demonstrates the recognition (i), liking (ii) and frequency of eating (iii) of food pictures. In this assessment, 98.2 ± 0.5 % high calorie food pictures and 96.5 ± 0.9 % low calorie food pictures (P=0.205) were correctly recognised by the volunteers [Figure 4.14 (i)]. The result also showed that volunteers have a tendency to significantly like (P=0.055) high calorie foods more than low calorie foods However, volunteers rated that they consumed low calorie foods more compared to high calorie foods, but this was not significant (P=0.306).

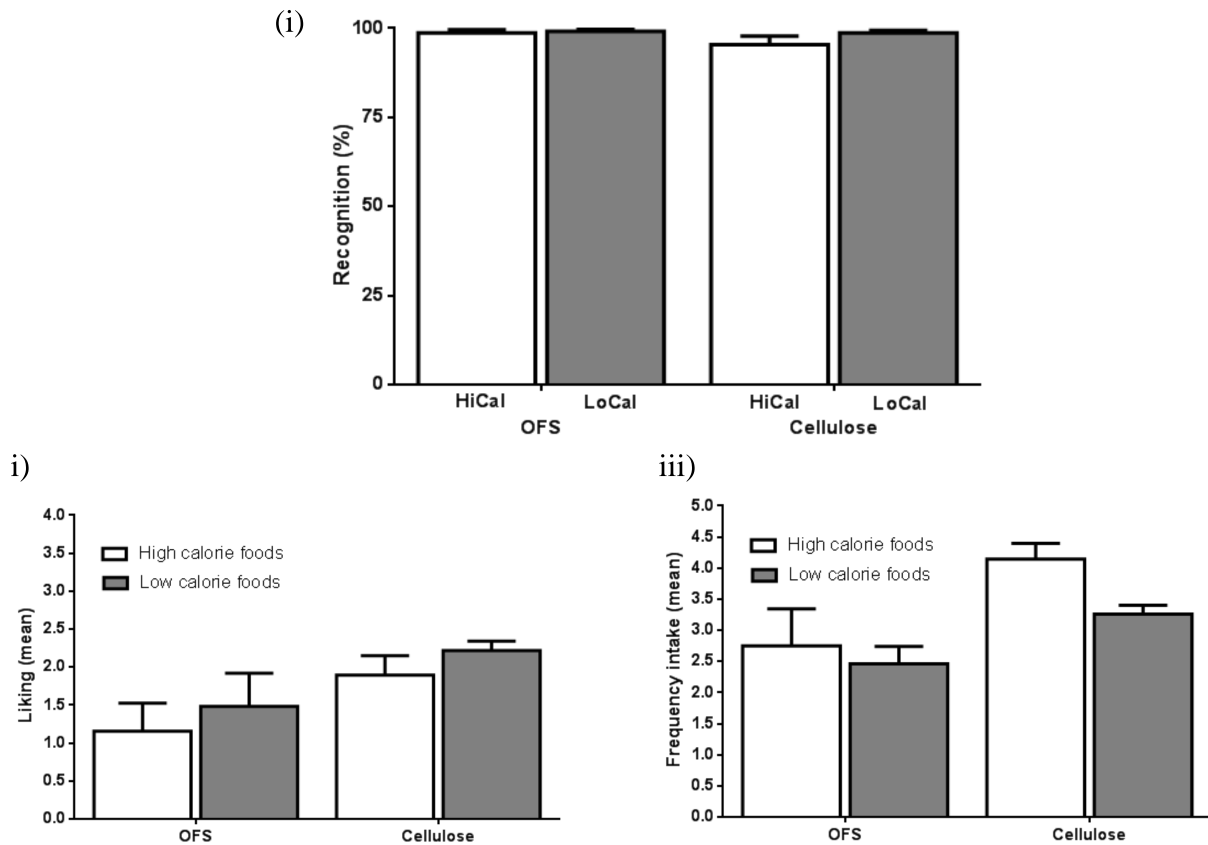


Figure 4.14 Recognition (i) liking (ii) frequency of eating of food pictures in oligofructose or cellulose groups. Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.5 Subjective Appetite Scores

Hunger Score

Figure 4.15 describes the hunger scores in $tAUC_{150mins}$. Cellulose had a tendency to reduce hunger score within the group (P=0.081) and compared with oligofructose (P=0.054). No significant effect was demonstrated in the oligofructose group (P=0.670), oligofructose (baseline: 838.2 ± 96.6 cm*min and post: 804.1 ± 108.2 cm*min) and cellulose (baseline; 697.8 ± 145.3 cm*min and post: 471.1 ± 47.8 cm*min).

Pleasant Score

Oligofructose supplementation had no significant effect on reducing subjective pleasant score compared to baseline (P=0.875) (baseline; 636.4 ± 138.6 cm*min and post: 658.4 ± 189.1 cm*min) whilst intake of cellulose showed a trend to reduce pleasant scores compared to baseline (P=0.082) (baseline; 734.0 ± 136.5 cm*min and post: 478.9 ± 59.3 cm*min). However, no significant difference between the treatment was demonstrated (P=0.183) (Figure 4.15).

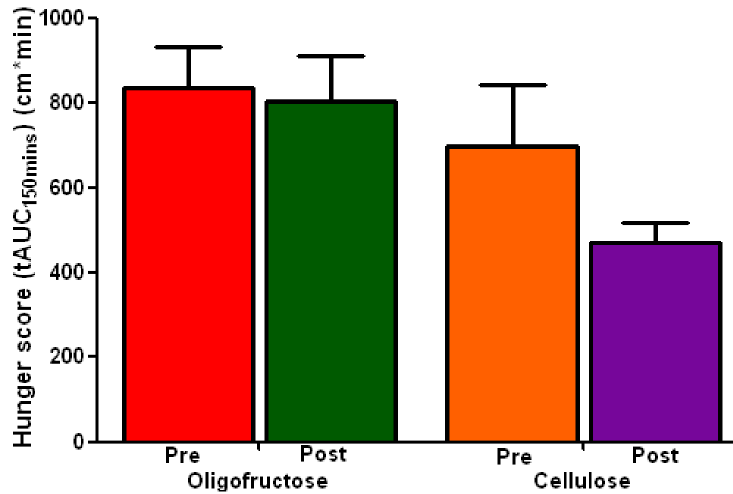
Volume Intake Score

Intake of cellulose has been shown to have a tendency to reduce volume food intake compared to oligofructose (P=0.063). In addition, it was also shown to significantly reduce volume intake scores when compared to baseline (P=0.029) (baseline; 696.5 ± 132.9 cm*min and post: 425.2 ± 61.3 cm*min) [figure 4.6] but no significant effect was demonstrated in the oligofructose group (P=0.437), (baseline; 847.2 ± 78.7 cm*min and post: 778.6 ± 116.1 cm*min) (Figure 4.15).

Fullness Score

Intake of oligofructose has been shown to increase fullness score compared to baseline, but this did not reach statistical significance (P=0.402) (baseline; 51.9 ± 25.1 cm*min and post: 92.4 ± 57.8 cm*min). No significant difference was also demonstrated in the cellulose group (P=0.563) (baseline; 51.8 ± 28.5 cm*min and 38.2 ± 13.6 cm*min). No significant difference between the group was also found (P=0.268) (Figure 4.15).

ii)



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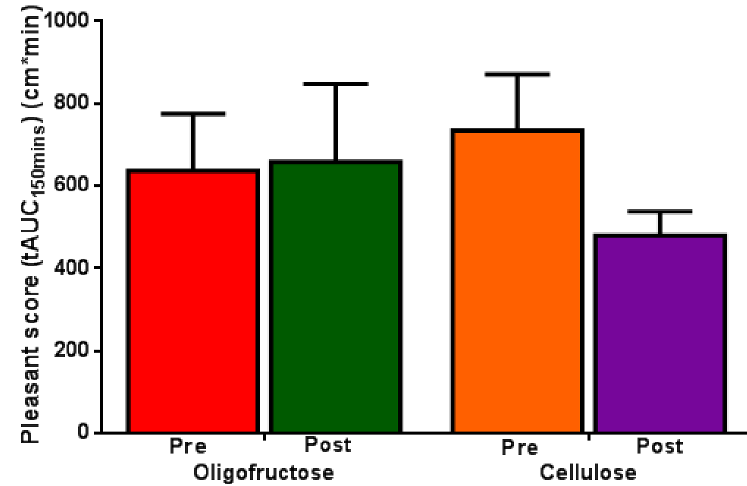

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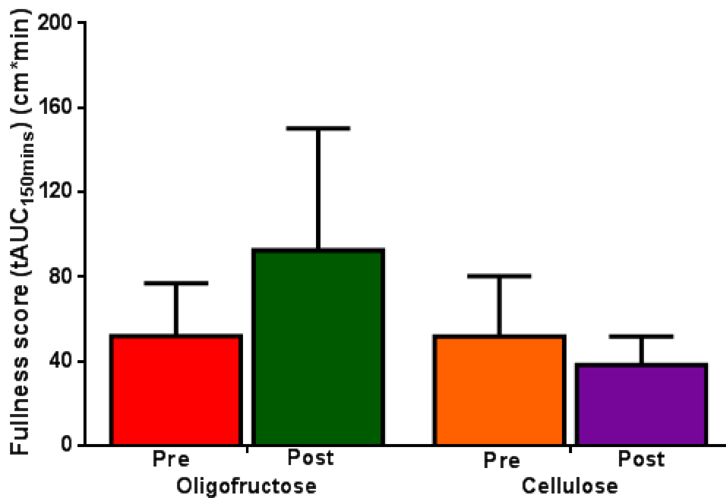

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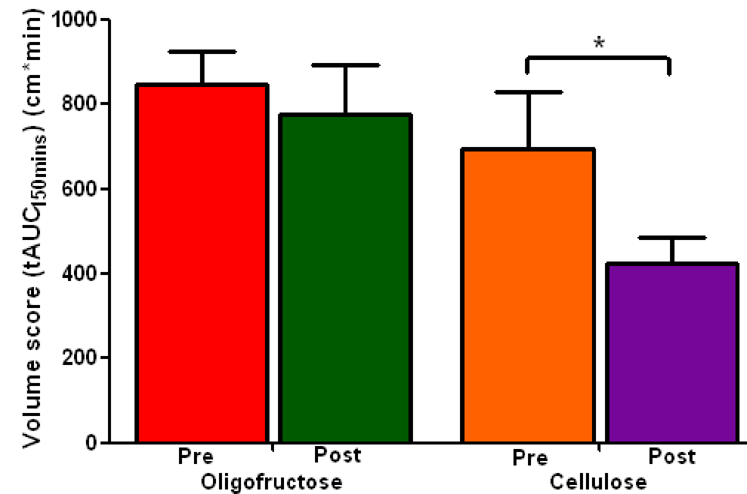


Figure 4.15 Subjective hunger, pleasant, volume intake and fullness scores in oligofructose or cellulose groups ($tAUC_{150mins}$). Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.5.1 Side Effects

Figure 4.16 demonstrates the subjective scores of sickness, anxiety, stress and sleepiness. Inclusion of oligofructose in the diet had no significant effect on sickness, anxiety, stress and sleepiness scores compared to cellulose supplementation (P=0.095, P=0.114, P=0.352 and P=0.424 respectively).

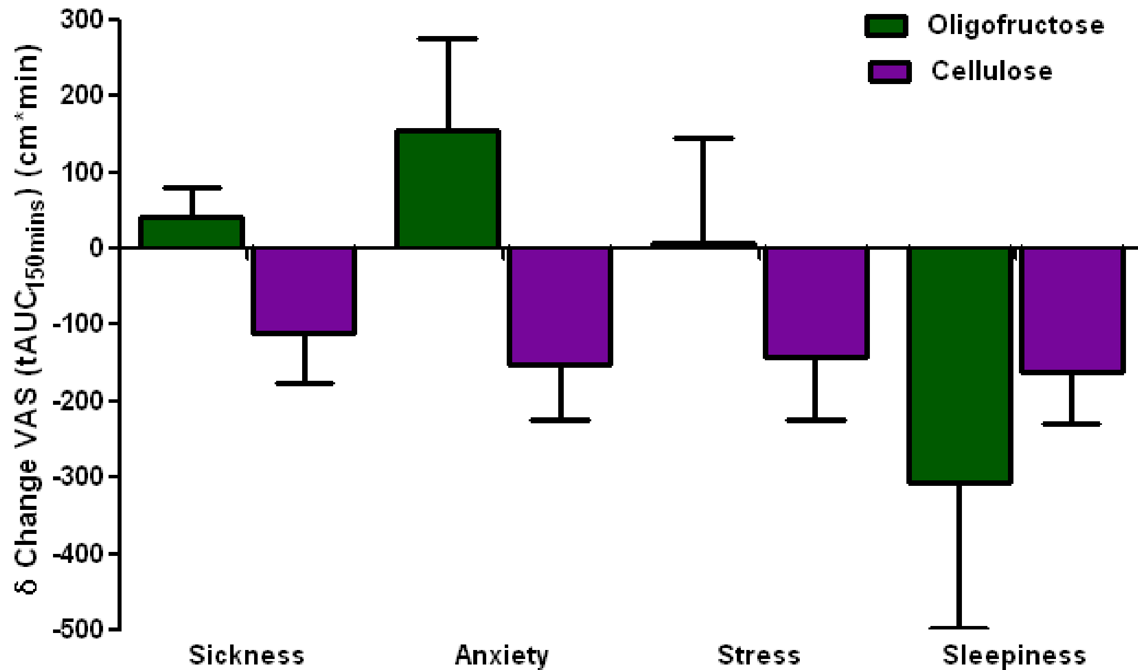


Figure 4.16 Change in subjective sickness, anxiety, stress and sleepiness scores in oligofructose or cellulose groups. Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.6 Gut Hormones

In this section, two types of gut hormones were assessed, GLP-1 and PYY which was obtained at 40, 55, 70 and 150 minutes at baseline (visit 2) and supplementation (visit 5). Two volunteers in the cellulose group had a problem with cannulation which resulted in haemolysed samples, therefore only eight volunteers were involved in this group analysis (oligofructose=4, cellulose=4).

4.4.6.1 Plasma GLP-1 Concentrations

Figure 4.17 demonstrates plasma GLP-1 (i) and $tAUC_{150mins}$ plasma GLP-1 (ii). The result showed that intake of oligofructose and cellulose had no significant effect on increasing plasma GLP-1 within each group (oligofructose: P=0.342 and cellulose: P=0.283). No significant effect was also demonstrated between the groups (P=0.198).

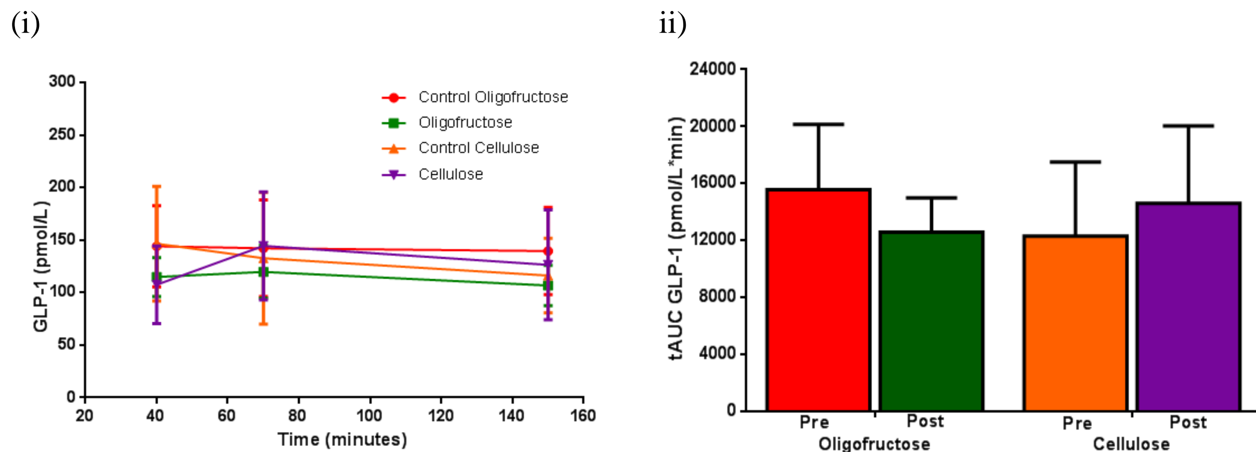


Figure 4.17 Fasting plasma GLP-1 levels in time course (pmol/l) (i) and $tAUC_{150mins}$ (ii) in oligofructose or cellulose groups. Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.4.6.2 Plasma PYY Concentrations

Figure 4.18 shows circulating plasma PYY levels in time course (i) and $tAUC_{150mins}$. Intake of oligofructose had no significant effect on increasing $tAUC_{150mins}$ PYY levels compared to baseline (P=0.730). The same observation was demonstrated within cellulose supplementation (P=0.683), mean ± SEM oligofructose [baseline; 2213.9 ± 117.5 and post: 3365.6 ± 456.0 pmol/l*min) and cellulose (baseline: 2143.2 ± 496.3 and post: 1925.1 ± 118.3 pmol/l*min]. No significant effect was also found between these groups (P=0.762).

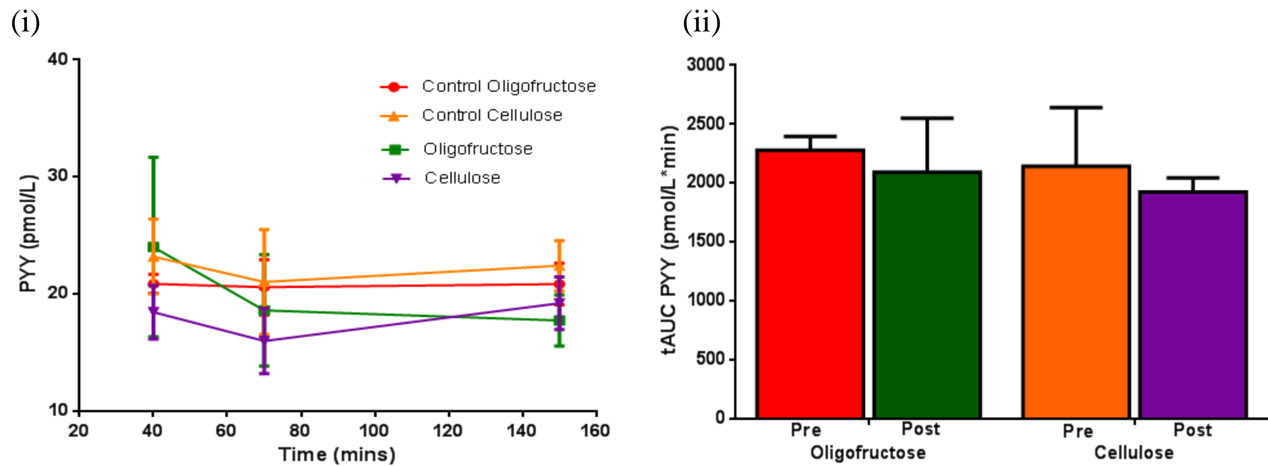


Figure 4.18 Fasting plasma PYY levels in time course (pmol/L) (i) and $tAUC_{150mins}$ (ii) in oligofructose or cellulose groups. Results are expressed as supplementation – baseline study day. Post-supplementation brain scans (between day 36-42 supplementation period) were performed after 5 hours ingestion of oligofructose or cellulose. Data is expressed as mean ± SEM. Oligofructose (n=4), cellulose (n=6).

4.5 Discussion

This preliminary study performed in humans is the first to investigate the effect of supplementing fermentable carbohydrate on BOLD fMRI activation. It was hypothesised that oligofructose supplementation would increase circulating plasma PYY and GLP-1 levels, leads to a reduction in BOLD signals in reward ROIs following viewing visual food stimuli, reduce food appeal rating and suppress subjective appetite scores. However, the results of this study did not support this hypothesis. Oligofructose supplementation showed no significant effect in pre-selected a *priori* ROIs (NAc, amygdala, OFC, hippocampus, insula and vACC) following viewing of visual food cues, PYY and GLP-1 levels, appeal ratings or VAS scores.

This is surprising as adding oligofructose to the diet has previously been shown to significantly increase plasma PYY and GLP-1 circulating levels, reduce energy intake and body weight in both rodents (Cani et al., 2004; Cani et al., 2005b; Cani et al., 2007b; Delzenne et al., 2005; Kok et al., 1998) and human studies (Cani et al., 2009; Parnell and Reimer, 2009; Verhoef et al., 2011). In addition, reductions in energy intake, body weight and body fat in rodents fed resistant starch and oligofructose-enriched inulin have also been shown to associate with increased hypothalamic activation signals particularly in the VMN and PVN (So et al., 2007) as well as in the ARC in MEMRI experiments (Anastasovska et al., 2012) .

In contrast, adding cellulose in the diet reduced BOLD activation in the ROIs: NAc, amygdala, hippocampus and insula. A significant reduction was also demonstrated in the OFC when viewing high calorie foods *vs.* object images. Additionally, reduced activations in OFC, amygdala and insula were also demonstrated after viewing food *vs.* objects images. The effect on reducing BOLD signals activation in cellulose group also maybe related with reduced hunger and volume food intake of VAS scores. However, no significant modulation on food appeal rating as well as circulating PYY and GLP-1 levels were found in both groups.

The lack of oligofructose effects on increasing circulating plasma PYY and GLP-1 levels could be because this study was conducted in the fasted state. In this study, measurements of plasma PYY and GLP-1 were performed following a 12 hour fast. Hence, it might be possible that oligofructose requires interaction with nutrients to modulate its effect on gut hormone release.

This suggestion is supported by the result obtained from the appetite study visit (figure 2.13, Chapter 2). The result showed that the effect of oligofructose on fasting plasma PYY and GLP-1 levels in the baseline visit (day 0) was no different compared with plasma fasting PYY and GLP-1 on post-supplementation visit (day 56). Similarly, Parnell and Reimer also showed no significant modulation on fasting plasma gut hormones after oligofructose supplementation (Parnell and Reimer, 2009). In contrast, intake of oligofructose with meal during breakfast leads to rise of postprandial plasma PYY levels at 120 minutes and subsequently peaked at 260 minutes compared with cellulose supplementation.

In this study, volunteers were scanned approximately six hours (12.30 hours) following oligofructose/cellulose intake in order to allow for the supplements to reach large intestine and subsequently be fermented by the gut bacteria (Molis et al., 1996; Rumessen et al., 1990). It was hypothesised that this time frame would allow oligofructose to increase released of circulating plasma gut hormones and in turn reduce BOLD activation following exposure to food stimuli and reduce hunger rating. However, it seems that this hypothesis was not proven. Based on these results, it is postulated that intake of 10 g (15.8 kcal) oligofructose (with water), without meals stimulation at seven am prior to scanning led to insufficient production of gut hormone secretion. This might be the reason why oligofructose failed to reduce BOLD activation in ROIs. Perhaps, the effect of oligofructose on reducing BOLD reward ROIs can be clearly demonstrated if the effect was compared between fasted versus postprandial supplementation visits. This is based on the result from the appetite study that intake of oligofructose with meal during breakfast significantly reduced energy intake at four hours thereafter (Section 2.4.1.3, Chapter 2).

It is still not entirely clear what caused the reduced BOLD activation in the reward ROIs in the cellulose group. Nevertheless, cellulose has been suggested to exert its role on satiation via three mechanisms, namely colonic fermentation, decrease transit time and bulking effects (Eastwood et al., 1973; Topping and Clifton, 2001; Wrick et al., 1983). However, It is unlikely that cellulose exerts it effect on BOLD activations via colonic fermentation as cellulose is mainly known as a low fermentation agent (Sunvold et al., 1995) compared to oligofructose. The distinct effect on increasing postprandial colonic fermentation between these two fibres has been shown very clearly using the breath hydrogen test performed on the appetite study day (Figure 2.12, Chapter

2). Instead of being a fermentation agent, cellulose has been shown to have a pronounced effect on shortening transit time (Cummings, 1984; Hillman et al., 1983; Wrick et al., 1983). Cellulose is the main structural component in plant cell walls. Adding cellulose in the diet reduces transit time as it escapes nutrient and absorption process, absorbs water from the colonic contents leading to increase faecal bulking. Therefore, it is suggested that cellulose, via its transit time and bulking effects, reduced BOLD reward in ROIs by activating intestinal vagal afferent to the hypothalamus and brainstem, which can then subsequently signals to the limbic regions, including the reward ROIs.

To date, no known study has investigated the effect of isolated dietary fibres on brain reward regions to study appetite control. Therefore, it is not possible to compare the findings with other studies. However, our observation that intake of cellulose reduces BOLD activation in brain reward regions is similarly demonstrated by other gut hormone infusion studies. Administration of anorectic hormones, $PYY_{3\text{-}36}$ (Batterham et al., 2007) and $PYY_{3\text{-}36}$ + $GLP\text{-}1_{7\text{-}36amide}$ (De Silva et al., 2011) have been shown to reduce activation in the hypothalamus (Batterham et al., 2007), OFC (Batterham et al., 2007; De Silva et al., 2011) and insula (De Silva et al., 2011) following viewing food cues stimulation and resting state experiment. Interestingly, both of these studies showed that the reduced activation in reward ROIs associated with a reduction of *ad libitum* energy intake. In this study, energy intake was not measured. Therefore, it is remains unknown whether the effect of cellulose on ROIs would lead to reduced energy intake. Nevetheless, cellulose supplementation showed a tendency to reduce hunger and volume scores compared to oligofructose. However, as the effect of cellulose on reducing BOLD activation and subjective appetite were demonstrated in a small study, this observation needs to be further clarified in a larger cohort.

Despite the promising findings demonstrated in the cellulose group, there are a number of limitations that should be taken into consideration before firm conclusions on the role of fibres on brain responses to food cues can be made. Firstly, this study was designed as a substudy of oligofructose intervention study which mainly looked into appetite, energy intake, and body weight. Therefore, the initial sample size (n=22) was not powered to fMRI signal change effects but it was determined based on the postprandial circulating plasma PYY levels. In addition,

powering this type of study based on expected changes in fMRI signal is difficult mainly because not many studies has been done in obese volunteers to investigate the effect of ROI BOLD signal changes in combination with other investigation (e.g. investigate the effect of oligofructose on brain activation and body weight at the same time).

Unfortunately, as the study developed, technical problems with the scanner were encountered, which resulted in only 10 out of 22 volunteers (oligofructose=4, cellulose=6) completing both of the baseline and supplementation brain scanning sessions. In contrast to other fMRI studies (Batterham et al., 2007; De Silva et al., 2011; Rothemund et al., 2007), the sample size of this study was comparatively low. Therefore, until the result of this study can be confirmed and re-investigated using a large sample size, the effect of cellulose on neuronal activity demonstrated in this study remains as a preliminary finding. However, the result of this study can be evaluated as a pilot study and used to power future investigation.

In this study, instead of whole-brain analysis, only several *priori* ROI regions were selected for the analysis. It might be possible that this has resulted in potentially overlooking activation in other regions. The primary reason for this was to limit the statistical tests to a few ROIs in order to control type I error during the analysis as this is only a small study of oligofructose on brain activity. In this study, the six pre-selected priori ROI regions; NAc, amygdala, OFC, hippocampus, insula and Acc were determined based on the activation map produced from food vs. object contrast which has been used to map with signal activation in another fMRI nutritional intervention study (section 4.6.2.4.1).

In order to assess whether the effect of dietary supplementation on ROIs activation was related to non-specific changes, control tasks such as object vs. blurred pictures contrast and an audio-motor-visual task were exposed to volunteers. An audio-motor-visual task mainly looked for non-specific changes in lingual gyrus, temporal gyrus and precentral gyrus. The result suggested that the effect of supplementation on ROI activation was not related to non-specific modifications as no significant difference was demonstrated when viewing blurred vs. object images as well as in the temporal gyrus, precentral gyrus and lingual gyrus regios following the AMV task. Including a control task in fMRI studies is important as it has been showed that

certain dietary components have the ability to alter fMRI BOLD signal activations (Laurienti et al., 2003).

In conclusion, supplementing oligofructose had no significant effect on reducing BOLD fMRI signal change following visual food-cues stimulation. In contrast, cellulose significantly reduced activation in the OFC and to a lesser extent reduced activation in other ROIs. These effects are also possibly leads to a trend towards reducing subjective hunger and volume intake scores demonstrated in the cellulose group. However, modulation in ROIs did not associate with circulating PYY and GLP-1 levels. The lack of effect of oligofructose on reducing fMRI BOLD signals possibly because it requires interaction with nutrients to exert its effect on inducing gut hormones secretion, thus reducing neuronal activation. This is because oligofructose has been shown to augment post-prandial PYY levels following intake with food/meal. Therefore, it might be possible that lack of effect on BOLD signals is related to the absence of gut hormone secretion. However, as this is only a small study with limitations including a relatively small number of volunteers, it is not possible to make a firm conclusion of the effect of dietary fibre on brain responses to food images. Therefore, confirmation of these findings needs to be performed in a larger cohort.

Chapter 5

The Development of Propionate Carrier Molecule as a Method of Understanding the Role of Propionate in Energy Homeostasis

5 Background

5.1 INTRODUCTION

The results from Chapter 2 to Chapter 4 suggest that fermentable carbohydrate, oligofructose has no significant effect in promoting weight loss in overweight / obese volunteers consuming high dose supplementation. Here, the effect of supplementing the end product of fermentable carbohydrate, SCFA on appetite and energy intake is explored.

It has been hypothesised that SCFAs, the main products generated from colonic fermentation of dietary fibres may have a significant role in modulating appetite and body weight. SCFAs are postulated to alter energy homeostasis by stimulating the release of anorectic gut hormones from the enteroendocrine L-cells possibly by binding to its receptors, FFAR2 and FFAR3 (Karaki et al., 2008). Therefore, increasing colonic production of SCFAs may have beneficial metabolic effects. Among the SCFAs, propionate is of interest due to its potential effect in modulating body composition (Berggren et al., 1996) and adipose tissue metabolism (Ge et al., 2008; Hong et al., 2005). Furthermore, recent evidence showed that propionate potentially involved in the stimulation of leptin secretion in adipocytes (Al-Lahham et al., 2010b; Xiong et al., 2004), therefore raising its potential as an anti-obesity treatment. This chapter will explore the role of propionate on gut hormone release and its effect on appetite.

5.1.1 Propionate

Propionate is a SCFA with three carbons and is the second most abundance colonic SCFA produced after acetate. Following its production in the colon, propionate is absorbed by the colonyctes and rapidly transported to the liver and the periphery. It was reported that 90% of the total propionate production is metabolised in the liver and the remaining 10% is circulated to the periphery (Wong et al., 2006). Propionate is predominantly produced from bacterial fermentation of dietary fibres in the colon. Besides endogenously produced by colonic microbial fermentation, certain foods also contain small amounts of propionate. Nevertheless, these foods are also produced from microbial fermentation, particularly propionibacteria (Fernandez-Garcia and McGregor, 1994) such as cheese,

yogurt, soured cream, sourdough bread and milk. Propionate has also been used as a preservative (E280) due to its role as fungal and bacterial growth suppressor (Levison, 1973).

Interestingly, studies have shown that L-rhamnose, a hexose sugar also able to increase serum propionate levels by four times compared with lactulose and glucose in both acute and long term studies (Vogt et al., 2004a; Vogt et al., 2004b). This suggested that L-rhamnose is potentially a propionate producer. In addition of natural food source, some studies also supplemented propionate directly to food products, such as breads (Dakin et al., 2004; Darzi et al., 2012; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Todesco et al., 1991), pasta (Frost et al., 2003) and also beverage (Ruijschop et al., 2008) in order to increase endogenous propionate production. In addition, this method also allow for a known amount of propionate to be delivered for metabolic activity. However, unlike propionate-derived colonic fermentation, propionate produced as part of the food products or added as a fermentative agent is likely to be absorbed in the small intestine, therefore may have different metabolic effects compared with propionate generated from colonic bacterial fermentation. To date, no known oral ingestion method has been shown to successfully increase propionate-derived colonic fermentation.

5.1.1.1 The Production of Propionate

During the colonic fermentation, gut microbiota degrade fermentable carbohydrates such as inulin-type fructans and resistant starch to propionate mainly via two distinct pathways. Substrates that contain pentoses undergo pentose phosphate pathway whilst substance with hexoses configuration are metabolised via Embden-Myerhoff-Parnas glycolytic pathway or alternatively, they can also be converted to 6-phospho-gluconate before entering pentose phosphate pathway for degradation. Both of these pathways generate pyruvate, a primary intermediary for propionate production. Pyruvate is then rapidly converted to propionate either via succinate decarboxylase pathway or the acrylate cycle (Cummings, 1981; Miller and Wolin, 1996; Prins, 1977; Wong et al., 2006) (figure 5.1).

