The effect of increased colonic propionate production on appetite regulation and energy homeostasis in humans

A thesis submitted for the degree of Doctor of Philosophy, Imperial College London

Habeeb Ali Alhabeeb

2017

Section of Investigative Medicine

Division of Diabetes, Endocrinology and Metabolism

Imperial College London

Abstract

Propionate is a short chain fatty acid produced in the colon via fermentation of undigested foods. Elegant *in vitro* and *in vivo* studies have shown that increased production of propionate in the gut can reduce body weight and energy intake, and improve glucose homeostasis. These positive effects have been attributed to increased levels of the gut hormones, PYY and GLP-1, increased energy expenditure and improved beta-cell function. Furthermore, bariatric surgery increases levels of colonic propionate and this likely contributes to the huge weight loss seen with this treatment. Additionally, other short chain fatty acids and the consumption of fermentable carbohydrates to increase levels of short chain fatty acids can delay gastric emptying and affect the central regulation of appetite. Taken together, these findings suggest propionate could have several beneficial health effects in humans.

Inulin-propionate ester was developed to deliver propionate to the colon. Effects of acute delivery of propionate to the colon with a single dose of inulin-propionate ester was investigated in healthy human volunteers on energy intake, subjective ratings of appetite and satiety and plasma glucose and insulin levels. Potential mechanisms for any effects were explored by assessing plasma levels of PYY and GLP-1, gastric emptying using ^{13}C-octanoic acid, energy expenditure using indirect calorimetry, and central effects using fMRI. In addition, encapsulated sodium propionate was used to assess delivery to the small intestine on appetite and glucose and insulin levels.

Acutely increasing colonic levels of propionate significantly reduced energy intake, increased satiety, increased levels of PYY and GLP-1, and reduced anticipatory food behaviour in humans. No effects were observed on gastric emptying, plasma levels of glucose and insulin and energy expenditure. In addition, delivery of propionate to the small intestine did not affect appetite or plasma levels of glucose and insulin.

Colonic propionate significantly reduced energy intake and reduced appetite likely through increasing levels of GLP-1 and PYY and reducing anticipatory food behaviour. In addition, propionate does not affect appetite or glucose and insulin levels via the small intestine. As obesity is a growing epidemic, it is likely that interventions that increase colonic propionate levels could prevent weight gain or drive weight loss by regulating energy homeostasis.

Declaration of Contributors

The majority of the work presented in this thesis was performed by the author. All collaborations and assistance are described below. The inulin-propionate ester (IPE) used in chapters 3 to 6 was provided by Dr Douglas Morrison and Professor Tom Preston at the University of Glasgow.

Chapter 3

The experiments in this chapter were performed in collaboration with Dr Edward Chambers. I collected and analysed data and performed assessments of GLP-1, PYY, insulin, glucose, breath hydrogen and energy intake. Dr. Chambers recruited for this study.

Chapter 4

The experiments in this chapter were performed in collaboration with Dr Edward Chambers. The calculations of the gastric emptying half-life data was performed by Professor Tom Preston at the University of Glasgow. I recruited all volunteers to this study, collected and analysed data for breath hydrogen, gastric emptying and VAS.

Chapter 5

The fMRI experiments in this chapter were performed in collaboration with Claire Byrne, Dr Edward Chambers, Navpreet Chhina and Dr Tony Goldstone, who assisted with data analysis and interpretation. The collection of data was supported by the Clinical Imaging Facility, Imperial College London staff: Julie Fitzpatrick and Dr Albert Busza. I recruited volunteers to the study and collected and analysed data (PYY, GLP-1, Glucose, insulin, VAS, breath hydrogen).

Chapter 6

The experiments in this chapter were also performed in collaboration with Dr Edward Chambers. I measured the energy expenditure, substrate oxidation, breath hydrogen, VAS and recruited all the volunteers.

Chapter 7

The experiments in this chapter were performed in collaboration with Dr Chong Lim. My part in this study involved collecting the data and coordinating study visits. I measured glucose, insulin, VAS and recruited some of the volunteers.

Copyright Declaration

Acknowledgements

I would like to thank Professor Gary Frost and Dr Edward Chambers for their constant support and encouragement. I am also grateful to all the colleagues in the department for their helpful discussions and support. I am also thankful to my family and friends for their support during my PhD.

I am grateful to the subjects who kindly volunteered for the studies, without whose participation much of this work would have been impossible.

This thesis presents independent research funded by Imperial College and supported by grants from the MRC, BBSRC, NIHR, an Integrative Mammalian Biology Capacity Building Award, a EuroCHIP grant and is supported by the NIHR Biomedical Research Centre Funding Scheme.

Abbreviations

AgRP	Agouti related peptide
ACC	Anterior cingulate cortex
AMV	Auditory motor visual
AMY	Amygdala
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARC	Arcuate nucleus
ATP	Adenosine triphosphate
AUD	Auditory cortex
BOLD	Blood-oxygen level dependent
BMI	Body Mass Index
BSA	Bovine Serum Albumin
CART	Cocaine- and amphetamine-regulated transcript
CHO	Carbohydrate
DLPFC	Dorsolateral prefrontal cortex
DM	Dextrin maltose
DMN	Dorsomedial nucleus of the hypothalamus
DPP-IV	Dipeptidyl peptidase IV
DS	Dorsal striatum
ED	Energy density
EE	Energy expenditure
EI	Energy intake
FC	Fermentable carbohydrate
FFAR2	Free fatty acid receptor 2
FFAR3	Free fatty acid receptor 3
fMRI	Functional magnetic resonance imaging
GE	Gastric emptying
GI	Gastrointestinal

GLP-1	Glucagon-like peptide-1
GPCR	G-protein coupled receptor
HE	High energy
HFCS	High fructose corn syrup
HIP	Hippocampus
HPLC	High performance liquid chromatography
INS	Insula
IPE	Inulin-propionate ester
IRMS	Isotope ratio mass spectrometry
LE	Low energy
LHA	Lateral hypothalamic area
MOT	Motor cortex
NAcc	Nucleus Accumbens
NDC	Non digestible carbohydrate
NDNS	National Diet and Nutrition Survey
NPY	Neuropeptide Y
OFC	Occipital frontal cortex
OFS	Oligofructose
PEP	Phosphoenolpyruvate
PFC	Prefrontal cortex
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
PYY	Peptide tyrosine tyrosine
REE	Resting energy expenditure
ROI	Region of interest
RQ	Respiratory quotient
SACN	Scientific Advisory Committee for Nutrition
SCD	Sudden cardiac death
SCFA	Short chain fatty acid

VAS	Visual analogue scales
VIS	Visual cortex
VMN	Ventromedial nucleus of the hypothalamus
VP	Ventral pallidum
VS	Ventral striatum
VTA	Ventral tegmental area
WHO	World Health Organization

Table of Contents

List of Figures

List of Tables

Chapter 1 Introduction

1.1 Obesity

1.1.1 Definition

The World Health Organisation (WHO) characterise obesity and being overweight, as the excessive or abnormal accumulation of body fat which may impact upon health (World Health Organization, 2015). More commonly, obesity is classified through the body mass index (BMI). The BMI is calculated based on the mass of the person in kg divided by the height of the person in m^2 to give an approximation of the total amount of body tissue (muscle, bone and fat). The BMI is then categorised as underweight, normal, overweight or obese. A BMI of over 25 kg/m^2 defines a person as overweight and a BMI of over 30 kg/m^2 defines a person as obese.

Obesity is a rapidly growing worldwide epidemic, which now affects over 600 million adults and 42 million children under the age of 5 worldwide (World Health Organization, 2015). Approximately 8% of deaths in Europe can be attributed to obesity and diseases related to obesity (Public Health England, 2007). In 2007, obesity cost the UK economy approximately £15.8 billion, including £4.2 billion in costs to the National Health Service. Further costs to the economy come from diseases associated with obesity impacting upon day-to-day lives and preventing sufferers from going to work (Public Health England, 2007).

The excess accumulation of body fat is likely caused through a constant and complex relationship between genetic predisposition and the environment. The rise in obesity is foremost likely linked to modern food production and wider availability of energy-dense or ‘fast’ foods. In addition to this, amounts of exercise and energy expenditure have declined due to the modern sedentary urban lifestyle (McPherson et al., 2007).

As well as changes in eating habits, several monogenic and polygenic genes have been identified which regulate food intake and energy balance. Mutations in these genes can affect the central regulation of food intake and energy homeostasis, resulting in the accumulation of excess body fat. It is likely these genetic mutations are also contributing to the rise in obesity cases (Choquet and Meyre, 2011).

1.1.2 Relationship between obesity and non-communicable diseases

It is well documented that there is a relationship between obesity and chronic diseases, hence the imminent need to reduce levels of obesity. Excess body fat increases the risk of cardiovascular disease. Specifically high blood pressure,hyperlipidaemia, atherosclerosis (Re, 2009). (McGill et al., 2002) have all been linked to being overweight or obese. Additionally, obese individuals are more likely to have an increased risk of heart attack and sudden cardiac death (SCD) (Plourde et al., 2014). Individuals suffering from obesity also have an increased risk of suffering from a stroke. This is because most overweight people have high cholesterol, high blood pressure and high levels of fats in their blood which all increase the susceptibility of having a stroke (Mitchell et al., 2015). Additionally, obese individuals are more likely to develop type 2 diabetes due to its link to insulin resistance, (Lois and Kumar, 2009). Obesity also increases the risk of developing cancers through various linked mechanisms (Calle et al., 2003, Wolin et al., 2010). It is also known that there is a strong association between depression and obesity (de Wit et al., 2010).

1.1.3 Current treatments and solutions for obesity

The long-term management of obesity can be complicated. Treatments aim to decrease weight to a normal BMI and reduce the risk of serious disease, so long-term health is not impacted. In particular, the prevention of any future weight gain after weight loss is very important. Treatments should take into account that weight regain is common and may hinder the overall weight maintenance and loss process. A solution which enables weight loss and maintenance of a normal weight to avoid any long-term health conditions needs to be rapidly sought.

Lifestyle changes

Commonly, the first method to address excessive body fat is targeting diet and exercise. Many studies have shown that exercising for 30 minutes, 5 times a week can stop weight gain and encourage weight stability(Office of Disease Prevention and Health Promotion, 2008). A common contributor to obesity is a poor unbalanced diet (normally too much sugar and fat), so specifically, diets should be changed to reduce the fat and sugar intake and encourage eating less, more often. Overweight people can improve their health and prevent risk of chronic disease by changing diet and exercising more even if initially they do not lose weight. Over a 12 month period, a combined approach between physical activity and diet has been found to be more effective for weight loss rather than doing exercise or diet alone(Johns et al., 2014). Evidence on whether lifestyle interventions successfully reduce and maintain weight loss is controversial. Some studies show

lifestyle interventions to be effective for long-term weight loss(Tuomilehto et al., 2001), however the majority of studies indicate that lifestyle interventions alone are unable to sustain weight loss and regain. In one study, more than half of the participants in the study gained weight within the first twelve months after lifestyle interventions, only one in four successfully avoided weight gain over three years, and less than one in twenty lost and maintained weight successfully(Crawford et al., 2000). In another study, looking at weight regain a year after lifestyle interventions, less than 8% had continued to lose weight (>5%), 59% had maintained their weight, and 33% had regained weight. The main challenge is understanding how to intervene in an obesogenic environment to ensure weight loss but also prevent weight regain (Weiss et al., 2007).

Pharmacological interventions

Several pharmacological approaches to target weight loss have appeared on the market over the past several decades. Regrettably most of these have since been withdrawn because of substantial side effects. For example, the fenfuramines were a major anti-obesity drug targeting the serotonin system launched in 1980 but were withdrawn in 2004 due to substantial cardiovascular side effects (Rothman and Baumann, 2009). In recent years, the involvement of several neuropeptides in regulating appetite and food intake has been discovered. It is likely that new therapeutics will act to target these. Currently, GLP-1 receptor agonists are used in type 2 diabetes but may also promote weight loss and may be a new class of anti-obesity drug used in the future (Pucci and Finer, 2015). However, administration of these drugs which act my modulating levels of neuropeptides may be problematic, as studies so far show they can cause severe nausea and this needs to be accounted for (De Silva and Bloom, 2012). Principally, the safety of these new anti-obesity drugs continues to be a high concern and is hindering treatment of obesity in a pharmacological manner, so non-pharmacological interventions may be the treatments of the future (McGavigan and Murphy, 2012).

Bariatric Surgery

Bariatric surgery using gastric bands or gastric bypasses is often used to treat obesity as a last resort when other methods have failed to promote weight loss. These kinds of surgery are only recommended for patients with a BMI of over 35 kg/m2. In this situation patients have a much higher risk of getting type 2 diabetes, cardiovascular disease and premature death.

Studies have shown that successful gastric bypasses and bands can increase a patient's life by an average of 2.5-12.0 years (Pontiroli and Morabito, 2011). This is in addition to dramatically reducing levels of type 2 diabetes and cardiovascular complications(Sjostrom et al., 2004), and encouraging a stable lower weight (Buchwald et al., 2004).

This information is all positive, however, in the USA, an alternative study found that 3 years after their bariatric surgery most patients had only lost, on average, 38% of their excess body weight, so may not be as dramatically effective as often thought. With this type of surgery, a 50% or greater loss of the preoperative excess body weight would be expected. This amount of weight loss, however, is still higher than other strategies, as this amount of weight loss then enables the patient to be at a low enough weight to reverse or ameliorate any weight-related comorbidities (Balsiger et al., 1997).

The potential mechanism of how bariatric surgery may help encourage weight loss in obesity is not entirely clear. Studies have shown that levels of the gut hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) are increased in patients in the months following a Roux-ex-Y gastric bypass and it may be these that are involved in the weight loss and maintenance process (Borg et al., 2006). In addition, after a Roux-ex-Y gastric bypass, propionate levels are significantly increased (Zhang et al., 2009), and this may encourage the weight loss observed. Surgery carries many risks, so other treatments that may modulate levels of these gut hormones (if they are indeed causing the weight loss) with fewer or no risks should be developed.

As diet therapy often fails, bariatric surgery is successful, but limited to only a small group of patients and a lot of drug treatments have failed because of severe side effects. Methods or a combination of methods which are simple to administer and carry little or no risks need to be developed to aid the future treatment of obesity (Pucci and Finer, 2015).

1.2 Appetite regulation

1.2.1 **The gut-brain axis in appetite regulation**

Appetite and energy homeostasis are regulated by complex interactions between the gastrointestinal tract and central nervous system. This is more commonly known as the gut-brain axis (Dockray, 1988). Communication between the brain and the gut is bidirectional, with function of the gastrointestinal tract, as well as, brain control of satiety being affected. This communication enables maintenance of energy homeostasis.

1.2.2 **The hypothalamus, brainstem and vagus nerve**

Within the brain the major sites of appetite regulation are the hypothalamus and brain stem. These receive and integrate hormonal and neuronal signals concerning appetite. There are several defined but highly connected regions of the hypothalamus which are involved in energy homeostasis. These include the lateral hypothalamic area (LHA), dorsomedial nucleus (DMN), ventromedial nucleus (VMN), paraventricular nucleus (PVN) and the arcuate nucleus (ARC).

The ARC is anatomically well located (near the median eminence where there is an incomplete blood-brain-barrier) to receive hormonal and neural signals from the gut, pancreas and adipose tissue. The ARC also receives direct input from the brainstem. The brainstem is neurally innervated by the vagus nerve, the gastrointestinal tract, the abdominal viscera and the mouth (Young, 2012). Mechanosensitive and chemosensitive neurons connected to the vagus nerve are located within the intestinal muscles and mucosa. This is an important appetite regulating pathway from the gut to the brain (Berthoud et al., 1995). The vagus nerve also likely mediates the effects of gut hormones on the brain when they are produced from enteroendocrine cells (Abbott et al., 2005).

1.2.3 **Anorexigenic and orexigenic neuropeptides**

Pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) peptide are anorexigenic neuropeptides. CART and POMC expressing neurons are found in the lateral ARC (Elias et al., 1998). Injection of CART reduces energy intake (Lambert et al., 1998) and fasting reduces CART levels in the ARC, demonstrating its anorexigenic effect (Kristensen et al., 1998). Similarly, POMC is a precursor of α-melanocyte stimulating hormone which is important in energy homeostasis because of its anorexigenic effect (Tsujii and Bray, 1989). Humans with mutations in the melanocortin receptor can suffer from morbid obesity (Yeo et al., 1998).

Agouti-related peptide (AgRP) and neuropeptide Y (NPY) are orexigenic neuropeptides which are abundantly expressed in the lateral ARC (Broberger et al., 1998). Injection of NPY increases food consumption and decreases energy expenditure (Billington et al., 1991). Similarly, injection of AgRP also causes increased food consumption and body weight gain (Small et al., 2001).

The balance between orexigenic and anorexigenic signaliing maintain energy homeostasis. Any dysfunction or disruption in these signalling systems can lead to increased energy intake and weight gain, with a potential risk of obesity and associated diseases.