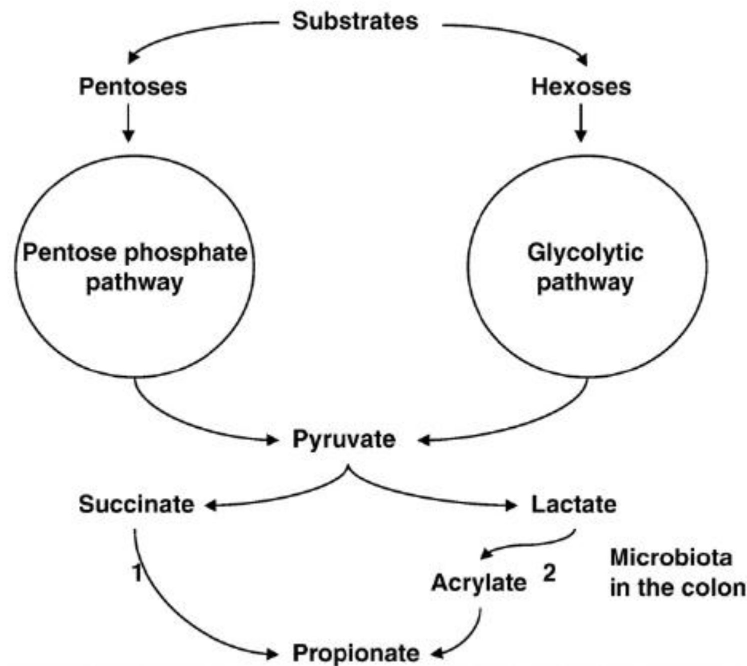


Figure 5.1 Colonic production of propionate by gut microbiota. Hexoses and pentoses derived from bacterial colonic fermentation of complex carbohydrates, protein or lipids are subsequently metabolised into pyruvate which is then either undergo succinate decarboxylation pathway or involved in the acrylate pathways. Diagram is adapted from (Al-Lahham et al., 2010a).

5.1.2 Metabolic Effects of Propionate

5.1.2.1 Propionate and Glucose Metabolism

From the large intestine, propionate is transported to the liver for glucose and lipid metabolism. In ruminants, propionate is known as the main gluconeogenic substrate (Leng et al., 1967; Wiltrout and Satter, 1972) whilst in humans, it is the only SCFA that is able to generate glucose (Al-Lahham et al., 2010a). In gluconeogenesis, propionate has two distinct roles; a substrate and also an inhibitor. As a substrate for gluconeogenesis, propionate enters kreb cycle through succinyl-CoA, which is then converts to oxaloacetate to produce glucose. The gluconeogenic effect of propionate in humans has been demonstrated in Wolever *et al.* (Wolever et al., 1991). In contrast to stimulate glucose production, propionate exerts its role as a gluconeogenesis inhibitor by inhibiting activation of pyruvate carboxylase, an enzyme that stimulates the conversion of pyruvate to oxaloacetate,

therefore reducing glucose production. In addition, methyl malonyl-CoA and propionyl-CoA, propionate's metabolites are also involved in gluconeogenesis by decreasing the activation of pyruvate carboxylase, hence inhibiting glucose production (Chan and Freedland, 1972; Wong et al., 2006) (figure 5.2). However, the effect of propionate in regulating blood glucose levels in humans is still unclear as data in the literature is inconsistent (Laurent et al., 1995; Todesco et al., 1991).

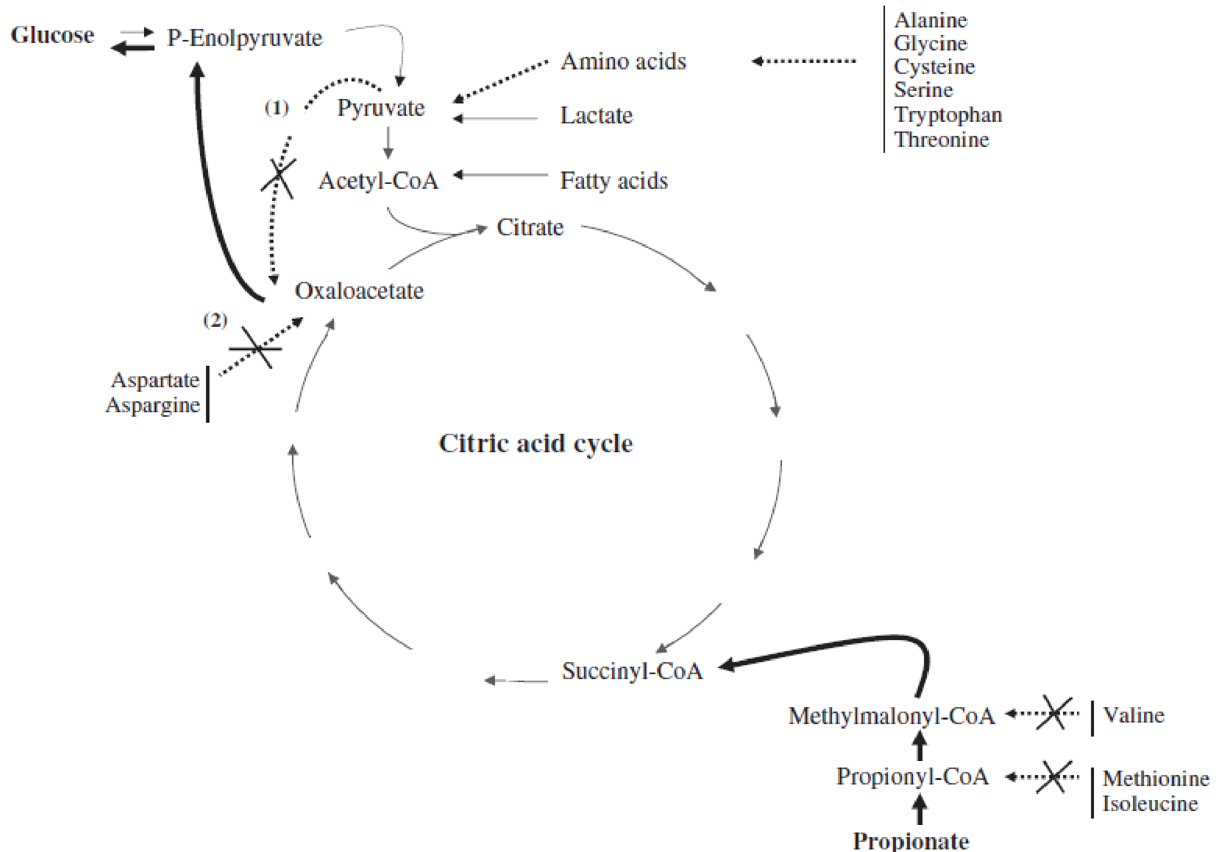


Figure 5.2 The role of propionate in gluconeogenesis, from (Verbrugghe et al., 2011). Propionate stimulates glucose production by entering citric acid cycle at the level of succinyl-CoA whilst it suppresses glucose production via its intermediates, methylmalonyl CoA and succinyl-CoA which inhibit the release of pyruvate carboxylase, the enzyme that modulate the conversion of pyruvate to glucose.

5.1.2.2 Propionate and Lipid Metabolism

Evidence from hepatocyte studies suggest that propionate may have a significant role in reducing fatty acid synthesis (Demigne et al., 1995; Lin et al., 1995; Nishina and Freedland, 1990).

Furthermore, several studies also showed that propionate has a hypocholestrolemic effect (Chen et al., 1984; Illman et al., 1988). The direct mechanism of how propionate reduces cholesterol is still not fully understood, but it is suggested that addition of propionate suppresses the release of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, an enzyme that involved in hepatic cholesterol synthesis (Bush and Milligan, 1971; Topping and Pant, 1995). Nevertheless, the role of propionate on lowering postprandial lipid levels in humans is controversial as studies showed no significant effect on plasma lipid levels after intake of 103 mmol/day and 78 mmol/day propionate for one week and seven weeks (Todesco et al., 1991; Venter et al., 1990). Nevertheless, Wolever *et al.* demonstrated that combination infusion of propionate and acetate was able to suppress the conversion of acetate to acetyl-CoA, a precursor for cholesterol synthesis (Wolever et al., 1991) and therefore reduced serum free fatty acid levels (Wolever et al., 1989). Indeed, addition of propionate into radiolabeled acetate infusion significantly reduced the conversion of acetate into triglycerides and lowered blood cholesterol levels compared when acetate was infused alone (Wolever et al., 1995). This finding showed that propionate has a promising role in modulating lipid metabolism. However, further investigations are required to confirm this observation.

5.1.2.3 Propionate and Insulin Sensitivity

The role of propionate on reducing free fatty acid and cholesterol levels mentioned in section 5.1.2.2, leads to a suggestion that propionate is a potential candidate for improving insulin sensitivity. Indeed, improved insulin sensitivity following lowering free fatty acid levels has been discussed in other studies (Boden, 2002; Boden and Shulman, 2002). Free fatty acids are generated from lipolysis in visceral adipose tissue and its secretion have been related with insulin resistance (Kennedy et al., 2009; Micha and Mozaffarian, 2009; Sharma and Chetty, 2005). Furthermore, the effect of propionate on insulin sensitivity might also relate with the role of SCFA receptors, FFAR2 and FFAR3 in stimulating adipogenesis and lipogenesis and suppressing lipolysis in adipocyte (section 5.1.2.4). Supplementing propionate in rodents (Ximenes et al., 2007) and human diets' (Darwiche et al., 2001; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996) also showed to reduce circulating insulin levels.

5.1.2.4 Propionate and Adiposity

SCFAs have been suggested to have a potential role in regulating adiposity after evidence showed that their receptors, FFAR2 and FFAR3 are found in adipocytes cells (Al-Lahham et al., 2010b; Brown et al., 2003; Ge et al., 2008; Hong et al., 2005; Le Poul et al., 2003; Xiong et al., 2004) (Please refer to Chapter 1 for the characteristic and physiological role of these receptors). Studies using 3T3 cell lines and primary mouse adipocytes cell cultures showed that acetate and propionate modulated adiposity by suppressing lipolysis and stimulating adipogenesis via FFAR2. Interestingly, PPARγ2, a pre-adipocyte differentiation marker was also shown to be stimulated following SCFAs' treatment (Ge et al., 2008; Hong et al., 2005). Although propionate has been shown to have a promising role in modulating adiposity, there is a disagreement of the availability of FFAR3 in the adipocytes in the literature. This is because after Xiong *et al.* confirmed the presence of FFAR3 mRNA expression in human and mouse adipose tissue in 2004, Hong *et al.* failed to show the same effect as Xiong *et al.* although the same techniques were applied (Hong et al., 2005; Xiong et al., 2004). Therefore, the availability of FFAR3 in adipocyte tissues is yet to be fully established.

5.1.2.5 Propionate and Leptin Expression

Recent evidence also showed that SCFAs, particularly propionate potentially plays a role in stimulating leptin secretion in rodents, bovine and human adipocytes (Al-Lahham et al., 2010b; Hong et al., 2005; Lee and Hossner, 2002; Soliman et al., 2007; Xiong et al., 2004). However, Al-Lahham *et al.* demonstrated that the levels of propionate needed to induce leptin secretion in *in vitro* human adipocytes explants are relatively high compared to the levels produced endogenously by the colonic fermentation, therefore questioning whether this is achievable *in vivo* (Al-Lahham et al., 2010b). In another experiment, Xiong *et al.* showed that propionate stimulated leptin production (increased by 2 fold) potentially via its receptor, FFAR3 in mice which were orally feed by gavage. However, the stimulated leptin levels were insufficient to reduce food intake. Nevertheless, it is interesting to note that the propionate supplemented in this study was in the physiological range therefore raising a possibility to be applied endogenously (Xiong et al., 2004). Taken together, these findings suggest that propionate may have a pronounced role in stimulating leptin expression, however the actual mechanism of how propionate regulates this process is yet to be determined.

5.1.3 Effects of Supplementing Propionate on Gut Hormones Released, Satiety and Food Intake

Studies have suggested that adding propionate in the diet leads to increased satiety sensation (Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Ruijschop et al., 2008), which is suggested to be due to a delay of gastric emptying (Liljeberg and Bjorck, 1996). In addition, Frost *et al*. agreed that adding 3 g of sodium propionate (31 mmol) into pasta not only delayed gastric emptying, but also increased plasma GLP-1 release and lowered postprandial glucose levels. Surprisingly, inclusion of propionate into sourdough bread administered to 12 healthy volunteers did not alter either appetite or energy intake in an acute test meal study (Darzi et al., 2012) possibly due to lower amount of propionate supplemented (2.3 mg/g propionic acid and 2.98 mg/g calcium propionate). In contrast to other studies (Darwiche et al., 2001; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Todesco et al., 1991), this study supplemented low amount of propionate in order to investigate the effect of palatable propionate bread as it has been suggested that high supplementation of propionate could caused nausea (Frost et al., 2003), which could be a confounding factor in reducing energy intake and appetite. Therefore, Darzi *et al*. suggested that the effect of propionate on energy intake and appetite scores demonstrated in these studies (Darwiche et al., 2001; Frost et al., 2003; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Todesco et al., 1991) were influenced by the unpalatable propionate taste, not a metabolic effect.

Recently, infusion of 20 mmol (peripheral) and 60 mmol (rectal) acetate in six hyperinsulinemic female patients has been shown to increase PYY and GLP-1 secretion. However, no effects on adiponectin and ghrelin secretion were demonstrated (Freeland and Wolever, 2010). In contrast, rectal infusion of 54 or 90 mmol of mixed SCFA (37.8 and 63 mmol acetate respectively) showed no effect on stimulating GLP-1, PYY and oxyntomodulin release (Ropert et al., 1996). These discrepancies might be due to different rates of infusion administered, which subsequently influenced the gut hormones secretion. In these studies, Freeland *et al*. infused 300 ml in 8 mins (37.5 ml/min) whilst Ropert *et al*. administered 180 ml in 60 mins (3 ml/min) (Freeland and Wolever, 2010; Ropert et al., 1996).

Despite SCFAs supplementation being shown to modulate appetite and gut hormones secretion, there are not many studies that have investigated SCFAs role on body weight. There is currently only one human study to investigate the role of SCFA on body weight. In this study, a low dose (15 ml or equivalent to 12.5 mmol acetate) or high dose (30 ml or equivalent to 25 mmol acetate) apple cider were supplemented in 105 obese Japanese volunteers for 12 weeks. The result showed that including acetate in a diet significantly reduced body weight, BMI, body fat and waist-hip ratio compared to the control (Kondo et al., 2009). In animals, supplementing propionate (4.3 % w/w) and butyrate (5 % w/w) in mice diet have been shown to suppress body weight gain in four weeks study (Lin et al., 2012). These encouraging results highlight the potential role of SCFAs supplementation as an anti-obesity treatment. However, this effect needs to be strengthened with extensive investigations to support this observation.

5.1.3.1 Challenges in delivering SCFAs to the large intestine

Although including SCFAs into diet have been shown to positively affect energy homeostasis, there are some drawbacks that might limit potential use as a food supplement. As previously mentioned, current available methods in delivering SCFAs to the gut are either via oral feeding and rectal infusion. Rectal infusion, although successfully able to deliver a high amount of SCFAs to the colon, is not a practical method to use both in clinical and free-living individual monitoring. Therefore, oral feeding is the only practical and suitable method to deliver the SCFAs to the gut (Bonnema et al., 2010; Karalus et al., 2012). However, oral supplementation with high fibre diets, although potentially demonstrated to suppress appetite and improve body composition, is related to gastrointestinal side effects, which can discourage food intake, thus this can confound the effect on appetite and energy intake. Inclusion of SCFAs *per se* in oral supplementation showed to potentially suppress appetite and body weight, the main challenge is the side effect (nausea), poor organoleptic issues and its short circulating plasma half-life (Darzi et al., 2011; Frost et al., 2003; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996).

The challenge is how to modulate energy homeostasis with SCFA without generating the adverse side effect. Therefore, a new route is needed to ensure increased colonic SCFA production

without the negative side effects associated with high fibre diets or oral supplementation of SCFAs. In order to achieve this goal, a collaboration with Dr Douglas Morrison and his colleagues from Scottish Universities Environmental Research Centre (SUERC), Glasgow, Scotland UK), we have created a novel delivering system whereby, propionate is bound to a carrier molecule (i.e. fermentable carbohydrate, inulin). This bound propionate can only be cleaved by colonic enzymes, which ensures that the conjugated propionate is released only after it arrives in the colon and the carrier is then digested by the gut microbiota. It is therefore proposed that by supplementing food with this novel propionate carrier molecule (PCM) will generate high colonic levels of propionate to stimulate FFAR2/FFAR3 receptors on L-cells to release gut hormones PYY and GLP-1 and increase satiety. The propionate carrier molecule could therefore be used as an effective treatment for obesity and its associated co-morbidities.

5.2 AIMS & HYPOTHESIS

5.2.1 AIMS

To develop an effective method of increasing colonic propionate levels in order to stimulate PYY and GLP-1 production leading to suppressed appetite and body weight. To achieve this objective, the following parameters were measured:

- Breath hydrogen release as a surrogate marker of colonic fermentation
- Plasma anorectic gut hormones, GLP-1 and PYY
- Subjective appetite scores using VAS
- Energy intake during *ad libitum* meal.

5.2.2 HYPOTHESIS

Delivering propionate, to the colon using a novel carrier molecule would stimulate the release of postprandial GLP-1 and PYY levels, affect appetite and decrease energy intake at an *ad libitum* meal.

These sections onwards will discuss four different propionate carrier molecule pilot studies which are the first investigations performed in humans.

5.3 STUDY 1: A first-in-man study to evaluate the safety, tolerability, colonic fermentation and gut hormones of Propionate Carrier Molecule: A single-blinded, pilot study.

Aim: To investigate the tolerability of 10 g propionate carrier molecule and its effect on colonic fermentation, GLP-1 and PYY levels and energy intake.

Hypothesis: Supplementing the diet with 10 g propionate carrier molecule would be well-tolerated in humans, increases colonic fermentation and trigger the release of anorectic gut hormones.

5.3.1 Materials

5.3.1.1 Propionate Carrier Molecule

The propionate carrier molecule (PCM) supplemented in this study was formulated and synthesized by Dr. Douglas Morrison from SUERC, Scotland, UK. This molecule is synthesized by chemically joining propionate to some of the carboxyl groups of fructose units via a novel conjugating system. In this conjugated molecule, PCM with a degree of esterification (DE) 0.25 was used in this study. This would allow 10% propionate to be bound to inulin. This means that in 10g PCM supplemented into a diet, one gram of propionate was added to nine grams inulin. Following supplementation, PCM is brought to the colon intact where the conjugated linkage is digested by the colonic enzymes. Inulin is then fermented by the gut microbiota. The exact methodology for the production of the PCM is currently in the process of getting intellectual property pattern. Hence, the exact method of this product is protected.

5.3.1.2 Dietary Treatments

PCM was added to wholemeal bread rolls which each roll contained ten grams PCM. The study control was also made from wholemeal bread, but without addition of PCM. The bread rolls weighted 110 g and were provided by Premier Foods, Holgran-Hovis division (Lichfield, Staffordshire, UK). The rolls were formulated to have the same taste and energy content of 320 kcal or 1339 kJ/100g and were stored at -20ºC before distributed to volunteers for home supplementation. The ingredient of the bread rolls are described below in Table 5.1.

Table 5.1 Ingredient of the control and propionate carrier molecule bread rolls

Ingredient	Weight (g) / bread roll	%
Bakers pride (flour)	57.6	52.3
Zippy +	0.6	0.5
Water	39.4	35.9
Block yeast	1.7	1.6
Salt	0.9	0.8
PCM (Treatment visit only)	9.8	8.9
Total	110	100

5.3.1.3 Volunteers

As this is the first time that PCM is investigated in human, a small pilot study was performed in six volunteers (four men and two women), aged between 21 to 65 years old and BMI in the range of 25 to 35 kg/m^2 following approval from Hammersmith, Queen Charlotte's and Chelsea Research Ethic Committee (08/H0707/99) (Registration No: NCT00750438). Written informed consent was obtained from each volunteer before the study began. Volunteers were recruited through poster advertisements at Hammersmith Hospital, St. Mary's Hospital, Charing Cross Hospital and the South Kensington campus of Imperial College London. Volunteers had a stable weight (not gain or loss weight more than three kg prior the start of the study), were free from any chronic diseases and were not on any prescription medication.

5.3.2 Methods

5.3.2.1 Study Design

5.3.2.1.1 Screening Session

Potential volunteers were first asked to attend a screening session in which their weight and height were measured, blood pressure was monitored, heart checked using an ECG and a small amount of blood for full blood count, urea and electrolytes, liver function tests, thyroid function tests and blood glucose levels were obtained. In order to access their dietary intake, volunteers were asked to complete DEBQ (van Strien et al., 1986) and SCOFF questionnaires (Morgan et al., 1999). Volunteers who were identified as restrained dieters from the questionnaires were not enrolled into the study. The study was performed in accordance with the declaration of Helsinki.

5.3.2.1.2 Before the Study Day

Volunteers were given verbal and written instruction to refrain from drinking alcohol, undertaking strenuous physical activity or abnormal energy intake for 24 hours before the study sessions. They were asked to eat a standardised evening meal and commencing a 10-12 hour fast before attending appetite study day the next morning. Only water was allowed to be consumed during this time. They were asked to complete a food diary 24 hours before each study day to ensure the standardised meal was consumed. The food diaries were analysed using Dietplan6 (Forestfield Software Ltd, West Sussex, UK).

5.3.2.1.3 Assessment Day

Volunteers were asked to attend two feeding study sessions at Sir John McMichael Centre, Hammersmith Hospital at 0845 after been fasted for 10 – 12 hours. The study days commenced at 0900 and finished at approximately 1500. An intravenous cannula was inserted into antecubital vein for blood sampling throughout the study day. Meals, hydrogen breath tests and serial blood samples were administered at defined time points (the parameters are described in the section 5.3.2.1.3.1, 5.3.2.1.3.2 and 5.3.2.1.3.3). Volunteers were asked to remain seated during the study with minimal physical activity. Study day one was the control study day where no supplement was given. Before volunteers were discharged home, they were given five bread

rolls containing ten grams of PCM for home consumption. They were advised to consume one roll per day by dividing it into three equal portions to eat with their breakfast, lunch and dinner. Five days PCM supplementation was performed in order for the gut to adapt with the new diet and reduce any potential gastrointestinal side effects. On day seven (study day 2), ten grams PCM was supplemented in the bread rolls and served during breakfast. The rest of the study protocol was similar to the study day one. Compliance was checked by counting the uneaten bread rolls returned on the second study day. An overview of the study is depicted in figure 5.3.

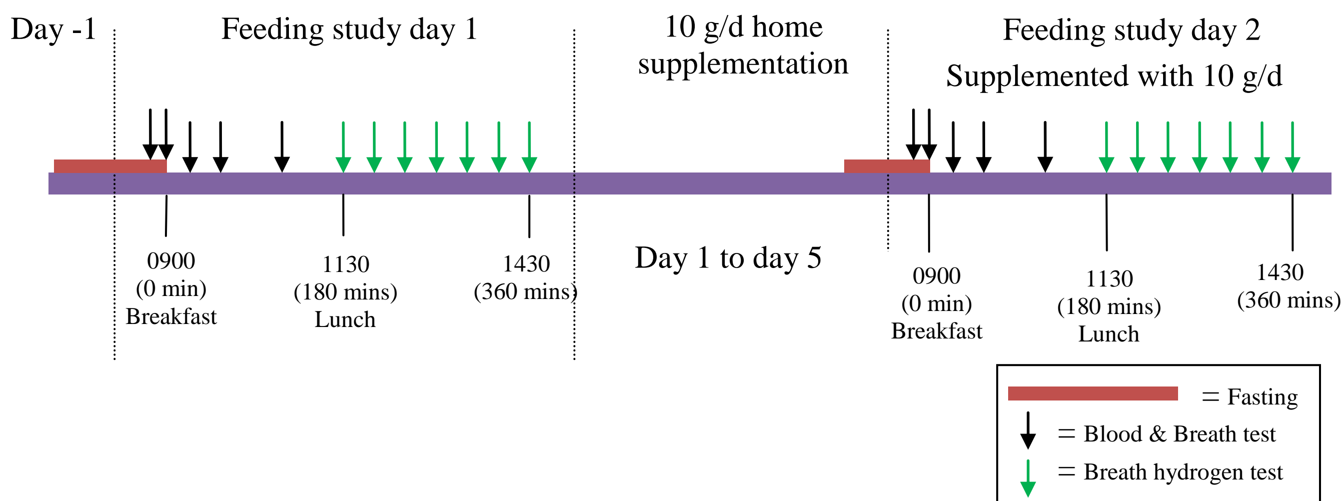


Figure 5.3 The schematic diagram of propionate carrier molecule pilot study. Volunteers were asked to attend two appetite study days; the control study day and 10 g PCM supplementation study day. A day after attending the control study day, volunteers started their 10 g/day PCM supplementation at home for 5 days. They completed their study by attending the supplementation study day on day 7 in which 10 g of PCM was administered. On each appetite study day, blood and breath hydrogen test were obtained throughout of the study period. The time course of the study were the baseline -10 and 0 mins whilst 30, 60, 120, 180, 210, 240, 270, 300, 330 and 360 mins for postprandial measurement.

5.3.2.1.3.1 Study Meals

Standardised breakfast and lunch were served at 0 and 180 minutes respectively. Meals supplied during the study days were based on choices made during the screening visit and these were applied for both of the study days (table 5.2). During the meal intake, volunteers were instructed to finish both of their breakfast and lunch within 10 and 30 minutes respectively.

Table 5.2 Meals options and macronutrients composition on the study days.

	Carbohydrate	Protein	Fat	Energy (kcal)
Breakfast				
150 ml semi-skimmed milk	7.05	5.10	2.55	69.00
250 ml Tropicana orange juice	22.50	1.75	-	107.50
110 g propionate ester / control roll	62.90	15.30	2.70	352.00
6.5 g butter	0.01	0.03	5.31	47.91
Cereals (chosen by volunteers)				
• 30 g Nestle Cookie crisp	24.03	1.92	1.02	113.10
• 30 g Nestle Nesquick	23.73	2.19	1.14	113.70
• 30 g Nestle golden nugget	25.20	2.10	0.45	113.40
10 g PCM bread rolls				320 kcal
Lunch (One of the ready meal below)				
Ready meals (Sainsbury's)				
• Spaghetti Bolognese	64.70	28.90	21.10	141.0
• Tomato & mozzarella pasta bake	72.00	20.20	12.80	120.0
• Chilli con carne	50.00	34.70	11.30	98.00

5.3.2.1.3.2 Breath Hydrogen Assessment

Breath hydrogen was assessed in the same method applied in the previous oligofructose study (section 2.3.2.2.3). The timepoints were -10, 0, 30, 60, 120, 180, 210, 240, 270, 300, 330 and 360 minutes.

5.3.2.1.3.3 Gut Hormones Analysis

Blood sampling for gut hormones PYY and GLP-1 were drawn and collected into lithium heparin tubes containing aprotinin (200 µl/7.5 ml blood). Each sample was then spun at 4000g at 4°C for 10 minutes and the plasma separated and divided into aliquots before immediate storage at -80°C. Gut hormones, PYY and GLP-1 were analysed using radioimmunoassay. Please refer to section 2.3.2.2.5 for the RIA methodology.

5.3.2.1.3.4 Gastrointestinal side effect assessment

In order to assess tolerability of the supplement, gastrointestinal side effects of stomach discomfort, nauseous, bloating, flatulence, heartburn, belching and diarrhoea scores were measured at 120 minutes using VAS (section 2.3.2.2.2).

5.3.2.1.4 Statistical Analysis

Data are presented as means ± SEM. Before statistical analyses were performed, data was checked for Gaussian distribution using the Kolmogorov-Smirnov test. All the data involved in this study are normally distributed. The area under the curve for breath hydrogen, plasma GLP-1 and PYY were calculated using the trapezoid rule. Differences between PCM and the control treatment for $tAUC_{360mins}$ breath hydrogen levels, $tAUC_{120min}$ postprandial GLP-1 and PYY levels as well as side effects assessment were assessed using a student two-tailed paired *t-test*. Statistical significance is defined by a P value of 0.05 or less and all statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego CA, USA).

5.3.3 Result

5.3.3.1 Volunteers Characteristics

Six participants (male=4 and female= 2) with mean age 36.8 ± 5.8 years (age range: 21 – 55 years) and BMI 30.5 ± 1.4 kg/m^2 were enrolled into this study, all of which completed both of the study days.

5.3.3.2 PYY and GLP-1 Analysis

Figure 5.4 shows $tAUC_{120min}$ plasma PYY (i) and GLP-1 levels (ii). Consumption of PCM supplementation on day seven showed a trend towards higher $tAUC_{120mins}$ PYY levels (P=0.059) (5884.4 ± 534.7 pmol/l*min) compared to the control group (4873.0 ± 494.3 pmol/L*min). In contrast, no significant effect of treatment (P=0.218) on plasma GLP-1 levels was demonstrated.

i) ii)

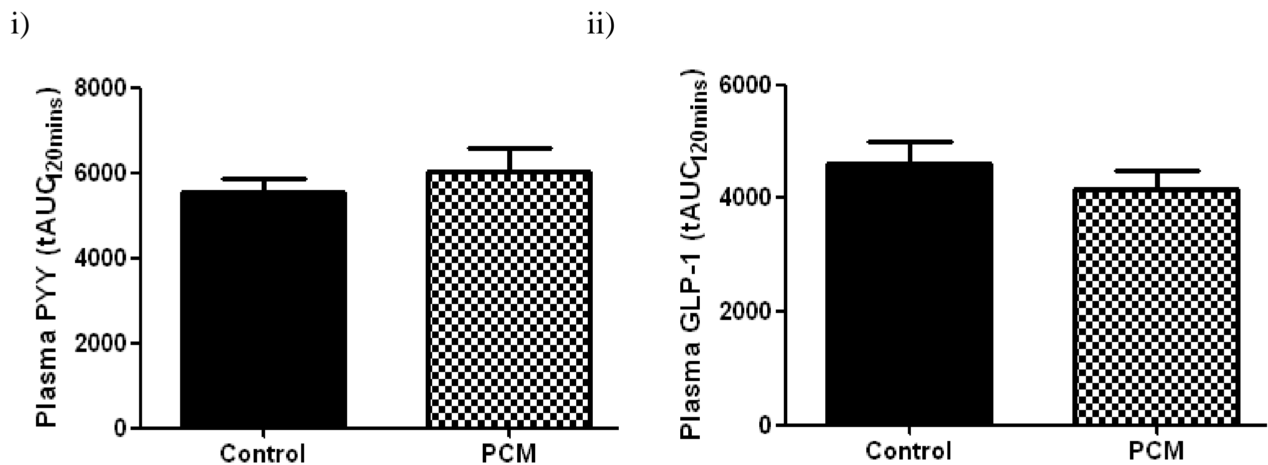


Figure 5.4 $tAUC_{120min}$ postprandial plasma PYY (i) and $tAUC_{120min}$ GLP-1 (ii) levels following intake of 10 g of PCM and control supplementations. The doses were supplemented to volunteers at 0 min. Standardised breakfastand lunch were provided at 0 and 180 mins respectively. Data is expressed as mean ± SEM, (n = 6).

5.3.3.3 Breath Hydrogen Analysis

Figure 5.5 demonstrates the breath hydrogen levels and $tAUC_{360mins}$ following intake of PCM and the control treatments. Intake of PCM significantly increased $tAUC_{360mins}$breath hydrogen excretion (P=0.048) compared to the control treatment. Fasting breath hydrogen levels were increased with PCM (PCM: 8.9 ± 4.5 ppm) but this was not significant (P=0.270) when compared with the control treatment (control: 2.4 ± 1.6 ppm). Intake of PCM consumption also led the breath hydrogen to peak at 270 minutes and remained in the higher levels until the end of the assessment timepoint. Contradictory, breath hydrogen following intake of control treatment resulted the levels remained under 15 ppm throughout the study period, mean ± SEM (36.7 ± 10.6 ppm and 12.2 ± 6.6 ppm (PCM and control respectively).

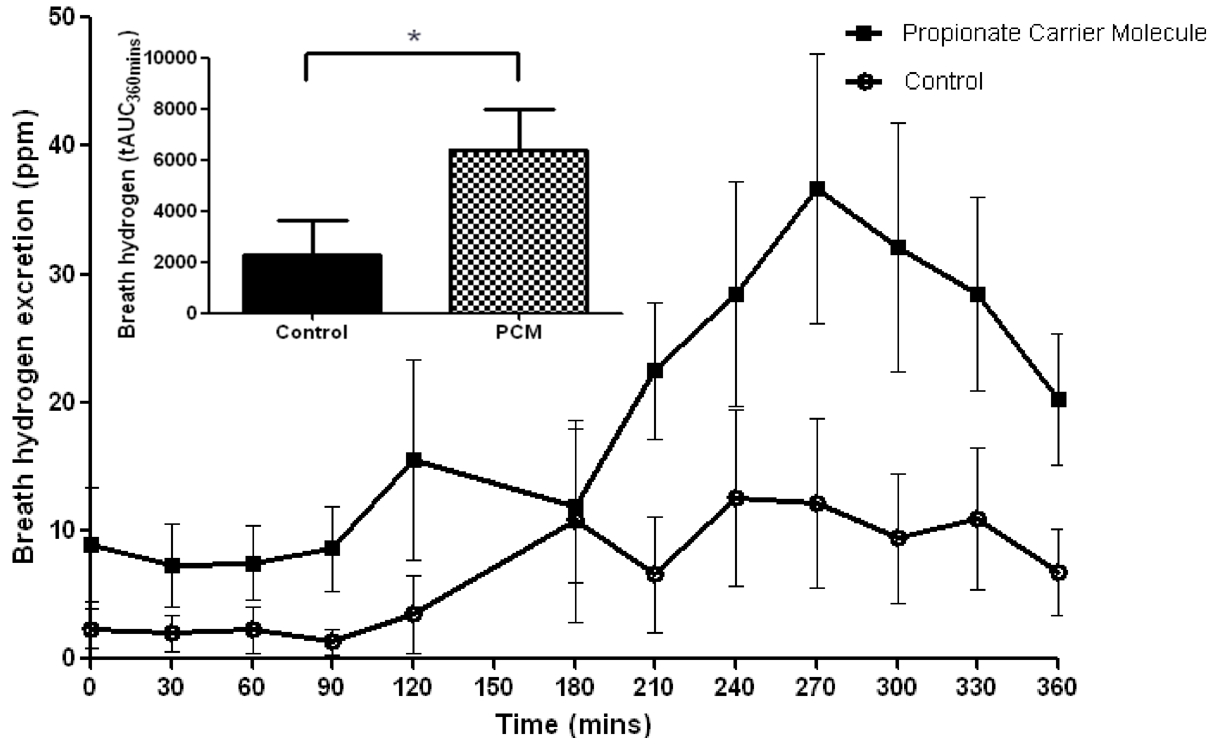


Figure 5.5 Breath hydrogen excretion (ppm) and $tAUC360_{mins}$ (inset) following intake of 10 g of PCM and control supplementations. The doses were supplemented to volunteers at 0 min. Standardised breakfast and lunch were provided at 0 and 180 mins respectively. Data is expressed as mean ± SEM, (n = 6). *P=0.048 PCM *vs.* control.

5.3.3.4 Side Effects Assessment

PCM supplementation was well-tolerated when rated at 120 mins with low cases of gastrointestinal side effects were reported. Ratings of stomach discomfort (P=0.625), nauseous (P=0.289), bloated (P=0.708), flatulence (P=1.000), heartburn (P=0.500), belching (P=0.177) and diarrhoea (P=0.500) were not significantly difference between the treatments (Figure 5.6).

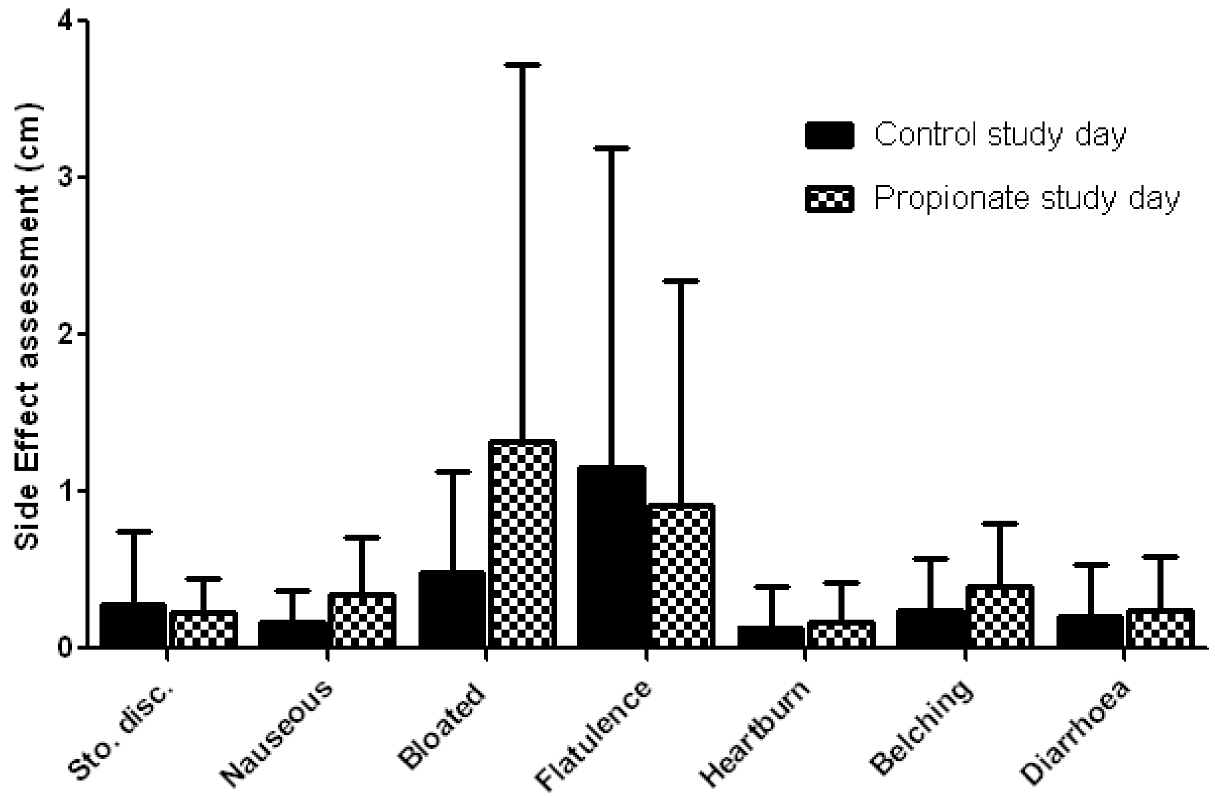


Figure 5.6 Assessment of GI side effects; stomach discomfort, nausea, bloating, flatulence, heartburn, belching, diarrhoea and bowel habits following 2 hours intake of 10 g PCM and control. The doses were supplemented to volunteers at 0 min. Standardised breakfastand lunch were provided at 0 and 180 mins respectively. Data is expressed as mean ± SEM, (n = 6)

5.3.4 Discussion

Propionate carrier molecule (PCM) is a novel SCFA colonic delivery method and its effect in humans has not yet been investigated. Therefore, the aim of this study was to investigate tolerability and its ability to deliver propionate directly to the human gut. In addition, its effect on postprandial gut hormones levels was also explored. The findings from this study will then be used in an investigation looking at the effect of PCM on appetite and energy intake in overweight and obese volunteers (Study 2).

The result of this study is consistent with the hypothesis that supplementing 10 g PCM for five days in six overweight volunteers was generally well-tolerated with no significant gastrointestinal side effect. Only one volunteer scored moderate on bloating scores. Evidence showed that increasing fibre intake in the diet could be associated with bloating and flatulence (Bonnema et al., 2010; Cani et al., 2006a; Karalus et al., 2012). However, these side effects were usually reported following high fibre intakes (>20 g), whilst less effects were demonstrated with fibre dose less than 15 g/day (Kaur and Gupta, 2002). Another issue was that some volunteers reported that intake of PCM bread rolls associated with bitter taste. It is speculated that the bitter taste could be attributed to the unbound propionate which was released during the binding of propionate to inulin. The palatability of the PCM will be improved in the next study.

Breath hydrogen analysis showed that adding PCM to the diet of volunteers significantly increased $tAUC_{360min}$ breath hydrogen levels compared to the control. In addition, the levels peaked at 270 minutes with mean value of 37 ppm, suggesting that PCM took approximately five hours to reach the colon to be fermented by the gut microbiota. Interestingly, after peaking at 270 minutes, breath hydrogen excretion levels seemed to be maintained at that level until the end of the study period. Hence, it might be possible that the fermentation activities were still occurring after six hours PCM ingestion. In contrast to PCM treatment, hydrogen excretion remained at a low level ($\leq$ 10 ppm) throughout the experiment in the control group. Piche *et al*. suggested that the elevation of breath hydrogen levels of more than 10 ppm from the baseline as an indicator of fermentable carbohydrate reaching the cecum thus activating the colonic bacterial fermentation (Piche et al., 2003). This observation highlights the potential of SCFA-carrier complex system to be used as a mediator to deliver specific SCFA to the colon. However, this observation remains to be investigated.

Evidence suggests that increased hydrogen excretion following fibre intake is associated with an increase in PYY and GLP-1 released (Cani et al., 2009; Frost et al., 2003; Gee and Johnson, 2005; Piche et al., 2003; Verhoef et al., 2011). In the present study, diet containing PCM showed a trend towards increasing $tAUC_{120mins}$ plasma PYY levels, but no significant effect on plasma GLP-1 was observed. It might be possible that the SCFA-carrier complex provided in this study is insufficient to increase colonic SCFA levels to a level that would increase gut hormone release. Nevertheless, it is might be possible that five days intake of PCM is not long enough to elevate plasma GLP-1 release compared to plasma PYY secretion. In contrast to our study, Cani *et al*. and Verhoef *et al*. showed an increase of plasma GLP-1 levels after 13 and 16 days of fermentable carbohydrates supplementation (Cani et al., 2009; Verhoef et al., 2011). Also, the fact that this study involved a small number of volunteers (n=6) might have resulted in insufficient power to detect changes in gut hormone release.

As a conclusion, this acute pilot study suggested that diet containing PCM was well-tolerated by volunteers, increased colonic fermentation activities and increased plasma PYY secretion. Further investigation is warranted to determine the possible effect of the PCM on appetite control.

5.4 STUDY 2: The Effects of Propionate Carrier Molecule on Colonic Fermentation, Gut Hormones Release and Appetite: A Controlled, Randomised, Double-Blind, 5 weeks study.

Aim: To investigate the effect of supplementing 10 g propionate carrier molecule on colonic fermentation, postprandial GLP-1 and PYY levels, appetite and energy intake.

Hypothesis: Diet containing propionate carrier molecule would significantly increase PYY and GLP-1 and subsequently suppress appetite and energy intake.

5.4.1 Materials

5.4.1.1 Dietary Treatments

In addition to PCM, inulin was used as a positive control in this study. Inulin, Beneo HP, Raftiline batch HPHPD7BPD was acquired from Sudzucker AG Mannheim, Ocshenfurt, Germany. In order to increase palatability, sourdough bread with added sugar was used in this study. In contrast to the previous study, the amount of PCM was reduced from 10 g to 5 g in each roll (table 5.3). Therefore, volunteers were required to take two rolls per day in order to have 10 g supplementation. In addition, the amount of propionate supplemented in the PCM was increased from 10% to 20% (2 g of propionate and 8 g of inulin was provided).

Table 5.3 Ingredient of the control (inulin) and PCM containing bread rolls (g)

Ingredient	Weight / bread roll (g)	%
Flour - goldcrest	95.2	86.5
Raftiline HP inulin / PCM	6.7	6.1
Sugar	2.9	2.6
Block yeast	2.9	2.6
Salt	1.5	1.4
Zippy plus	1.0	0.9
Total	110	100

5.4.1.2 Sample Size Calculations

The sample population was calculated using the Russ Lenth power calculator (Lenth, 2009). The primary outcome was plasma PYY release with subsequent reduction in appetite feelings and energy intake. A difference of 20 pmol/l with standard deviation of 18 pmol/l in each group was considered as a significant impact on appetite. Based on a power of 90% with p value of 0.05, it was estimated that 20 volunteers were needed to reach the significant. To allow for a drop-out, 26 volunteers were recruited.

5.4.1.3 Volunteers

Healthy overweight volunteers aged between 21 to 65 years old and BMI of 25 to 35 by advertising in Hammersmith, St. Mary's, Charing Cross hospital and Imperial South Kensington's campus. 26 volunteers were involved in which each of them giving their written informed consent prior the start of the study. Volunteers were comprised of local population, Imperial College of London students and six of them were the volunteers from the previous PCM pilot study. Before the study began, approval was sought from Hammersmith, Queen Charlotte's and Chelsea Research Ethic Committee (08/H0707/99) (Registration No: NCT00750438) and the study was performed in accordance with principles of the Declaration of Helsinki. Volunteers' details are described in section 5.4.3.1.

5.4.2 Methods

5.4.2.1 Study Design

5.4.2.1.1 Randomisation

Volunteers were randomly assigned to PCM or inulin group by a colleague who was not involved in this study using a sealed envelope system.

5.4.2.1.2 Assessment Day

Volunteers arrived at Clinical Unit, Sir John McMichael Centre, Hammersmith Hospital at 0830 in the morning after 10-12 hours overnight fast on the same day every week for five weeks. Upon arrival, a cannula was placed in an antecubital vein for the duration of the study. In each

visit, the study day started at 0900 and finished around 1500 (360 minutes). Similar with previous study, hydrogen breath tests, VAS and blood were collected at -10, 0, 30, 60, 90, 120, 180, 210, 240, 270, 300 and 360 minutes (figure 5.8). In visit one, volunteers went through an acclimatization study day in order for them to familiarise with the study protocol. Visit two was the control feeding study with no supplement. After completion of the control study day, volunteers were randomised to receive either 10 g/day of inulin or 10 g/day PCM contained in bread rolls, to be taken at home for six days before having the next study day the following week. During the home supplementation, they were asked to take two rolls a day. In visit three, volunteers had an appetite study day supplemented with inulin / PCM during breakfast and lunch based on what they received in the randomization process. After the study day three, they had a washout period for seven days. Visit four was a second control feeding study day, hence no treatment was provided. Next, the volunteers were swapped their supplementation to the other treatment group; so those who had taken PCM previously now took inulin and vice versa. At visit five, the final study day, volunteers had either inulin or PCM during the breakfast and lunch according to what they received in the previous six days (Figure 5.7).

5.4.2.1.2.1 Study Meals

The same meals as the previous study were given to volunteers (refer to Section 5.3.2.1.3.1).

5.4.2.1.2.2 Breath Hydrogen Assessment

The same method has been used with the pilot study (Refer to Section 2.3.2.2.3).

5.4.2.1.2.3 Gut Hormones Analysis

Blood sampling for gut hormones PYY and GLP-1 were drawn at -10, 0, 30, 60, 90, 120, 180, 210, 240, 270, 300 and 360 minutes and collected into lithium heparin tubes containing aprotinin (200 µl/7.5 ml blood). Each sample was then spun at 4000g at 4°C for 10 minutes and the plasma separated and divided into aliquots before immediate storage at -80°C. Gut hormones, PYY and GLP-1 were analysed using RIA. Please refer to Section 2.3.2.2.5 for the methodology.

5.4.2.1.2.4 Subjective Appetite Scores

The same method has been used with the study 1. Please refer to Section 2.3.2.2.2.

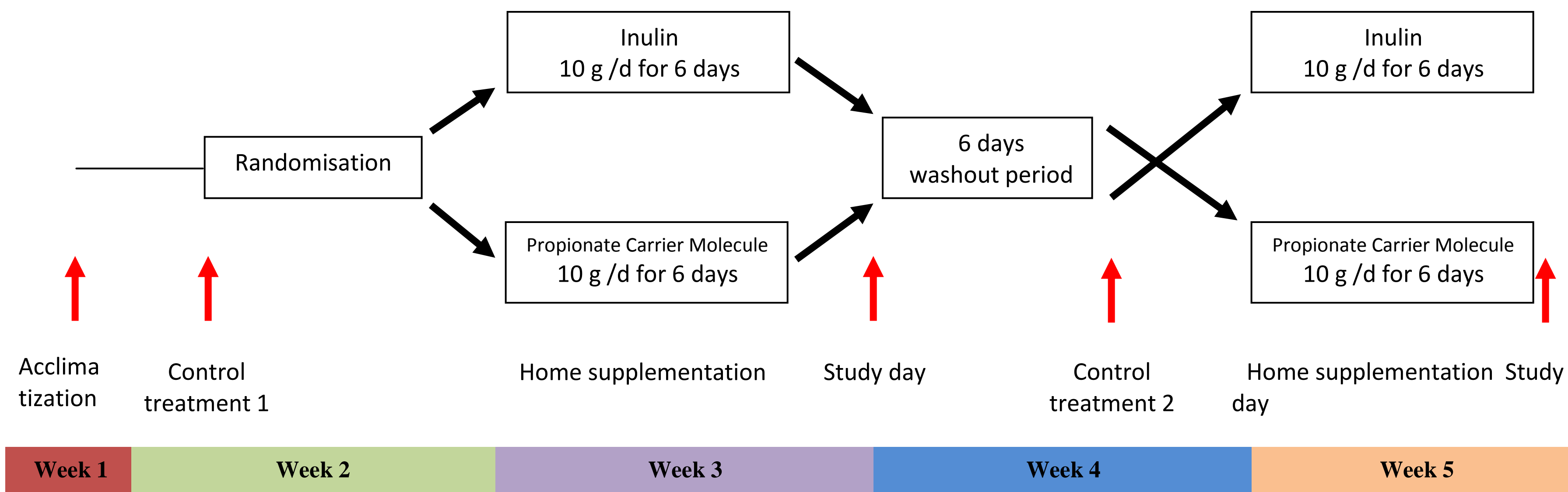


Figure 5.7 The study design. This randomised, double-blind and crossover study consists of 5 study visits; the acclimatization study day (visit 1), control study day (visit 2), Supplementation study day 1 (visit 3), control study day (visit 4) and supplementation study day 2 (visit 5). Volunteers were randomised to the treatment arrangement after they attended visit 2. The randomisation was performed using envelope system by a colleague of our department who was not involved in the study. After the randomisation process, volunteers were given the supplementation to take it at home for 6 days before attended their test supplementation study day on visit 3. A week after volunteers had their washout period, they continued the study by having the second supplementation on day 17 to day 23. Volunteers who had PCM on first supplementation would have inulin on second supplementation, and vice versa.

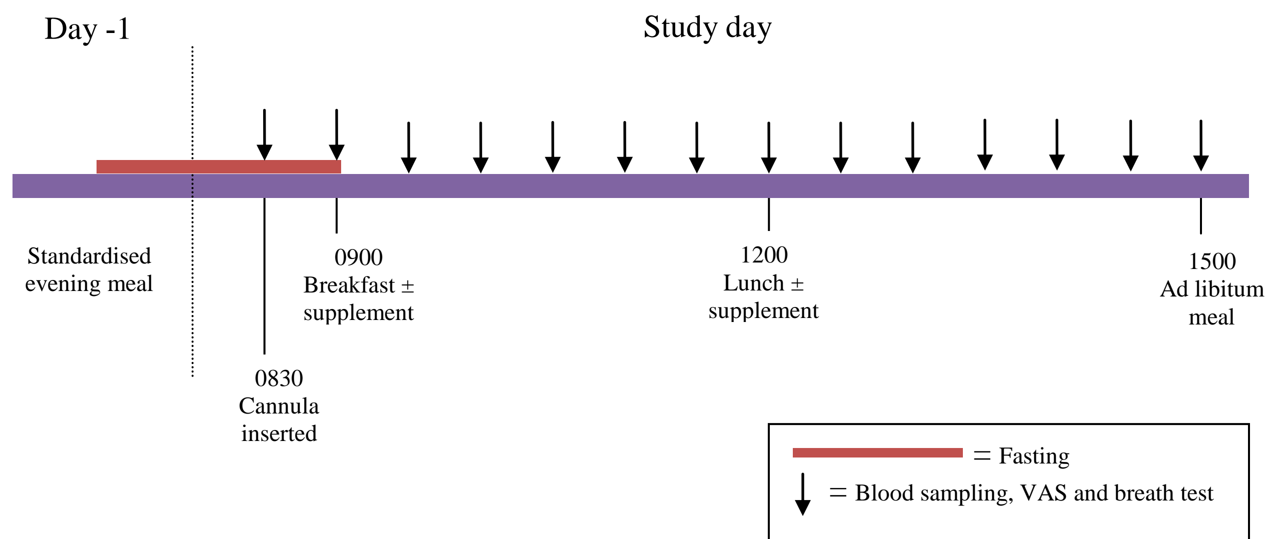


Figure 5.8 Protocol of the study day. Volunteers were attended the test study days after had their 10 – 12 hours fasting. Prior to fasting, they were asked to eat a standardised evening meal and finished eating before 2100 but water was exceptionally allowed until the next morning. Upon arrival, cannula was inserted and followed with baseline blood sampling, VAS and breath hydrogen test measured. After they had their breakfast, the blood sampling, VAS and breath hydrogen test were continuously accessed every 30 minutes throughout the study period. Lunch was provided at 180 minutes (1200) and *ad libitum* energy intake assessment was performed at 360 minutes (1500). Supplementation of either inulin or PCM was included in the breakfast and lunch on visit 3 and visit 5 according to the randomization group.

5.4.2.1.3 Statistical Analysis

Data are presented as mean ± SEM. Prior the analyses, data were checked for Gaussian distribution using the D'Agostino & Pearson omnibus normality test. When compared the treatment between all groups, tAUC was calculated using the trapezium rule for breath hydrogen, postprandial GLP-1 and PYY and VAS result and compared to each group using repeated measures one way ANOVA. Postprandial time course for hydrogen breath test was compared using one-way repeated measures ANOVA with Bonferroni post hoc test as the data is significant. Differences between post-supplementation and baseline of energy intake were assessed using a student two-tailed paired *t-test*. A P value of 0.05 or less was considered significant. Statistical analysis was performed on Graph Prism 5 (GraphPad Software, Inc., La Jolla, USA).

5.4.3 Results

5.4.3.1 Volunteers characteristics

Twenty six volunteers were recruited in this study but only twenty volunteers (male=5 and female=15) completed this study. The mean ages were 41.0 ± 2.9 years (21 – 66 years) and BMI were 30.3 ± 0.6 kg/m^2. Two volunteers withdrew from the study due to personal reasons and four volunteers who had energy intake more than two standard deviations away from the population mean were also excluded.

5.4.3.2 Energy Intake

Including PCM into volunteers' diets significantly reduced energy intake (P<0.05) by 18.8% compared to the baseline visit (392.2 ± 59.4 kcal [control PCM] and 318.3 ± 57.5 kcal[PCM]) whilst a reduction of 18.1% was also demonstrated in the inulin group when compared to the baseline visit (270.4 ± 43.9 kcal [inulin] and 330.1 ± 47.5 kcal [control inulin]) (Figure 5.9).

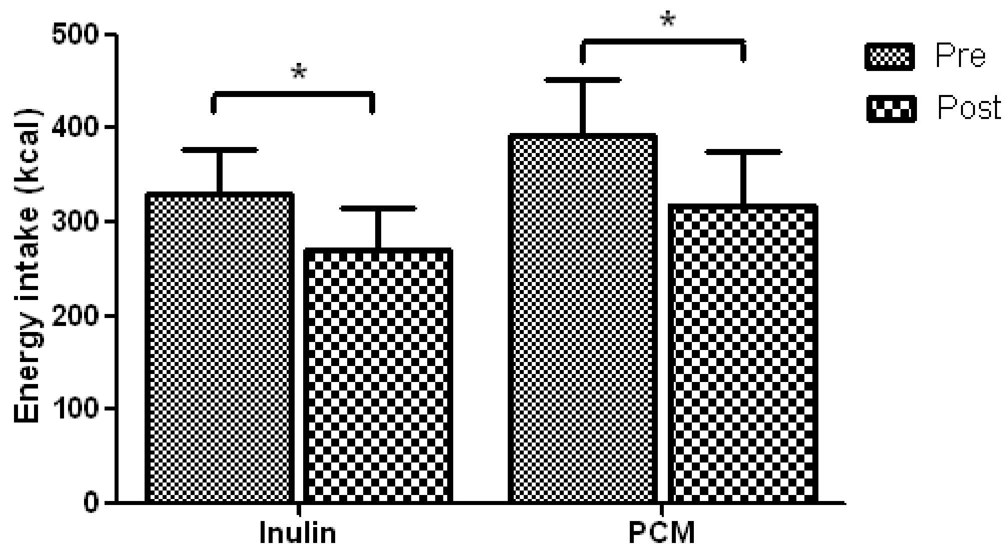


Figure 5.9 ***Ad libitum*** **energy intake assessment (kcal) following intake of 10 g PCM and inulin supplementations.** Bread rolls-containing 5 g of either PCM or inulin was served during standardised breakfast and lunch at 0 min and 180 mins whilst *ad libitum* meal was provided at 360 mins. Control inulin and control PCM are the study days without any supplementation and performed prior the treatments study days. Data is expressed as mean ± SEM, (n = 20).*P<0.05 inulin and PCM *vs.* control

5.4.3.3 Evening Meal Assessment

This analysis was performed in order to investigate potential variations that might influence energy intake on the appetite study day. The result showed that volunteers in both groups increased their energy intake during post-supplementation evening meals. In addition, volunteers in the inulin group significantly increased energy intake (P=0.046) (Mean ± SEM; 874.3 ± 79.9 kcal [control inulin], 1038.2 ± 80.7 kcal [inulin], 845.2 ± 94.6 kcal [control PCM] and 1028.8 ± 87.8 kcal [PCM]) (Figure 5.10).

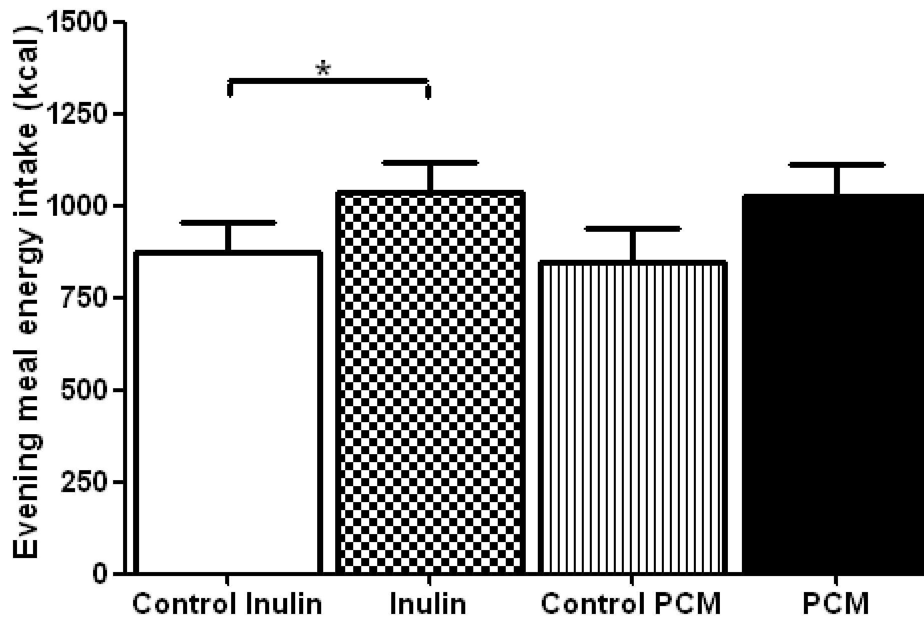


Figure 5.10 Evening meal energy intake assessment (kcal) following intake of 10 g PCM and inulin supplementations. Bread rolls-containing 5 g of either PCM or inulin was served during standardised breakfast and lunch at 0 min and 180 mins whilst *ad libitum* meal was provided at 360 mins. Control inulin and control PCM are the study days without any supplementation. Data is expressed as mean ± SEM, (n = 20).*P<0.05 inulin *vs.* control.

5.4.3.4 Subjective Appetite Scores

Supplementing PCM in the volunteers' diet had no significant effect for $tAUC_{360mins}$ fullness (P=0.109), $tAUC_{360mins}$ hunger (P=0.086), $tAUC_{360mins}$ prospective food intake (P=0.076) and $tAUC_{360mins}$ desire to eat (P=0.105) compared to other treatments (Figure 5.11 i) – iv))

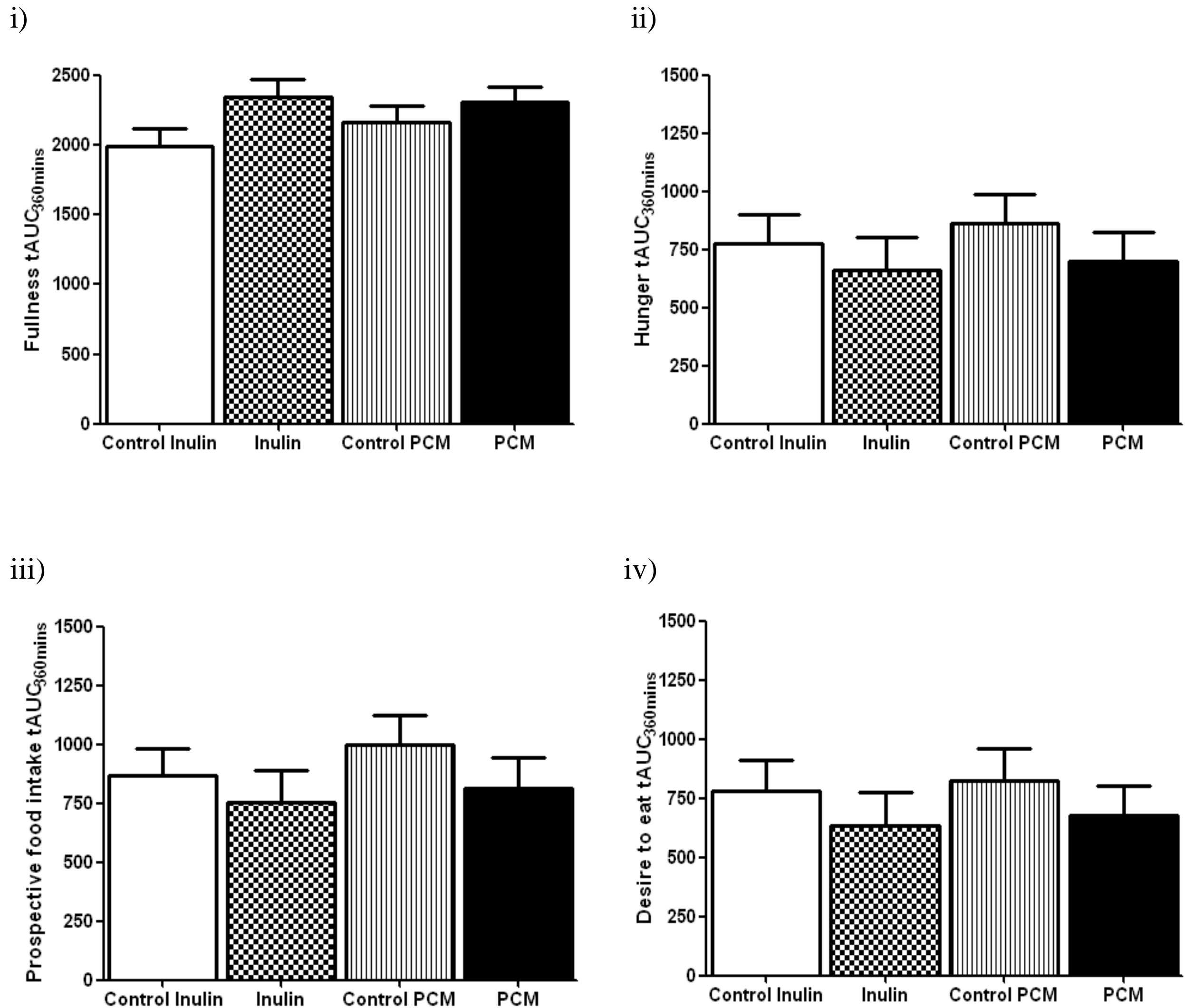


Figure 5.11 Subjective appetite scores accessed by VAS following intake of 10 g PCM and inulin supplementations i) $tAUC_{360min}$ fullness scores ii) $tAUC_{360min}$ hunger scores iii) $tAUC_{360min}$ prospective food intake scores iv) $tAUC_{360min}$ desire to eat scores. Bread rolls-containing 5 g of either PCM or inulin was served during standardised breakfast and lunch at 0 min and 180 mins whilst *ad libitum* meal was provided at 360 mins. Control inulin and control PCM are the study days without any supplementation. Data is expressed as mean ± SEM, (n = 20).

5.4.3.5 Gut Hormones Analysis

5.4.3.5.1 GLP-1 Analysis

Postprandial plasma GLP-1 levels peaked at 210 minutes after ingestion of all treatments (figure 5.12). No significant effect was demonstrated in $tAUC_{360mins}$ (P=0.389) with mean ± SEM $tAUC_{360mins}$ scores of (72140.2 ± 5043.0 pmol/L*min [control inulin], 71826.6 ± 4001.5 pmol/L*min [inulin], 72107.1 ± 3870.9 pmol/L*min [control PCM] and 67746.6 ± 3892.2 pmol/L*min [PCM]) (Figure 5.12 [inset]).