1.2.4 **Using functional magnetic resonance imaging to measure brain activity in appetite regulation**

Functional magnetic resonance imaging (fMRI) is a non-invasive technique used to measure activity in the brain under certain conditions. fMRI enables us to detect differences in regions of brain activity between treatments and in response to different tasks. The principles of fMRI rely on differences in the levels of oxygen in the blood entering the brain. Areas with more activity require more oxygenated blood, and oxygenated blood behaves differently in a magnetic field to deoxygenated blood. The measurement of oxygen use, blood volume and blood flow is termed the blood-oxygen-level-dependent (BOLD) signal.

The BOLD signal is detected in an fMRI scanner using radio waves and a magnetic field. Radio waves are fired at the area of the brain being studied and this causes movement of the electrically charged protons found in the nuclei of hydrogen atoms. When the magnetic field is switched on, the protons are all aligned, but when a burst of radio waves is fired, the protons are knocked out of alignment. When this burst of radio waves stops, the protons begin to realign, and as this is happening they release signals which the scanner detects. Protons from the areas with the most oxygenated blood produce the strongest signals, and indicate the most brain activity. A 3D image is produced from these signals and then areas of activity are mapped in squares called voxels which are representative of groups of thousands of neurons. To increase levels of oxygenated blood to the areas of interest in a study, specific relevant tasks are performed. For example, when looking at appetite regulation, activity in the hypothalamus and brainstem can be mapped in response to

food images or smells and non-food images or smells. This can then be used alongside treatments to assess any modulation of brain activity (Killgore et al., 2003, Beaver et al., 2006, Goldstone et al., 2009).

1.3 Gut Hormones

1.3.1 **Background**

In response to food intake several physiological responses occur. One of these of particular interest is the production of gut hormones from the gastrointestinal tract. The presence of food in the gut is detected by enteroendocrine L-cells which produce gut hormones upon stimulation, and these gut hormones can then affect the gut-brain axis. The amount of gut hormones produced can be modulated by the anticipation of food, gastric distention, and the nutrient composition of the food. These gut hormones affect gut function, are able to modulate further energy intake and cause satiation (De Silva and Bloom, 2012). A wide array of gut hormones are responsible for these processes but in this introduction, Peptide YY (PYY) and Glucagon-like Peptide 1 (GLP-1) will be the main focus.

1.3.2 **PYY**

PYY also known as pancreatic peptide YY3-36 or peptide tyrosine tyrosine, is a short peptide made up of 36 amino acids. PYY is closely related to NPY and Pancreatic polypeptide and they share structural homology. PYY is a ligand for the G- protein coupled receptors Y_1, Y_2, Y_4, Y_5 and Y_6 (Blomqvist and Herzog, 1997). Upon feeding, large amounts of PYY are produced in the L-cells of the colon and ileum, with smaller concentrations also being produced in the stomach. Levels of PYY in the plasma are low when in a fasted state. After food intake, PYY begins to be released after 30 minutes, and the highest levels are detected 1 to 2 hours postprandially. PYY is known to remain in the plasma for up to 6 hours after a meal (Adrian, 1985). In addition, PYY levels in the brain are higher in a fed rather than fasted state. PYY plays an important role in regulating appetite which will be discussed in section 1.3.4. (De Silva and Bloom, 2012).

1.3.3 **GLP-1**

GLP-1 is the biologically active peptide product form of the selectively cleaved 160 amino acid long proglucagon. GLP-1 binds to the G-protein coupled receptor GLP-1 receptor (GLP-1R) (Dillon et

al., 1993). GLP-1 is produced in the L-cells of the small intestine in response to food intake (Holst, 2007). Levels of GLP-1 in the plasma show a biphasic response postprandially with an initial peak between 30 and 60 minutes and a second peak at around 120 minutes when the body produces fatty acids (Snyder et al., 2008). It is important to note that GLP-1 has a very short half-life of 2 minutes as it is rapidly degraded by dipeptidyl peptidase IV (DPP-IV) (Kieffer et al., 1995). This needs to be considered when developing anti-obesity drugs targeting GLP-1. Despite this, GLP-1 still plays an important role in appetite regulation and this will be discussed further in section 1.3.4. (De Silva and Bloom, 2012).

1.3.4 **Role of PYY and GLP-1 in appetite regulation.**

PYY and GLP-1 are heavily involved in the regulation of energy intake and appetite. Obesity has been linked to lower circulating basal levels of PYY and meal-stimulated levels of PYY in adult and children (Batterham et al., 2003, Roth et al., 2005). Several studies have shown that increasing levels of PYY can dramatically decrease food intake in both obese and non-obese individuals. PYY dose-dependently reduces energy intake by 32% in healthy humans (Degen et al., 2005) and by 33% in obese individuals over a 24 hour period (Batterham et al., 2002). Increasing levels of GLP-1 shows similar effects on food intake to PYY. GLP-1 dose-dependently reduces energy intake in rats (Schick et al., 2003), and infusing GLP-1 in healthy individuals significantly increases subjective ratings of satiety and significantly decreases food intake (Flint et al., 1998).

GLP-1 and PYY are able to affect appetite control by modulating anorexigenic and orexigenic signalling in the brain. PYY and GLP-1 released by the enteroendocrine L-cells in the gut affects the ARC, vagus nerve and brainstem to control energy intake. PYY and GLP-1 decrease NPY and AgRP neuronal activity and increase activity of POMC and CART neurons thereby inhibiting food intake (Bewick, 2012). In addition, infusion of PYY and GLP-1 significantly reduced the BOLD signal in areas involved in food reward and response in humans (De Silva et al., 2011).

A schematic diagram linking the gut hormones to traditional anorexigenic and orexigenic signalling is shown in Figure **1.1**. The exact mechanisms of how GLP-1 and PYY affect energy homeostasis are not entirely clear so need to be explored further.

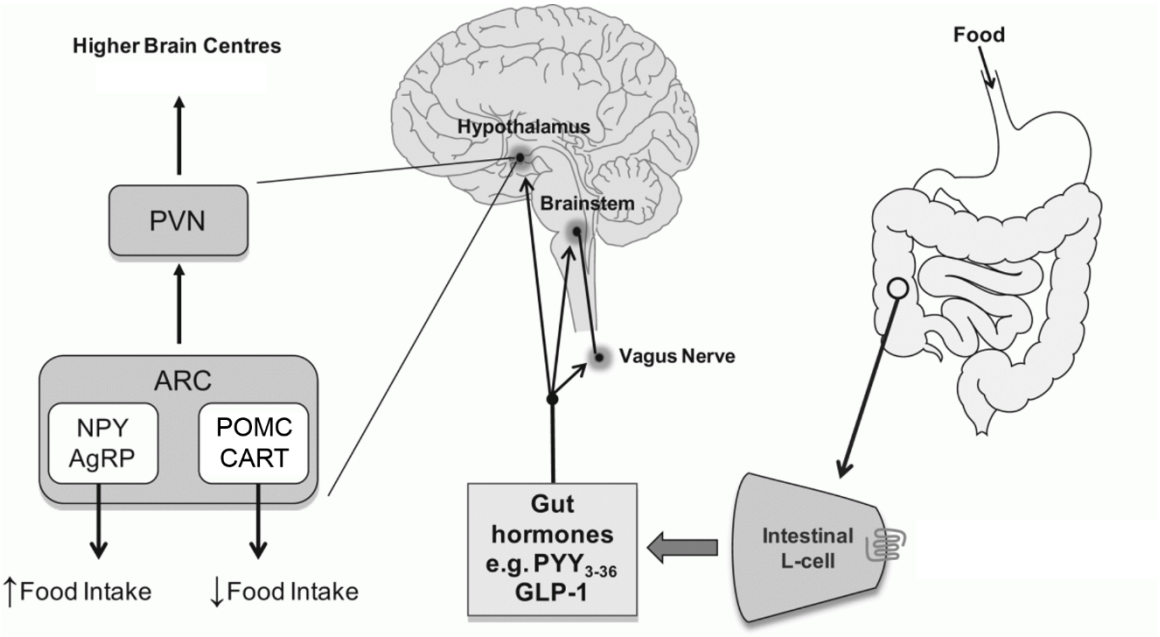


Figure 1.1: Diagram showing the effect of gut hormones on orexigenic and anorexigenic signalling in appetite regulation.
In response to food reaching the intestine, entereoendocrine L cells produce PYY and GLP-1. These gut hormones signal to the hypothalamus, brainstem and vagus nerve to modulate traditional orexigenic and anorexigenic signals in the ARC of the hypothalamus to control food intake. Activity of the orexigenic neuropeptides, NPY and AgRP are decreased and activity of the anorexigenic neuropeptides, POMC and CART are increased to reduced overall energy intake. In addition, activity in the PVN and higher brain centres is also modulated in response to food. Diagram adapted from (Bewick, 2012).

1.4 Non-digestible carbohydrates

1.4.1 **Definition**

Non-digestible carbohydrates (NDC) are mainly defined as carbohydrates that are resistant to digestive enzymes in the stomach, small intestine and are generally not digested before reaching the colon (Darzi et al., 2011).

1.4.2 **Classification and sources of non-digestible carbohydrates**

Plant foods are major sources of NDC in the diet (Slavin, 1987). The most classic examples of NDC are dietary fibre, inulin, and resistant starch, plus other relatively small components that are associated with NDC in the plant cell walls, for example, lignin, are also classed as NDC (Scientific Advisory Committee on Nutrition, 2014). The main two classes of NDC are oligosaccharides and polysaccharides. Oligosaccharides have a degree of polymerisation between 3 and 9 and are divided into the subgroups: α-glucans; which can be digested in the small intestine and non α-glucans; which all tend to reach the caecum. Polysaccharides have a degree of polymerisation of

10 or greater and are divided into the subgroups: starch (α-glucans) and non-starch polysaccharides (non α-glucans) and these all tend to reach the caecum undigested.

1.4.3 **Role of NDC and obesity**

As obesity continues to rise, it could be partially or wholly attributed to changes in eating patterns over the last 50 years. In particular, the typical Western diet now contains more fat than in previous years (Scientific Advisory Committee on Nutrition, 2014). In addition to this, there have been large changes in the quality and quantity of carbohydrates (CHO) that are consumed in the Western diet (Briefel and Johnson, 2004, Grooms et al., 2013). In most Western countries the major sources of NDC are grains, starchy vegetables, legumes and fruits. However, these are poor quality sources of NDCs as they only supply 1-3g of NDC per serving, which is very low (Slavin, 1987).

The UK National Diet and Nutrition Survey (NDNS) from 2008 to 2012 published in 2014 by Public Health England identified that the typical UK adult diet contains excessive added sugars, saturated fat and not enough fruit and vegetables. Of particular concern is the finding that UK adults only consume on average less than 14g of fibre per day whilst the dietary reference value recommends at least 18g/day. In response to this, a recent draft report on fibre and CHO consultation from the Scientific Advisory Committee for Nutrition (SACN) recommended increasing the guideline adult daily total fibre intake to 30g/day in the UK (Scientific Advisory Committee on Nutrition, 2014). These imbalances in most Western adults' diets are likely linked to increased cases of obesity.

1.4.4 **Beneficial effects of NDC on body weight**

NDC have several benefits towards health and may also encourage weight loss. Several population studies suggest there is an inverse relationship between consuming NDCs and weight gain (Liu et al., 2003, Ludwig et al., 1999) and that there is also an inverse correlation between consuming NDCs and fat mass (Kromhout et al., 2001, Nelson and Tucker, 1996, Tucker and Thomas, 2009).

Studies have shown that an increased intake of NDC is able to increase satiety and decrease appetite. NDC have a low energy density which is important in controlling energy intake (Slavin and Green, 2007). A high intake of NDCs is able to reduce overall energy intake. In one study, consuming 14g of NDC reduced overall *ad libitum* food intake by 10% and caused significant

average weight loss of almost 1.9 kg over a 3 month period. Therefore it is clear that NDC have a beneficial impact on both energy intake and body composition (Howarth et al., 2001).

The mechanisms underlying the effect of NDCs on weight gain and food intake may be by several different routes. It is speculated that NDCs could replace high caloric food from the diet, may prevent macronutrients being absorbed in the gut, may alter the environment inside the gut or cause increased levels of gastric distension (Heaton, 1973).

1.4.5 **Processing of NDC in the colon to produce short chain fatty acids**

NDC are resistant to digestion in the stomach and small intestine and travel relatively unprocessed to the large intestine when they undergo fermentation by specific bacteria. The NDC are broken down over several stages in the colon. In the colon there are several specific regions and each region has its own specialist microbiota, dependent on pH and which digestive secretions are present, leading them to work together as a community. An illustration of these regions and their microbiota can be seen in Figure **1.2**.

The bacteria possess various different enzymes to break down respective parts of the NDC before short chain fatty acids (SCFAs) are then produced in the final stages. For example; the epithelial layer of the gut normally contains minimal bacteria when in a healthy state. The diffuse mucin layer contains specialist colonisers, including *Akkermansia muciniphilia*, before the main digestion and breakdown of NDC in the gut-liquid phase in the lumen where there is a diverse microbial community. Starch and plant-cell containing foods are digested by specialised primary colonisers, including several species of *Ruminococcus*. This fermentation by the bacteria produces metabolisable energy (~2 kcal/g), gases (CO_2, H_2, and CH_4) and SCFAs.

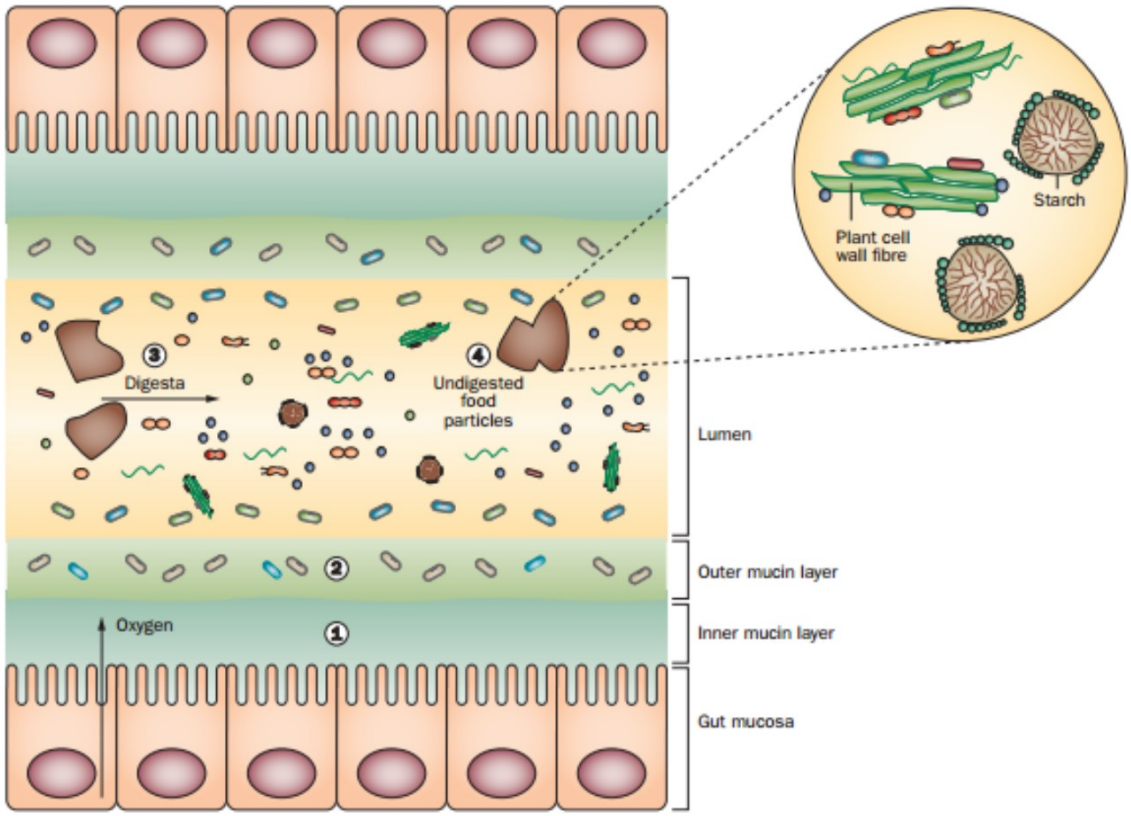


Figure 1.2: Schematic diagram showing the specific populations of microbiota that inhibit the large intestine.
The several specific regions of the colon are each inhabited by their own specialist microbiota, which work effectively together as a community. 1) In the epithelial layer and inner mucin layer there is minimal colonisation of microbiota; 2) the diffuse mucin layer is inhabited by specialist colonizers; 3) the gut-liquid phase in the lumen hosts diverse microbiota; 4) the gut-substrate particles phase in the lumen hosts specialised primary colonisers. Figure taken from (Flint et al., 2012).