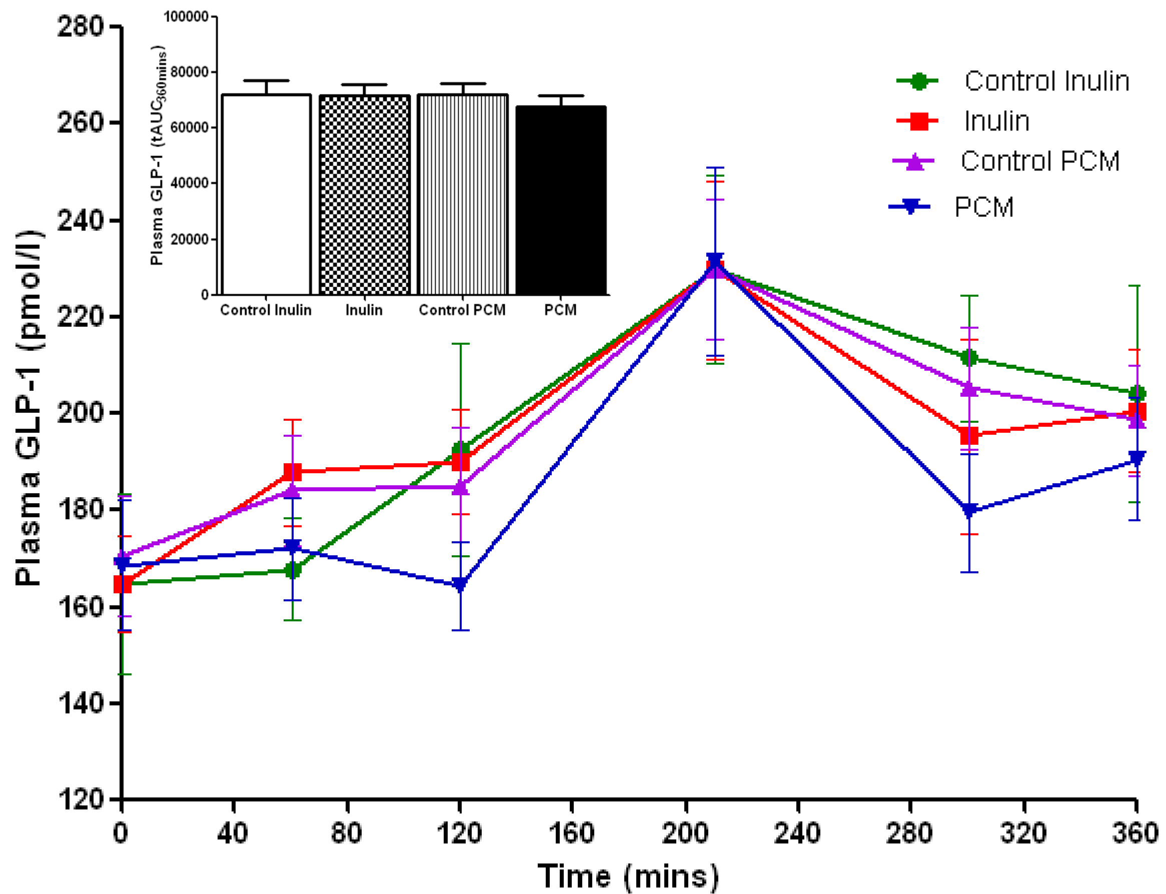


Figure 5.12 Postprandial plasma GLP-1 levels (pmol/L) and $tAUC_{360min}$ (inset) following 10 g PCM and inulin supplementations. Bread rolls-containing 5 g of either PCM or inulin was served during standardised breakfast (0 min) and lunch (180 mins) whilst *ad libitum* meal was provided at 360 mins. Control inulin and control PCM were the study days without any supplementation. Data is expressed as mean ± SEM, (n = 20).

5.4.3.5.2 PYY Analysis

Administration of PCM in volunteers' diet had no significant effect on $tAUC_{360mins}$ plasma PYY compared to other treatments (P=0.739) (Figure 5.13). $tAUC_{360mins}$ scores of the treatments are 7068.6 ± 613.7 pmol/L*min [control inulin], 7098.6 ± 557.9 pmol/L*min [inulin], 7396.7 ± 484.7 pmol/L*min [control PCM] and 6754.0 ± 607.8 pmol/L*min [PCM] (Figure 5.13 [inset]).

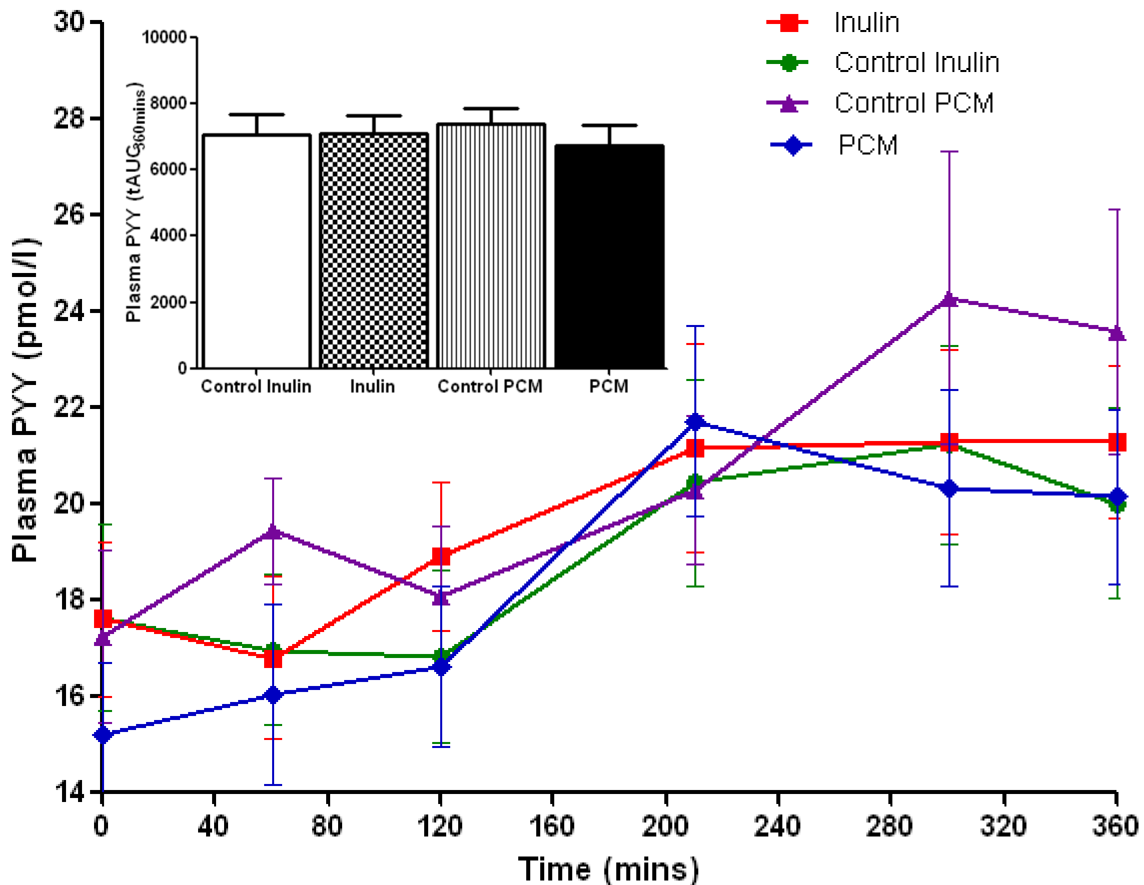


Figure 5.13 Postprandial plasma PYY levels (pmol/L) and $tAUC_{360min}$ (inset) following intake of 10 g PCM and inulin supplementations. Bread rolls-containing 5 g of either PCM or inulin was served during standardised breakfast (0 min) and lunch (180 mins) whilst *ad libitum* meal was provided at 360 mins. Control inulin and control PCM were the study days without any supplementation and performed prior the treatments study days.Data is expressed as mean ± SEM, (n = 20).

5.4.3.6 Breath Hydrogen Analysis

Inulin significantly increased (P<0.0001) breath hydrogen excretion compared with other treatments. Inulin also increased $tAUC_{360min}$ compared to other treatments but no significant difference (P=0.259) was demonstrated (Figure 5.14) (mean ± SEM; 3291.8 ± 589.2 ppm*min [control inulin], 5514.8 ± 1042.8 ppm*min [inulin], 4045.7 ± 833.2 ppm*min [control PCM] and 4389.8 ± 1007.0 ppm*min [PCM].

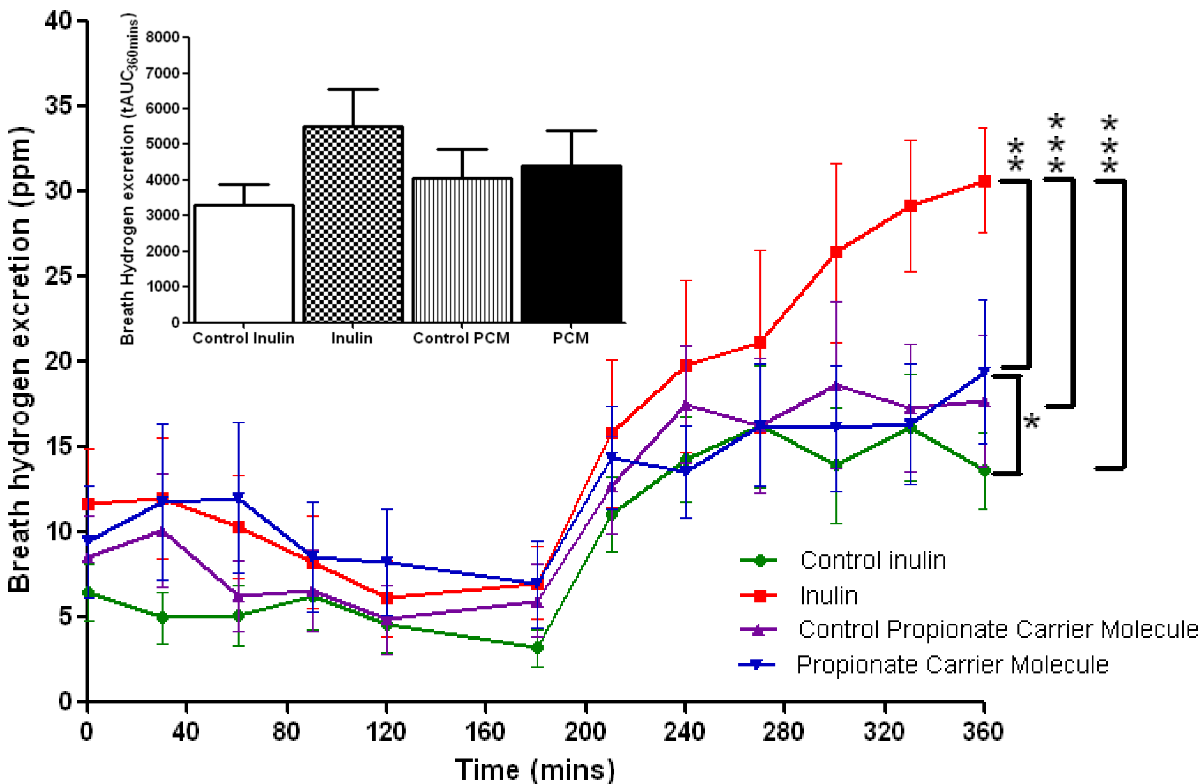


Figure 5.14 Breath hydrogen test (ppm) and $tAUC_{360min}$ (inset) following intake of 10 g PCM and inulin supplementations. Bread rolls-containing 5 g of either PCM or inulin was served during standardised breakfast (0 min) and lunch (180 mins) whilst *ad libitum* meal was provided at 360 mins. Control inulin and control PCM were the study days without any supplementation. Data is expressed as mean ± SEM, (n = 20). *P<0.05 PCM *vs.* control inulin, **P<0.01 inulin *vs.* PCM, ***P<0.001 inulin *vs.* control inulin and control PCM.

5.4.4 Discussion

From Study 1, it is now known that PCM was safely delivered to the colon and subsequently involved in the colonic fermentation activities. Furthermore, PCM also increased postprandial plasma PYY levels compared to the control treatment. The current study showed that adding 20% PCM into volunteers' diet reduced *ad libitum* energy intake compared to baseline, but had no significant difference in breath hydrogen release, plasma PYY and GLP-1 levels and appetite sensations when compared to the inulin group.

In contrast to Study 1, inclusion of 20% PCM in this study did not significantly alter breath hydrogen levels when compared to baseline study day, but the inulin group did significantly increase breath hydrogen levels within the group. However, there was no significant difference between the treatments. Adding inulin-type fructans in the diet have consistently been shown to increase colonic fermentation in other studies (Cani et al., 2009; Fernandes et al., 2011; Piche et al., 2003). In this study, it is suggested that lack of effect of PCM to significantly increase breath hydrogen levels is because the amount of inulin in the PCM group was less than the amount provided in the inulin group. In PCM group, only 8 g of inulin was provided as the remaining 2 g was propionate. In contrast, inulin group provided 10 g inulin for the colonic fermentation. Therefore, this might explain the non-significant effect of PCM on breath hydrogen levels.

Moreover, it is also possible that splitting the 10 g dose into 5 g during breakfast and lunch has influenced colonic fermentation activities. It was hypothesized that splitting the dose would prolong the fermentation activities thus subsequently elevated plasma GLP-1 and PYY released. However, the hypothesis was not proven as no significant effect on breath hydrogen released was showed. In the pilot study, 10 g PCM supplemented during breakfast took 270 mins to reach the colon and subsequently ferment by the gut bacteria. Therefore, it might be possible that splitting the dose has diluted PCM levels and consequently minimised the effect of PCM. Therefore the twice daily dose used in this study may not be the most effective method to increase fermentation activities. However, there is currently insufficient evidence available in the literature to elucidate an optimal dosing regimen for fermentable carbohydrate. Peters *et al*. reported that no effects on satiety and energy intake when administered three types of equicaloric test bars containing either 0.9 g β-glucan, 8 g fructooligosaccharides or combination of both

fibres in two study days (Peters et al., 2009). In contrast, Cani *et al*. showed that supplementation of 8 g of oligofructose twice daily enhance satiety and decreased hunger at meal and dinner for two weeks (Cani et al., 2006a). This raises the question as to whether the dose supplied was insufficient to increase colonic propionate and modulate gut hormones release.

An increase in colonic fermentation is suggested to correlate with elevation of gut hormones release (Piche et al., 2003). In this study, the lack of effect of PCM on increasing colonic fermentation mirrored the non significant effect on postprandial plasma PYY and GLP-1 levels. Surprisingly, adding inulin in the diet also had no significant effect on plasma GLP-1 and PYY levels. The result of this study was opposite to a study on overweight volunteers which showed that PYY, but not GLP-1 was significantly increased after 12 weeks intake of 21 g/day oligofructose supplementation (Parnell and Reimer, 2009), whilst Cani *et al*. showed that supplementing 16 g/day significantly increased PYY and GLP-1 released in healthy subjects (Cani et al., 2009). These studies showed that perhaps there is still no consensus on the effect of fermentable supplementation on GLP-1 and PYY in the literature. Furthermore, this discrepancy may be related to different methodologies and supplementation periods applied when comparing the studies. In addition, PCM is a novel supplement, therefore the effect possibly would not be the same with other dietary fibres.

In this study, assessment of appetite rating and energy intake were the main endpoints. Intake of inulin and PCM showed to have a similar trend in increasing fullness, reducing hunger, prospective food intake and desire to eat sensations within each group, but no significant difference was found between the treatments. Meanwhile, energy intake assessment consumed at *ad libitum* meal was significantly decreased in both groups, but no significant difference when compared between the groups. However, the reduction of energy intake demonstrated in inulin group might have been confounded by a significant increase of evening meal intake prior attending the study day. Indeed, the effect of evening meals on baseline hunger as well as plasma PYY and GLP-1 have been discussed in details in Chandarana *et al*. (Chandarana et al., 2009). In future, standardised ready meals will be provided in the future studies in order to control for this confounding issue. However, the finding of this study seems to have a similarity with the observation demonstrated in rodent studies in our lab. In this rodent study, Wistar rats were fed

5%, 10% and 20% of their total diet with PCM for 10 days and were compared to cellulose and inulin treatment. The result exhibited that whilst 5% PCM showed no effect on food intake and body weight, 10% PCM was able to reduce food intake compared to cellulose, but the effect was not different when compared to inulin (Peters, 2010). This result suggested that 10% PCM possibly has similar effect with inulin in modulating energy homeostasis. A lack of PCM effect on appetite may be because of several methodological reasons. The dose used in the present study was different from Study 1. In Study 1, 10% PCM was used whilst in this study, 20% PCM was supplemented. The dose was increased from 10% to 20% in assuming that this will increased propionate delivery to the colon, increased the fermentation activities to secrete gut hormones secretion and ultimately altered appetite and energy intake. However, it seems that the hypothesis was not proven as there was no clear evidence of change in appetite rating, plasma PYY and plasma GLP-1 levels.

Splitting the PCM dose from 10 g per roll taken once at breakfast to five grams per bread roll taken at breakfast and lunch might also contribute to the lack of effect of PCM on appetite. The dose was split to five grams per bread roll in order to improve palatability as it was reported that intake of 10 g per roll in study 1 was associated with bitter taste. In addition, as the control, inulin is a natural plant product with a slight sweet taste (Niness, 1999), imbalance taste between PCM and inulin was improved by adding 2.85 g sugar in each bread roll. Although this step has improved the bread palatability, adding sugars in the bread rolls has resulted in increased caloric content compared to the normal bread roll supplemented on the baseline visit (352 kcal in the inulin or PCM supplemented bread rolls compared to 240 kcal in the normal brown bread rolls). Therefore, it is possible that the discrepancies of the bread rolls caloric content might have affected energy intake assessed during the *ad libitum* meal and increased the caloric intake during evening meal.

Following the potential effect of PCM on reducing energy intake demonstrated in this study, further investigation on the effect of supplementing different DE of PCM on modulating appetite and energy intake will be performed. In the next study (Study 3), the methodological issues that were experienced in this study such as the dose levels and method of delivering PCM will be investigated.

5.5 STUDY 3: The Effects of Propionate Carrier Molecule on Colonic Fermentation, Gut Hormones Release and Appetite: A Dose Optimization Study

Aim: To investigate the degree of esterification of propionate carrier molecule needed to have the effective effect on stimulating plasma PYY and GLP-1 release, reduce subjective appetite and energy intake.

Hypothesis: Increase degree of esterification of propionate carrier molecule significantly increase gut hormones, PYY and GLP-1 and subsequently suppresses appetite and energy intake.

5.5.1 Materials

5.5.1.1 Dietary Treatments

In this study, PCM was provided to volunteers in three different DE; 0.25, 0.5 and 0.8. This is to provide different amount of propionate in each substance; 0.25 contains 10% propionate per PCM, 0.5 has 20% propionate per PCM and 0.8 provided 30% propionate per PCM. PCM and inulin were provided to volunteers in white, powdered form and has a similar appearance.

5.5.1.2 Volunteers

Nine healthy volunteers (8 males and 1 female) aged between 21 to 65 years old and BMI of 25 to 35 were recruited in this study. They were recruited through advertising in Hammersmith Imperial College London and Hammersmith Hospital. Volunteers were given their written informed consent prior the start of the study. The study was approved from Hammersmith, Queen Charlotte's and Chelsea Research Ethic Committee (08/H0707/99) (Registration No: NCT00750438) and performed in accordance with principles of the Declaration of Helsinki. Volunteers' details are described in table 5.7. The inclusion and exclusion of this study were the same as Study 1 and Study 2.

5.5.2 Methods

5.5.2.1 Study Design

In this single-blinded, randomised study, volunteers were received 10 g inulin and 10 g PCM with 10%, 20% and 30% propionate daily for three days and followed with four days washout period every week for four weeks.The first two days were the run-in period in which they needed to take 5 g PCM twice on day 1 and 10 g PCM once on day 2. During this period, volunteers were given a choice whether to mix it into a drink or with their foods as long as the supplementation was not cooked or heated in high temperature. Appetite assessment study day was performed on day three. All the study visits were performed in Clinical Unit, Sir John McMichael Centre, Hammersmith Hospital (Figure 5.15).

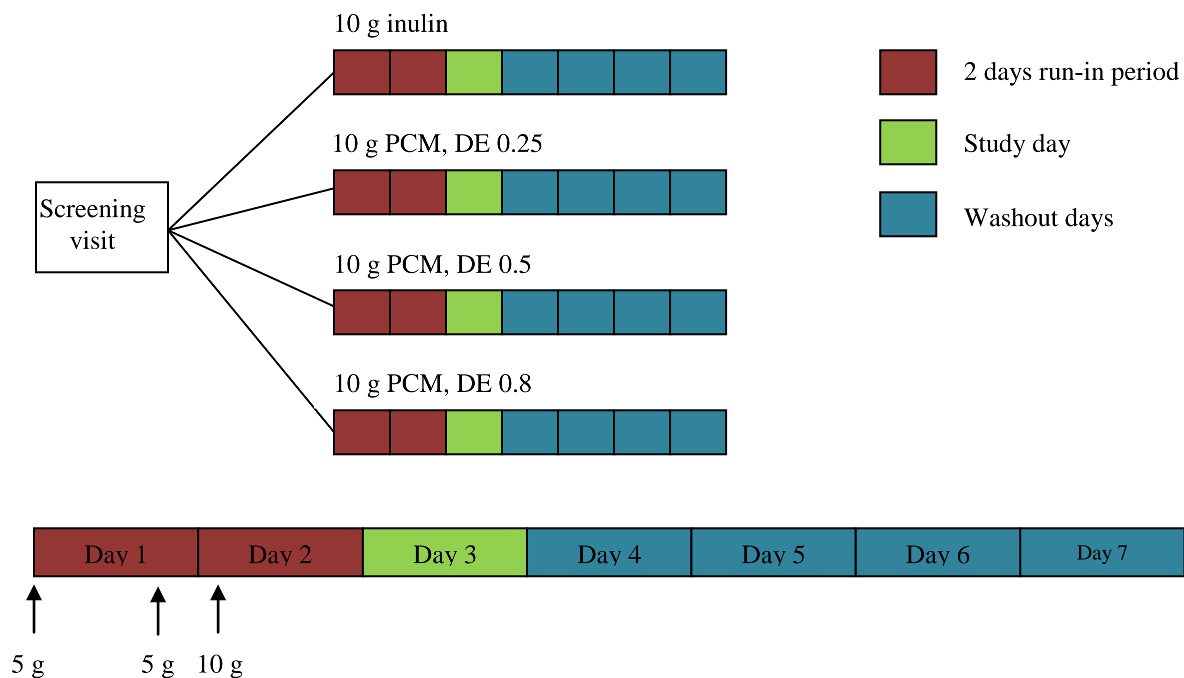


Figure 5.15 Dose optimization study design. Volunteers were asked to attend 4 study visits; 10 g inulin, 10 g PCM with DE 0.25, 10 g PCM with DE 0.5 and PCM with DE 0.8. 2 days before attending study visits, volunteers take 5 g PCM on the morning and the evening on day 1 and 10 g PCM on the morning of day 2. Study day was held on day 3. After the study day, volunteers were had their washout study days for 4 days before continued the following supplementation period.

5.5.2.1.1 Free-Living Supplementation Period

5.5.2.1.2 Appetite Assessment Study Day

On each study visit, a cannula was inserted in the forearm for blood sampling throughout the day. The investigations started at 0900 to 1700 and took approximately 420 minutes to complete. Similar with study 1 and study 2, breath hydrogen test, VAS, energy intake assessment and blood withdrawal were performed. A day before the study day, volunteers were instructed to complete 24 hours food diary to check whether the standardised foods are taken in every visit.

5.5.2.1.2.1 Study Meals

After baseline measurements, volunteers were provided with standardised meals consists of breakfast, lunch and an *ad libitum* meal. The preference of these meals was made by volunteers during screening session and their choices were applied for all of the study visits. Breakfast was provided at 0 minute with an addition of 10 g of inulin and 10%, 20% and 30% PCM on 4 consecutive weeks. A lunch was served at 180 minutes. At 420 minutes, an *ad libitum* spaghetti bolognaise was provided for energy intake assessment. Volunteers were instructed to eat until comfortably full and the meal was weighed before and after administration (please refers to Table 5.4 for macronutrient and energy composition).

Table 5.4 Macronutrient composition and energy content of standardised breakfast, lunch and *ad libitum* meal provided during the study days.

	CHO	Protein	Fat	Energy (kcal)
Breakfast				
130 ml semi-skimmed milk	6.50	4.42	2.21	63.70
300 ml whole milk	14.10	9.60	10.80	192.00
40 g Chocolate nesquik	31.84	1.68	1.28	150.40
30 g Rice crispies	26.10	1.80	0.30	114.90
10 g supplement (PCM or Inulin)				320
Lunch				
400 g Sainsburys Chicken and Mushroom Pie	13.20	28.10	11.90	356.00
***Ad libitum* meal (g)**				
Spaghetti Bolognaise	12.30	7.90	7.00	-

5.5.2.1.2.2 Breath Hydrogen Assessment

Breath hydrogen was assessed in the same method applied in the pilot study (Section 2.3.2.2.3). However, in this study, breath hydrogen was accessed at every hour at -10, 0, 60, 120, 180, 240, 300, 360 and 420 minutes.

5.5.2.1.2.3 Gut Hormones Analysis

Blood sampling for PYY and GLP-1 were drawn at -10, 0, 15, 30, 60, 90, 120, 180, 210, 240, 300, 360 and 420 minutes, which were then analysed using radioimmunoassay. Please refer to Section 2.3.2.2.5.

5.5.2.1.2.4 Glucose assay

Glucose samples were analysed in the Department of Clinical Biochemistry, Hammersmith Hospital. Analyses of the glucose were performed using an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK). Glucose assay sensitivity was 0.3 mmol/L with an intra-assay coefficients of variation of 1.0% .

5.5.2.1.2.5 Insulin Assay

Blood sampling for insulin were drawn at -10, 0, 15, 30, 60, 90, 120, 180, 210, 240, 300, 360 and 420 minutes and were determined by using iodine-125 RIA kits (Millipore, Missouri, USA). The methods are described in the Section 2.3.2.2.6.

5.5.2.1.2.6 Subjective Appetite Scores

Subjective appetite scores used the same method described in the section 2.3.2.2.2 but in different timepoints which were -10, 0, 30, 60, 90, 120, 180, 240, 300, 360, 420 minutes.

5.5.2.1.3 Statistical Analysis

Data are presented as the mean ± SEM. Before the analyses were performed, data were checked for Gaussian distribution using the Kolmogorov-Smirnov test. Postprandial time course for hydrogen breath test, postrandial GLP-1 and PYY and VAS were compared using one-way repeated measures ANOVA with Bonferroni post hoc test if the data is significant. When compared the treatment between all groups, AUC was calculated using the trapezium rule for breath hydrogen, postprandial GLP-1 and PYY and VAS result and compared to each group using repeated measures one way ANOVA. Total mean energy intake in each group as well as change from baseline (with inulin as baseline) was also determined by using one way ANOVA. A P value of 0.05 or less was considered significant. Statistical analysis was performed on Graph Prism 5 (GraphPad Software, Inc., La Jolla, USA).

5.5.3 Results

5.5.3.1 Volunteers characteristics

Table 5.5 characterises the demographic data for the volunteers that participated in this study. This is the first time dose optimization study using PCM was performed in humans, therefore power calculation based on the previous PCM studies could not be done. All of the volunteers were involved in the appetite study day and home supplementation.

Table 5.5 Baseline characteristics of the study volunteers

Characteristics	Numbers
Completed (n)	9
Withdrawn (n)	0
Sex (n)	
• Male	8
• female	1
Age	26.3 ± 2.0
Age range	21 – 39
BMI (kg/m^2)	23.9 ± 0.7

Values are expressed as mean ± SEM

5.5.3.2 Energy intake

Supplementing PCM into volunteers' diet showed to have a dose response effect to reduce energy intake with 30% PCM showed as the highest reduction compared to inulin [Figure 5.16 i) and ii)]. In addition, change from baseline showed a reduction of 4.3% for 10% PCM, 7.5% for 20% PCM and 15.9% for 30% PCM. However, there was no significant differences between the treatments (P=0.208). The mean energy intake of the treatments was 1120.0 ± 131.7 kcal [inulin], 1061.0 ± 135.6 kcal [10% PCM], 1034.0 ± 124.2 kcal [20% PCM] and 930.4 ± 124.2 kcal [30% PCM]).

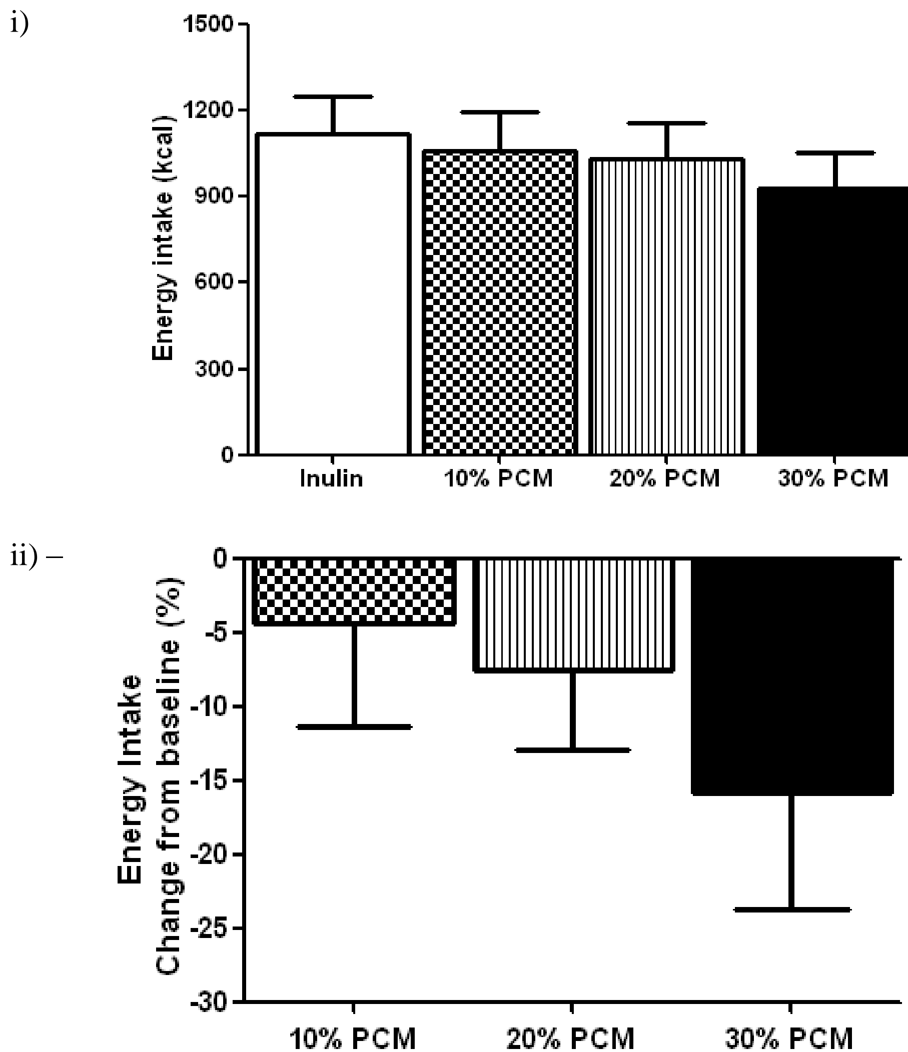


Figure 5.16 ***Ad libitum*** **energy intake assessment (kcal) (i) and change from baseline (kcal) (ii) following intake of 10g inulin, 10% PCM, 20% PCM and 30% PCM.** PCM was supplemented in volunteers' meal at breakfast (0 min). Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.5.3.3 Subjective Appetite Scores

Including 10%, 20% and 30% PCM in the diet tended to have dose-response effect on increasing $iAUC_{420mins}$ fullness scores, reducing hunger and prospective food intake but these were not statistically significant when compared to inulin (P=0.398, P=0.757 and P=0.826 respectively) [Figure 5.17 i) - vi)]. No significant difference was also found in $iAUC_{420mins}$ pleasant to eat scores (P=0.991),

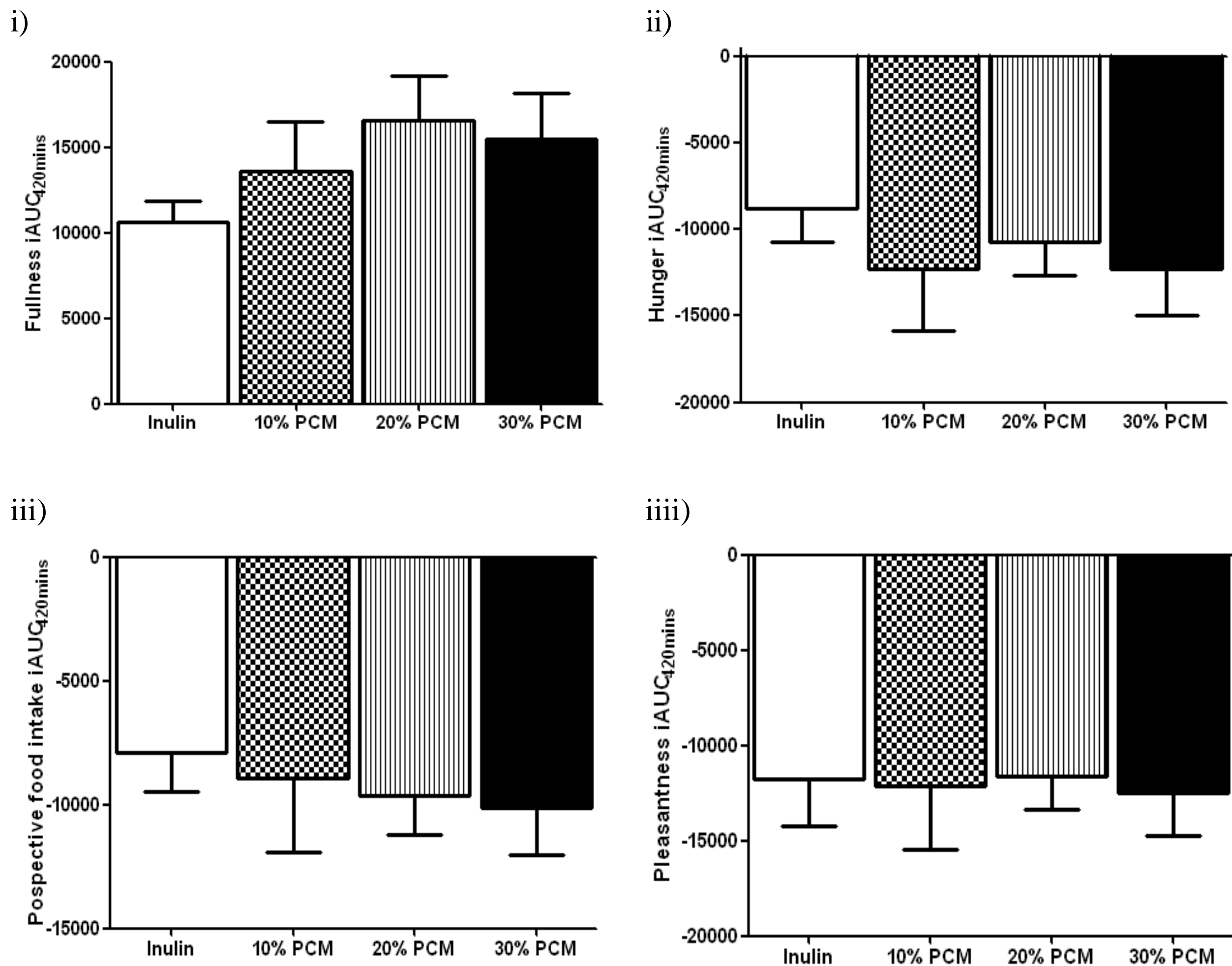


Figure 5.17 Subjective appetite ratings following intake of inulin, 10% PCM, 20% PCM and 30% PCM. i) fullness scores ii) $iAUC_{420min}$ for fullness scores iii) hunger scores iiii) $iAUC_{420min}$ for hunger scores v) prospective food intake scores vi) $iAUC_{420min}$ for the prospective to eat scores vii) pleasant to eat scores viii) $iAUC_{420min}$ for pleasant to eat scores. PCM was supplemented in volunteers' meal at breakfast (0 min). Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.5.3.4 Breath Hydrogen Analysis

Supplementing different dose of PCM increased breath hydrogen excretion starting from 180 minutes ingestion with 10% PCM showed the highest released of breath hydrogen. However, this was not significant when compared to inulin (Figure 5.18). Inulin significantly increased breath hydrogen analysis (P=0.006) and $tAUC_{420mins}$ excretion levels compared to all treatment (P=0.015). Mean tAUC/time of breath hydrogen excretion for inulin 10% PCM, 20% PCM and 30% PCM are 28.4 ± 3.0 ppm/mins, 22.7 ± 3.2 ppm/mins, 17.1 ± 1.5 ppm/mins and 20.7 ± 2.1 ppm/mins.