SCFAs are produced via very specific biochemical pathways as shown in the schematic diagram in Figure **1.3**. Bacteria metabolise monosaccharides to form phosphoenolpyruvate (PEP) via two main pathways; the glycolytic pathway and the pentose-phosphate pathway (Cummings, 1981, den Besten et al., 2013b, Miller and Wolin, 1996). PEP is converted into pyruvate and acetyl-CoA, which are further converted to other products, in particular, the SCFAs (Flint et al., 2012, Macfarlane and Macfarlane, 2003).

Acetate is produced by the oxidative decarboxylation of pyruvate and hydrolysis of acetyl-CoA, or by using formate. This is catalysed by several species of microbiota including *Bifidobacteria*. Butyrate is produced by the condensation of two molecules of acetyl-CoA, and is catalysed by several microbiota including *Clostridia* (Cummings, 1981, Jorgensen et al., 1997). Propionate is produced via carbon dioxide fixation which forms succinate which is then decarboxylated or via the reduction of acrylate and lactate (Blackburn and Hungate, 1963, Paynter and Elsden, 1970). This

formation of propionate is triggered by several species of microbiota including the *Bacteroides*. These SCFAs are then further processed by specialist colonies of bacteria including *E. rectale/roseburia* and *Faecalibacterium prausnitzii* (Flint et al., 2012). The SCFAs serve many functions, but predominantly they are utilised in the Kreb's cycle to produce energy in the form of adenosine triphosphate (ATP) (Al-Lahham et al., 2010).

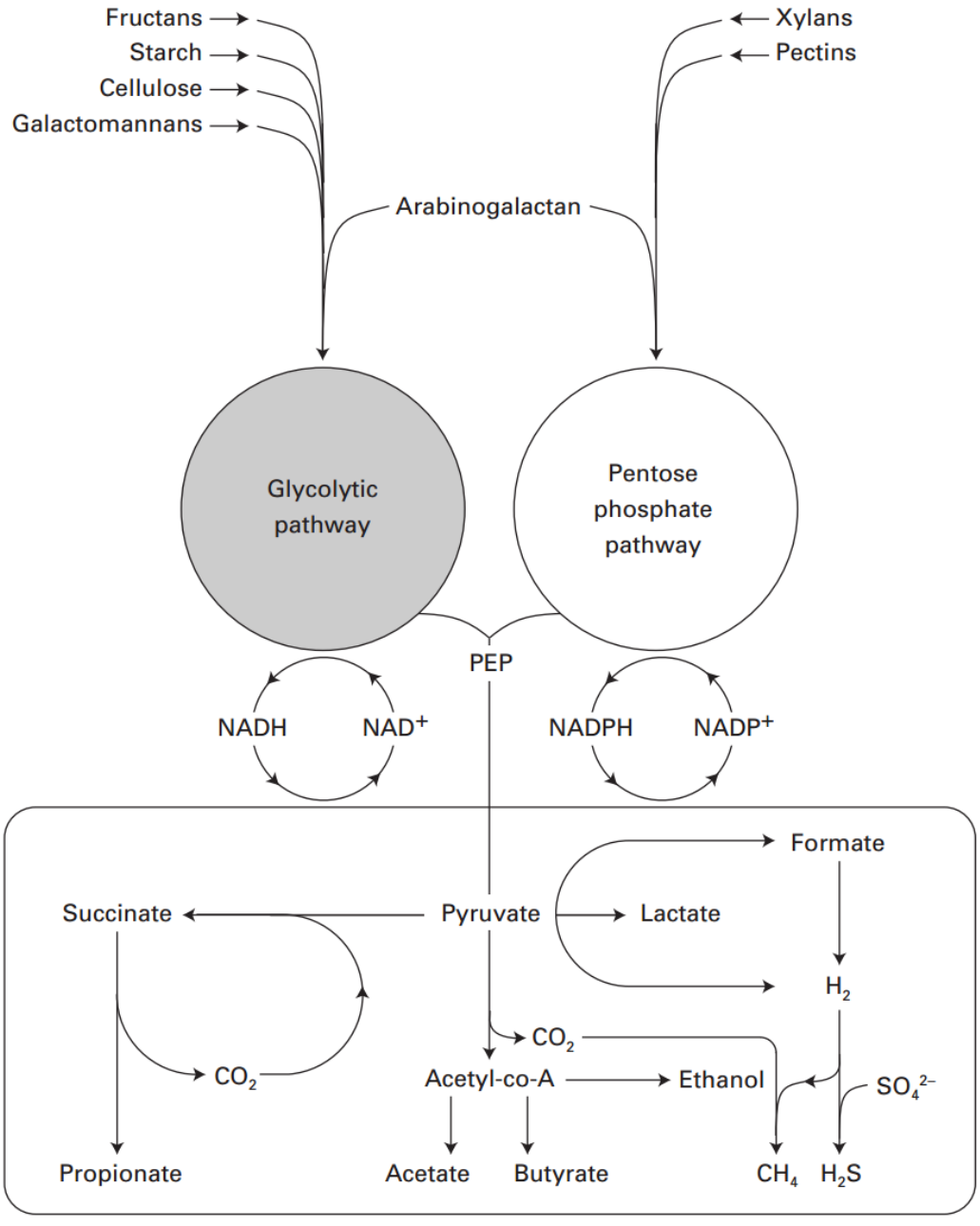


Figure 1.3: Schematic diagram showing the biochemical pathways of SCFA production
Monosaccharides are metabolised to form phosphoenolpyruvate (PEP) via two main pathways; the glycolytic pathway and the pentose-phosphate pathway. PEP is converted into pyruvate and acetyl-CoA, which are further converted to form the SCFAs. Acetate is produced by the oxidative decarboxylation of pyruvate and hydrolysis of acetyl-CoA, or by using formate. Butyrate is produced by the condensation of two molecules of acetyl-CoA. Propionate is formed via carbon dioxide

fixation forming succinate which is then decarboxylated or through the reduction of acrylate and lactate. Taken from (Macfarlane and Macfarlane, 2003).

As fermentation of NDC in the colon produces SCFAs, this use of NDC to yield SCFAs needs to be further explored, as it may be this aspect which is directly relevant to weight loss and preventing weight regain. Studies in both humans and animals have shown that NDC directly increase levels of SCFAs in the colon, and this may have implications for weight and energy homeostasis (Le Blay et al., 1999, Tarini and Wolever, 2010). This concept will be explained further in section 1.5.

1.5 SCFAs

1.5.1 **Definition**

SCFAs are carboxylic acids with a short aliphatic tail less than 6 carbon atoms in length.

1.5.2 **SCFAs in the human colon**

Over 80% of SCFAs present in the human colonic lumen are in the form of acetate (C_2), propionate (C_3) and butyrate (C_4) in the approximate molar ratio 60:25:15 (Cummings et al., 1987). The chemical structure of the SCFAs can be seen in Figure 1.4.

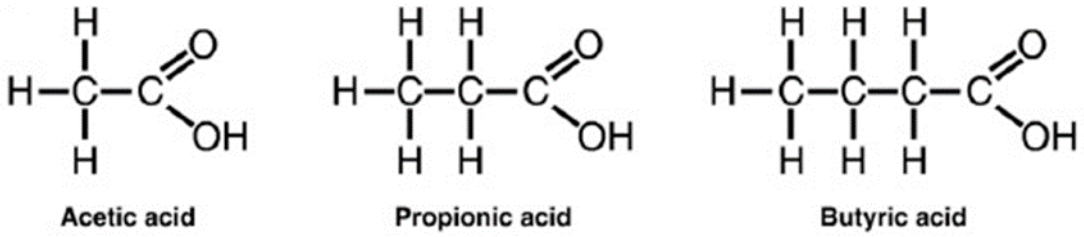


Figure 1.4: The chemical structures of the SCFAs
Acetic acid (acetate), propionic acid (propionate) and butyric acid (butyrate). (Figure taken from (Al-Lahham et al., 2010).

1.5.3 **SCFA receptors**

SCFAs are endogenous ligands for the previously orphaned G-protein coupled receptors GPR41 and GPR43 which are now better known as free fatty acid receptor 3 (FFAR3) and free fatty acid receptor 2 (FFAR2), respectively (Brown et al., 2003, Le Poul et al., 2003). FFAR2 and FFAR3 are only 33% homologous in their amino acid identities so are found in different tissue regions, hold different physiological roles and have different affinities for the different SCFAs. FFAR2 has an

equal affinity to acetate, butyrate and propionate but FFAR3 has the highest affinity for propionate ≥ butyrate> acetate.

FFAR3 and FFAR2 have different G-protein coupling specificities. Both FFAR3 and FFAR2 activate $G_{i/o}$ proteins, but FFAR2 can also activate G_q proteins (Le Poul et al., 2003, Brown et al., 2003). FFAR3 is coupled to $G_{i/o}$, and its activation is linked to gut microbiota-related energy sensing in the sympathetic nervous system and colon (Samuel et al., 2008). FFAR2 has been found to be only coupled to $G_{i/o}$ in the adipose tissue, but activates G_q proteins in the colon (Vijay-Kumar et al., 2010). The highest expression of FFAR3 is in immune cells but is also found in high levels in the bone marrow, adipose tissue, pancreas, colon and spleen, whereas, FFAR2 has the highest expression in adipose tissue but is also found in high levels in some immune cells, the spleen, pancreas, bone marrow, and lymph nodes (Brown et al., 2003, Le Poul et al., 2003, Nilsson et al., 2003). As FFAR2 is expressed in the colon (Karaki et al., 2006) this is likely important to link SCFA to appetite regulation. Specifically, FFAR2 is found on the luminal side of L-cells in the human colon (Karaki et al., 2008). Importantly, activation of FFAR2 in the colon by propionate has been shown to stimulate the release of the gut hormones GLP-1 and PYY (Psichas et al., 2015), which will be discussed in further detail in Section 1.6.

1.5.4 **SCFA effects on appetite regulation**

There is some evidence that SCFAs may directly affect appetite regulation in the brain. In a study in rats, acetate modulates the signal intensity measured using manganese-enhanced MRI in the hypothalamus to reduce food intake (Frost et al., 2014). Furthermore, fermentable CHO given to mice regulates neuronal activity in the appetite centres of the brain. The potential mechanism behind this could be through increased levels of SCFAs in the gut (Anastasovska et al., 2012).

1.6 Linking SCFA to PYY and GLP-1 and appetite regulation

A clear effect of both the gut hormones and SCFAs on energy homeostasis have been described in sections 1.3.4 and 1.5.4, respectively. However, it is likely a relationship between SCFAs and gut hormones which culminate in these effects.

In the gut, the SCFAs and gut hormones are found in similar locations which could enable SCFAs to exert effects on gut hormone production. This could then subsequently modulate appetite and

energy intake. As described earlier, the SCFAs are endogenous ligands for FFAR3 and FFAR2 (Brown et al., 2003, Le Poul et al., 2003). There is a high expression of FFAR2 and FFAR3 in the L-cells which produce GLP-1 (Tolhurst et al., 2012, Kaji et al., 2011) and PYY (Tazoe et al., 2009). In addition, stimulation of cultured human colonic L-cells causes the production of GLP-1 and PYY, and their expression is colocalised in these cultures (Habib et al., 2013).

Propionate added to human colonic cultures increases the activity and stimulation of L-cells. In addition, propionate and acetate significantly increase the production of GLP-1 in these cultures (Tolhurst et al., 2012). Furthermore, *in vivo* in rats, propionate significantly increases the release of GLP-1 and PYY through activation of FFAR2 (Psichas et al., 2015). This effect of SCFAs on the release of gut hormones is further corroborated in humans where ingestion of propionate significantly increases the levels of the gut hormones GLP-1 and PYY postprandially (Chambers et al., 2014).

These studies broadly show the capacity for SCFAs, in particular, propionate, to increase PYY and GLP-1 and affect orexigenic and anorexigenic signalling to maintain energy homeostasis. However, this relationship should be explored further to understand the specific mechanisms and its potential for controlling food intake and appetite in both normal and obese individuals.

1.7 Other potential mechanisms by which SCFAs may affect appetite regulation: gastric emptying

1.7.1 **Definition**

Gastric emptying (GE) is defined as the movement of food from the stomach to the small intestine. The gastrointestinal tract has a number of breaks including the duodenal, jejunal and ileal break that control the passing of food through the tract. The main role of the ileal break is to control the speed of food movement and subsequent gastric emptying.

1.7.2 **Role of PYY and GLP-1 in gastric emptying**

PYY and GLP-1 have been implicated in slowing gastric emptying. In an animal study, PYY stimulates the ileal break and decreases the speed of gastric emptying (Wisen and Hellstrom,

1995). In humans, injection of PYY also decreases the rate of gastric emptying (Savage et al., 1987). It has been shown that PYY binds to neuropeptide receptor 2 on vagal afferents to slow gastric emptying and suppress appetite (Lenard and Berthoud, 2008). GLP-1 similarly delays gastric emptying. Infusing GLP-1 slows the rate of GE by 3 times compared to saline in humans (Hellström et al., 2006). This effect of GLP-1 on GE has also been shown to be dose-dependent (Little et al., 2006).

1.7.3 **SCFA effects on gastric emptying**

As described in section 1.7.2; studies have shown that the gut hormones PYY and GLP-1 can inhibit GE. In addition, levels of GLP-1 and PYY can be increased by SCFAs as described in section 1.6. There are a few studies in animals suggesting that SCFAs could delay GE. Pigs administered with a 60:30:10 SCFA mixture of acetate, propionate and butyrate, respectively, show slowed gastric emptying (Cuche and Malbert, 1999). In a follow up study the authors concluded that SCFA likely inhibit distal gastric motility by the release of an inhibiting factor, which is likely PYY (Cuche et al., 2000). The effect of SCFAs on GE are further corroborated in human studies where supplementation of bread with sodium propionate significantly slowed gastric emptying measured by plasma levels of paracetamol (Liljeberg and Bjorck, 1996).

There is, however, some uncertainty as to how SCFAs affect GE. One explanation could be that SCFAs produced in the GI tract may reduce peristaltic activity and trigger tonic activity to slow GE. Also, when SCFAs are in the terminal ileum there could be an interaction with the colon contents to cause contraction and slowing of gastric motility. Another plausible explanation could be a combined neuro-hormonal sensory mechanism whereby SCFAs increase levels of PYY and GLP-1 and it is the effect of these hormones rather than the SCFAs alone which slow gastric emptying (Cherbut, 2003). The link between SCFAs, specifically, propionate, PYY and GLP-1 and gastric emptying are still not entirely clear so this area requires further research to understand the precise mechanisms.

1.8 Other potential mechanisms by which SCFAs may affect energy homeostasis: energy expenditure

1.8.1 **Definition**

Overall energy expenditure (EE) is defined as the basal metabolic rate plus the thermic effect of food plus any energy lost through physical activity. In humans we normally measure this by indirect calorimetry. This enables us to calculate the respiratory quotient which in turn provides us with an estimate of the basal metabolic rate based on the amount of CO_2 eliminated divided by the amount of O_2 consumed.

1.8.2 **Effects of PYY and GLP-1 on EE**

Gut hormones may mediate effects on EE, however very few studies on this exist, and the conclusions are controversial. One study showed PYY infusion in humans significantly increases fat oxidation and EE (Sloth et al., 2007). However, in an earlier study infusing GLP-1 in humans actually decreased EE and CHO oxidation (Flint et al., 2000). Therefore, there is currently no clear consensus on the effects of PYY and GLP-1 on EE and fat oxidation, and future work is needed in this area.

1.8.3 **Effects of SCFAs on EE**

The balance between EE and energy intake affects overall appetite and weight gain. Several studies exist that suggest SCFAs may increase EE. In rodents, propionate increases the rate of oxygen consumption through increased sympathetic activity and FFAR3 activation (Kimura et al., 2011). Increased adaptive thermogenesis, fat oxidation and mitochondrial activity are also found when mice are supplemented with butyrate and this further supports that SCFAs may affect energy expenditure (Gao et al., 2009).

In parallel with this, our laboratory carried out a small pilot study, where 14 healthy humans were supplemented with propionate or a control on two consecutive days and EE was measured. No differences in EE were observed in the fasted state, however a trend for increased EE ($p=0.07$) in the propionate group compared to the control group was found. The effects of propionate on EE in a fed state are unknown and should be explored.

The SCFA receptors FFAR2 and FFAR3 are also likely to be involved in the control mechanisms of energy expenditure. Mice lacking FFAR3 have a significantly decreased EE compared to their WT counterparts (Kimura et al., 2011, Bellahcene et al., 2013). Propionate given to WT mice

significantly increases oxygen consumption, whereas this effect is not seen in mice without FFAR3 (Kimura et al., 2011). In addition, the overexpression of FFAR2 in mice increases overall levels of EE (Kimura et al., 2013).

The role of SCFA, principally propionate and FFAR2 and FFAR3 in energy expenditure in these animal studies is interesting. However it remains to be determined whether energy expenditure in humans can also be affected by the SCFA propionate or fermentable carbohydrates. At present, a gap in our knowledge here exists as no studies regarding this have yet been conducted.