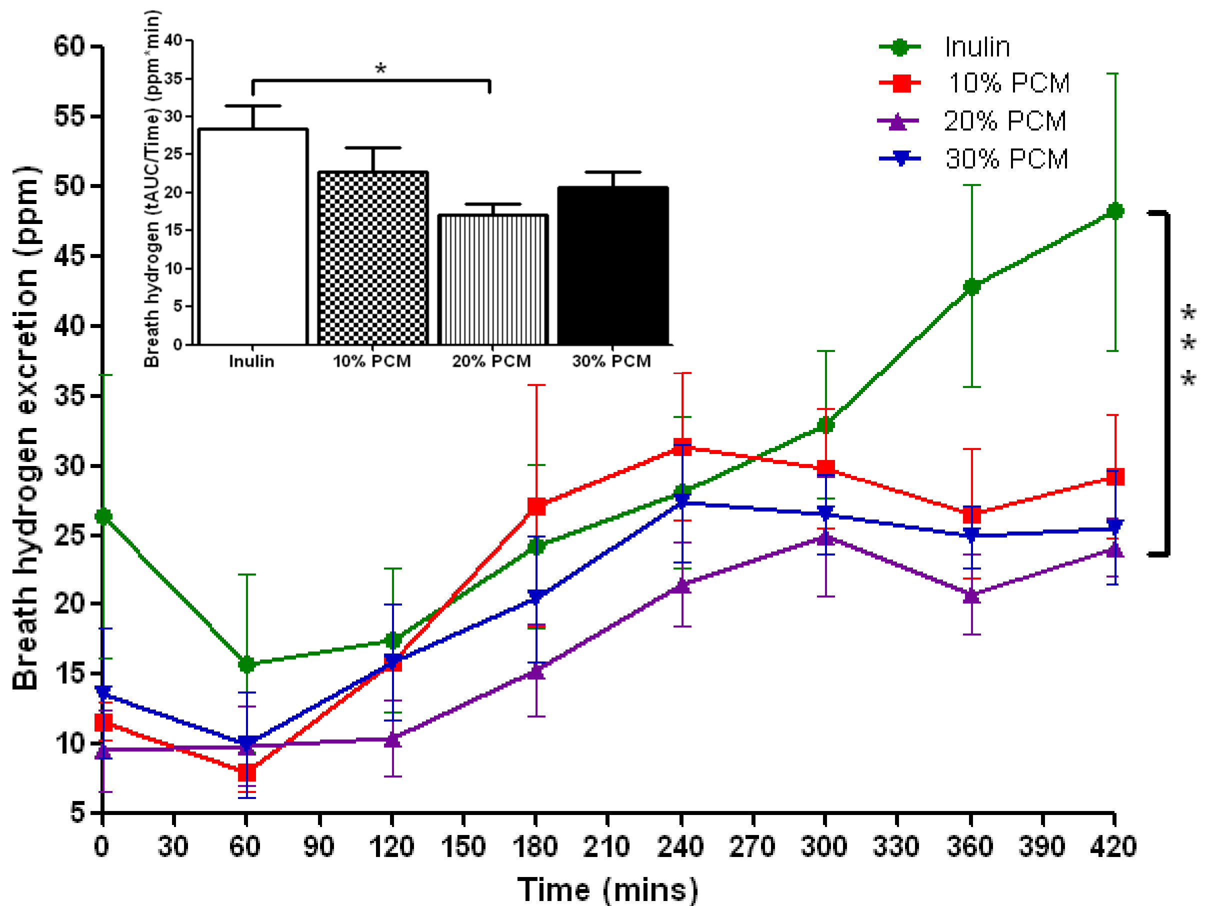


Figure 5.18 Hydrogen breath test (ppm) and $tAUC_{420mins}$ (inset) following 7 hours intake of inulin, 10% PCM, 20% PCM and 30% PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9). *P=0.015, *P<0.05 inulin *vs.* 20% PCM, ***P<0.001 inulin vs. 30% PCM.

5.5.3.5 Plasma Metabolites

5.5.3.5.1 PYY Analysis

In this analysis, only dose PCM 30% was selected as this dose has been shown to reduce energy intake at optimum levels compared to other dose. Inclusion of 30% PCM in the diet showed to increase plasma PYY levels and peaked at 300 minutes after ingestion of the supplement (Figure 5.19). $iAUC_{420mins}$ for plasma PYY secretion after intake of 30% PCM was significantly increased compared to inulin supplementation (P=0.047) (mean ± SEM; 358.6 ± 490.3 pmol/L*min [inulin] and 1818.0 ± 397.8 pmol/L*min [30% PCM] (Figure 5.19 [inset]).

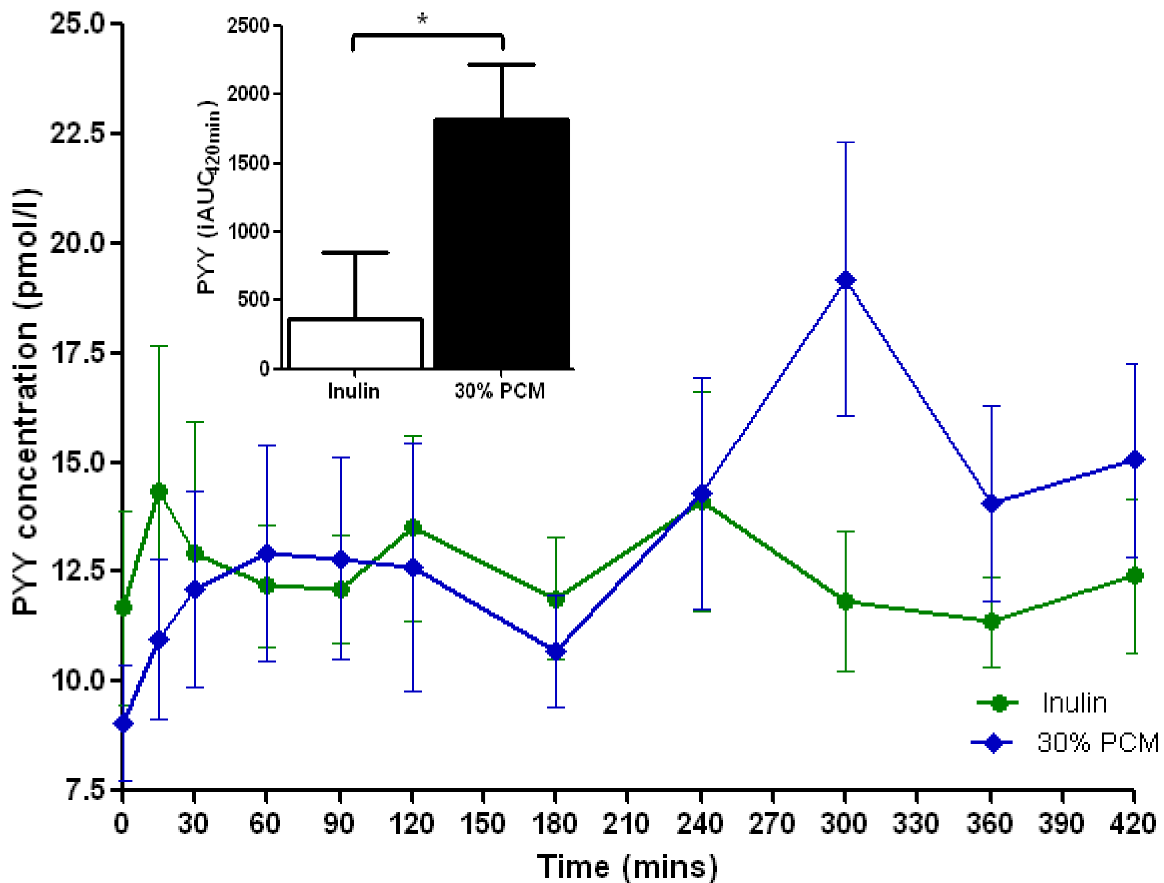


Figure 5.19 Postprandial PYY levels (pmol/L) and $iAUC_{420mins}$ (inset) following intake of inulin, 10% PCM, 20% PCM and 30% PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).*P<0.05 30% PCM *vs.* inulin.

5.5.3.5.2 GLP-1 Analysis

Similar with PYY analysis, only 30% PCM dose was selected for this analysis. Addition of 30% PCM showed no significant effect on increasing GLP-1 levels compared to inulin treatment (P=0.338) (Figure 5.20). $iAUC_{420mins}$ for plasma GLP-1 secretion after PCM supplementation was not significant when compared to inulin (P=0.109) (mean ± SEM; 4244.0 ± 683.8 pmol/L*min [Inulin] and 2130.0 ± 875.3 pmol/L*min [30% PCM] (Figure 5.20 [inset]).

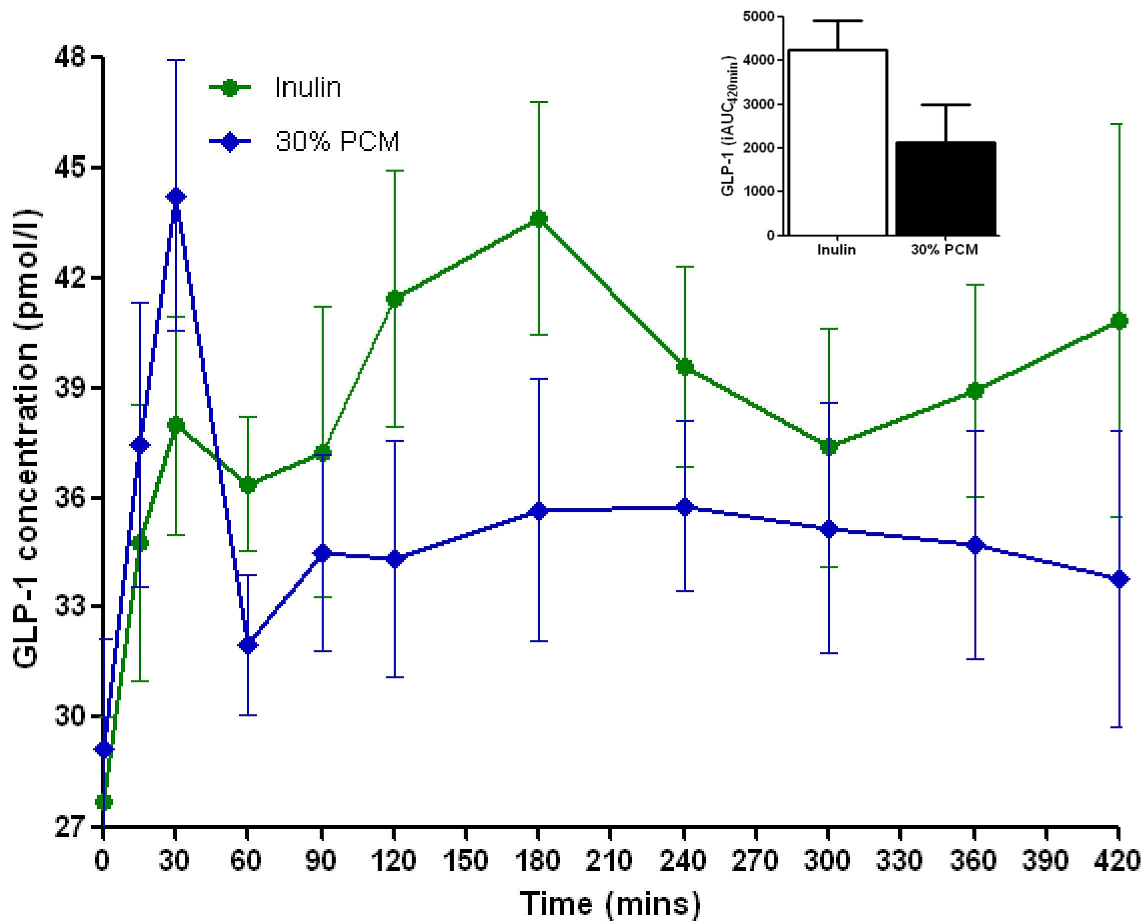


Figure 5.20 Postprandial GLP-1 levels (pmol/L) and $iAUC_{420min}$ (inset) following intake of inulin, 10% PCM, 20% PCM and 30% PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.5.3.5.3 Plasma glucose

Inclusion of different dose of PCM showed no significant effect on reducing postprandial glucose levels compared to inulin group (P=0.972) (Figure 5.21). tAUC/Time for the plasma glucose response between 0 and 420 minutes after addition of PCM was not significantly different between the treatments (P=0.506) (mean ± SEM; 5.5 ± 0.2 mmol/L*min [inulin], 5.4 ± 0.2 mmol/L*min [10% PCM], 5.5 ± 0.1 mmol/L*min [20% PCM] and 5.4 ± 0.3 mmol/L*min [30% PCM]) (Figure 5.20 [inset]).

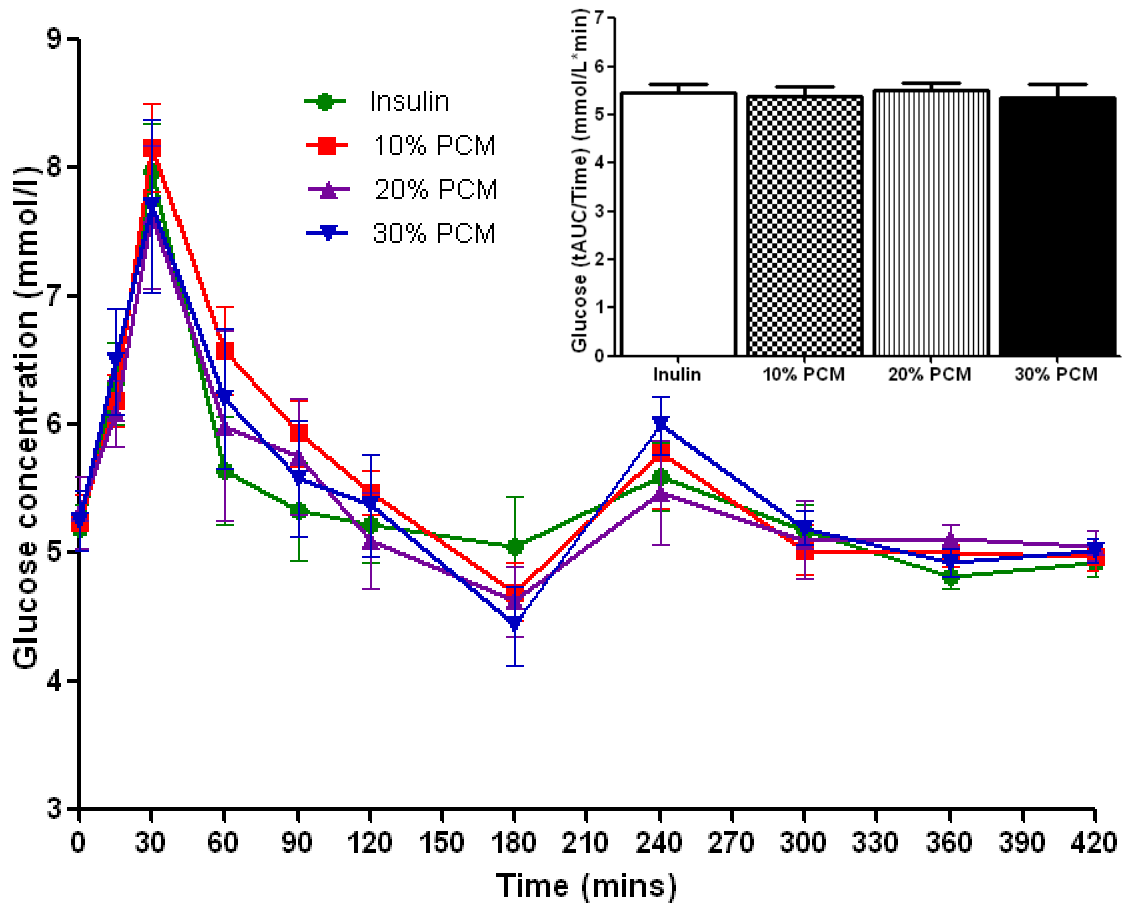


Figure 5.21 Postprandial glucose levels (mmol/L) and $tAUC_{420min}$ (inset) following intake of inulin, 10% PCM, 20% PCM and 30% PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.5.3.5.4 Insulin Analysis

Supplementing PCM into volunteers' diet showed a trend towards reduction insulin levels (P=0.060) when dose levels were increased. 30% PCM showed to reduce insulin levels 30 minutes and 240 minutes after ingestion compared to other treatment (Figure 5.22). tAUC/Time (mean ± SEM; 63.3 ± 8.6 uU/ml*min [inulin], 59.0 ± 5.9 uU/ml*min [10% PCM], 52.8 ± 5.2 uU/ml*min [20% PCM] and 48.8 ± 5.7 uU/ml*min [30% PCM] (Figure 5.22 [inset]).

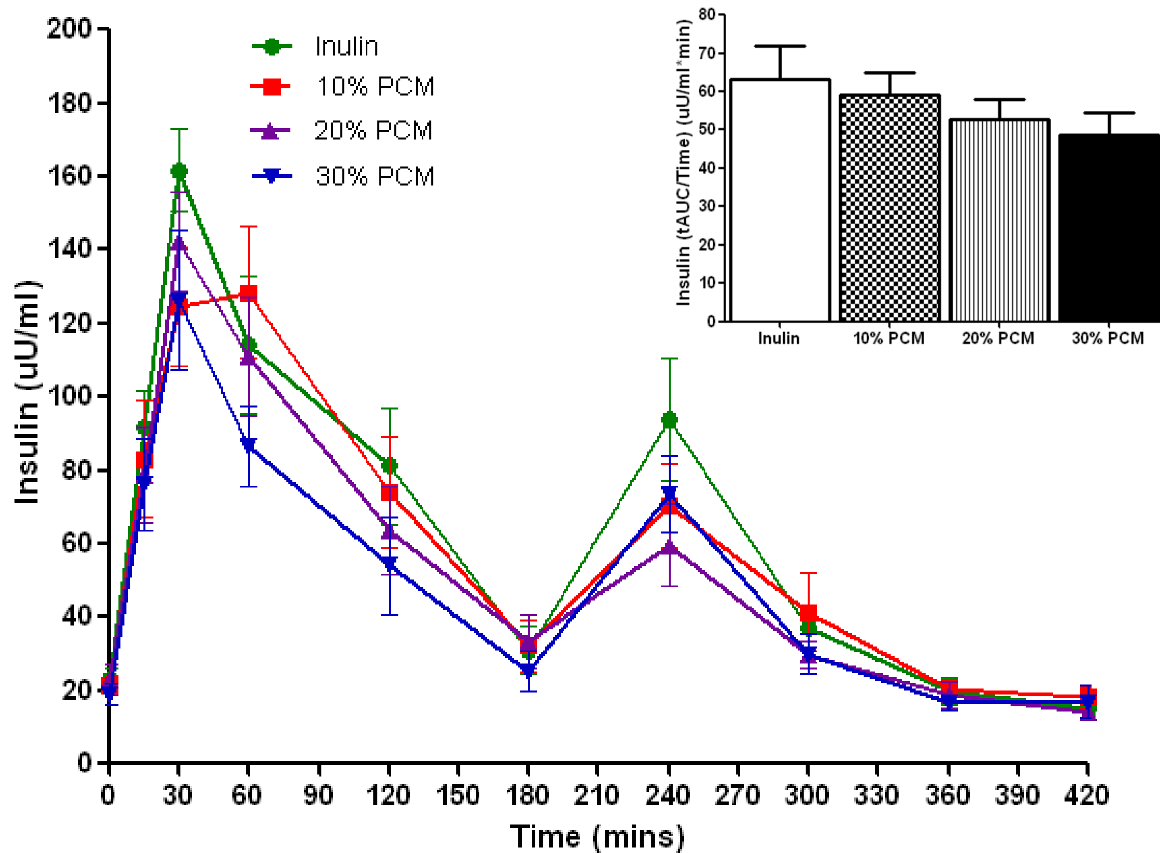


Figure 5.22 Postprandial insulin levels (uU/ml) and $tAUC_{420min}$ (inset) following intake of inulin, 10% PCM, 20% PCM and 30% PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.5.4 Discussion

The aim of the present study was to investigate the optimum dose of propionate needed to affect appetite and energy intake. In this study, propionate was added as 10% (1 g), 20% (2 g) and 30% (3 g) of total weight of PCM. The result of this study supported the hypothesis that the higher the amount of propionate supplemented in the PCM, the greater effects it has on energy intake. 30% propionate/PCM showed the most pronounce effect on reducing energy intake and significantly increased plasma PYY secretion when compared to inulin, but the supplement had no significant effect on plasma GLP-1 secretion. The beneficial effect of 30% PCM on plasma PYY possibly due to the additional concentration of propionate (3 g) to trigger plasma PYY released compared to inulin alone in inulin group. A trend towards dose response effect can also be seen on reducing insulinemia, appetite ratings and energy intake. However, increasing DE of PCM has no effect on breath hydrogen secretion and plasma glucose levels.

Theoretically, supplementing inulin and propionate to the colon would increase the release of GLP-1 secretion. However, this observation was not found in this study. From these three studies, it seems that there was no relationship between PCM and plasma GLP-1 secretion. In contrast to PCM, inulin has been consistently shown to increase plasma GLP-1 secretion in rodents and humans (Cani et al., 2004; Cani et al., 2005a; Cani et al., 2005b; Cani et al., 2006b; Cani et al., 2007b; Delzenne et al., 2005; Delzenne et al., 2007; Verhoef et al., 2011). It might be possible that there is unknown interaction/mechanism that might have limited PCM to increase GLP-1 levels. However, this warrants further investigation. Evidence from the literature shows that peripheral GLP-1 infusion reduces postprandial blood glucose in healthy subjects, non-insulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus (Gutniak et al., 1992; Nathan et al., 1992). It is suggested that GLP-1 reduced postprandial glycaemia via several mechanisms, including suppressing the release of glucagon and insulin secretion (Gutniak et al., 1992; Komatsu et al., 1989; Ritzel et al., 1995), delay gastric emptying (Wettergren et al., 1993) and also enhancing glucose delivery or disposal in peripheral tissues (D'Alessio et al., 1994; D'Alessio et al., 1995; Morales et al., 1997). Based on these findings, it might be possible that the lack of PCM effect on postprandial GLP-1 levels resulted in no significant effect on reducing postprandial glycemia in this study. Moreover, propionate is a gluconeogenic substrate (Bergman, 1990) and it has been demonstrated to increase blood glucose levels in both humans

and animals studies (Kley et al., 2009; Verbrugghe et al., 2011; Wolever et al., 1991; Remesy et al., 2004). Therefore, this might be the reason why there was no significant effect on lowering blood glucose levels. In contrast to these studies, some studies able to show the hypoglycaemic effect of propionate (Berggren et al., 1996; Darwiche et al., 2001; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Lin et al., 2012; Todesco et al., 1991).

Interestingly, including PCM into the volunteers' diet has been shown to reduce plasma insulin levels and that the largest reduction was demonstrated with 30% propionate/PCM dose when compared to inulin as a baseline. Similar observations have been reported in other human (Darwiche et al., 2001; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996) and mice (Lin et al., 2012) studies. Darwiche *et al*. suggested that addition of oral propionate on reducing insulin levels could possibly be related to its physiological role in reducing gastric emptying rate (Darwiche et al., 2001). Furthermore, it is also suggested that the effect of propionate on stimulating adipogenesis and suppressing lipolysis might also contribute to improved insulin sensitivity (Allen et al., 2009; Al-Lahham et al., 2010a; Ge et al., 2008; Hong et al., 2005). However, although 30% propionate/PCM significantly increased plasma PYY levels compared to inulin, this dose does not associated with increase fermentation activity as shown in study 1. The lack of effect of PCM on stimulating colonic fermentation was possibly related to the increased amount of propionate in the PCM molecule that reduced the amount of inulin available for colonic fermentation, For example, 10% PCM provided 9 g inulin and 1 g propionate whilst 30% propionate provided 7 g inulin and 3 g propionate, thus the increased amount of bound propionate has reduced the amount of inulin in the PCM molecule to be fermented by the gut microbiota.

In conclusion, the results of this study suggest that PCM has potential effects on suppressing appetite, energy intake and reduced insulin levels. The highest dose tested in this study, 30% propionate/PCM, has shown the highest effect on modulating energy intake, possibly through increasing plasma PYY. However, the next question that needs to be answered is whether 10 g PCM is the optimum and effective dose to suppress appetite and energy intake. Therefore, in the next study, the effect of 5 g, 10 g and 15 g PCM doses will be investigated in single-blinded,

controlled and five weeks using 30% propionate/PCM that has been characterised from this study.

5.6 STUDY 4: The Effects of Propionate Carrier Molecule on Colonic Fermentation, Gut Hormones Release and Appetite: A Dose Escalating study

Aim: To determine the optimal dose of propionate carrier molecule to increase plasma PYY secretion, reduce subjective appetite and energy intake.

Hypothesis: Increase doses of propionate carrier molecule would have a dose-response effect on appetite regulation and that the highest dose of propionate carrier molecule will have the most significant effect on postprandial plasma PYY levels and subsequently suppresses appetite and energy intake.

5.6.1 Material

5.6.1.1 Dietary Treatments

30% propionate/PCM supplementation used in this study was prepared in the similar method with PCM doses in the study 3 (Section 5.5.1.1). The supplement was provided by Dr. Douglas Morrison, SUERC, Scotland, UK).

5.6.1.2 Volunteers

Nine healthy volunteers (8 males and 1 female) aged between 21 to 61 years old and BMI of 25 to 35 were recruited in this study. They were recruited through advertising in various sites of Imperial College London. Volunteers were given their written informed consent prior the start of the study. Before the study began, approval was sought from Hammersmith, Queen Charlotte's and Chelsea Research Ethic Committee (08/H0707/99) (Registration No: NCT00750438) and the study was

performed in accordance with principles of the Declaration of Helsinki. Volunteers' details are described in Table 5.6.

5.6.2 Methods

5.6.2.1 Assessment Day

Volunteers were asked to attend four study visits.The first visit is a baseline study day, therefore no supplementation was administered. On the second visit, five grams of PCM was provided and the dose was increased by five grams in every visit until they received 15 g on visit 4. Each study visit took 420 minutes to complete. In each visit, breath hydrogen test, VAS, energy intake assessment and blood withdrawal were performed (Figure 5.23). Home supplementation was started a day after baseline study day. During this period, they were asked to take PCM supplementation for six days in order to allow for gut adaptation before the effect of PCM dose was evaluated at the end of supplementation which was on day 7 (Figure 5.24).

5.6.2.1.1 Study Meals

Meals were provided in the similar protocol with study 3 (Section 5.5.2.1.2.1)

5.6.2.1.2 Breath Hydrogen Assessment

Breath hydrogen was assessed in the same method applied in the oligofructose study (Section 2.3.2.2.3). However, in this study, breath hydrogen was accessed at every hour began at -10, 0, 60, 120, 180, 240, 300, 360 and 420 minutes.

5.6.2.1.3 Gut Hormones Analysis

Blood sampling for insulin, glucose and gut hormones PYY were drawn at -10, 0, 15, 30, 60, 90, 120, 180, 210, 240, 300, 360 and 420 minutes and the levels were determined using radioimmunoassay. Methods are discussed in details in Section 2.3.2.2.5.

5.6.2.1.4 Glucose assay

Glucose assay were analysed in the Department of Clinical Biochemistry, Hammersmith Hospital using an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK). Glucose assay sensitivity was 0.3 mmol/L with an intra-assay coefficient of variation of 1.0%.

5.6.2.1.5 Insulin Assay

Insulin assay was performed by using iodine-125 RIA kits (Millipore, Missouri, USA). Please refer to Section 2.3.2.2.6 for more detail.

5.6.2.1.6 Subjective Appetite Scores

Subjective appetite scores used the same method described in the section 2.3.2.2.2 but in different timepoints which were -10, 0, 30, 60, 90, 120, 180, 240, 300, 360, 420 minutes and last assessment was performed after meal.

5.6.2.2 Statistical Analysis

Data are presented as the mean ± SEM. Prior the analysis, Gaussian distribution was used to check for data normality using the Kolmogorov-Smirnov test. Time course for breath hydrogen test, VAS and postprandial plasma metabolites; glucose, GLP-1 and PYY were compared using two-way repeated measures ANOVA with treatment and time as within-subjects factors. tAUC was measured using the trapezium rule for breath hydrogen test, plasma glucose, GLP-1 and PYY, VAS before divided with the overall timepoints (tAUC/Time) and compared using one way ANOVA. Comparison between treatments were assessed using repeated measures ANOVA if normally distributed and Friedman test if the data is not normally distributed. A P value of 0.05 or less was considered significant. Statistical analysis was performed using graph prism 5 (GraphPad Software, Inc., La Jolla, USA).