1.9 Other potential mechanisms by which SCFAs may affect appetite: pre-colonic effect of propionate

In this introduction I have described how SCFAs, and where relevant, propionate, have been shown to be involved in regulating appetite. The mechanism is likely through increased production of GLP-1 and PYY by enteroendocrine L-cells. However there may actually be an effect of propionate on appetite regulation before reaching the colon.

Previous work in our laboratory has shown that ~75% of propionate is delivered to the colon, whilst ~25% is released prior to reaching the colon (Chambers et al., 2014). Appetite is also significantly reduced before the propionate has reached the colon. This indicates that there is probably a pre-colonic effect of propionate on appetite. To corroborate this, Liljeberg, et al., 1995 also showed that the addition of 0.5g of propionate to a meal, increased satiety 45 minutes after consumption, and this is well before the propionate would be able to affect appetite via colonic L-cells. The full spectrum of short-term effects of propionate are currently unknown and need to be explored.

1.9.1 **SCFAs and insulin and glucose regulation**

Insulin and glucose are tightly controlled in normal individuals and may also influence appetite regulation. Due to the relationship between obesity and diabetes; increasing insulin sensitivity and glucose tolerance is an important direction of anti-obesity therapeutics. Consumption of NDCs and subsequent SCFA production can increase insulin sensitivity, independently of their effects on weight and adiposity (Robertson et al., 2003, Robertson et al., 2005). Acetate activation of FFAR2 has been shown to increase insulin secretion in vitro (McNelis et al., 2015). Both FFAR2/3 are found

in beta cells in the pancreas and can modulate insulin secretion (Priyadarshini and Layden, 2015, Priyadarshini et al., 2015).

In addition, SCFAs may play a role in glucose regulation. FFAR2 and FFAR3 receptor knockout mice show impaired glucose tolerance in vivo and an increased body weight (Tolhurst et al., 2012) Oral sodium propionate (9.9g in white bread) or placebo for 1 week significantly lowered glucose levels in healthy humans (Todesco et al., 1991). In addition, 20 healthy women given a daily dose of 7.5g sodium propionate for 7 weeks showed a significantly decreased fasting blood glucose and increased insulin sensitivity (Venter et al., 1990). Likewise, mice supplemented for 12 weeks with acetate, butyrate or propionate in their diets, showed an improved insulin sensitivity and glucose tolerance, likely as a results of improved β-cell function (den Besten et al., 2015).

However, some human studies have also shown an absence of any effect of the SCFAs on glucose tolerance and insulin sensitivity. In one human study, 6 healthy volunteers were given rectal infusions with mixed doses of SCFAs. There was no significant effect on glucose and insulin (Wolever et al., 1989). Similarly, in another human study, 6 healthy volunteers given mixed SCFA doses over a 3 hour period, showed no effect on glucose or insulin with this acute treatment (Laurent et al., 1995). Studies showing an effect of SCFAs on insulin sensitivity and glucose are intriguing, but further work in humans is required in order to confirm these possible benefits.

1.10 Summary

In this introduction I have described the role of SCFAs and gut hormones on energy homeostasis, which may be relevant for advancing anti-obesity therapeutics. The exact mechanism of SCFAs, in particular, propionate, on energy homeostasis still remains unclear, therefore, the work presented in this thesis attempts to address this gap in our knowledge.

1.11 Hypotheses

My main hypothesis was that increasing colonic propionate would reduce energy intake. To explore this hypothesis I designed experiments to understand the following objectives and further hypotheses:

- Increasing levels of propionate to the colon would reduce food intake and increase levels of PYY and GLP-1.
- Increasing levels of propionate to the colon would improve glucose tolerance and insulin sensitivity.
- Increasing levels of propionate to the colon would delay gastric emptying.
- Increasing levels of propionate to the colon would increase EE and fat oxidation in the postprandial period.
- Increasing levels of propionate to the colon would reduce the BOLD signal in brain areas implicated in food reward and appetite.
- I also hypothesised that propionate would have a pre-colonic effect when delivered to the small intestine to decrease appetite and increase satiety, whilst improving glucose tolerance and insulin sensitivity.

1.12 Aims of the thesis

The work presented in this thesis has the following specific aims:

1. To investigate the effect of increasing levels of propionate in the colon on:
    a. Plasma levels of GLP-1, PYY, insulin, and glucose
    b. Appetite, satiety and nausea
    c. Food intake
    d. Energy expenditure and fat oxidation
    e. Gastric emptying
    f. The central nervous system

2. To investigate the pre-colonic effects of propionate by delivering propionate to the small intestine on:
    a. Appetite, satiety and nausea
    b. Insulin and glucose levels

Chapter 2 Methods

This chapter describes the methods shared between some of the results chapters used to obtain the results presented in the following chapters of this thesis. Methods which are specific to individual chapters are described in the individual chapter.

2.1.1 Production of inulin-propionate ester

The inulin-propionate ester (IPE) was developed by Dr Douglas Morrison from the University of Glasgow. The IPE is 3g of propionate bound to 7g of inulin via an ester linkage. 10g of IPE delivers 2.36 g of propionate to the colon after 0.49 g of losses in the intestine (Chambers et al., 2014) as was produced at the University of Glasgow as follows. The IPE was made by reacting inulin with propionic anhydride (0.8 L/Kg inulin) in water at a pH of 8-8.5 and a reaction temperature of < 20 ^{o}C. The remaining unreacted propionate was removed by filtration through activated carbon columns twice whilst keeping the reaction mixture at pH 2. Spray drying was used to recover the product as fine amorphous crystals.

To establish how much free propionate the final product contained, 100 mg of product was dissolved in 2 mL water containing 10mM butyric acid as the internal standard. To calculate free propionate, 200 µL of the solution was treated with 100 µL concentrated orthophosphoric acid followed by immediate ether extraction (1 mL). To calculate the total propionate (free + bound), 200 µL of the solution was treated with a further 100 µL of concentrated orthophosphoric acid for 1 hour at 80°C before extraction with 1 ml ether. Propionate and butyrate in the ether extracts were quantified by gas chromatography with flame ionisation detection 6. The yield of propionate was quantified relative to the internal standard and the amount of free propionate calculated by the ratio (free/total) as a percentage. The degree of esterification was also quantified using this analysis by using the yield of bound propionate (total – free) per gram of ester to calculate moles of propionate yielded per mole of inulin-propionate ester.

The inulin propionate ester produced had a degree of esterification of 0.74 ± 0.02, therefore on average 24.6 ± 0.67% (0.74 out of maximum of 3 per monosaccharide unit) of all hydroxyl groups were replaced by an ester group. The amount of free propionate was 2.57 ± 0.26 % of the total propionate highlighting that more than 97% of propionate was bound to the inulin. Solubilisation

in acid at pH 1-2 (similar to the pH in the stomach) released less than 1% of the bound propionate and a temperature of >80ºC is necessary to release the bound propionate

2.1.2 Colonic delivery assessment

Delivery of the propionate ester or inulin control to the gut was measured by using a breath hydrogen test. Breath hydrogen was detected from subjects at several time points during the study visits after receiving the IPE or inulin with a handheld hydrogen monitor - Gastro+ Gastrolyzer® (Bedfont Scientific Ltd, Kent, UK). Bacteria in the intestinal lumen of the large or small intestine act on carbohydrates that enter the gut resulting in the production of hydrogen (H_2). This H_2 diffuses into the blood stream and then to the lungs where it can then be detected in expiratory air. H_2 is accurately measured in parts per million (ppm) in expiratory air revealing breakdown of carbohydrate in the intestine.

2.1.3 Assessment of gut hormones

GLP-1 in plasma samples was detected using a sensitive and specific radioimmunoassay developed in house (Kreymann et al., 1987). A rabbit specific antibody to GLP-1 was used in this assay. ^{125}I-GLP-1 was produced using the iodogen method (Wood et al., 1981) and purified by high pressure liquid chromatography (HPLC). The assay was performed in 700 µL of 0.06 M phosphate buffer (pH 7.3) containing 0.3% bovine serum albumin (BSA) and 0.3% TWEEN-20 (Sigma, UK). GLP-1 standards were diluted in a range from 0.125 fmol/ml to 0.0125 pmol/ml. 100 µl of standards and samples were assayed for 4 days at 4ºC before separation of free and bound GLP-1 using charcoal absorption. Samples were centrifuged at 2500 rpm at 4ºC for 20 min. Bound GLP-1 in the supernatant was carefully separated and collected in fresh tubes. Labelled GLP-1 was counted using a gamma scintillation counter (LB2111 Multi Crystal Gamma Counter, Berthold Technologies, Germany) for 240 seconds. GLP-1 concentrations in samples were calculated from the standard curve.

PYY in plasma samples was detected using a sensitive and specific radioimmunoassay developed in house (Adrian, 1985). A rabbit specific antibody to PYY was used in this assay. [1]

^{25}I-PYY was produced using the iodogen method (Wood et al., 1981) and purified by HPLC. The assay was performed in 350 µL of 0.06 M phosphate buffer (pH 7.3) containing 0.3% bovine serum albumin (BSA) and 0.3% TWEEN-20 (Sigma, UK). PYY standards were diluted in a range from 0.5

fmol/ml to 0.05 pmol/ml. 100 µl of standards and samples were assayed for 3 days at 4°C before separation of free and bound PYY by immunoprecipitation using sheep anti-rabbit antibody (Pharmacia Diagnostics, Sweeden). 100 µl of secondary antibody was incubated for 1 hour at RT. After incubation with secondary antibody 500 µl of 0.01% triton x-100 (Sigma, UK) and 100 µl of 10% Poly(ethylene glycol) (Sigma, UK) was added and then samples were centrifuged at 2500 rpm at 4°C for 30 min. Bound PYY in the supernatant was carefully separated and collected in fresh tubes. Labelled PYY was counted using a gamma scintillation counter for 240 seconds, as previously described. PYY concentrations in samples were calculated from the standard curve.

2.1.4 Assessment of appetite, satiety and nausea

To investigate effects on appetite, satiety and nausea, 100 mm visual analog scales (VAS) were used at several time points during the study visit. Subjects completed the VAS before each blood sample to measure the gut hormones. For example, subjects were asked; “how hungry do you feel right now?” and “how strong is your desire to eat?”. The distance from the left of the 100 mm line to where the subject marked was calculated to give a score.

2.1.5 Assessment of insulin

To investigate effects on insulin levels we used a human insulin radioimmunoassay kit (Millipore U.K.). The assay works on the principle of a fixed concentration of labelled tracer antigen being incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited. Unlabelled antigen on the sample is added to the system allowing competition between labelled tracer and unlabelled antigen for limited constant binding sites. Bound tracer will decrease as the concentration of unlabelled antigen in the sample could be measured after separating antibody-bound from free tracer and counting one or both fractions. A standard curve enables the amount of antigen in the sample to be calculated from a known concentration of unlabelled antigen. The protocol was followed according to the manufacturer’s instructions, in brief, standards were prepared in provided tubes in the concentration range of 0 to 100 µU/mL. Standards, samples and controls were loaded in duplicate. Radiolabelled ^{125}I-Insulin was added to all tubes and human insulin antibody was add to appropriate tubes. These were vortexed and incubated overnight at room temperature. The precipitation reagent was added to appropriate tubes and vortexed and incubated for 20 mins in 4°C. Tubes were then centrifuged for 20 mins at 4°C at

2000 $g_{(av)}$. Supernatant was decanted and tubes were counted in a gamma counter for 1 min to calculate the amount of human insulin (μU/mL) in our samples.

2.1.6 Assessment of glucose

To investigate effects on levels of glucose at the time points described in the study design section above. Glucose amounts were measured directly in blood plasma using an automated clinical chemistry analyser - the Abbott Architect ci8200 analyser (Abbott Diagnostics, USA).

2.1.7 Statistical analysis

All data were analysed for statistical significance using Graphpad Prism 6.0 software. All data were checked for normality. Data were analysed using a paired t-test when comparing control to IPE, by ordinary one-way analysis of variance (ANOVA) when comparing three or more groups defined by one factor or by using a two-way ANOVA when comparing three or more groups defined by two factors. Statistical significance was determined when $p<0.05$.

Chapter 3 Exploring the effect of increasing colonic propionate on acute energy intake and its relationship to gut hormones and glucose homeostasis.

3.1 Background

In recent decades there has been growing evidence that fermentable carbohydrates (FC) and subsequent SCFA production can modulate appetite regulation. FC are fermented in the colon to produce SCFAs (Tarini and Wolever, 2010). SCFAs are then able to activate FFAR2 and FFAR3 on intestinal L cells which are able to produce the anorectic gut hormones, PYY and GLP-1, when stimulated (Tolhurst et al., 2012, Psichas et al., 2015). PYY and GLP-1 also play important roles in appetite regulation (Bewick, 2012). However, the specific mechanisms of this relationship between SCFAs and gut hormones in humans, and its utility to alter food intake in both normal and obese individuals remains unclear.

Despite the known benefits of increasing colonic concentrations of SCFAs reviewed in Chapter 1, high doses of SCFAs given orally have been associated with causing nausea in humans and have been described as unpalatable (Frost et al., 2003). We sought to understand whether modification of propionate by linking it to inulin with an ester linkage solves these side effects of nausea and unpalatability. To increase colonic propionate I will use IPE, the development of which, was previously described in the methods chapter.

Previous work by our laboratory has shown that IPE is able to reach the colon. ^{13}C isotope methodology and breath hydrogen testing (Bond et al., 1975) were used to determine the time course of release of the bound propionate from the Inulin-propionate ester in 9 healthy volunteers. 100 mg of the bound propionate was labelled with 13C and following ingestion of 10 g IPE, breath hydrogen (a marker of colonic fermentation) began to rise after 180 min and peaked at 240 min post-ingestion. More than 80% of the 13C recovered in breath over 24 hours appeared coincident with and after breath H2 onset, suggesting delivery of the majority of the tracer to the colon. It was also estimated that 10 g of IPE delivered 2.36 g propionate to the colon after accounting for small intestinal losses (0.49 g). The isotopic data showed that propionate released from the IPE appeared in the blood and was thus available systemically (Chambers et al., 2014).

The effect of using IPE to increase colonic levels of propionate on energy intake in humans has not yet been reported, so the work presented in this chapter explores this. In addition, should IPE reduce energy intake, little is known about this mechanism so we also sought to further understand the effect of IPE on plasma levels of GLP-1 and PYY, as well as, insulin and glucose in this study. We were also interested in whether inulin-propionate ester is able to avoid the undesired side effects of nausea and unpalatability associated with other SCFA studies.

3.2 Hypotheses

- I hypothesise that increasing levels of propionate to the colon would reduce food intake and increase levels of PYY and GLP-1.
- I also hypothesise that inulin-propionate ester would reduce subjective appetite and may alter insulin sensitivity and glucose tolerance.

3.3 Aims and Objectives

In this chapter we sought to investigate the effect of increasing levels of propionate in the colon by providing subjects with a single dose of 10 g of IPE on:

- Food intake
- Plasma levels of GLP-1, PYY, insulin, and glucose
- Subjective appetite and satiety
- Subjective nausea

3.4 Methods

3.4.1 **Study Design**

This study was carried out in a double–blind, crossover manner, with two visits, a week apart. Subjects abstained from strenuous exercise and alcohol 24 hours before the study day and consumed the same meal between 19:00 and 20:00 the evening before. They fasted overnight and arrived at Hammersmith hospital at 8:30am on each visit. A cannula was inserted into a forearm vein and baseline blood samples were collected at -10 and 0 min. Following the 0 min sample, subjects were served a standardised breakfast (398 kcal; 71 g CHO, 8 g fat, 10 g protein) containing

either 10 g of IPE, or 10 g inulin control. At 180 min a standardised lunch (356 kcal; 34 g CHO, 12 g fat, 28 g protein) was provided, and at 420 min food intake was measured in subjects. Postprandial blood samples were taken at 15, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min and collected into heparin-coated tubes containing 0.2 ml of aprotonin (Bayer, UK). Plasma was separated immediately by centrifugation at 4°C and then stored at -70°C until analysed. Subjective hunger, satiety, and nausea were monitored with the use of 100 mm VAS. Subjects were asked to complete the VAS before each blood sample. A schematic diagram of the study design can be seen in Figure 3.1.