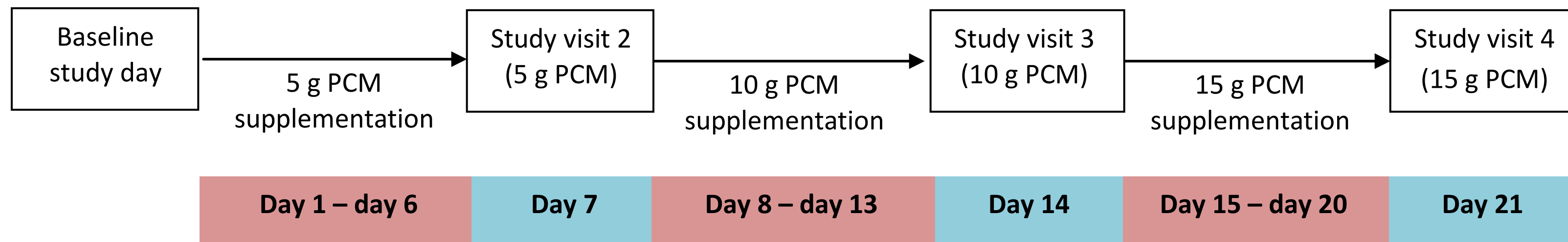


Figure 5.23 The schematic diagram of dose escalating study day. In 4 continuous weeks, volunteers were asked to take 0 g, 5 g, 10 g and 15 g of PCM in increasing order. Each dose has to be taken in 7 days which was divided into 6 days self-supplementation at home before they were asked to attend the appetite study day on day 7.

Figure 5.24 The schematic diagram of study day protocol. Volunteers were expected to attend their study days at 0900 after 10 – 12 hours fasting. Upon arrival, they were cannulated and followed with baseline VAS, H_2 breath test and blood pressure measurements. Standardised breakfast containing PCM dose of 0 g, 5 g, 10 g and 15 g were served at 0 minute every week respectively. Postprandial measurements were started at 15 minutes postprandially and continuously performed every 30 minutes until the end of the study day. At 180 minutes, standardised lunch was provided and they were given 15 minutes to finish eating their meals. An *Ad libitum* was served at 420 minutes for energy intake assessment.

5.6.3 Results

5.6.3.1 Volunteers characteristics

Table 5.6 characterises the demographic data for volunteers that participated in this study. This is the first time dose escalating study using PCM was performed in human, therefore power calculation based on the previous PCM studies could not be done. All of the volunteers were involved with 0 g, 5 g, 10 g and 15 g PCM study day and home supplementation

Table 5.6 Baseline characteristics of the study volunteers

Characteristics	Numbers
Completed (n)	9
Withdrawn (n)	0
Sex (n)	
• Male	8
• Female	1
Age	32.4 ± 5.2
Age range	21 – 61
BMI (kg/m^2)	25.6 ± 1.6

Values are expressed as mean ± SEM

5.6.3.2 Energy intake

PCM doses of 10 g and 15 g showed to reduce energy intake assessed at 420 minutes compared to other treatments [Figure 5.25 i) and ii)]. However, there was no significant different (P=0.340) demonstrated between the treatments (mean ± SEM; 1179.7 ± 156.9 kcal [0 g], 1199.1 ± 174.2 kcal [5 g], 1080.5 ± 163.8 kcal [10 g],1056.7 ± 168.9 kcal [15 g]).

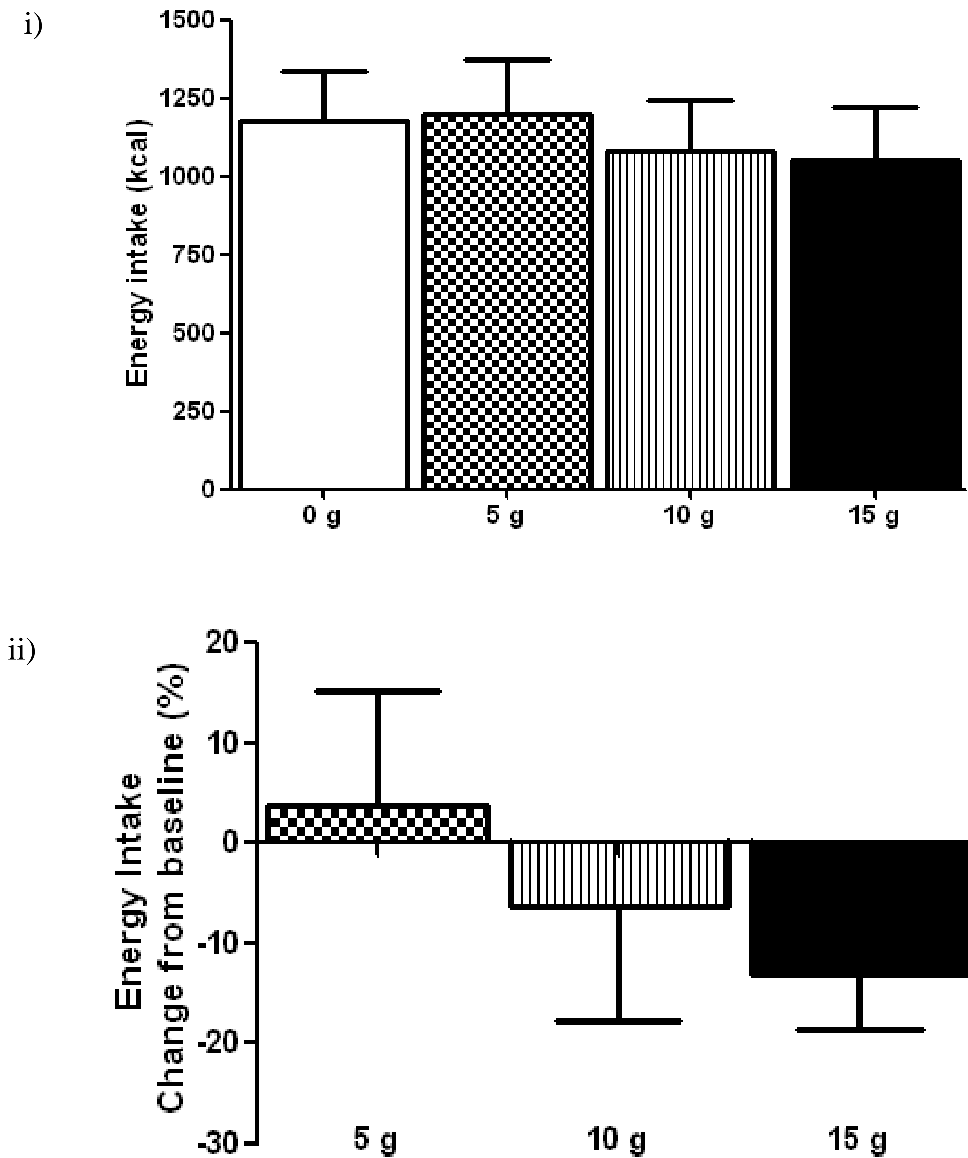


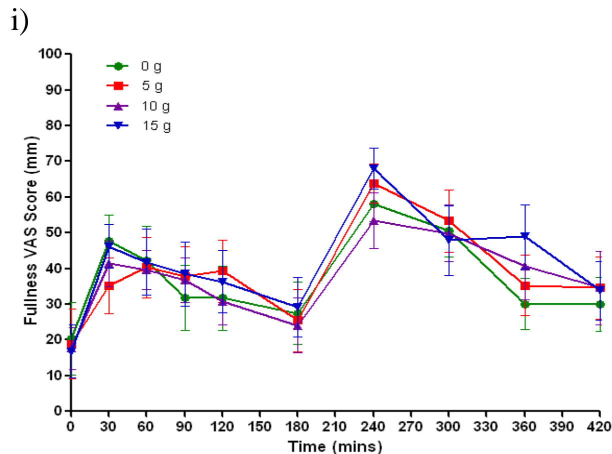
Figure 5.25 *Ad libitum* energy intake assessment (kcal) (i) and change from baseline ii) following intake of 0 g, 5 g, 10 g and 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.6.3.3 Subjective Appetite Scores

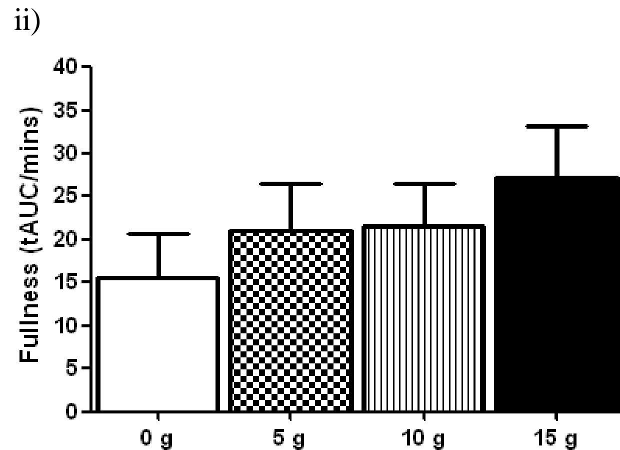
5.6.3.3.1 Appetite assessment

Administration of PCM with 0, 5, 10 and 15 g showed to increase fullness, reduced hunger, pleasantness and prospective food intake scores but there were no significant different between these treatments (P=0.271, P=0.176, P=0.768 and P=0.222) [Figure 5.26 i), iii), v), and vii)]. However, tAUC/mins prospective food intake scores were significantly decreased (P=0.030) with 10 g PCM (mean ± SEM; -3.6 ± 3.8 mm/mins [0 g], -10.7 ± 4.8 mm/mins [5 g], -16.3 ± 4.6 mm/mins [10 g] and -15.0 ± 5.4 mm/mins [15 g]) when compared to the baseline. In addition, dose of 10 g PCM also reduced tAUC/mins hunger and pleasantness scores compared to other doses, but no significant difference was showed, hunger (mean ± SEM; -11.2 ± 6.0 mm/mins [0 g], -12.1 ± 5.7 mm/mins [5 g], -22.7 ± 5.3 mm/mins [10 g] and -12.1 ± 4.2 mm/mins [15 g]), pleasant to eat food (mean ± SEM; -15.1 ± 4.1 mm/mins [0 g], -14.2 ± 4.8 mm/mins [5 g], -19.1 ± 5.6 mm/mins [10 g] and -14.8 ± 5.5 mm/mins [15 g]) whilst dose of 15 g was found to increase tAUC/mins fullness scores but no significant difference was found (mean ± SEM; 15.7 ± 5.1 mm/mins [0 g], 21.0 ± 5.5 mm/mins [5 g], 21.6 ± 5.0 mm/mins [10 g], and 27.1 ± 6.0 mm/mins [15 g]) [Figure 5.26 ii), iiii), vi), and viii)]).

i)



ii)



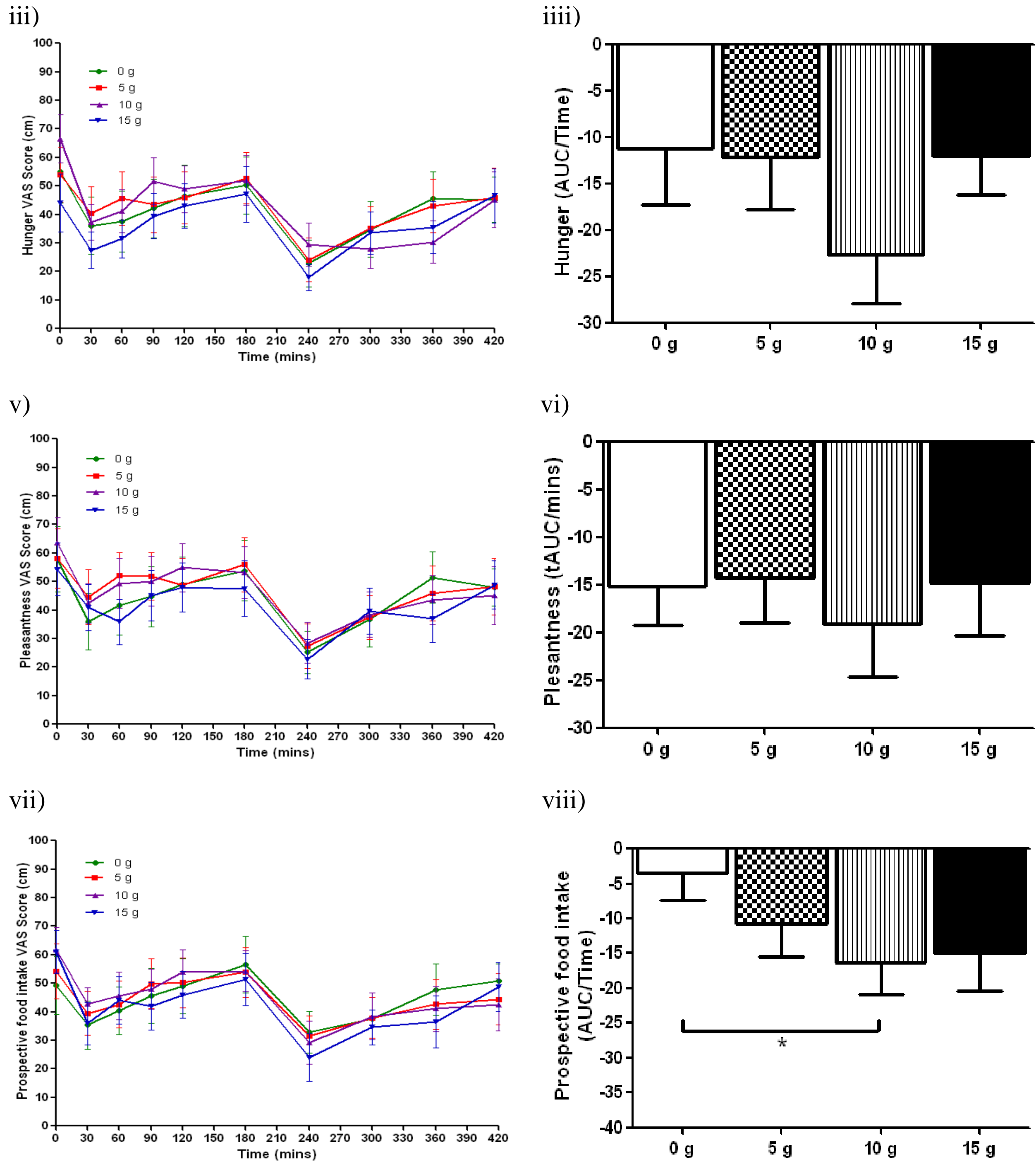


Figure 5.26 Subjective appetite ratings assessed by VAS following intake of 0 g, 5 g, 10 g and 15 g PCM i) fullness scores ii) tAUC/mins for fullness scores iii) hunger scores iiii) tAUC/mins for the hunger scores v) pleasant to eat scores vi) tAUC/mins for pleasant to eat scores vii) prospective food intake scores viii) tAUC/min for prospective to eat scores. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).*P<0.05 0g *vs.* 10g PCM.

5.6.3.3.2 Side effects assessment

In analysing potential side effect scores following PCM consumption, we asked volunteers to mark 1 = mild effect, 2 = medium and 3 = severe effect. PCM supplementations were well-tolerated with low cases on GI side effects were reported and showed no dose response effect. However, the most reported cases upon PCM supplementations were bloating and flatulence (Figure 5.27). Surprisingly, the highest reported cases on these side effects was after intake of 10 g dose (n=5) compared to higher dose of 15 g (n=3 and 2 respectively) and 20 g (n=2) supplementation. Nevertheless, these side effects were reported as mild effect.

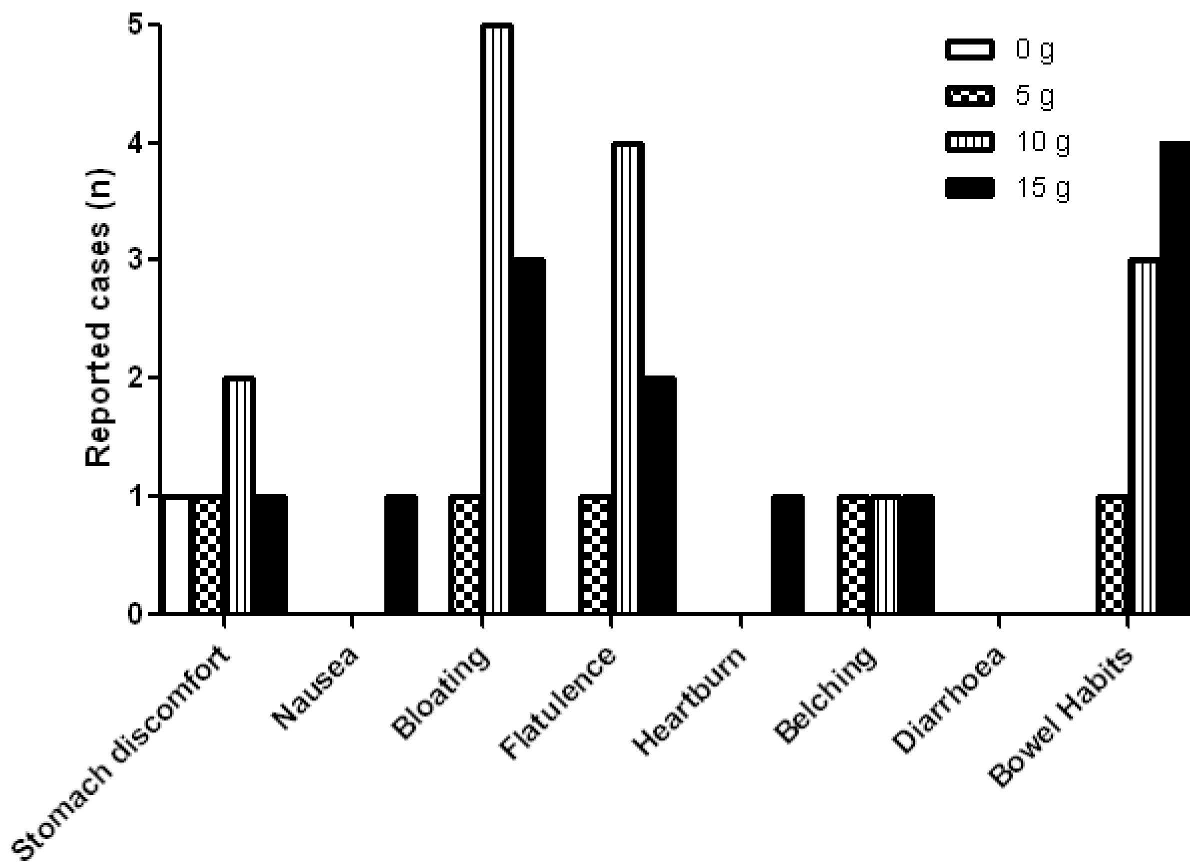


Figure 5.27 Assessment of GI side effects; stomach discomfort, nausea, bloating, flatulence, heartburn, belching, diarrhoea and bowel habits following intake of 0 g, 5 g, 10 g and 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.6.3.3.3 Supplementation assessment

Assessment of supplementation administered at 0 minute after the breakfast meal showed no significant difference in pleasant (P=0.971), bitter (p=0.504), tasty (P=0.569), sweet (P=0.814), sick (P=0.2781) and salty (P=0.227) scores between these doses (Figure 5.32). However, volunteers scored reduce pleasantness, tasty, sweet and increase bitterness and saltiness as the doses were increased. However, these scores were not significant.

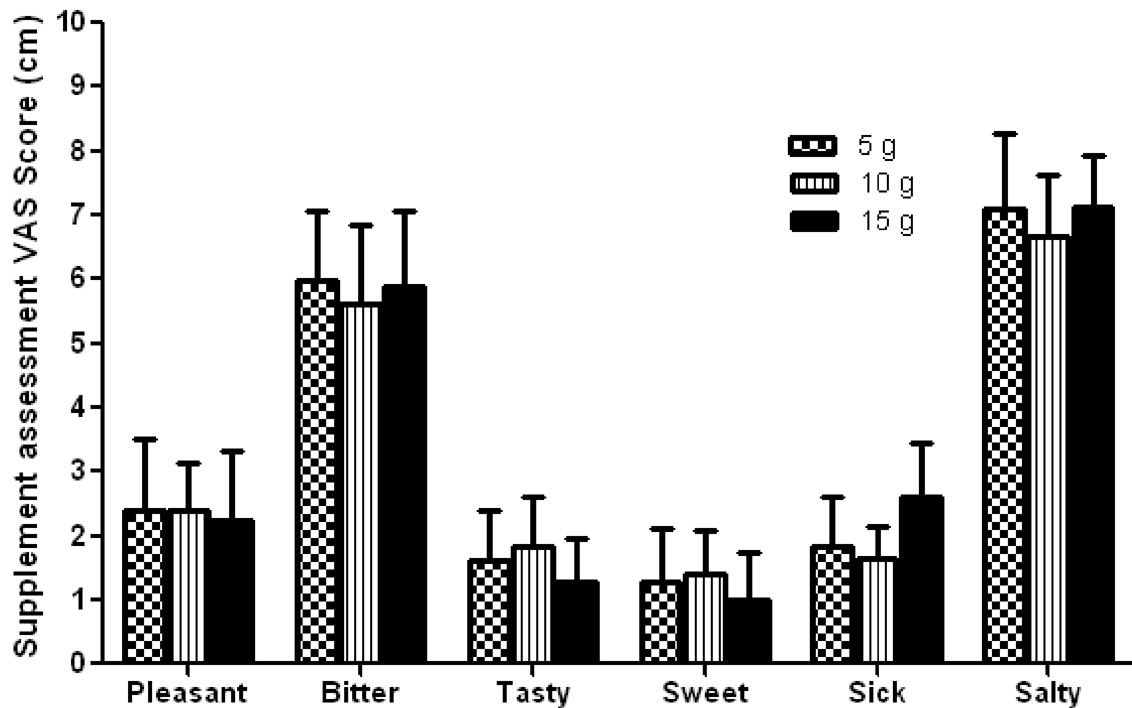


Figure 5.28 Supplement assessment following intake of 0 g, 5 g, 10 gand 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum*meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.6.3.4 Plasma Metabolites

5.6.3.4.1 PYY Analysis

Addition of PCM supplementations showed no significant effect on increasing plasma PYY levels. In this analysis, we were interested to investigate the effect of high doses of PCM on PYY secretion, therefore, plasma samples of 5 g study day was not performed (Figure 5.29). tAUC/Time for plasma PYY secretion after PCM supplementation was not significantly different between each others (P=0.732) (Mean ± SEM; 22.4 ± 2.0 pmol/L*min [0 g], 24.2 ± 3.4 pmol/L*min [10 g] and 22.7 ± 3.1 pmol/L*min [15 g] (Figure 5.29 [inset]).

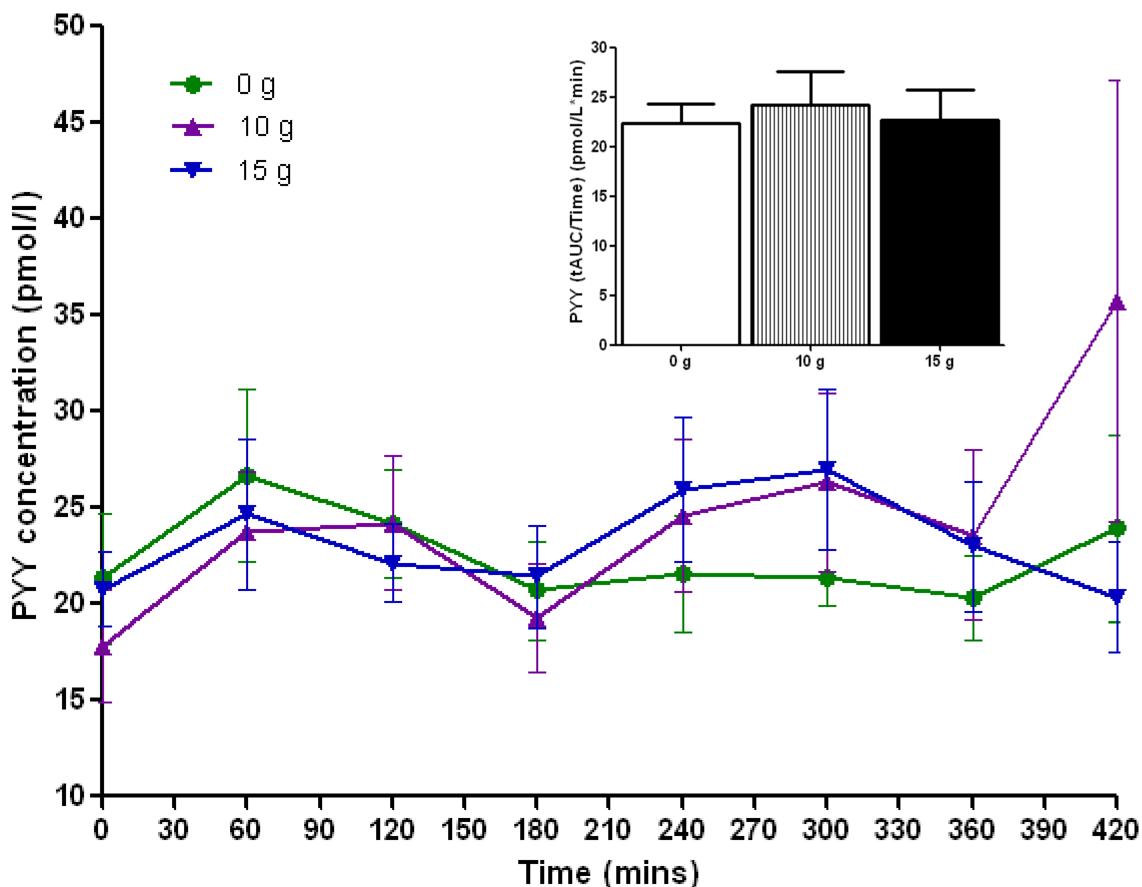


Figure 5.29 Postprandial PYY levels (pmol/L) and $tAUC_{420min}$ (inset) following intake of 0 g, 5 g, 10 g and 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.6.3.4.2 Glucose Assay

Postprandial plasma glucose peaked at 30 minutes after breakfast and again increased for 60 minutes after lunch (Figure 5.30). tAUC/Time for the plasma glucose response between 0 and 420 minutes after addition of PCM were not significantly different between the treatments (P=0.158) (mean ± SEM; 4.8 ± 0.3 mmol/L*min [0 g], 5.0 ± 0.2 mmol/L*min [5 g], 4.9 ± 0.2 mmol/L*min [10 g] and 5.0 ± 0.2 mmol/L*min [15 g] (Figure 5.30 [inset]).

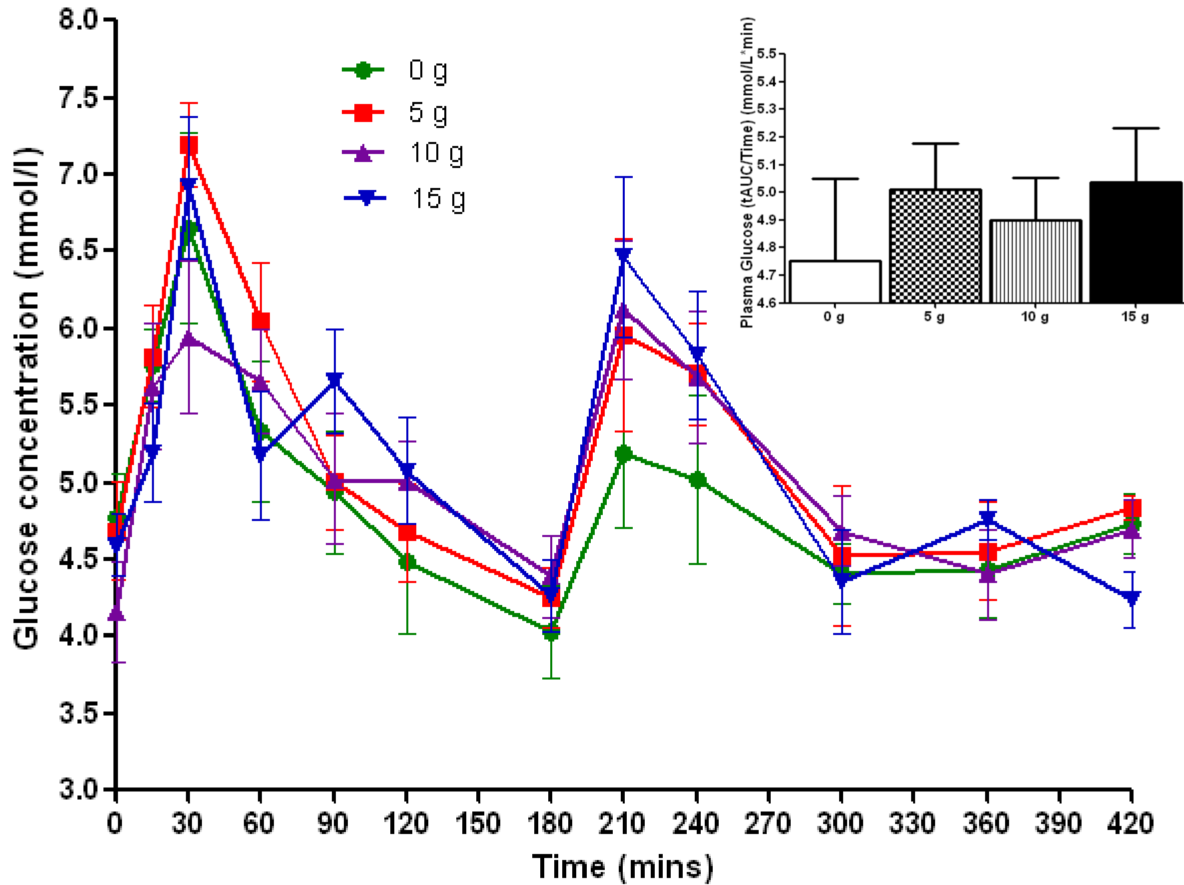


Figure 5.30 Postprandial glucose levels (mmol/L) andtAUC$_{420min}$ (inset) following intake of 0 g, 5 g, 10 g and 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.6.3.4.3 Insulin Assay

Postprandial insulin levels peaked at 30 minutes after breakfast with the highest levels showed by 15 g PCM dose and 30 minutes after lunch with 5 g released the highest insulin levels but this was not significantly different compared to other treatments (P=0.051) (Figure 5.31). tAUC/Time (mean ± SEM; 40.5±5.3 uU/ml*min [0 g], 46.7 ± 5.9 uU/ml*min [5 g], 38.8 ± 5.0 uU/ml*min [10 g] and 38.2 ± 5.7 uU/ml*min [15 g]) (Figure 5.31[inset]).

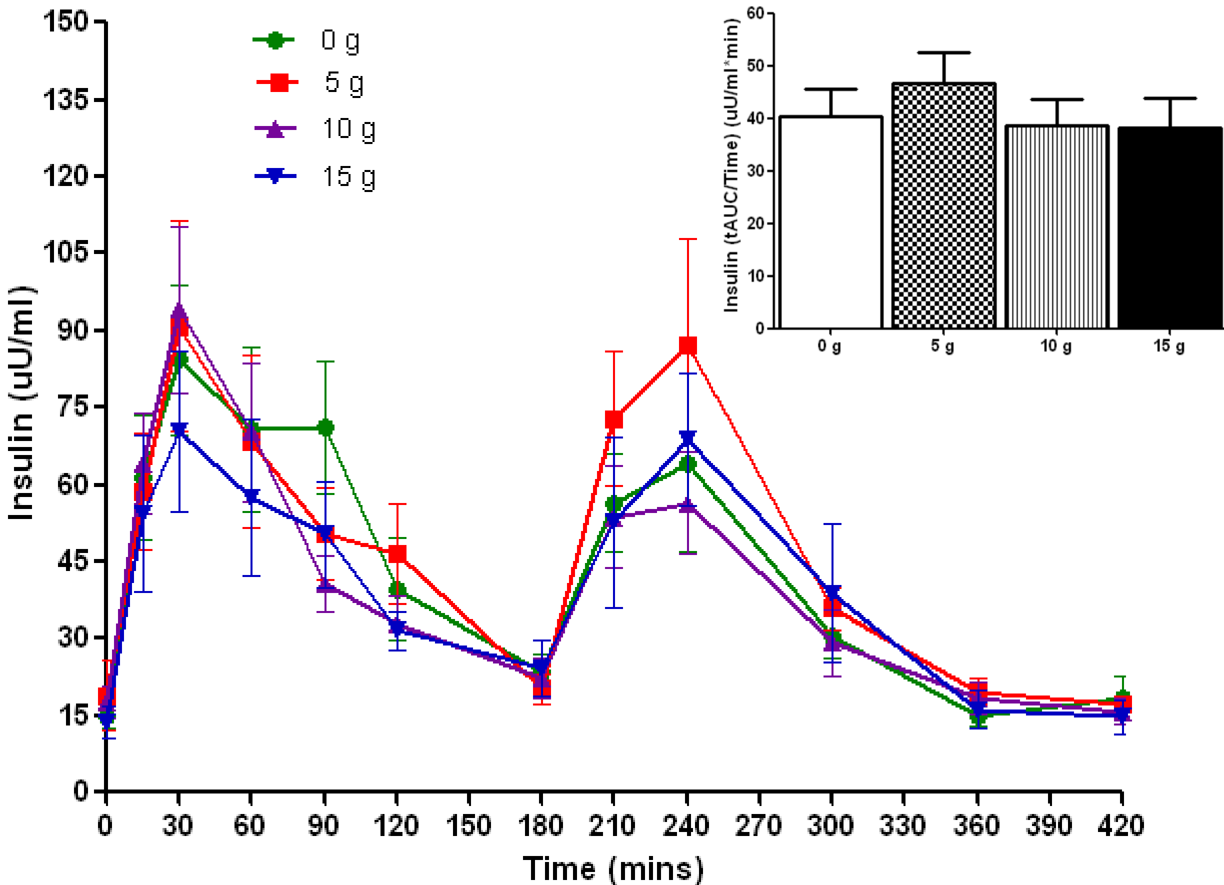


Figure 5.31 Postprandial insulin levels (uU/ml) and $tAUC_{420min}$ (inset) following intake of 0 g, 5 g, 10 g and 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised breakfast, lunch and *ad libitum*meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).