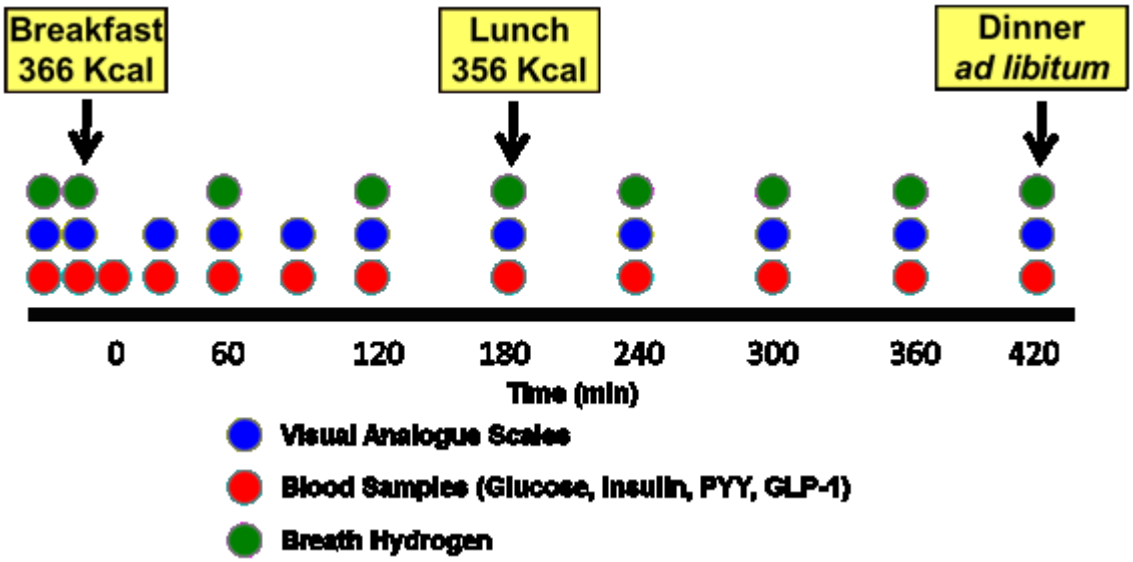


Figure 3.1: **Schematic diagram showing the study design for food intake study.**
20 healthy volunteers were recruited. At both visits, subjects in a single blind randomised trial received IPE or inulin (control). Assessment of gut hormones GLP-1 and PYY, glucose and insulin was made through blood tests and appetite was assessed by VAS. Breath hydrogen was measured to detect fermentation of inulin in the colon. Food intake was measured by weighing the amount consumed when given free access to food at 420 mins.

3.4.2 **Power Analysis**

This is a pilot study in a new area and therefore a power calculation was not possible. However, similar studies using SCFAs to alter food intake in humans used 16 volunteers in a cross-over design and showed a 6% reduction in food intake over a 24 h period, so we expect this number in our study to provide a similar result (Darzi et al., 2014).

3.4.3 **Study participants**

Study participants were recruited according to the following criteria. The inclusion criteria were a body mass index (BMI) of 20 to 35 kg/m^2 and 21 to 65 years of age. The exclusion criteria were smoking, substance abuse, pregnancy, use of medications, a change in body weight > 5 kg in the previous 3 months, medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, thyroid function and liver function). All subjects provided informed, written consent prior to starting the study, which was approved by the Hammersmith and Queen Charlotte's Research Ethics Committee (08/H0707/99). The study was carried out in accordance with the Declaration of Helsinki. A flow diagram of recruitment to this study can be seen in Figure **3.2**.

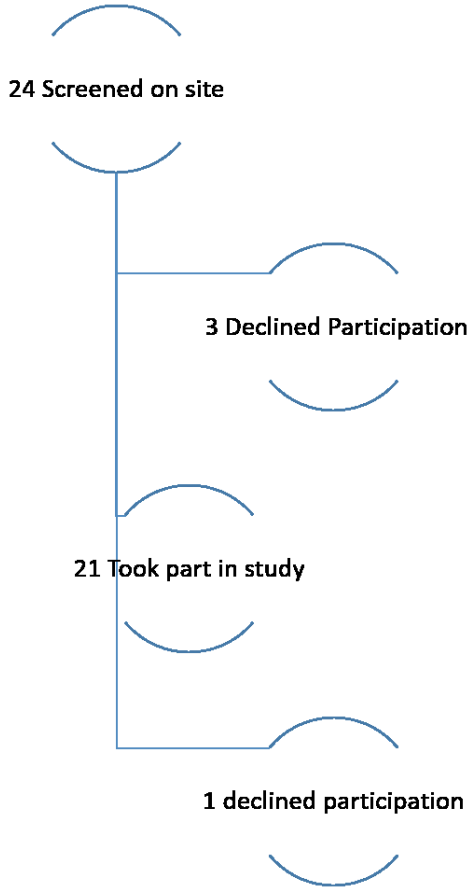


Figure 3.2: Schematic diagram showing the recruitment of participants to the food intake study
The number of telephone screenings, on site screenings, participants and exclusions throughout can be seen for this study.

3.4.4 **Intervention**

The IPE was developed by Dr Douglas Morrison from the University of Glasgow. The IPE is 3g of propionate bound to 7g of inulin via an ester linkage which aims to increase palatability compared to propionate alone as described in the methods chapter. 10g of IPE delivers 2.58 g of propionate to the colon after 0.49 g of losses in the intestine (Chambers et al., 2014). The control in this study was 10 g of inulin.

3.4.5 **Measurement of energy intake**

To investigate the effect of IPE or inulin on energy intake (EI), free access to food via a buffet meal was provided 7 hours after the 10g of IPE or inulin was given. Subjects were told to eat until they felt comfortably full and the amount of food eaten was calculated by weighing the amount of food at the start and after the subject had eaten and calculating the difference.

3.4.6 **Subjective ratings of appetite, satiety and nausea**

To investigate the effect of IPE or inulin on appetite, satiety and nausea, 100 mm VAS were used at several time points during the study visit as described in the methods chapter.

3.4.7 **Radioimmunoassays for GLP-1 and PYY**

To investigate the effect of IPE or inulin on the gut hormones GLP-1 and PYY blood samples were taken at several time points (15, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min) after receiving a standardised breakfast with inulin control or IPE and collected as described in the methods chapter. Levels of GLP-1 and PYY were assessed by an in-house radioimmunoassay as described previously in the methods chapter.

3.4.8 **Radioimmunoassay for Insulin**

To investigate the effect of IPE or inulin on levels of insulin, blood samples were taken at several time points (15, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min) after receiving a standardised breakfast with inulin control or IPE and collected as described in the methods chapter. Levels of insulin were detected in the plasma by radioimmunoassay as described in methods chapter.

3.4.9 **Measurement of glucose**

To investigate the effect of IPE or inulin on levels of glucose were measured using an automated clinical chemistry analyser - the Abbott Architect ci8200 analyser (Abbott Diagnostics, USA).

3.4.10 **Marker for fermentation of inulin**

Delivery of the IPE or inulin control to the gut was measured by using a breath hydrogen test as described in the methods chapter.

3.4.11 **Statistical Analysis**

Food intake, breath hydrogen, GLP-1, PYY, Insulin and glucose were analysed by paired t-test. VAS was analysed by two-way ANOVA.

3.5 Results

3.5.1 **Study Participants**

In this study 20 healthy adult volunteers were recruited. Characteristics of the volunteers are listed in Table 3.1.

Table 3.1: Characteristics of participants in the effect of propionate ester on food intake study.

Participants' Characteristics	**Mean ± SD**
Age (years)	30.8 ± 9.7
Weight (kg)	75.0 ± 13.5
BMI (kg/m^2)	25.4 ± 3.6
Gender	15 men and 5 women

3.5.2 **Inulin-Propionate ester is fermented in the colon**

Figure **3.3** shows the response curve for the production of breath hydrogen. After 240 min breath hydrogen increased significantly from the baseline value ($p<0.05$) with both IPE and inulin. This significant increase in breath H_2 is a marker for IPE and inulin being delivered to the colon.

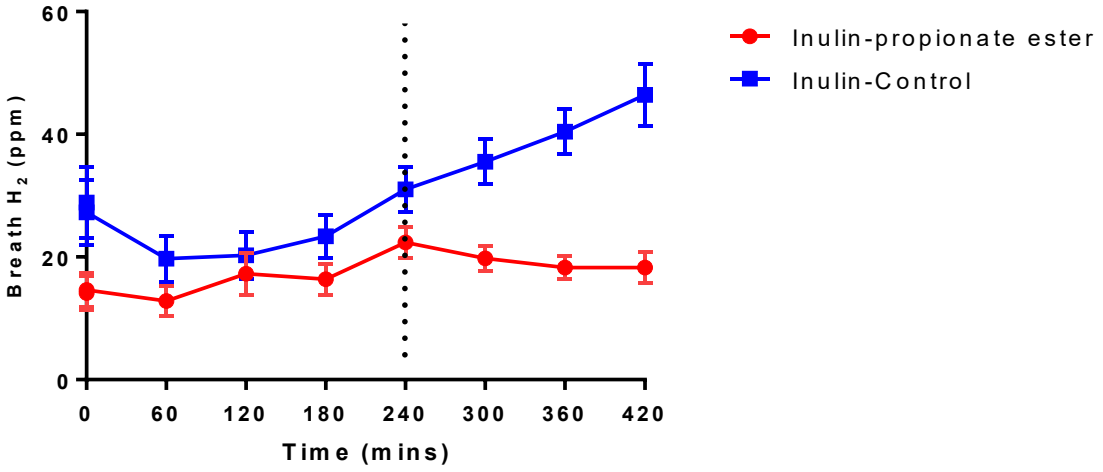


Figure 3.3: Breath hydrogen over time for subjects given inulin control or IPE.
At 240 min the breath hydrogen response in the IPE (red) and inulin (blue) trials identifies the arrival of the IPE or inulin at the colon.

3.5.3 **Inulin-Propionate ester decreases food intake**

Twenty healthy subjects were given 10 g of IPE or inulin control in this double-blind, randomised, crossover trial. Food intake after the IPE or inulin control was measured by the provision of free access to an unlimited buffet meal 7 hours after the supplement was given, and told to eat until comfortably full. We found that consumption of 10 g of Inulin-propionate ester significantly reduced food intake in subjects by 14% compared to 10 g inulin. The mean food intake with IPE was 1013 ± 94 Kcal, whilst food intake with inulin was 1175 ±103Kcal (Figure **3.4**, $p<0.01$). Individual data for food intake can also be seen in Figure **3.4**.

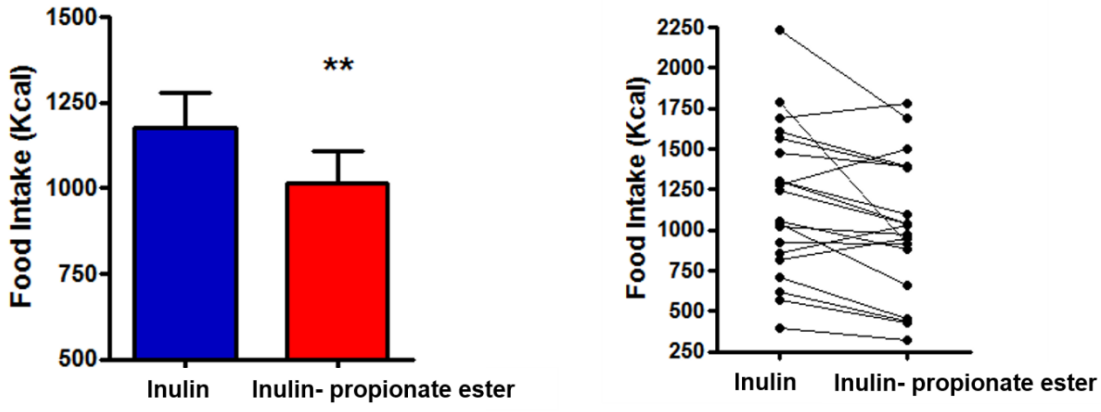


Figure 3.4: **Effect of 10g IPE vs. inulin (control) on food intake in healthy humans.**
Bar chart shows food intake in Kcal for subjects receiving Inulin-propionate ester or control (n = 20) (**p=0.0095) Bars show mean ± SEM. Individual data for food intake with IPE or inulin is also shown. Plot shows the amount of food intake in Kcal with IPE or inulin control. Points show values plotted for individuals (n=20).

3.5.4 **Inulin-Propionate ester increases GLP-1**

Blood samples were taken from the twenty volunteers every hour for 7 hours after 10 g of IPE or inulin control to detect plasma levels of GLP-1. We found that consumption of 10 g of IPE significantly increased levels of GLP-1 (Figure **3.5**). Breath hydrogen increases at 240 mins signalling fermentation of the inulin and IPE (section 3.5.2), therefore any changes in GLP-1 were assessed after this point where the inulin or IPE had reached the colon. Mean GLP-1 levels for ΔAUC240-420min were 3495 min × pmol/L with control compared to 10801 min × pmol/L with the IPE ($P<0.05$) between 240-420 min.

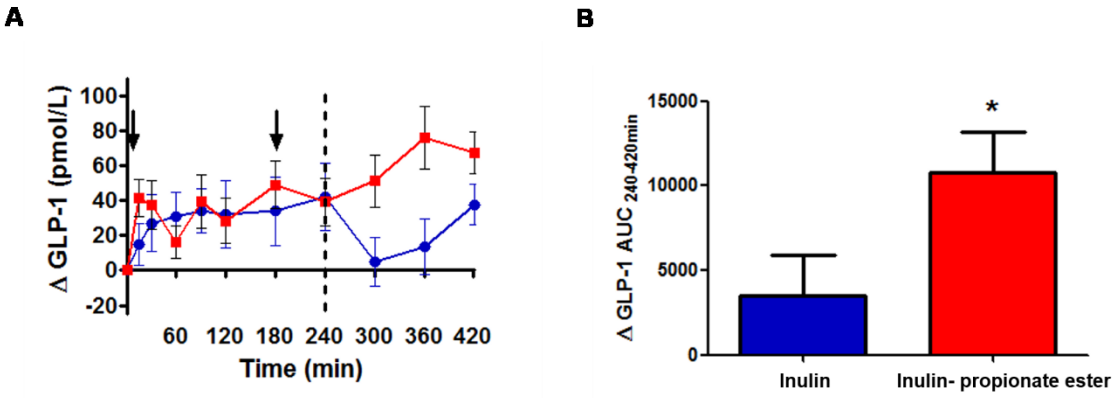


Figure 3.5: Effect of 10g IPE vs. inulin (control) on levels of GLP-1 in healthy humans.
(A) Line graph shows plasma levels of GLP-1 (pmol/L) over time (n = 20). Arrows indicate the timings of the standardised breakfast (0 min) and lunch (180 min). The dashed line is the time that breath hydrogen starts to increase in both trials, indicating delivery of the IPE to the colon. Red shows IPE, blue shows inulin control. (B) Bar chart shows mean ΔGLP-1 AUC240-420min for subjects receiving IPE or control (n = 20) (p=0.0329). Bars show mean ± SEM.

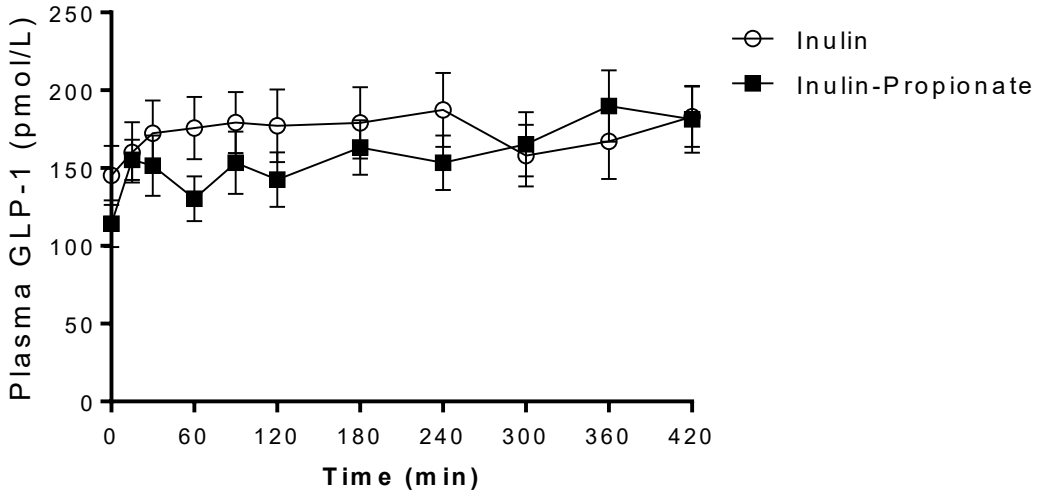


Figure 3.6: The total Effect of 10g IPE vs. inulin (control) on levels of GLP-1 in healthy humans.

3.5.5 **Inulin-Propionate ester increases PYY**

We also sought to understand the effect of IPE on levels of PYY in humans. Again, blood samples were taken from the twenty volunteers every hour for 7 hours after 10 g of IPE or inulin control to detect levels of PYY. We found that consumption of 10 g of IPE significantly increased levels of PYY (Figure **3.7**). The mean PYY ΔAUC 240-420min with control was 429 min × pmol/L vs. 3349 min × pmol/L with propionate ester (P<0.05).

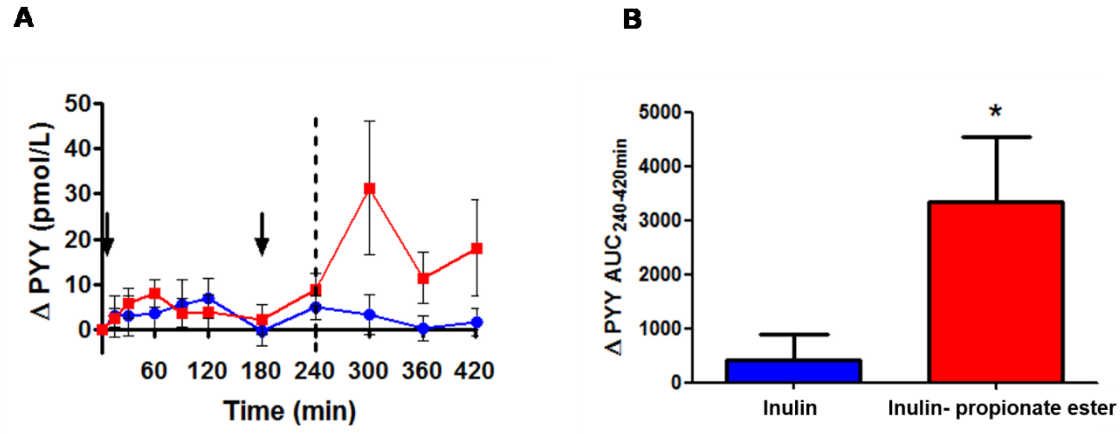


Figure 3.7: **Effect of 10g IPE vs. inulin (control) on levels of PYY in healthy humans.**
(A) Line graph shows plasma levels of PYY (pmol/L) over time (n = 20). Arrows indicate the timings of the standardised breakfast (0min) and lunch (180min). The dashed line is the time that breath hydrogen starts to increase in both trials, indicating delivery of the IPE to the colon. Red shows IPE,

blue shows inulin control. (B) Bar chart shows mean ΔPYY AUC240-420min for subjects receiving IPE or control (n = 20) (p=0.0410). Bars show mean ± SEM.

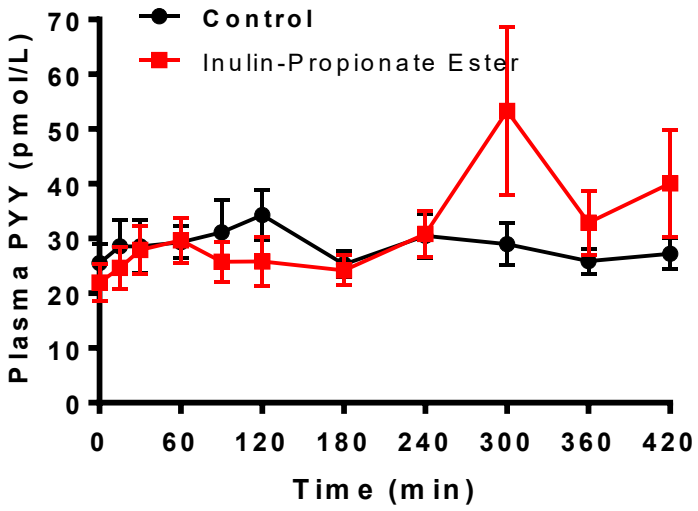
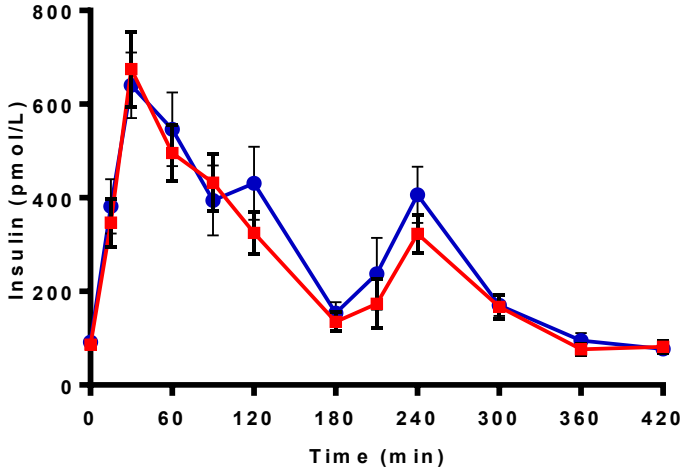


Figure 3.8: The total Effect of 10g IPE vs. inulin (control) on levels of PYY in healthy humans.

3.5.6 **Inulin-Propionate ester does not affect insulin levels**

We also sought to understand the effect of IPE on levels of insulin in humans. Again, blood samples were taken from the twenty volunteers every hour for 7 hours after 10 g of IPE or inulin control to detect levels of insulin. We found that consumption of 10 g of inulin-propionate ester does not significantly affect levels of insulin, ($p>0.05$, Figure **3.9**). The mean peak insulin level was 161.7 ± 11.1 µU/mL with the control whilst the mean peak insulin level with IPE was 126.2 ± 19.1 µU/mL. The mean AUC for 0-420 min with inulin was 20043.8 ± 2333 and was 18005.7 ± 1674.6 with IPE.

A



B

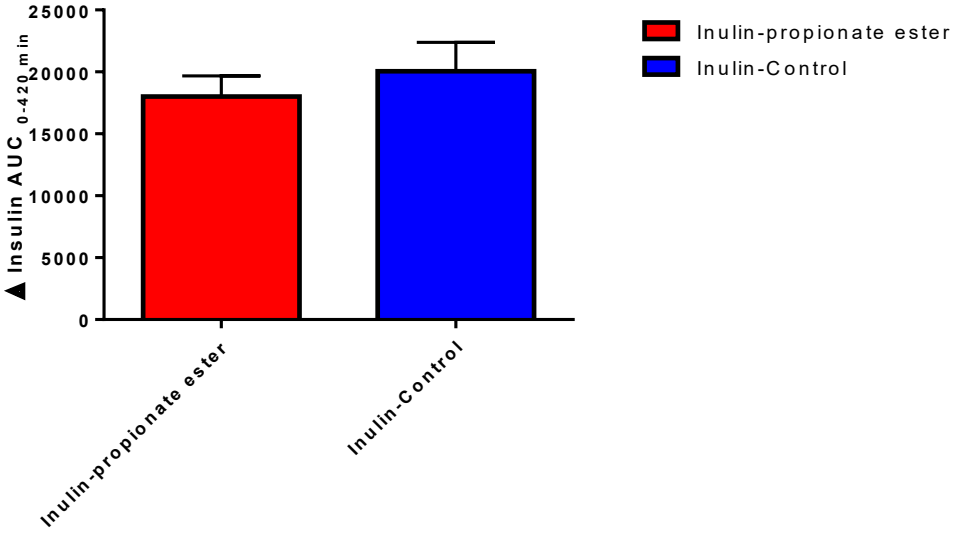


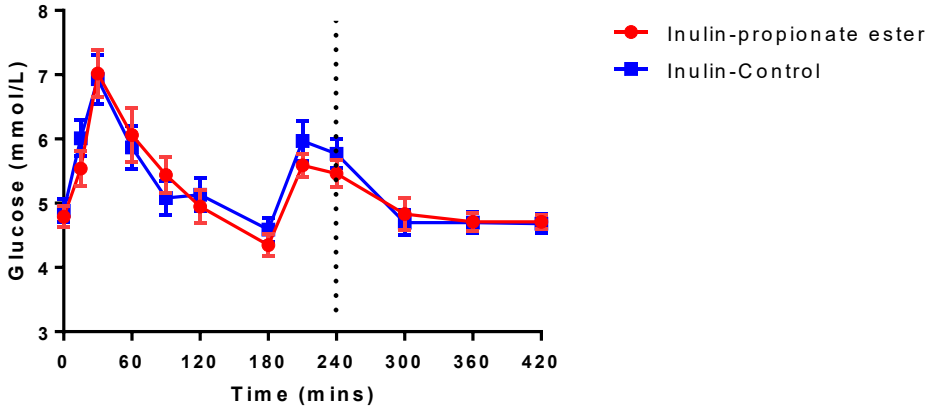
Figure 3.9: Inulin-Propionate ester supplementation does not affect insulin levels
(A) Line graph shows plasma levels of insulin (pomol/L) over time (n=20). Arrows indicate the timings of the standardised breakfast (0 min) and lunch (180 min). 10 g control or 10 g IPE were provided with breakfast at 0 min. Data show mean ± SEM ($p>0.05$). (B) Bar chart shown mean Δ Insulin AUC 0-420 min for subjects receiving IPE or inulin (n=20) ($p>0.05$) Bars show mean ± SEM.

3.5.7 **Inulin-Propionate ester does not affect glucose levels**

We also sought to understand the effect of IPE on levels of glucose in the volunteers. Again, blood samples were taken from the twenty volunteers every hour for 7 hours after 10 g of IPE or inulin control to detect levels of glucose. We found that consumption of 10 g of IPE does not significantly affect levels of glucose ($p>0.05$, Figure **3.10**). The mean peak glucose level was 6.93± 0.39 mmol/L

with control and the mean peak glucose level with IPE was 7.02 ± 0.37 mmol/L. The mean AUC for 0-420 min with inulin was 2167.7 ± 64.7 and was 2146.5 ± 72.0 with IPE.

A



B

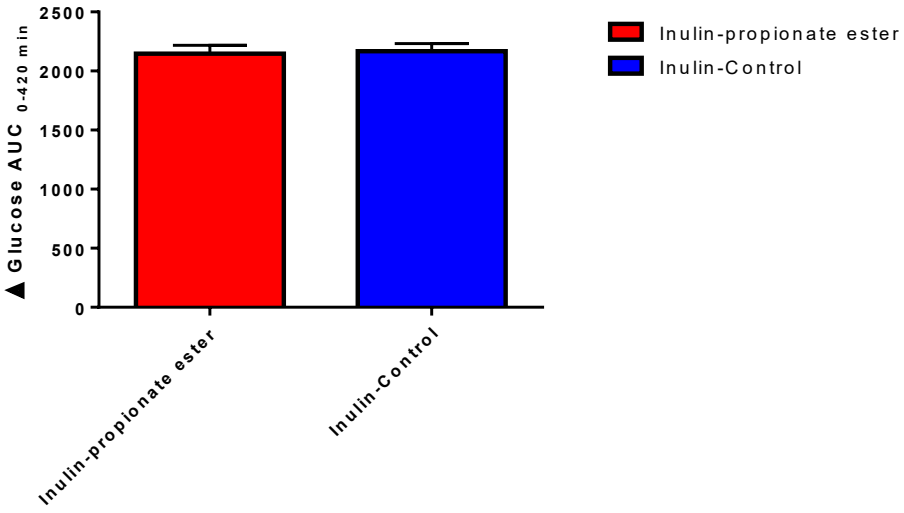


Figure 3.10: IPE supplementation does not affect postprandial glucose levels.
(A) Line graph shows plasma levels of glucose (mmol/L) over time (n = 20). Arrows indicate the timings of the standardised breakfast (0 min) and lunch (180 min). 10 g control or 10 g inulin-propionate ester were provided with breakfast at 0 min. Data show mean ± SEM ($p>0.05$). (B) Bar chart shows mean Δ glucose AUC 0-420 min for subjects receiving IPE or inulin (n=20) ($p>0.05$) Bars show mean ± SEM.

3.5.8 **Inulin-Propionate ester does not affect subjective ratings of appetite or satiety**

We sought to understand the effect of IPE on satiety and appetite in humans. VAS were used to measure satiety and appetite. The twenty volunteers completed VAS every hour for 7 hours after 10 g of IPE or inulin control to detect levels of satiety and appetite. We found that consumption of 10 g of IPE does not affect levels of satiety and appetite as measured by responses to the questions – "How hungry do you feel right now?", "How Pleasant Would It Be To Eat Right Now?", "How full do you feel right now ?" (Figure **3.11**).

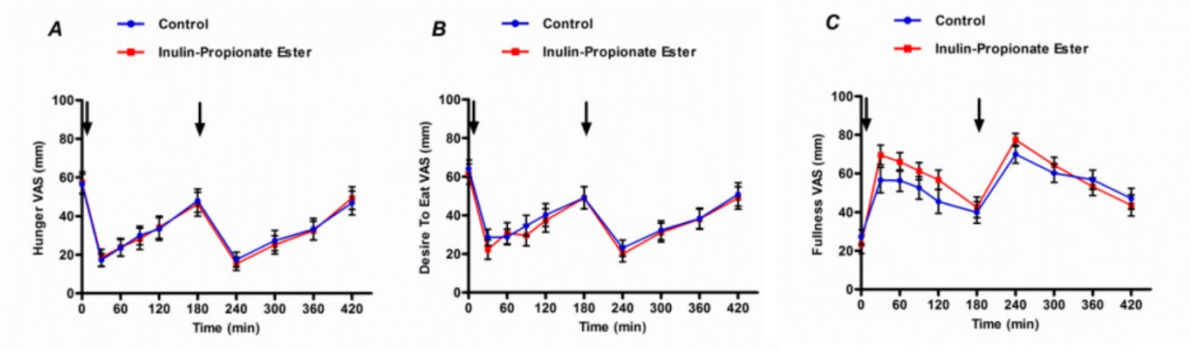


Figure 3.11: Increasing colonic propionate does not affect subjective ratings of appetite and satiety.
Graphs show A. Hunger, B. Desire to Eat, C. Fullness. Ratings were made using 100 mm visual analogue scales (VAS), with ends of the scale signposting extremes (e.g. 0 mm equalling not at all hungry and 100 mm being extremely hungry). Black arrows indicate standardized meals. 10 g IPE or 10 g control were provided with breakfast at 0 min. Data points are presented as means ± SEM (n=20). Blue is control, red is IPE.

3.5.9 **Inulin-Propionate ester does not cause nausea**

We also sought to understand the effect of IPE on nausea in humans. VAS were used to measure nausea in the twenty volunteers every hour for 7 hours after 10 g of IPE or inulin control to detect any sickness associated with IPE. We found that consumption of 10 g of inulin-propionate ester does not affect levels of nausea in response to the question "Do you feel sick right now?" (Figure **3.12**).

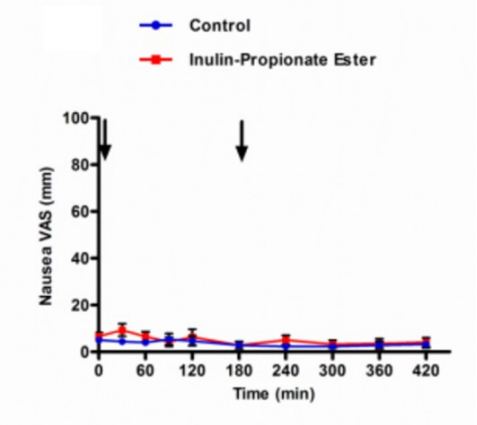


Figure 3.12: Increasing colonic propionate does not affect subjective ratings of nausea. Graph shows subjective ratings of Nausea. Ratings were made using 100 mm VAS with ends of the scale signposting extremes (e.g. 0 mm equalling not at all nauseous and 100 mm being extremely nauseous). Black arrows indicate standardized meals. 10 g IPE or 10 g control were provided with breakfast at 0 min. Data points are presented as means ± SEM (n=20). Blue is control, red is IPE.

3.6 Summary of results

The results presented in this chapter have shown the effects of acutely supplementing humans with IPE on food intake, levels of the gut hormones GLP-1 and PYY, nausea and insulin and glucose levels. We found that:

- IPE significantly decreased food intake in healthy humans by 14%.
- IPE significantly increased plasma levels of PYY and GLP-1 in healthy humans.
- IPE does not affect subjective ratings of appetite despite reducing energy intake.
- IPE does not significantly affect subjective ratings of nausea.
- IPE does not affect plasma levels of insulin and glucose.

3.7 Discussion

3.7.1 **Inulin-Propionate ester decreased food intake**

In this study we demonstrate for the first time in humans that increasing colonic propionate by providing 10g of IPE with breakfast significantly decreases energy intake when offered free access to food for lunch on the same day. Specifically, food intake was decreased with consumption of IPE by 14%. This is the first time colonic delivery of an individual SCFA using an oral method has been shown to affect appetite. Other studies have reported that increases in SCFAs from fermentable CHO decrease energy intake. This finding matched our original hypothesis that IPE would decrease energy intake because SCFAs are known to increase levels of the gut hormones GLP-1 and PYY (Psichas et al., 2015) which may reduce energy intake. Our findings corroborate those of others in the field showing the effects of SCFAs on energy intake. For example, energy intake is significantly reduced in rats given acetate intraperitoneally (Frost et al., 2014). Furthermore, our study is concordant with the use of NDC to reduce energy intake, likely through fermentation in the colon raising colonic levels of SCFAs. For example, in a human study looking at the effects of increasing colonic fibre on energy intake. 33 male subjects visited 3 times, a week apart, and were given a breakfast containing 0g of inulin, 24g of inulin or 24g of Lupin-kernel fibre with 0g of inulin. After these breakfasts all energy intake on that day and the following day was measured. EI on the day of the 24g inulin containing breakfast was reduced by 1521kJ - approximately 14% less compared to the 0g inulin breakfast (Archer et al., 2004). It is interesting that this study shows a 14% reduction in energy intake like we found in our study with IPE in a similar number of volunteers also over an acute period. This suggests that acutely increasing colonic SCFAs can consistently achieve a 14% energy intake reduction. It would be interesting to know whether this energy intake reduction by SCFAs would also be sustainable in a more long-term study.