5.6.3.5 Breath Hydrogen Analysis

Breath hydrogen excretion has been used as a marker for bacterial fermentation activities in the gut. Including different doses of PCM significantly increased (P=0.011) the breath hydrogen excretion compared to control study day (Figure 5.32). In the first 3 hours, 15 g PCM was pronouncedly increased the breath hydrogen excretion compared to other treatments with two of the volunteers in this group showed an early rise in the first of two hours PCM ingestion, but the levels seem to maintain after 180 minutes until the end of the study day.The excretion of hydrogen was found to be peaked at 240 minutes with 15 g PCM showed the highest effect, but there was no significant difference between the treatments (P=0.438). However, when compared to control treatment, 15 g of PCM showed a trend towards higher breath hydrogen excretion (p=0.007). Mean tAUC/time of breath hydrogen excretion for 0 g, 5 g, 10 g and 15 g are 15.9 ± 1.5 ppm*min, 21.9 ± 1.3 ppm*min, 19.5 ± 1.1 ppm*min, and 21.6 ± 1.8 ppm*min respectively.

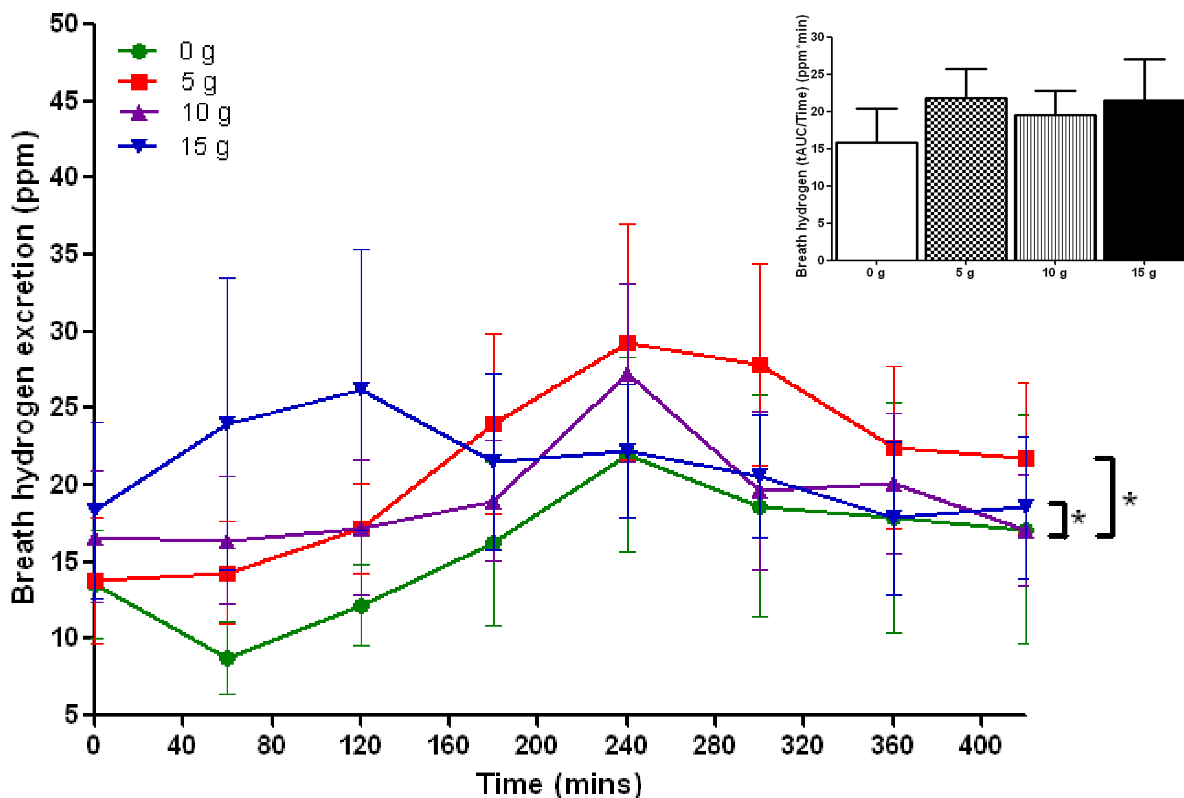


Figure 5.32 Breath hydrogen test (ppm) and $tAUC_{420min}$ (inset) following intake of 0 g, 5 g, 10 gand 15 g PCM. PCM was supplemented in volunteers' meal during breakfast. Standardised meals for breakfast, lunch and *ad libitum* meal were provided at 0, 180 and 420 minutes respectively. Data is expressed as mean ± SEM, (n = 9).*P<0.05 0 g *vs* 5g PCM, *P<0.05 0g *vs* 15 g PCM.

5.6.4 Discussion

In this dose escalation study, the effect of increasing 30% propionate/PCM doses on plasma PYY released, subjective appetite and energy intake were investigated. The results revealed that10 g PCM significantly reduced prospective food intake whilst 15 g PCM was pronouncedly increased fullness score. Moreover, the result also showed a dose response effect on reducing energy intake with 15 g PCM was showed the highest effect on reducing energy intake compared to inulin, but no significant difference in group analysis. However, it is a challenge to elucidate the optimal dose of fibre on appetite, without causing adverse gastrointestinal symptoms or unpalatable taste. Although high intake of fibres were commonly related to gastrointestinal side effects, intake of 15 g PCM in this study seemed to be well tolerated with only mild effects being reported. However, the main concern with 15 g PCM was the palatability in which high ratings on salty and bitter taste were scored. The salty and bitter tastes of PCM were derived from free, unbound propionate which was released during the conjugation process of propionate to inulin. Therefore, it might be possible that unpleasant tastes experienced by volunteers could influence volunteers' appetite and subsequently reduced energy intake.

A study suggested that the unpalatable taste due to high dose of oral propionate is a potential confounder in appetite studies as it could induce the released of gut hormones such as GLP-1 (Frost et al., 2003) and delay gastric emptying (Darzi et al., 2012; Frost et al., 2003) which subsequently reduced food intake and hunger. Furthermore, it is also has been shown to increase nausea (Frost et al., 2003). Unfortunately, plasma GLP-1 was not measured in this study therefore it is not known whether 15 g PCM can influence GLP-1 secretion. Plasma GLP-1 was not measured in this study because results from the three previous studies showed no significant effect on plasma GLP-1, therefore plasma PYY secretion was the main focus in this study. Interestingly, it seems that plasma PYY released was not confounded by the unpleasant taste of 15 g PCM as 10 g of 30% propionate/PCM has been shown to increase plasma PYY levels compared to other doses.

Studies showed that increased release of PYY is related to increase satiety. However, this relationship was mostly exhibited after administration of intravenous infusion (Batterham et al., 2002; Batterham et al., 2003; Degen et al., 2005; Sloth et al., 2007) whilst the satiety effect after

oral feeding of dietary fibre is still unclear (Juvonen et al., 2011; Weickert et al., 2006). Nevertheless, the present study revealed that there is a potential association between plasma PYY released and satiety after oral feeding, but because this study involved a small number of volunteers, this observation remained to be further investigated.

Similar to Study 3, PCM supplementation had no significant effect on reducing glucose as compared to other oral propionate studies (Darwiche et al., 2001; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Todesco et al., 1991). However, Darzi *et al.* suggested that the effect of oral propionate on reducing glucose and insulin levels in these studies (Darwiche et al., 2001; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Todesco et al., 1991) were confounded by the unpalatable taste of propionate and not due to the metabolic effect (Darzi et al., 2012). On the other hands, 10 g of 30% propionate/PCM showed a similar trend on reducing tAUC/time insulin levels as demonstrated in study 3 with average levels of 40-50 μU/ml*min. However, there was no significant effect when compared to 5 g and 15 g PCM. In agreement to our study, Darzi *et al*. also found no significant effect in reducing postprandial glucose and insulin levels after intake of propionate-rich bread (Darzi et al., 2012). Furthermore, Wolever *et al*. showed that rectal infusion of propionate in six healthy volunteers significantly increased peripheral glycaemia compared to acetate and saline infusion (Wolever et al., 1989) which highlighted the role of propionate as a gluconeogenic precursor in ruminants (Yost et al., 1977) and cats (Kley et al., 2009; Verbrugghe et al., 2011). It seems that the role of propionate on postprandial glycaemia and insulinemia in humans are still unclear and need further exploration.

Although this study showed that PCM supplementation led to increase colonic fermentation, this observation was only estimated using breath hydrogen test as a surrogate marker. To date, breath hydrogen test is the only practical method to access colonic fermentation activities. This is because currently no method available to directly access colonic SCFAs measurement. Therefore, measuring colonic SCFAs in humans remains a challenge as not many evidence available to show how these molecules are released, absorbed and metabolised in the literature. The closest available methods to estimate the production of SCFAs are via blood and faecal sample, but the result might not reflect the actual colonic SCFAs production (Topping and Clifton, 2001). In investigating the role of fermentable carbohydrates on appetite and energy

intake, it is one of the focuses to estimate the production of propionate from the fermentation and whether the external supplementation would actually help in triggering the endogenous SCFAs to stimulate gut hormone release. In the context of PCM supplementation, it is important to investigate whether or not the molecule was delivered or reached the colon. This perhaps can be investigated using stable isotope technique as suggested by Topping and Clifton (Topping and Clifton, 2001). To date, there are currently limited SCFAs studies using this method to investigate the endogenous SCFAs production possibly due to difficulties in developing the method. In this technique, a carbohydrate is radiolabelled, supplemented in a diet and that the excretion is observed via peripheral venous plasma or excreted air from breath hydrogen test (Topping and Clifton, 2001). Therefore, this will be one of the aspects that will be investigated in the future study.

In conclusion, this study suggested that PCM has a potential to be developed as anti-obesity treatment. The main challenge in supplementing PCM particularly in high doses is the presence of unpleasant pungent taste as demonstrated in the dose of 15 g which can be a potential confounding factor in reducing energy intake. 10 g of 30% propionate/PCM seems to be the most suitable and effective dose in reducing energy intake with minimal adverse effects. It is postulated that PCM potentially suppressed energy intake via PYY stimulation although this was not significant when compared to other treatments. It is possibly that the short duration of study and underpowered sample size that actually influenced the lack effect of PCM on anorectic gut hormones. Alternative methods of supplementing SCFAs, the intravenous and rectal infusion, although has been shown to elevate plasma PYY and GLP-1 (Freeland and Wolever, 2010), is not a practical method to apply as it requires certain equipment and medical training. Most importantly, the fact that propionate able to stimulate the release of PYY showed that it is not impossible to increase gut hormones production via external stimulation/supplementation. However, this warrants further investigations. If successful, oral supplementation of SCFAs can be developed as a future treatment for obesity.

Chapter 6

General Discussion

This thesis describes studies that were designed to investigate the role of fermentable carbohydrates in stimulating gut hormone release and its effect on appetite and energy balance. It is hypothesised that the fermentable carbohydrate, oligofructose and a novel propionate carrier molecule (PCM) could increase circulating plasma PYY and GLP-1, decrease subjective appetite and energy intake, thus leading to a reduction of body fat and body weight. The investigations in Chapter 2 – Chapter 5 have suggested that oligofructose increased plasma PYY, reduced appetite and energy intake in healthy overweight volunteers. However, effects on postprandial plasma PYY and energy intake were not significant when compared to cellulose supplementation. There was also no suppression on energy intake or body weight following eight weeks supplementation at home. Following the lack of effect of fermentable carbohydrate on modulating appetite and body weight, a SCFA, propionate (an end product of fermentable carbohydrate) was supplemented directly to the colon, by attaching the molecule to a carrier, an inulin. The result showed that addition of PCM in the diet led to increased plasma PYY and reduced energy intake. This interesting finding highlighted the potential role of this novel molecule as a new target for anti-obesity treatment.

Unlike the current study, other research groups have shown that oligofructose play a significant role in regulating appetite and body weight in both animals and human studies (Cani et al., 2005a; Cani et al., 2006a; Cani et al., 2009; Parnell and Reimer, 2009; Zhou et al., 2009). Fermentable carbohydrates are postulated to modulate energy homeostasis by stimulating the release of gut hormones, PYY and GLP-1. Investigation in rodents showed that including oligofructose in the diet increased proximal and medial colonic GLP-1_{7-36} and portal PYY, GLP-1_{7-36} amide and GLP-2 levels (Cani et al., 2007b; Delzenne et al., 2005). Interestingly, increased GLP-1 secretion also has been linked with increased GLP-1 expressing cells following differentiation of the L-cells in the proximal colon (Cani et al., 2007b). The significant modulation of gut hormones following oligofructose intake has led to suppressed food intake, attenuated body fat and body weight gain (Cani et al., 2005b; Cani et al., 2007b). In this study, it is hypothesised that intake of 30 g/day oligofructose would increase circulating PYY and GLP-1 levels, thus this would decrease appetite, energy intake, body weight and adiposity following a six week supplementation period (following 2 weeks run-in period). In this study, dose supplementation was determined based on preliminary results from an oligofructose dose-finding

study (Pedersen, 2010). This dose-finding study suggests that oligofructose has a dose-dependent effect in increasing plasma PYY and reducing energy intake with doses of 35 g and 55 g/day having the most pronounced effect. However, as doses of more than 30 g/day related to gastrointestinal side effects, a dose of 30 g/day was selected as a supplementation dose in this study.

The findings of Chapter 2 and 3 show that intake of oligofructose significantly increased plasma PYY in the oligofructose group but not in the cellulose group. However, the rise following oligofructose supplementation was not significant when compared to the cellulose group. In addition, although oligofructose significantly reduced subjective hunger, no significant effect on energy intake was demonstrated when compared to cellulose. A similar observation was seen in the assessment of appetite following an eight week free-living supplementation period. No significant change on energy intake was found, although there was a tendency for oligofructose to reduce subjective hunger. These results suggest that the increased plasma PYY levels following oligofructose intake are able to suppress appetite, but were insufficient to alter energy intake. The lack of effect of oligofructose to reduce energy intake (assessed by food diaries) during the free-living supplementation period also mirrored the non-significant effect of oligofructose on body weight, total, regional and ectopic body adiposity. The result of this study is in contrast with earlier oligofructose human studies which showed that increased PYY (Cani et al., 2009; Parnell and Reimer, 2009; Verhoef et al., 2011), GLP-1 (Cani et al., 2009; Verhoef et al., 2011) as well as reduced ghrelin plasma levels (Parnell and Reimer, 2009) have resulted in significant suppression of energy intake in healthy lean volunteers (Cani et al., 2009; Verhoef et al., 2011) and reduced body weight in overweight adults (Parnell and Reimer, 2009). The discrepancy between the current result and these studies could be due to several methodological differences.

Firstly, an eight week supplementation period in this study possibly is not long enough for the gut to adapt with oligofructose supplementation to stimulate L-cells to increase production of PYY and GLP-1 levels. Other oligofructose studies were performed for a duration of 12 weeks (Antal et al., 2008; Parnell and Reimer, 2009) and one year (Abrams et al., 2007). Secondly, it might be possible that the sample population of this study (22 volunteers) had low statistical

power to detect small differences between the dietary treatments. The sample size of this study was determined based on power calculation that used AUC postprandial PYY levels from an oligofructose dose response study as a main outcome (Pedersen, 2010). In contrast, other oligofructose studies (Abrams et al., 2007; Antal et al., 2008; Parnell and Reimer, 2009) have investigated the effect of oligofructose on body weight maintenance in larger scale sample sizes, 33 volunteers (Antal et al., 2008), 48 volunteer (Parnell and Reimer, 2009) and 97 volunteers (Abrams et al., 2007). Fourthly, whilst the earlier oligofructose studies (Antal et al., 2008; Cani et al., 2006a; Cani et al., 2009; Parnell and Reimer, 2009; Verhoef et al., 2011) compared the effect of oligofructose with a digestible carbohydrate, maltodextrin, the effect of oligofructose in this study was compared with a non-fermentable carbohydrate, cellulose. However, 13g of maltodextrin was also added to cellulose in order to match the caloric content with oligofructose. The main challenge in comparing the effect of two types of fibre in appetite studies is that fibres have their own physico-chemical properties that promote satiety at different parts of the gut (Howarth et al., 2001). In the case of cellulose, it mainly acts as a bulking agent in the gut and also in shortening transit time, whilst oligofructose exerts it effect in the colon through bacterial fermentation. Therefore, it is postulated that this results in non-significant effect on appetite-related outcome measures. Currently there is lack of evidence comparing the effect of fermentable carbohydrates with non-fermentable carbohydrates on appetite and energy balance. Howarth *et al*. showed that three weeks supplementation with fermentable carbohydrates, β-glucan and pectin had no significant effect on appetite, body weight or fat loss (Howarth et al., 2003) when compared with the non-fermentable carbohydrate, methylcellulose. However, three weeks fibre intake in this study might be too short to evaluate the effectiveness of fermentable carbohydrate on regulating appetite and body weight loss.

Interestingly, recent evidence suggested that the effect of resistant starch on reducing energy intake and body fat maybe related with activation in the brain. In animals, rats that reduced weight following intake of resistant starch also have been shown to increase activation in hypothalamic VMH and PVN in MEMRI-imaging study (So et al., 2007). In mice, adding oligofructose-enriched inulin in the diet led to significant increased activation in the ARC and reduced energy intake and body weight compared to a control group (Anastasovska et al., 2012).

The SCFAs, the end products of colonic fermentation of fibres have also been shown to cross the BBB (Conn et al., 1983; Song et al., 2009; Anastasovska et al., 2012).

Based on these studies, fermentable carbohydrates and their products, SCFAs, potentially have a significant role in central neuronal processing and higher CNS. However, to date, the effect can only be found in rodents as no investigation of SCFAs or fermentable carbohydrates have been performed in humans. In this study, the effect of supplementing oligofructose on brain activation was investigated following task-responsive signal changes in non-homeostatic regions. In humans, task-responsive signal changes are mostly studied in non-homeostatic regions as it is impossible to study this type of investigation in hypothalamus and brainstem due to its susceptibility to signal drop out due to the air sinuses during the image acquisition.

It is hypothesised that intake of oligofructose reduces brain activation in the NAc, amygdala, OFC, hippocampus, insula and vACC following food image stimulation. However, the result of this study did not support the hypothesis as no significant change in BOLD activation in the aforementioned ROIs was demonstrated following high calorie foods vs. objects stimulation in the oligofructose group. Surprisingly, activation in these regions was reduced after intake of cellulose and a significant effect was demonstrated in the OFC. Interestingly, a significant reduction of BOLD signal in the OFC following cellulose intake was similarly demonstrated when PYY_{3-36} or combination of PYY_{3-36} and $GLP\text{-}1_{7-36}$ were infused in healthy lean volunteers following stimulation with high calorie foods vs. objects and during resting experiment (Batterham et al., 2007; De Silva et al., 2011). Infusion of PYY and GLP-1 has previously been shown to reduce hunger and appetite (Batterham et al., 2003; Flint et al., 1998; Gutzwiller et al., 1999). Hence, this suggests that reduced BOLD activation in pre-selected ROIs following cellulose supplementation is related with a satiation effect. However, as energy intake was not measured in this study, this effect cannot be confirmed. Nevertheless, there was a significant reduction in VAS volume food intake and a trend toward a reduced hunger score in cellulose group. No significant effect on subjective appetite was shown in the oligofructose group. This finding conflict with the results observed on the appetite assessment study day as oligofructose, but not cellulose was shown to significantly suppress subjective appetite.

Despite the encouraging finding of the effect cellulose on brain reward activation, this result cannot be used to conclude its role in modulating neuronal signals as a small number of volunteers (oligofructose=4, cellulose=6) were involved in this study. Nevertheless, as this is the first time the role of fermentable carbohydrate has been investigated on human neuronal brain activation, therefore it can be used as a preliminary observation and for powering sample size for future investigation. Therefore, in the future, It would be interesting to reinvestigate this study in a longer supplementation period (>12 weeks) using a larger cohort.

As fermentable carbohydrates were unable to affect appetite and energy balance, the role of fermentable carbohydrates main product, the SCFAs, was investigated in this study. SCFAs have been proposed to play a significant role in energy homeostasis. This is because SCFAs may be able to induce the enteroendocrine L-cells to stimulate the production of gut hormones, PYY and GLP-1. It is postulated that SCFAs induce the effect by binding to its receptor, FFAR2 and FFAR3 (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003) which has been found in various tissues including the intestines (Karaki et al., 2008; Tazoe et al., 2008) and adipocytes (Al-Lahham et al., 2010b; Brown et al., 2003; Ge et al., 2008). In the intestine, FFAR2 and FFAR3 can be found in L-cells where it has been found to be co-localised with cell expressing PYY and GLP-1. Therefore, it is possible that the L-cells sense SCFAs through the receptors, thus subsequently release PYY and GLP-1.

Based on the potential role of SCFAs in increasing gut hormones secretion, many studies have attempted to use SCFAs as dietary supplements and investigated their role in reducing appetite and energy intake. However, not all studies have been able to show an effect. Darzi *et al*. suggested that propionate had no significant effect in reducing appetite or energy intake (Darzi et al., 2012). SCFAs are volatile fatty acids. Therefore, it might be possible that they have been absorbed into the circulation before it reached the gut. Moreover, intake of SCFAs has also been related with nausea and poor palatability (Darzi et al., 2011; Frost et al., 2003). In contrast to earlier SCFA studies which directly included SCFA in volunteers' diet (Darzi et al., 2012; Frost et al., 2003; Liljeberg et al., 1995; Liljeberg and Bjorck, 1996; Todesco et al., 1991), propionate in this study was chemically attached to a carrier, inulin (a long molecular weight of oligofructose) via a conjugated linkage. This linkage can only be cleaved by enzymatic process in the gut, thus ensuring that propionate will only be released

in the colon whilst inulin will subsequently be fermented by the gut bacteria. It is postulated that this system would increase colonic SCFAs to increase PYY and GLP-1. It is hypothesised that intake of PCM will deliver propionate directly to the gut, increase colonic SCFAs and stimulate plasma PYY and GLP-1 release, thus leading to a reduction of hunger and energy intake in healthy normal and overweight volunteers.

The development of PCM for appetite regulation was investigated in four small pilot studies in Chapter four of this thesis. In study 1, as this is the first time PCM was investigated in humans, the tolerability of 10 g PCM with a low degree of esterification (a degree of propionate dose is added into the complex molecule), 0.25 or 10% propionate/PCM (or 1 g of propionate and 9 g of inulin) was investigated in six healthy overweight volunteers. The result supports the hypothesis that PCM with DE 0.25 was safely delivered to the colon (monitored by increase breath hydrogen test as a fermentation marker) with a tendency to increase plasma PYY levels. In addition, the dose was also well-tolerated by the volunteers.

To understand the effects of PCM on increasing plasma PYY levels, a randomised, double-blinded, crossover study was performed to investigate the role of PCM with DE 0.5 has on appetite and energy intake. The study design was strengthened by an increased sample size of 20 volunteers and DE was increased from 0.25 (10% propionate/PCM) to 0.5 (20% propionate/PCM) or 2 g propionate and 8 g inulin. It is hypothesised that this will stimulate higher production of PYY and GLP-1. However, the result was inconsistent with my hypothesis as 10g PCM with DE 0.5 had no significant effect on increasing plasma PYY and GLP-1 levels. However, it significantly reduced *ad libitum* energy intake when compared to inulin. Surprisingly, energy intake in the inulin group was also reduced by a similar magnitude with PCM but no significant difference was demonstrated between the groups. It can be postulated that the supplemented propionate was insufficient to trigger higher release of gut hormones to affect energy intake when compared to inulin. Based on the discrepancy between PCM 0.25 and PCM 0.5 on circulating PYY and GLP-1 and energy intake, a dose finding study was performed in study 3 to determine optimum levels of PCM to influence appetite regulation.

In study 3, the effect of 10g PCM with DE 0.25 (10% propionate/PCM), 0.5 (20% propionate/PCM) and 0.8 (30% propionate/PCM) on gut hormone release and energy intake was investigated in nine healthy lean volunteers and the effect was compared to inulin. It is hypothesised that PCM would have dose response effects on increased PYY and GLP-1 levels, suppressing appetite and energy intake. PCM with DE 0.8 (30% propionate/PCM) would have the most pronounced effect in reducing energy intake compared to other doses. These findings were consistent with the study hypothesis. PCM with different different DE have dose response effects on reducing energy intake with the highest effect being demonstrated by 0.8 PCM. The dose response effect of PCM has also been demonstrated in subjective fullness and prospective food intake VAS scores. Based on the promising effect of PCM with DE of 0.8 (30% propionate/PCM), this dose was selected to be used in the next dose-finding study.

This dose-escalation study aimed to investigate the effect of 5 g, 10 g and 15 g PCM with DE on plasma PYY levels, subjective appetite and energy intake. It is hypothesised that the higher the dose supplemented in diet, the greater effect can be seen on appetite, plasma PYY and energy intake. The result of this study is consistent with this hypothesis. PCM doses were demonstrated to have a dose dependant effect on energy intake. Both 10 g and 15 g doses reduced energy intake, but this was not significant. However, the main challenge with supplementing high dose of SCFAs in the diet is potentially related with pungent and bitter taste. In the context of PCM, 15 g was related to a high salty taste derived from unbound propionate that was released during conjugation propionate to inulin. Based on the result from this study, 10 g PCM with DE of 0.8 was selected as the optimum dose and this dose will be used in the future, double-blinded study in healthy overweight subjects. In the future, it would also be interesting to determine the levels of SCFAs in human system (blood, faeces and urine) produced from PCM supplementation using gas chromatography.

Conclusion

In conclusion, results from the oligofructose study showed that oligofructose has acute impact on human appetite but no role in modulating energy intake, body weight, adiposity and BOLD fMRI signal change following food cues stimulation over eight-week supplementation period. The exploration of new methods of delivering SCFAs directly to the colon may give an exciting opportunity to increase colonic SCFA and stimulate the production of PYY and GLP-1. Hence this highlights its potential to be developed as an appetite suppressant and long-term body weight treatment. This finding warrants further investigation in overweight/obese subjects to elucidate the interaction of increase plasma PYY levels on energy intake and body weight maintenance.

Future Work

In Chapter 2, it is suggested that intake of oligofructose has lead to increase plasma PYY concentration. Indeed, similar observation also has been demonstrated in other studies (Cani et al., 2009; Parnell and Reimer, 2009; Piche et al., 2003). However, the levels were not different when compared to cellulose supplementation. As previously discussed, PYY is one of the anorectic gut hormones which is released following stimulation by SCFAs' receptor, FFA2 and FFA3 in L-cells (Kaji et al., 2011; Karaki et al., 2006; Karaki et al., 2008) after bacterial fermentation of fermentable carbohydrates. Although many studies have shown that oligofructose supplementation leads to increase PYY and GLP-1 concentration, not many studies have related the released of PYY and GLP-1 concentration with SCFAs profile and colonial bacterial fermentation. Therefore, it would be interesting to investigate the link between changes in gut hormone levels, SCFA profile and colonic bacterial composition. This particularly important as inter-individual differences in colonic SCFA profile might be the contributor of lack of effect of oligofructose on inducing anorectic gut hormone release in this study. SCFA profile can be determined by gas chromatoghraphy as previously described (Fernandes et al., 2011; Vogt et al., 2004b). On the other hand, gut microflora composition has been shown to be differ between lean and overweight mice and humans (Ley et al., 2005; Ley et al., 2006; Turnbaugh et al., 2006). In addition, the 'obese gut microbiota' also has been suggested to have high efficient to harvest energy compared to lean microflora. Therefore, it is also interesting to investigate whether the obese/overweight volunteers in this study also have similar 'obese microbiota

pattern' and whether oligofructose supplementation able to change the gut microbiota composition in the obese volunteers following 8 weeks supplementation. To date, no known human study has investigated the effect of oligofructose on gut microbiota composition, SCFA profile and gut hormone release concurrently.

Based on the promising findings in Chapter 5, a randomised, controlled, parallel study was designed to investigate the effect of supplementing PCM with DE 0.8 (30% propionate/PCM) on appetite, body fat and body weight in healthy overweight/obese volunteers in the department of Investigative Medicine (protocol reference number:08/H0707/99) (ClinicalTrial.gov identification no: NCT00750438). In this study, the effect of 10 g/day PCM with DE 0.8 (30% propionate/PCM) on appetite regulation will be investigated by measuring circulating gut hormones levels, subjective appetite, energy intake, glucose tolerance, insulin sensitivity, body weight and body adiposity in volunteers with BMI of 25 to 40 kg/m^2 and aged between 30 to 65 years old) in the duration of 26 weeks. The effect of PCM will be compared with inulin. It is estimated that 60 volunteers (PCM=30 and inulin=30) are needed to detect significant effect between these treatment. This investigation will be performed in 13 visits which include an acclimatization appetite assessment visit prior the start of the study. Baseline visits were performed between visit 2-5 before volunteers are undergo self-living supplementation period for 24 weeks. During the supplementation period, they will be asked to attend five short study visits for body weight, waist and hip measurements as well as bioelectrical impedance for body fat quantification. Post-supplementation measurement will be performed similarly with baseline visit at weeks 11 – 13. Body adiposity will be determined using whole body MRI scanning. In addition, questionnaires of physical activity and energy intake (using food diaries) will also be monitored during this period.

Although SCFAs have been studied in energy homeostasis and appetite regulation, no work has yet been done to understand the exact mechanisms of how SCFAs distribute in the body. This is particularly interesting as SCFAs can possibly cross the BBB (Conn et al., 1983) and influence appetite directly via CNS. To date, specific methods for a direct assessment of bio-distribution and bio-kinetics of these SCFAs *in vivo* is still lacking. Therefore, it would be interesting to further elucidate the mechanisms and bio-distribution of PCM in the body. It is suggested that

this can be performed using the advanced imaging technique, positron emission tomography using radioisotope carbon-11 [^{11}C] to trace the distribution of SCFAs in the body. Based on the evidence of the physiological effect of propionate on energy homeostasis, it can be speculated that the highest levels of propionate can be located in the large intestine as it the centre for fermentation activities, followed by liver, brain, portal vein and to a lesser extent in the periphery. Therefore, it is hoped that the result of this study would lead to a clearer understanding of the effect of SCFA, particularly PCM on appetite regulation and body weight, thus raising its potential to be developed as an anti-obesity treatment.

Appendices

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Participant information sheet

Project title:

Effect of oligofructose on appetite, body composition, and gut hormones in healthy overweight subjects

Investigators: *Ms Norlida Mat Daud (PhD student),* ***Mrs Nurhafzan Ismail*** *(PhD student), Professor Jimmy Bell, Dr. Tony Goldstone, and Professor Gary Frost (Principle Investigator)*
Institution: *Imperial College London, Department of Investigative Medicine, Hammersmith Hospital Campus*

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish. Ask us if there is something that is not clear or if you would like more information.

If you do decide to take part in the study, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation. Thank you for taking the time to read this information sheet.

What is the study about?

The nutrients under investigation in this study are natural, harmless dietary fibres called oligofructose and cellulose. Oligofructose is a natural storage compound found in garlic, onion, Jerusalem artichoke, and chicory root. The most common source of oligofructose is chicory. Oligofructose is already used in many food products today. It provides 30-50% of the sweetness of table sugar and is often used as a low-calorie alternative to sugar and fat in dairy products and baked goods. Cellulose is a structural component of plant material and hence a part of a normal diet containing grains, fruit and vegetables. All bran is an example of a food item that is high in cellulose.

Like other dietary fibres, oligofructose and cellulose cannot be digested in the small intestine; hence almost all oligofructose and cellulose reach the large intestine. In the large intestine cellulose and oligofructose behave differently. Oligofructose is broken down by the gut flora producing several bio-active compounds which may improve the health of the host. Cellulose on the other hand acts mainly as a bulking agent. Regular intake of dietary fibres may also improve appetite regulation and thus may be useful in bodyweight management.

You will only receive one of the two dietary fibres mentioned above. The study is blinded which mean that you will not know which one of the fibres you will receive. Once you have completed the study you may ask the investigators which kind of dietary fibre you were provided with.

Appendix 1 – Patient information sheet (Oligofructose Study)

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This study is looking into the effect of a daily intake of 30g dietary fibre on appetite, food intake, body weight, appetite regulating hormones and sugar in the blood in healthy, overweight people. We will ask you to ingest 3 daily doses of a dietary fibre supplement with your normal diet for a total of 8 weeks and record your daily food intake and appetite feeling for a total of two weeks over a 9 week period. The daily dose of dietary fibre will be increased gradually over two weeks which should minimize any potential discomfort related to an increase in dietary fibre intake.

We will also be looking at the acute response to the dietary fibre supplements. For that reason we are going to ask you to come in for three appetite study session in our Clinical Investigation Unit. The total duration of the study will be approximately 10 weeks.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

Who can take part in the study?

To take in the study there are some inclusion criteria you need to fulfil; these are listed below. Please check this list carefully.

Inclusion criteria

- Healthy males and females aged 20-50
- BMI 25-35 kg/m^2
- Weight stable for three months prior to enrolment in study (weight change < 3 kg over a period of three months)
- Non-smokers
- No current or history of endocrine disease, gastrointestinal disease, kidney or liver diseases, cardiovascular disease, pancreatitis, or cancer
- Habitual dietary fibre ≤ 25g/day (as assessed by 3-day dietary record following screening)
- Hydrogen producers (will be tested at screening)

There are also a number of reasons why you may not be able to take part; these are the listed below:

Exclusion criteria

- Use of antibiotics less than three months prior to participation in the study
- Participation in other research studies in the previous three months
- Blood donation less than three months before participation in study
- Anaemia
- High blood pressure
- Pregnancy or breastfeeding

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- Substance abuse
- Vegan **or vegetarian** diet
- **Food intolerance or food allergies**
- Regular use of prebiotic, probiotic or synbiotic food items/ supplements
- Intense exercise undertaken for more than 5h per week
- Metallic or electronic implants e.g. pacemaker, cochlear ear implants, fixed dental braces
- Claustrophobia
- Depression

What do I need to do?

Overview

Before consuming the dietary fibre supplement you need to attend the clinical investigation unit and Robert Steiner Unit on four occasions. On the two first visits you will have MRI scans, for further details on these please turn to page 8. The following two visits are appetite study sessions (visit 3 and 4). Between visit 3 and 4 you will be asked to record everything you eat and drink for seven days. You will also be asked to complete some simple questionnaires on your appetite sensations and general wellbeing for those same seven days.

During or after the dietary fibre supplementation further MRI scans will be conducted at visit 5 and 7. Visit 6 will be another appetite study session (same as visit 3 and 4). Please see page 5 for an overview of the visits.

Screening procedure

Before you are enrolled in the study you need to come in for a screening interview. As part of the screening process, you will be asked to complete a screening questionnaire to determine your general health status and eating habits.

A small blood sample will be taken to check for anaemia. Furthermore you will be asked to blow into a handheld breath hydrogen monitor (hydrogen is produced when dietary fibres are broken down in the gut). Only those who produce hydrogen will included in the study. Your body weight, height, body composition and blood pressure will be measured. Body composition will be assessed by bio-electrical impedance. This is a painless procedure and only takes a few minutes. The impedance machine resembles a normal set of weighing scales.

To ensure that you are comfortable eating the food items served during the study you will be asked to taste some of the food items that will be used on the study sessions and rate how much you like these items. After the screening you will be asked to complete a three day dietary record and return this as soon as possible. This is to assess your normal dietary habits and usual dietary fibre intake.

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Screening Visit

- Interview and explanation of study
- Blood test (30 ml = 3 tablespoons) and breath hydrogen test
- Screening questionnaires: Personal details, Dutch Eating Behaviour Questionnaire, SCOFF, MRI safety check form
- Consent form

Visit 1 – Assessment of Body Composition

- Magnetic Resonance Imaging – baseline scan
- Blood sample (30 ml)

Visit 2 – Exploration of Neural Activation in the Brain

- Functional Magnetic Resonance Imaging – baseline scan
- Blood samples (80 ml) and questionnaires

Visit 3 – Appetite study day

- Blood sampling (30 ml)
- Visual Analogue Scales questionnaires (assessment of appetite and side-effect)
- Breath hydrogen monitoring
- Test meals

Baseline dietary record and appetite and side-effect assessment
(Day -7 to -1)

Visit 4 – Appetite study day (DAY 0)
Same procedure as on visit 3, total amount of blood withdrawn: 160 ml

Supplementation Run-in: DAY 1-14

- Gradual increase in daily intake of supplement to 30g per day

6 weeks of Supplementation: DAY 15-56

- 30g of supplement to be consumed daily with main meals (3 x 10g)
- Contact by phone and/or email four times over the six weeks

Visit 5 - Exploration of Neural Activation in the Brain
Same procedure as on visit 2
Visit takes place some time during week 4 of the supplement period (Day 36-42)

Dietary record and appetite and side-effect assessment
(Day 49-55)

Visit 6– Appetite study day – Post-intervention visit (DAY 56)
Same procedure as on visit 4

Visit 7 – Assessment of Body Composition

- Magnetic Resonance Imaging – post-intervention scan - same procedure as on visit 1

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Dietary fibre supplementation

The supplement will be provided in sachets (containing 10g portions). During the two first weeks following the second appetite study session (visit 4) in the hospital your daily intake should gradually be increased to 30g per day. On day 15 the actual supplementation period begins and this will last six weeks. From day 15 to 55 you will be asked to consume 3 x 10g of the provided supplement every day. The supplement is to be consumed with the main meals and can be mixed into most drinks, sprinkled on or mixed into food. The supplement must not be cooked and exposed to high temperatures (for example by adding it to freshly boiled water). Unused sachets must be returned to the investigators.

During the 6 week supplementation period the investigators will contact you by phone and email to ensure that the fibre supplementation has no untoward side-effects and ensure that instructions are followed and answer any questions you may have about the study.

Dietary records and questionnaires

A 7 day dietary record is to be completed prior to the visit 4 and at the end of the fibre supplementation period (day 49-55). In this record you write down everything you eat and drink, including snacks between meals.

On the same days appetite sensation and side-effects will be recorded using visual analogue scales. At the end of the day you will be asked questions like “How full did you feel after eating meals today?” and “How hungry did you feel between meals today?”. You will answer them by making a vertical mark on a ten centimetre line anchored with the terms “not at all” and “extremely”. Similarly, side-effects such as nausea, sickness, flatulence, bloating, diarrhoea, and general wellbeing will be assessed. The questionnaires are very simple and take no more than 5 minutes to complete.

Appetite study session in the research ward (day -8, 0, and 56)

24h before coming in for a study session you will be asked to abstain from strenuous exercise, alcohol and caffeine containing drinks. The evening before *every* study session you need to consume a standard meal (of your choice).

On the morning of the study sessions you will be asked to arrive fasted overnight (10 hour fast). You can drink as much water as you like during the fast. After explanation of the procedures and confirmation that you are happy to proceed, your body weight will be recorded. Blood pressure will be measured after 5 minutes rest in a seated position. A catheter will be inserted in a forearm vein facilitating blood sampling throughout the day. Two baseline blood samples will be withdrawn and then breakfast will be served; you will have 20 minutes to consume the meal. Blood sampling will continue regularly after breakfast and appetite questionnaires - Visual analogue scales (VAS) - are completed every time a blood sample is drawn. Breath hydrogen will be measured using handheld breath hydrogen monitors at selected time points throughout the session. About 4 hours after

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breakfast you will be served lunch. Breakfast and lunch will consist of the same food items on every study session. Seven hours after breakfast a large meal will be served. You can choose between chicken tikka masala, macaroni cheese and bolognaise bake. Please be aware that your choice applies to all three appetite study sessions. The meal is served in excess and you are asked to eat until comfortably full. You will be given a maximum of 30 minutes to consume the meal, and should eat alone and undisturbed. Reading material and TV, mobile phones, other electronic devices etc. should not be within reach during the meal. During the study session you will be asked to minimise physical activity. You can watch films or TV, bring a laptop, books, magazines and the like to keep you occupied during your stay in the ward. Water is allowed *ad libitum* though you will be requested not to drink less than 10 minutes prior to a blood sample and VAS completion.

During the study session you will be asked to minimise physical activity. You can watch films or TV, bring a laptop, books, magazines and the like to keep you occupied during your stay in the ward. Water is allowed freely though you will be requested not to drink less than 10 minutes prior to a blood sample and VAS completion.

Whole body MRI scans before and after taking the fibre supplement (visit 1 and 7)

You will attend the Robert Steiner MRI unit at Hammersmith Hospital after a 10h overnight fast. The study visit will last between 2-3 hours. You are asked to refrain from strenuous exercise and drinking alcohol the day before each visit. You will be asked to change into hospital clothes and complete a metal check form.

You will have your height and weight, waist and hips measured and a scanning technique which measures your amount of body fat and where it is stored i.e. around your waist or around your hips, called bio-electrical impedance analysis. This painless method involves lying still under a semi-tube base unit while a wireless belt is placed on your abdomen for around 30 seconds or measuring the electrical current from your body for 10 seconds. You will also be given a short questionnaire to complete about how physically active you are, your general health and family history of any illnesses.

You will have a measurement of the volumes of your body parts and the body fat percentages of each body section. This safe body volume imaging technique involves standing in an enclosed cubicle in front of a 3D scanner which uses white light. The measurement process takes no more than 10 seconds and does not use radiation. You will have a small plastic cannula tube inserted into a vein in one arm to take a blood sample to measure your blood sugar, fat and hormones. With your permission, we will also take a sample of DNA and RNA from blood or saliva to look for changes in your genes that may be involved in the how the body controls appetite and body weight.

You will then lie on the trolley in the scanner. During the scan you will lie supine or prone in the scanner and are automatically moved through the scanner. While in the scanner you will

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have access to a buzzer to sound an alarm, and will be able to hear and respond to instructions from the scanning console. You will be in the MR scanner for up to 1 hour.

Scanning will be performed on either the Philips 3.0 Tesla or Philips 1.5 Tesla MR scanners in the Robert Steiner MR Unit at the Hammersmith Hospital. None of the magnetic resonance imaging techniques to be used employs ionising radiation or intravenous contrast agents. Whole body anatomical MR scanning will be performed to determine total and regional fat volumes.

Functional MRI scans before and whilst taking the fibre supplement (visit 2 and 5)

You are asked to refrain from strenuous exercise and drinking alcohol the day before each visit. The evening prior to the scan you will be asked to have a standard evening meal. We will also ask you to keep a record of all food and drink consumed for one day before the visit, the day of the visit and for one day afterwards. If female, you may be asked to have the study visit at a particular time in your menstrual cycle since this can alter your appetite.

Similar to the whole body MRI scan you will attend the Clinical Investigation Ward and Robert Steiner MRI unit at Hammersmith Hospital after a 10h overnight fast. The study visit will last between 2 - 3 hours. After explanation of the procedure a doctor or a nurse will insert a small plastic tube into a vein in the arm to take a blood sample. With your permission, we will also take on one occasion a sample of DNA and RNA from blood or saliva to look for changes in your genes that may be involved in the how the body controls appetite and body weight.

You will also have your height, weight and body fat content measured again using a ‘bio-electrical impedance’ machine. This is a painless safe method which involves measuring the electrical current from your body and takes only about 5 minutes. You will also complete questionnaires about your eating habits, personality and mood on each study visit. These questionnaires should take about 30 minutes to complete on the first visit and 5 minutes to complete on the second visit.

After having completed the MRI safety check form and an appetite questionnaire (VAS) you will be asked to lie on a trolley and then be moved into the scanner. The scanner is made up of a large magnet with a central bore or tunnel; you will lie on the scanning couch inside the bore during the scan. The scanner uses changes in magnetic fields to obtain a precise picture of your brain. Your head will be placed in a padded head coil for support. The scanner makes a loud knocking noise when it is running, and therefore you will be asked to wear earplugs during the scan.

You will be asked to perform simple computer tasks whilst lying inside the MRI scanner. Food pictures and other pictures will be shown on a computer screen and with a hand-held response pad you can respond to the visual information. During the scan you will have an alarm bell to sound if you have any concerns. If you do not like being in the magnet for any reason, the scan can be immediately stopped at any time. The scan will take no longer than

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1 hour. After the scan further blood samples will be taken and you will be asked to complete another appetite questionnaire. The total volume of blood taken at this visit will be up to 80mL (5 tablespoons). At one of the visits you will also be asked to score how often and how much you usually like to eat the foods shown in the pictures using a visual analogue scale.

Expected duration of the study

The study will last about 10 weeks. You will be asked to attend the research ward on maximum 7 non-consecutive days. Some visits will only last about 1-3 hours, whereas the three appetite study sessions last from about 08.30 am to about 17.00.

Number of participants in this study

You will be one of approximately 20 volunteers who will participate in this study.

Are there any risks of participating in the study?

The risks for this study are low, as you will be checked that you meet the criteria for inclusion in the study prior to starting, and those with existing health problems will be unable to participate.

There maybe slight discomfort and possible bruising due to the insertion of the catheter for blood sampling. An experienced member of staff will insert the catheter and we will do our best to avoid these problems. The volume of blood collected in total during one study day is 30-160 ml which is considerably less than a typical blood donation. During the entire study (about 10 weeks) a total of 600 ml of blood will be withdrawn. This includes the blood sample taken at the screening visit. 600 ml is just over one pint (568 ml). For a normal, healthy person the blood sampling should pose no risk. The maximum amount of blood taken in one day will be 160 ml (=10.6 tablespoons) and this amount will be taken over the course of 8 hours.

When consuming high amounts of dietary fibre some people will experience increased wind, increases in bowel movements and/or bloating; these side-effects are expected to be temporary and mainly occur at the beginning of the study. The purpose of the supplementation run-in (day 1-14) is to minimise the occurrence of these side-effects. If you should experience any of these side-effects, they will not have any adverse effects on your health.

At the MRI visits anatomical scans of your brain and whole body will be collected. It should be noted that these scans cannot be viewed as a comprehensive health screening procedure. However, very rarely, unexpected information can be detected during MRI scans or from blood tests which may warrant further investigation. In this event, a report will be sent to your GP, who will arrange further tests and coordinate further care. Significant abnormalities may also preclude you from further participation in the study. Such detection has the benefit of starting treatment early but in a small number of cases this may have implications for your future employment and insurance schemes.

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What are the possible benefits of participating in the study?

You will not directly benefit from taking part, however the information we get from this study may help identify how different dietary fibres affect appetite. Full expenses will be paid to cover travel incurred during the participation in this study upon provision of valid receipts. You will also receive £300 for your inconvenience on completion of the study. The honorarium will be less, and at the discretion of the Principal Investigator, if you withdraw before completion of the study or if the study is terminated prematurely.

What if something goes wrong?

If you suffer an adverse event or deterioration in health as a result of your participation in this study, appropriate compensation will be paid to you through the Imperial College School of Medicine's "No Fault" Compensation Scheme.

Will my taking part in this study be kept confidential?

If you consent to take part in the study we may need access to your medical records. All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it. We will inform your GP of your participation in this study. We will also inform all other doctors treating you (for example, hospital specialists), unless you have any objection to us doing so.

General

The handling of blood will be carried out in accordance with the University policy on the donation and use of human specimens in teaching and research (March 1998-SP/03/98). You will be free to withdraw from the study at any time and do not need to give any reason for your withdrawal. You can file complaints or queries about the study and their participation at any time to the principal investigator Professor Gary Frost by phone, phone 0208383 8037 or by email: g.frost@imperial.ac.uk, Ms Norlida Mat Daud, e-mail: n.mat-daud08@imperial.ac.uk or **Mrs Nurhafzan Ismail, email: Nurhafzan.ismail08@imperial.ac.uk**.

Should you want to know the results of the study you can ask the investigators and they will send out the results written in lay terms. All information and data obtained from the study will be restricted to the investigators only and will be kept strictly confidential as required by the Data Protection Act (1998). All participant information will be coded. The Hounslow and Hillingdon Research Ethics Committee has given a favourable opinion on this study.

Thank you for taking the time to read this information sheet.

If you decide to take part in the study a copy of the information sheet and a copy of the signed consent form will be given to you.

THIS INFORMATION SHEET IS VALID FOR USE UNTIL **31/12/2011**.

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CONSENT FORM

Title of Project: **Effect of oligofructose on appetite, body composition and gut hormones in healthy overweight subjects**

Name of Researcher:

Please initial box

1. I confirm that I have read and understand the information sheet **version 3, date 1/09/09** for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that my images and sections of any of my medical notes may be looked at by responsible individuals from Imperial College London or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records and scans. ☐
4. I agree to the investigators contacting my general practitioner about my participation in the study and the results of any medical tests from my visits and scans. ☐
5. I give permission for my images to be used for research by responsible individuals from Imperial College London and Imperial College Healthcare NHS Trust so long as they do not contain identifying personal information. ☐
6. I agree for a DNA sample to be taken and stored to look for changes that may be involved in obesity and the control of blood sugar and appetite (this is optional - you do not have to consent to this if you do not want to). ☐
7. I agree to take part in the above study. ☐
8. I agree to be contacted again by the Investigators to participate in future research (this is optional - you do not have to consent to this if you do not want to). ☐

______________________	______________	______________________
Name of Patient	Date	Signature
______________________	______________	______________________
Name of Person taking consent (if different from researcher)	Date	Signature
______________________	______________	______________________
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 3. Food Preference Sheet

Food Preference

You will be given a serving of food to eat and you are asked to say how much you *like* or *dislike* it. Use the scale to indicate your attitude by checking at the point which best describes your feeling about the food. Keep in mind that you are the judge. You are the only one who can tell what you like. An honest expression of your personal feeling is important to the study.

- Like Extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike Extremely

Comments:

Appendix 4. SCOFF questionnaires

Initials:

ID:

Date:

1. Do you make yourself sick because you feel uncomfortably full?

   YES / NO

---

2. Do you worry you have lost control over how much you eat?

   YES / NO

---

3. Have you recently lost more than one stone in a 3 month period?

   YES / NO

---

4. Do you believe yourself to be fat when others say you are too thin?

   YES / NO

---

5. Would you say that food dominates your life?

   YES / NO

Appendix 5. Dutch Eating Behaviour Questionnaires

Participant code______________________ Date___/___/___

Please answer the following questions as carefully and honestly as possible.
Read each question and simply fill in the column which best applies to you.

	Never	Seldom	Sometimes	Often	Very often	Not relevant
1. If you have put on weight, do you eat less than you usually do?						
2. Do you have a desire to eat when you are irritated?						
3. If food tastes good to you, do you eat more than you usually do?						
4. Do you try to eat less at meal times than you would like to eat?						
5. Do you have a desire to eat when you have nothing to do?						
6. Do you have a desire to eat when you are depressed or discouraged?						
7. If food smells and looks good, do you eat more than you usually eat?						
8. How often do you refuse food or drink offered because you are concerned about your weight?						
9. Do you have a desire to eat when you are feeling lonely?						
10. If you see or smell something delicious, do you have a desire to eat it?						
11. Do you watch exactly what you eat?						
12. Do you have a desire to eat when somebody lets you down?						
13. If you have something delicious to eat, do you eat it straight away?						
14. Do you deliberately eat foods that are slimming?						
15. Do you have a desire to eat when you are cross?						
16. Do you have a desire to eat when you are approaching something unpleasant to happen?						
17. If you walk past the baker do you have a desire to buy something delicious?						
18. When you have eaten too much, do you eat less than usual the following days?						
19. Do you get a desire to eat when you are anxious, worried or tense?						
20. If you walk past a snack bar or café, do you have a desire to buy something delicious?						
21. Do you deliberately eat less in order not to become heavier?						
22. Do you have a desire to eat when things are going against you, or things have gone wrong?						
23. If you see others eating, do you have also the desire to eat?						
24. How often do you try not to eat between meals because you are watching your weight?						
25. Do you have a desire to eat when you are frightened?						
26. Can you resist eating delicious food?						
27. How often in the evening do you try not to eat because you are watching your weight?						
28. Do you have a desire to eat when you are disappointed?						
29. Do you eat more than usual when you see other eating?						
30. Do you take your weight into account when you eat?						
31. Do you have a desire to eat when you are emotionally upset?						
32. When preparing a meal are you inclined to eat something?						
33. Do you have a desire to eat when you are bored or restless?						

Appendix 6 – Three-Factor Eating Questionnaires

Please circle the response that you feel best describes you

ID#: Initials: Date:

	Part I	True	False
1.	When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	T	F
2.	I usually eat too much at social occasions, like parties and picnics.	T	F
3.	I am usually so hungry that I eat more than three times a day.	T	F
4.	When I have eaten my quota of calories, I am usually good about not eating any more.	T	F
5.	Dieting is so hard from me because I just get too hungry.	T	F
6.	I deliberately take small helpings as a means of controlling my weight.	T	F
7.	Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	T	F
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.	T	F
9.	When I feel anxious, I find myself eating.	T	F
10.	Life is too short to worry about dieting.	T	F
11.	Since my weight goes up and down, I have gone on reducing diets more than once.	T	F
12.	I often feel so hungry that I just have to eat something.	T	F
13.	When I am with someone who is overeating, I usually overeat too.	T	F
14.	I have a pretty good idea of the number of calories in common food.	T	F
15.	Sometimes when I start eating, I just can't seem to stop.	T	F
16.	It is not difficult for me to leave something on my plate.	T	F
17.	At certain times of the day, I get hungry because I have gotten used to eating then.	T	F
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.	T	F
19.	Being with someone who is eating often makes me hungry enough to eat also.	T	F
20.	When I feel blue, I often overeat.	T	F
21.	I enjoy eating too much to spoil it by counting calories or watching my weight.	T	F
22.	When I see a real delicacy, I often get so hungry that I have to eat right away.	T	F
23.	I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	T	F
24.	I get so hungry that my stomach often seems like a bottomless pit.	T	F
25.	My weight has hardly changed at all in the last ten years.	T	F
26.	I am always hungry so it is hard for me to stop eating before I finish that food on my plate.	T	F
27.	When I feel lonely, I console myself by eating.	T	F
28.	I consciously hold back at meals in order not to gain weight.	T	F
29.	I sometimes get very hungry late in the evening or at night.	T	F
30.	I eat anything I want, any time I want.	T	F
31.	Without even thinking about it, I take a long time to eat.	T	F
32.	I count calories as a means of controlling my weight.	T	F
33.	I do not eat some foods because they make me fat.	T	F
34.	I am always hungry enough to eat at any time.	T	F
35.	I pay a great deal of attention to changes in my figure.	T	F
36.	While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.	T	F

ID#: Initials: Date:

Part II				
37. How often are you dieting in a conscious effort to control your weight?	rarely	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 guild 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 unlikely	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 unlikely	moderately unlikely	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 (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never ‘giving in’), what number would you give yourself?	0 eat whatever you want, whenever you want 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	little like me	pretty good description of me	describes me perfectly

Appendix 7 – Visual Analogue Scales

Participant code___________ Date___/___/___

Answer the following questions by placing a vertical mark through the line for each question. Mark the line according to how you **feel right now**

How full do you feel?

Not at all |————————————————| Extremely

How hungry do you feel?

Not at all |————————————————| Extremely

How much food do you think you could eat?

Not at all |————————————————| Extremely

How strong is your desire to eat?

Not at all |————————————————| Extremely

Did you feel nauseous?

Not at all |————————————————| Extremely

Does your stomach hurt?

Not at all |————————————————| Extremely

Do you feel bloated?

Not at all |————————————————| Extremely

Did you experience problems with flatulence in the past half an hour?

Not at all |————————————————| Extremely

Appendix 8 – 3-day Food Diaries

FOOD RECORD

Read through these instructions and the example carefully once or twice before you start.

Please record **ALL** food and drink consumed at the time of eating and **NOT** from memory at the end of the day. Keep this record sheet with you throughout the day. You should include all meals and snacks, plus sweets, drinks etc. When recording food eaten at meals, please include any sauces, dressing or extras eg: gravy, salad dressing, pickles, as well as the main food.

If you do not eat a particular meal or snack simply draw a line across the page at this point.

Guidelines for describing food & drink:

1. Please give details of method of cooking eg: grilled, boiled, roasted.

2. Give as many details as possible about the type of food you eat:

   a) State brand name where applicable
      eg: 'Princes' sardines in tomato sauce OR
      'Sainsburys' half-fat Edam cheese.

   b) Name the type of biscuit, cake or cereal
      eg: Rich Tea, Madeira, Branflakes.

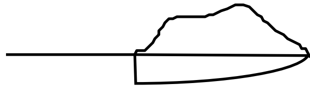
   c) Name the type of cheese, fish or meat
      eg: Cheshire cheese, haddock fillet, pork chop.

3. Suggestions for recording quantity of food and drink:

   a) For many foods such as vegetables, cereals and some fruit a household measure is adequate, state the number of teaspoons (tsp) or tablespoons (tbsp) or cups, and whether level, rounded or heaped.

Level

Rounded



Heaped

b) All convenience foods have their weight on the packaging and this can be quoted

eg: 150g carton Ski raspberry yoghurt OR
½ 15 oz can baked beans.

c) Bread, fruit loaves etc. Indicate the size of the loaf and the thickness of the slice

eg: 1 thick slice granary bread, small loaf.

d) Cheese, fish, meat. When possible, please weigh your portions of these foods. Otherwise describe as well as you can.

eg: 2 large thin slices ham OR
2 small lamb chops (no fat eaten) OR
Medium fillet of cod grilled with 1 tsp flora OR
Cube of cheddar cheese the size of a matchbox.

Remember to include everything you eat and drink including snacks and nibbles.
Please do not change what you normally eat just because you are filling in this record - Be Honest!

Look at the example of how to fill in you record - you may find this helpful.

THANK YOU VERY MUCH FOR YOUR HELP

DIETARY RECORD SHEET - EXAMPLE

Record **ALL** food and drink consumed during the day including snacks, nibbles, sauces and dressings.

Record method of cooking, type and quantity of food
eg: 6 tbsp boiled wholemeal spaghetti
2 egg sized roast potatoes.

DAY: Example DATE: 1st June 1994

MEAL/ SNACK	QUANTITY EATEN	DETAILS OF FOOD & DRINK	Leave Blank
Early Morning:	1 cup 1 tbsp	Tea with Skimmed milk	
Breakfast:	3 heaped tbsp ¼ pint 1 medium slice 1 tsp 2 mugs	Branflakes (Kellogg's) Skimmed milk for cereal & drinks Wholemeal bread (large loaf) Flora extra light margarine Coffee	
During Morning:	1 mug 1 tbsp 1 medium	Coffee with skimmed milk Apple (eaten with skin)	
Midday:	 4 medium slices 4 level tsp 2 thin slices 1 large 1 1 can (330ml)	Sandwiches: wholemeal bread (Allinsons) large loaf, sliced Flora extra light margarine Ham (no fat) Tomato Banana Diet Tango	
During Afternoon:	1 glass 25g pkt	Low Calorie squash made with concentrated squash KP roasted salted peanuts	
Evening Meal:	4 heaped tbsp 1 apple sized 3 tbsp 1 x 150g tub 1 glass 1 cup 1 tbsp	Chicken & mushroom casserole (home-made with skimmed milk in the sauce) Jacket potato Broccoli, boiled Shape raspberry yoghurt Half mineral water/half natural orange juice Tea with skimmed milk	
During Evening:			
Bedtime Snack:	1 mug 1 tsp ½ mug 2	Ovaltine made with: Ovaltine ordinary silver top milk, the rest water Rich Tea biscuits (Sainsburys).	

Appendix 9 – 8 Weeks Supplementation Record

SUPPLEMENTATION RECORD

Read through these instructions and the example carefully once or twice before you start.

You have been given ______ of sachets for the 8 weeks of supplementation.

You are advice to take:

1 sachet a day for the 1st week starting from _____________ to _____________

2 sachets a day for the 2nd week starting from ______________ to ____________

3 sachets a day for the 3rd week starting from ______________ to _____________

Please remember that:

1- **The supplement has to be consumed either with the main meals, mixed into drinks, sprinkled on or mixed into food.**
2- **The supplement must not be cooked and exposed to high temperatures (for example by adding it to freshly boiled water).**
3- **Unused sachets must be returned to the investigators.**

We would like you to record, what you eat or drink with the supplementation for 8 consecutive weeks. You should start on the morning of that day and continue for the six following days. **Please be alert to the date you need to increase your supplementation above**.

Record at the time of eating and **NOT** from memory at the end of the day. Keep this record sheet with you throughout the day.

Please do not change what you normally eat just because you are filling in this record - Be Honest!

Look at the example of how to fill in you record - you may find this helpful.

THANK YOU VERY MUCH FOR YOUR HELP

SUPPLEMENT RECORD SHEET

Record time and details of supplementation. E.g as below:

Time taken	Quantity taken	Details of food & drink (with what you take the supplement)	Leave blank
8.30 am	½ sachet	Sprinkle in 250 ml porridge	

WEEK 1 – WEEK 8

Balance sachets:

	Time taken	Quantity taken			Details of food & drink (with what you take the supplement)	Leave blank
		Breakfast	*Lunch*	*Dinner*		
Day 1						
Day 2						
Day 3						
Day 4						
Day 5						
Day 6						
Day 7						

Appendix 10 –fMRI study sheet

Date of scan: Initials: ID #: Hospital #:

Group: A B Visit no: 1 2

Food picture runs: AB CD EF GH (start at 11.30 +90)

MR personnel: Norlida Mat Daud/Nurhafzan Ismail/Tony Goldstone/Giuliana Durighel/Christina Prechtl/Sam Scholtz/Navpreet Chinna/Other:

Check buttons work in WordPad ☐

3T MRI #: IFIS hood / Data logging PC / (IFIS PC Folder#:

Attach: Respiratory belt (under arms) Pulse oximeter (L ring, red on pulp) Check audio cable

Time into Philips 3T scanner: Time out scanner:

Scans:	Test*	Rest*	Food x 2	Field	AMV*	T1	DTI	
Time (mins):	2	9	10 x 2		4	6	6	10
No. of vols:		192	192 x 2			114	(* = hunger rating included)	
Prep phase	Full	Auto		Auto	Auto (ensure CLEAR ON)			

Time of scan: /

T1/T2/T2W_FLAIR Anatomical scan: Parallel to AC-PC Line

fMRI scans: gradient epi BOLD, TR 3000, TE 30, FOV 280x220x143, 2x2x3.25 mm, FA 90, 44 slices ascending contiguous, SENSE 2. Slice pos: 30° to AC-PC Line, CLEAR ON
Add + 0.0926 to
Initial RL tilt degrees Initial *r[0] = - (parallel AC-PC)
-30° RL tilt degrees Adjusted *r[0] = - (30° to AC-PC)

Rest: 192 vols. Eyes shut, awake. Initial 6 vols discarded. **At end: Awake or Asleep**

Field map: spin echo TR 800, TE 20, flip 90, 3.25 x 2 x 2 mm, δTE 0 & 2.5

Food: 2 x 192 vols. Stimulus duration 2.5 sec; ISI 3.0 sec. ITI 0.5 sec; 3 conditions x 5 blocks x 6 pictures x 2 runs (high calorie, low calorie, household object) + 16 inter-condition blurred blocks per run. Block length 18 sec. Initial 6 vols to be discarded.

AMV: 114 vols. Auditory (story), visual (4Hz colour checkboard), motor (tap R index finger 1Hz)

DTI: 32 dir, b factor 1000, TR 13951, TE 59, 73 slices, SENSE 2.5, 224x224, 1.75x1.75x2mm, AC-PC line, no z-gradient correction, full prep, **PB-volume shim**

Hunger VAS button: **Problems:**
Test Rest AMV

☐ Download edat files x5 to memory stick ☐ Save edat files to Appetite folder

☐ Save LabChart Pro data to laptop ☐ Download LabChart Pro data to Appetite

☐ Upload MR scans to MRIdb ☐ Save MR scans to disc DVD #:

☐ Upload T1 T2 MR scans to PACS ☐ Export fieldmap raw data to Export/Appetite

☐ GP letter sent - 1st visit only ☐ Photocopy x2 (case folder, hospital notes)

☐ MRI T1 anatomical report ☐ Pcopy x3 (case folder, master file, hosp notes)

Appendix 11 – Example of Probiotic, Prebiotic and Synbiotic Products to Avoid During the Supplementation Period

Dairy:

- Muller Vitality drinks and yogurts
- Activia (drinks and yogurts)
- Benecol
- Flora proactive
- Actimel
- Yakult
- Ski yogurts
- Alpro soya yofu products
- Irish Creamy Probiotic yogurts
- Tesco natural defences yogurt
- Tesco probiotic dairy drinks

- + all other products containing bifidobacteria and/or added inulin/fructo-oligosaccharides

Cereals

- Weetabix Oatibix bitesize (Apple & Sultana and Original)
- Weetabix Oaty bars (White chocolate, Milk chocolate, Strawberry)
- Ryvita Goodness bars
- Sainsbury's cereal bars, Be Good to Yourself (Maple, Peach & Apricot)
- Alpen light cereal bars

- + all other products containing added inulin/fructo-oligosaccharides

Dietary Supplements containing bacteria cultures and/or high amounts of inulin/fructo-oligosaccharides

Beware of all "light", "low fat", reduced sugar" or "reduced calories" products where fat or sugar may be replaced by inulin or oligofructose

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