Similarly, energy intake in humans was also significantly reduced by 5% after consumption of 16g fibre per day over a period of 2 weeks (Cani et al., 2005). In this study 10 volunteers of both sexes were given 16g of oligofructose (OFS) or 16g dextrin maltose (DM) as a control for 2 weeks with a 2 week break and then 2 weeks of the alternative treatment. The EI was 5% lower with OFS period compared to DM. This suggests that supplementation with the fibre OFS can subtly decrease EI. However this modest reduction in EI of 5% may be due to a small sample size of 10 subjects or that OFS produces lower amounts of colonic SCFAs compared to inulin. In contrast a study using small doses of propionate with bread had no significant effect on EI compared to control (Darzi et

al., 2012). This was likely also confounded by the unpalatability of propionate in this study which we have designed the IPE to avoid.

Our findings that IPE can decrease EI adds to the growing body of evidence that increasing colonic SCFAs can impact on EI. This warrants further exploration of the use of SCFAs to reduce EI but it remains to be understood whether this will be a suitable long-term approach, whether this will be useful to obese individuals and whether increasing concentrations of propionate is the most relevant SCFA human target.

3.7.2 **Inulin-Propionate ester increased PYY and GLP-1.**

Here we have shown that 10g of IPE significantly increases plasma levels of GLP-1 and PYY in humans for the first time. This met our hypothesis that increasing propionate in the colon would increase the gut hormones GLP-1 and PYY. Some of the rationale for this was based on a study of 12 volunteers given a high dose of high fructose corn syrup (HFCS), a low dose of HFCS or 24g of inulin with a low dose of HFCS. The inulin group had significant increases in plasma levels of acetate, propionate and butyrate up to 6 hours after consumption (Tarini and Wolever, 2010). In addition FC significantly increase levels of PYY (Nilsson et al., 2013).

Our work showing increases in PYY and GLP-1 with IPE extends from a body of work implicating a link between FCs, SCFAs and the gut hormones GLP-1 and PYY. For example, L-cells from murine colonic cultures produce GLP-1 when stimulated with propionate (Tolhurst et al., 2012). In addition, *in vivo* in rats, propionate significantly increases PYY and GLP-1 via activation of FFAR2 (Psichas et al., 2015). Furthermore, this is important as SCFAs activate FFAR2/3 and levels of these receptors are also high in the L-cells which produce GLP-1 and PYY (Tolhurst et al., 2012, Tazoe et al., 2009).

Effects of propionate on food intake in this study may be through these plasma increases in GLP-1 and PYY. Several studies show a relationship between GLP-1, PYY and appetite regulation. In particular, injection of PYY can reduce energy intake by 33% in humans (Degen et al., 2005) and infusing GLP-1 can also decrease energy intake by 12% in humans (Flint et al., 1998). All these studies highlight the effect of increasing consumption of FC can increasing SCFA amounts which can then lead to increased plasma levels of GLP-1 and PYY.

We believe this decrease in food intake by 14% with IPE observed in this study is likely mediated by significant increases in the gut hormones GLP-1 and PYY. We hypothesise that the reduced energy intake may be through GLP-1 and PYY effects on slowing gastric emptying, or affecting the central nervous system, and this needs to be explored further. Further work in this thesis attempts to address these outstanding questions.

3.7.3 **Inulin-Propionate ester does not affect subjective ratings of appetite or satiety.**

Energy intake and satiation measured by free access to food was significantly reduced by 14% with ingestion of IPE. However, in this study, findings of subjective ratings of appetite and satiety using VAS were not affected by IPE. There is, however, obviously a physiological effect on satiation by the IPE as observed by this significant reduction in energy intake. It could be that, appetite and satiety were unchanged despite increased satiation with IPE, or it could be that VAS to measure appetite and satiety may not be the most sensitive and reliable measurement of appetite. Similarly another study showed that satiation was increased when measured by free access to food but subjective ratings of appetite were unaffected (Van Wymelbeke et al., 2001). This suggests that increased satiation can occur in the absence of altered appetite or satiety. In contrast, infusion of GLP-1 or PYY can increase satiety and satiation in humans (Batterham et al., 2003, Verdich et al., 2001). This suggests from our data that SCFAs may act via alternative mechanisms to increase satiation, as increases in satiety are not seen alongside increases in satiation.

3.7.4 **Inulin-Propionate eater does not cause nausea**

We showed that IPE does not cause nausea. This was a welcome finding as sodium propionate has previously been shown to be unpalatable and likely to nausea (Frost et al., 2003, Liljeberg et al., 1995). This suggests that creating IPE has overcome these side effects and should be the preferred method to increase colonic propionate.

3.7.5 **Inulin-Propionate ester does not affect glucose or insulin.**

In this study, acute supplementation of propionate did not alter plasma levels of glucose and insulin. This is not surprising as several other acute studies have shown that acute supplementation with propionate does not affect glucose or insulin. In one human study, 6 healthy volunteers were given rectal infusions with mixed doses of SCFAs (90mmol acetate + 30 mmol propionate or 180mmol

acetate + 60 mmol propionate). There was no significant effect on glucose and insulin (Wolever et al., 1989). Similarly in another human study, 6 healthy volunteers were given 36mmol of acetate or 12mmol of propionate or 36mmol of acetate + 12mmol of propionate or control over a 3 hour period, again there was no effect on glucose or insulin with this acute treatment (Laurent et al., 1995).

However, this does not rule out that propionate or other SCFAs cannot affect levels of insulin or glucose. It is likely that a more chronic provision of SCFAs is required to induce these changes. Oral sodium propionate (9.9g in white bread) or placebo for 1 week significantly lowered glucose levels (Todesco et al., 1991). In addition, 20 healthy women given 7.5g sodium propionate or placebo in capsules every day for 7 weeks showed a significantly decreased fasting blood glucose and increased insulin sensitivity (Venter et al., 1990). In addition, mice supplemented for 12 weeks with acetate, butyrate or propionate in their diets, showed an improved insulin sensitivity and glucose tolerance, likely as a results of improved β-cell function (den Besten et al., 2015).

Increasing levels of SCFAs through supplementing with NDC can also affect insulin sensitivity and glucose tolerance, however this does not identify specific effects of any of the SCFAs. In one human fibre study, significant effects on insulin and glucose were observed. 10 healthy volunteers given 30g a day of resistant starch or placebo as a control for 4 weeks showed significant improvements in insulin sensitivity (Robertson et al., 2005). Further, 48 overweight or obese individuals given 21g a day of oligofructose or placebo as a control for 12 weeks also showed significant decreases in insulin and glucose levels (Parnell and Reimer, 2009). However in a long-term fibre study in 12 healthy volunteers given increasing doses of 15g, 25g 35g, 45g and 55g of oligofructose per week no effect on insulin and glucose was found (Pedersen et al., 2013). Similarly in another fibre study with 22 overweight or obese volunteers given 30g of oilgofructose or 30g of cellulose as a control for 6 weeks, no effects on glucose or insulin were found either (Daud et al., 2014).

The difference between chronic and acute supplementation of SCFAs on insulin and glucose may be dependent on long term changes in several parameters. These also may differ dependent on whether an individual is obese or of a normal weight. Furthermore, SCFAs may alter glucose and insulin function through altered gluconeogenesis and lipogenesis in the liver (Canfora et al., 2015).

Our findings show no significant effect of IPE on glucose and insulin. However, this adds to a body of conflicting evidence of whether SCFAs do indeed alter glucose and insulin homeostasis. Due to these conflicts, further exploration of the use of SCFAs to regulate glucose and insulin is warranted. In particular, the effect of SCFAs on glucose tolerance and insulin sensitivity need to be understood both in short-term and long-term use of SCFAs.

3.7.6 **Limitations of this work**

The assessment of food intake by a buffet meal with free access to food has been shown to be a reproducible and reliable method (Allirot et al., 2012). The findings from this study are encouraging, but we must remember that this was only an acute intervention and it will be important to look at chronic effects on food intake. In addition, it will be important to investigate the effects on food intake in non-healthy individuals and larger sample sizes.

3.7.7 **Conclusions**

In this study we have shown for the first time in humans the effect of raising colonic propionate using 10 g of IPE on food intake and gut hormone levels. We have shown that 10 g of IPE significantly increases the gut hormones PYY and GLP-1 and significantly decreases food intake by an average of 14%. The exact mechanisms of these effects of increased colonic propionate on gut hormones and energy intake need to be elucidated, and further work in this thesis aims to address this.

Chapter 4 Exploring the effect of increasing colonic propionate on gastric emptying

4.1 Introduction

Gastric emptying is the process by which food is moved from the stomach to the small intestine through the gastrointestinal tract. The gastrointestinal tract has a number of breaks including the ileal, jejunal and duodenal break which control the movement of food from the stomach to the small intestine. The main role of the ileal break is to control the rate of food movement and subsequent rate of GE. Chemosensors and mechanoreceptors through the GI tract are stimulated as food passes through and can activate neural pathways to appetite centres. Slower gastric emptying results in greater activity of these receptors in the gut and activity in the brain thereby reducing appetite and increasing satiety (Yamada and Alpers, 2009).

The rate of GE can be increased or decreased depending on several variables. In particular, GE can be altered by the food content (Calbet and MacLean, 1997), the host's microbiota (Quigley, 2011), as well as, other parameters. In addition, the gut hormones PYY and GLP-1 have been associated with slowing GE in humans (Savage et al., 1987, Hellström et al., 2006). PYY activates the ileal break and slows the speed of GE *in vivo* in dogs (Wen et al., 1995). Similarly, PYY infusion in healthy humans decreases the rate of GE (Savage et al., 1987). PYY acts to slow gastric empting and reduce appetite by activating neuropeptide receptor 2 on vagal afferents (Lenard and Berthoud, 2008). Similar to PYY, GLP-1 can also affect gastric emptying. Infusing GLP-1 to healthy humans reduced the rate of GE three-fold compared to control (Hellström et al., 2006). This is likely as an effect of stimulation of the vagal nerve via GLP-1 (I˙meryüz et al., 1997). Importantly, these effects of PYY and GLP-1 on GE have been shown to be dose-dependent (Little et al., 2006).

Evidence also exists to suggest that SCFAs can increase levels of PYY and GLP-1 (Chambers et al., 2014) and chapter 3, and alongside this, evidence also exists that suggests that SCFAs can delay GE (Liljeberg and Bjorck, 1996). Infusing a mixture of the SCFAs; acetate, propionate and butyrate, in a ratio of 60:30:10, respectively, delayed GE in pigs (Cuche and Malbert, 1999). In a further study from the same group they found that PYY and GLP-1 also increased with the SCFA mixture which may have been the mechanism behind the delayed GE (Cuche et al., 2000). In addition, in a human study, volunteers fed bread containing sodium propionate showed a

significantly delayed GE measured by plasma levels of paracetamol (Liljeberg and Bjorck, 1996). The mechanism by which SCFAs may slow GE is not entirely clear. It could be that SCFAs produced in the GI tract may decrease peristaltic activity and trigger tonic activity to reduce GE. Alternatively, SCFAs may interact in the terminal ileum with colon content to cause contrition and reduce gastric motility (Cherbut, 2003). Finally, an explanation could be that it is a combined neuro-hormonal effect through SCFAs increasing levels of PYY and it is the effect of these hormones rather than the SCFAs alone that can delay GE (Cherbut, 2003). The relationship between SCFAs, in particular propionate, PYY and GLP-1 and GE are still not clear so this area needs further research.

The effect of using IPE to increase colonic levels of propionate on GE in humans has not yet been reported, so the work presented in this chapter explores this. In chapter 3, an acute does of IPE significantly decreased food intake, and in this chapter we will determine whether reduced energy intake was caused by a slowing of gastric emptying. In addition, in chapter 3, the gut hormones PYY and GLP-1 were significantly increased by 10g IPE, taken together with the evidence above, it is plausible that increased PYY and GLP-1 may cause a slowing of gastric emptying to reduce food intake. We were also interested in whether IPE is able to affect appetite and satiety, as no effects were identified in chapter 3.

4.2 Hypothesis

I hypothesise that increasing levels of propionate to the colon would slow GE to reduce appetite and satiety.

4.3 Aims and Objectives

In this chapter we sought to investigate the effect of increasing levels of propionate in the colon by providing subjects with a single dose of 10 g of IPE on:

- Gastric emptying
- Subjective appetite and satiety

4.4 Methods

4.4.1 **Study Design**

This study was carried out in a single–blind, crossover manner, in two visits, at least a week apart. Subjects had not had strenuous exercise and alcohol 24 hours before the two study days and had consumed the same meal between 19:00 and 20:00 the evening before. They fasted overnight and arrived at Hammersmith hospital at 8:30am on each visit. Following the 0 min sample, subjects were served a standardised breakfast (398 kcal; 71 g CHO, 8 g fat, 10 g protein) containing either 10 g of IPE, or 10 g inulin control. At 300 min when the propionate is fermented in the colon (Chambers et al., 2014) and as described in chapter 3 when PYY and GLP-1 are significantly increased, participants were served a standard lunch (354 kcal; 47 g CHO, 12 g fat, 12 g protein) with 100 mg ^{13}C-octanoic acid to be used to measure gastric emptying (Ghoos et al., 1993). Breath CO_2 was collected every 15 min from baseline before the standard lunch until 4 hours later by exhaling alveolar breath through a straw into Exetainers (Labco, Buckinghamshire, UK). $^{13}CO_2$ enrichment was determined by isotope ratio mass spectrometry (IRMS). The time to 50% AUC excretion of ^{13}C in breath ($T_{1/2}$) was calculated as a proxy for gastric emptying rate (Ghoos et al., 1993). Breath hydrogen was measured at -10, 30, 60, 90, 120, 150, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480, 510, 540 mins to determine arrival of IPE or inulin to the colon. A schematic diagram showing the study design for this investigation can be seen in Figure **4.1**.

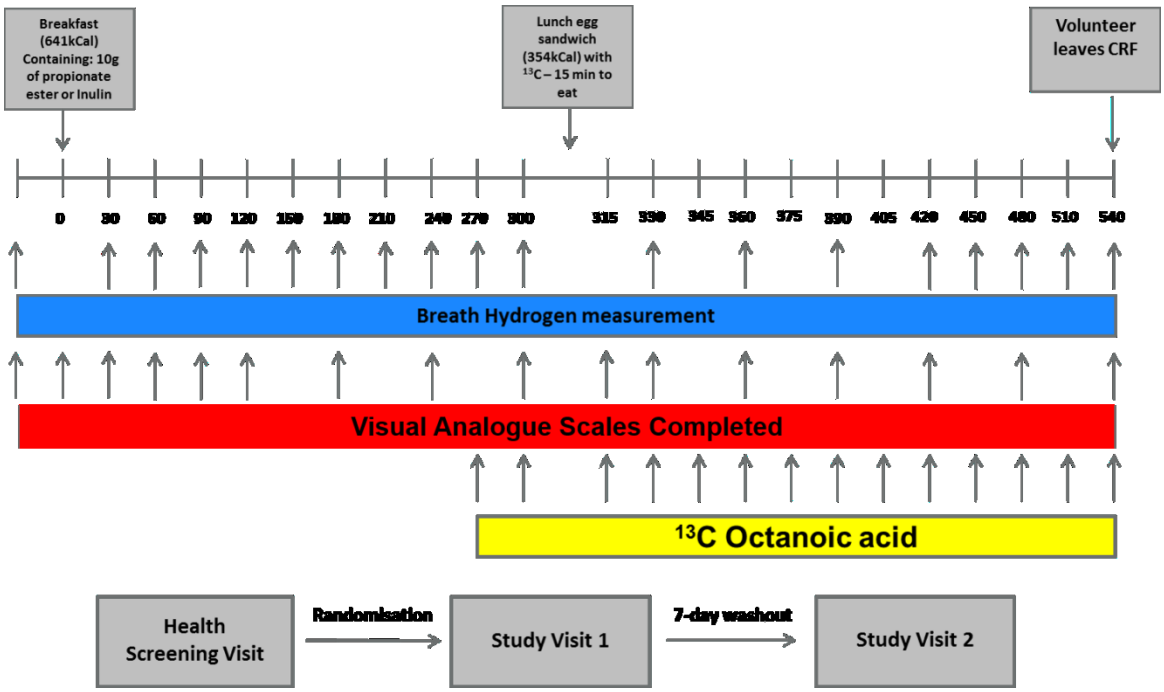


Figure 4.1: Schematic diagram showing the study design for the gastric emptying study. 14 healthy volunteers were recruited in this study. Volunteers would then visit on 2 occasions for test day 1 and then one week later for test day 2 randomly where they would receive a dose of inulin or inulin-propionate ester. The timeline shows the times when VAS were completed and the times when breath hydrogen was measured. As the gut hormones were released after 240 minutes in the previous investigation, a lunch containing ^{13}C Octanoic acid was provided to the volunteers after 300 minutes. This is used to measure gastric emptying because during metabolism levels of $^{13}CO_2$ can be measured in the breath. If there is delayed gastric emptying the time for the production of $^{13}CO_2$ will also be delayed.

4.4.2 **Study participants**

In this study 14 healthy adult volunteers were recruited. The inclusion criteria were a BMI of 20 to 35 kg/m^2 and 21 to 65 years of age. The exclusion criteria were smoking, substance abuse, pregnancy, use of medications, a change in body weight > 5 kg in the previous 3 months, medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, thyroid function and liver function). All subjects provided informed, written consent prior to starting the study, which was approved by the Hammersmith and Queen Charlotte's Research Ethics Committee (08/H0707/99). The study was carried out in accordance with the Declaration of Helsinki. A flow diagram of recruitment to this study can be seen in Figure **4.2**.

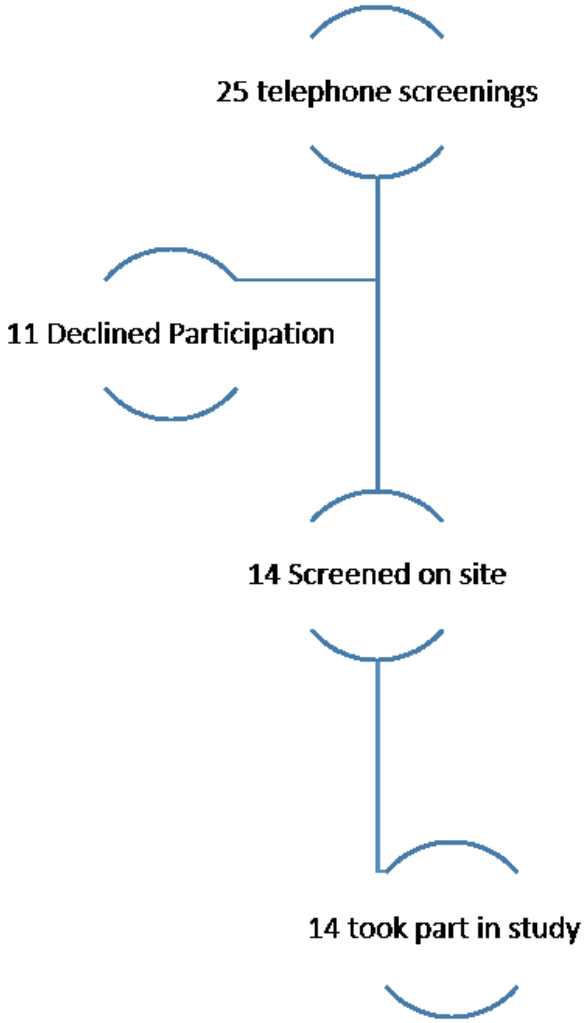


Figure 4.2: Schematic diagram showing the recruitment of participants to the Gastric emptying study.
The number of telephone screenings, on site screenings, participants and exclusions throughout can be seen for this study.

4.4.3 **Power analysis**

This is a pilot study in a new area and therefore a power calculation was not possible. However, similar studies using PYY to assess gastric emptying in humans used 7 volunteers and showed PYY slowed gastric emptying but this was measured by breath hydrogen technique. We expect recruiting a similar number of volunteers in our study to provide a similar result (Savage et al., 1987).

4.4.4 **Intervention**

The IPE was developed by Dr Douglas Morrison from the University of Glasgow. The IPE is 3g of propionate bound to 7g of inulin via an ester linkage which aims to increase palatability compared to propionate alone as described in the methods chapter. 10g of IPE delivers 2.36 g of propionate

to the colon after 0.49 g of losses in the intestine (Chambers et al., 2014). The control in this study was 10 g of inulin.

4.4.5 **Measurement of gastric emptying**

The ^{13}C-octanoic acid breath test was used to measure the time taken for gastric emptying in this study. The ^{13}C-octanoic acid breath test is a non-invasive, reproducible, stable isotope method for measuring gastric emptying (Ghoos et al., 1993). ^{13}C-octanoic acid is a radio labelled medium chain fatty acid which is retained in the solid phase of the meal in the stomach and is then metabolised normally in the body as an intermediate in the Krebs cycle where CO_2 with a ^{13}C label is produced as a by-product and taken to the lungs and breathed out and is measured in breath samples by a radioisotope count (Verbeke, 2009). By measuring the level of ^{13}C-octanoic acid that appears in breath samples we can calculate the time taken for the stomach to empty after eating. This is calculated by the Seigel curve fit to measure the half-life of recovery of ^{13}C. The half-life is the time taken to recover half of the original dose of ^{13}C. ^{13}C-octanoic acid was added to egg yolks and served in a sandwich at lunch 300 mins after supplementation with IPE or inulin was given. Breath samples were taken at several time points for approximately 17 hours after this to ensure complete metabolism of the ^{13}C. This method was chosen to measure gastric emptying in this study because it has been found to be more accurate with solids than using the paracetamol method to measure gastric emptying (Ghoos et al., 1993).

4.4.6 **Assessment of appetite and satiety**

To investigate the effect of IPE or inulin on appetite and satiety, 100 mm VAS were used at several time points during the study visit as described in the methods chapter.

4.4.7 **Statistical Analysis**

Breath hydrogen, mean data for gastric emptying and VAS were analysed by paired t-test. A pearson correlation coefficient was used to correlate times for gastric emptying with control and IPE.

4.5 Results

4.5.1 **Study Participants**

14 healthy volunteers which fit the inclusion criteria stated in the methods above were recruited to this study. Characteristics of the volunteers can be found in Table 4.1.

Table 4.1: Characteristics of participants in the effect of IPE on gastric emptying study.

Participants' Characteristics	Mean ± SD
Age (years)	32.1 ± 3.7
Weight (kg)	69.4 ± 3.5
BMI (kg/m^2)	24.0 ± 0.9
Gender	8 men and 6 women

4.5.2 **Inulin-Propionate ester significantly increases breath hydrogen**

Figure **4.3** shows the response curve for the production of breath hydrogen to detect the fermentation of IPE and inulin in the colon. After 240 min breath hydrogen increased significantly from the baseline value ($p<0.05$) with both Inulin-propionate ester and inulin. This significant increase in breath H_2 is a marker for Inulin-propionate ester and inulin being delivered to the colon and fermented.

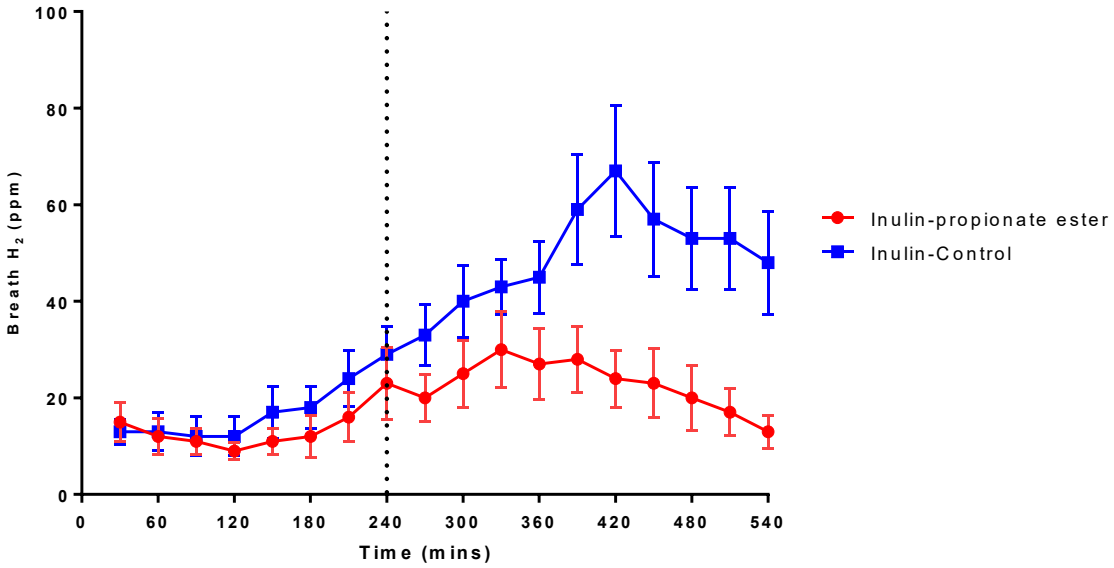


Figure 4.3: Inulin-Propionate ester significantly increases breath hydrogen.
The dashed line shows the time (240 min) at which the breath hydrogen response in the Inulin-propionate ester (red) ($p= 0.00227$) and inulin (blue) ($p= 0.000137$) trials significantly differs from baseline (0 min) identifying the arrival of the propionate or inulin at the colon (n=14).

4.5.3 **Inulin-Propionate ester does not affect gastric emptying**

10g of IPE did not significantly affect the time taken for gastric emptying (P>0.05). The mean time for gastric emptying with IPE was 185 ± 8 min whereas the mean time for gastric emptying with inulin was 180 ± 8 min (Figure **4.4**). In addition, the individual data is shown in Figure **4.5**, and there is no significant difference between the gastric emptying rate for inulin and IPE. The individual data is also shown as the majority of subjects (8 subjects) actually had increased gastric emptying in the IPE trial. To further corroborate these findings, correlating individual data for the time taken for gastric emptying with IPE with the time taken for gastric emptying with inulin show a similar time for gastric emptying on both trials, so if someone had delayed gastric emptying with IPE he/she would also have delayed gastric emptying with control (Figure **4.6**).

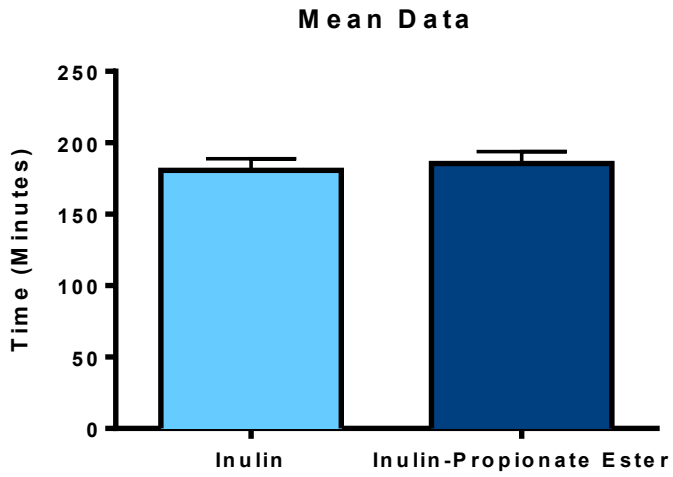


Figure 4.4: 10g of IPE does not significantly affect gastric emptying.
Group data for time taken for gastric emptying with IPE or inulin. Bar chart shows the time taken in minutes for gastric emptying with IPE or inulin control as measured by the half-life of the recovery of ^{13}C. (n=14) Bars show mean ± SEM (p=0.5082).

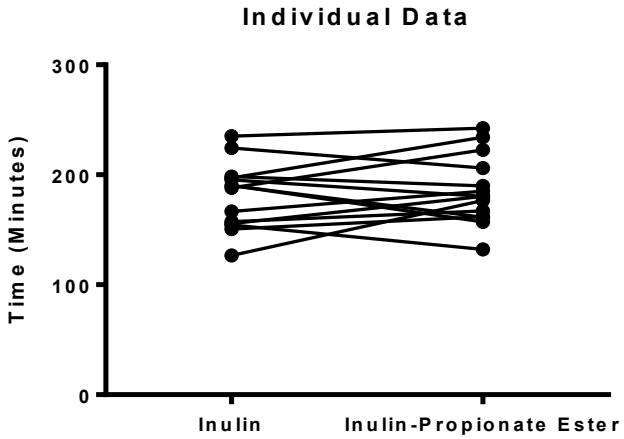


Figure 4.5: 10g of IPE does not significantly affect gastric emptying.
Individual data for time taken for gastric emptying with IPE or inulin. Plot shows the time taken in minutes for gastric emptying with Inulin-propionate ester or inulin control as measured by the half-life of the recovery of ^{13}C. (n=14) Points show values plotted for individuals.

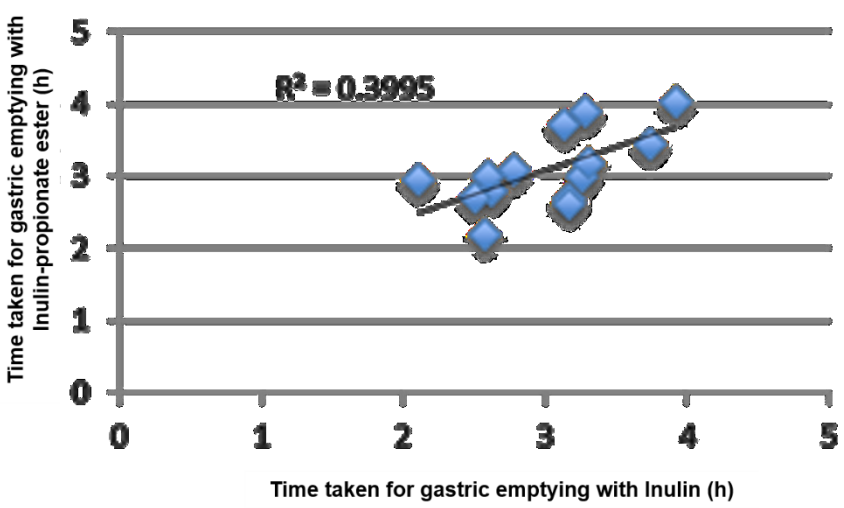


Figure 4.6: Time taken for gastric emptying does not differ between inulin and IPE.
Correlation of individual data for the time taken for gastric emptying with IPE with the time taken for gastric emptying with inulin. Scatter plot shows the time taken for gastric emptying with inulin plotted against the time taken for gastric emptying with IPE as measured by the half-life of the recovery of ^{13}C (n=14). P= 0.0153.

4.5.4 **Inulin-propionate ester significantly increases satiety**

Figure **4.7** shows the response curve for feelings of satiety in the two trials as well as mean group data for satiety. Subjects receiving IPE reported significantly ($p<0.05$) higher feelings of 'fullness'

compared to inulin. The mean AUC with inulin-propionate ester was 27572.7±1898 mm, whilst with inulin the mean AUC was 24295.7 ± 2554 mm.

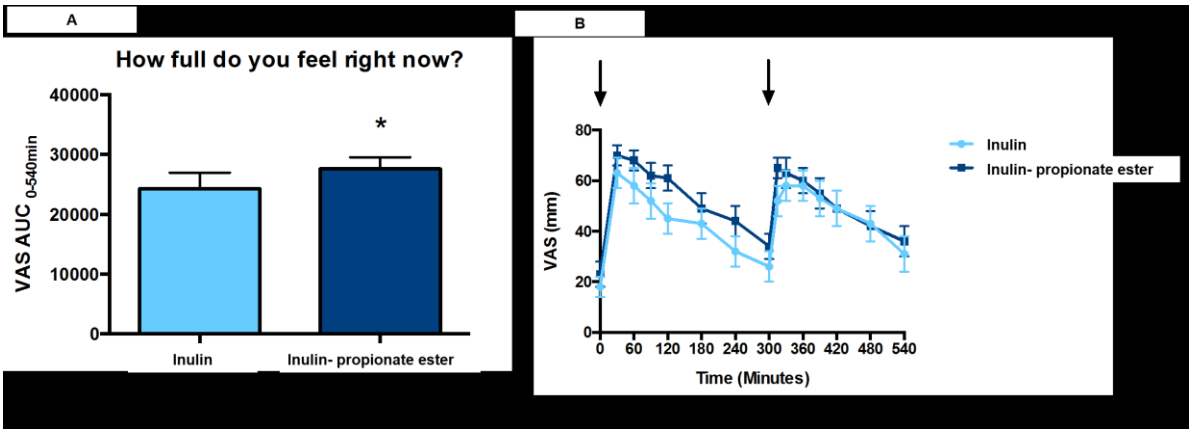


Figure 4.7: Inulin-propionate ester significantly increases satiety.
A) Bar chart shows mean AUC from 0 to 540 mins for VAS "How full do you feel right now?" for all subjects given IPE or inulin. Error bars show SEM. (n=14) (p= 0.044525). B) Fullness response curves for the two different trials as measured by VAS. Arrows show breakfast and lunch.

4.5.5 **Inulin-propionate ester significantly decreases appetite**

Figure **4.8** shows the response curve for the participants' desire to eat (appetite) and mean group data for appetite in the two trials. Participants' given Inulin-propionate ester showed a significantly ($p<0.05$) decreased 'desire to eat' compared to the control 'inulin' group. The AUC with Inulin-propionate ester was 17403.7 ± 2881 mm, while the AUC with inulin was 20043.7 ± 2873 mm.

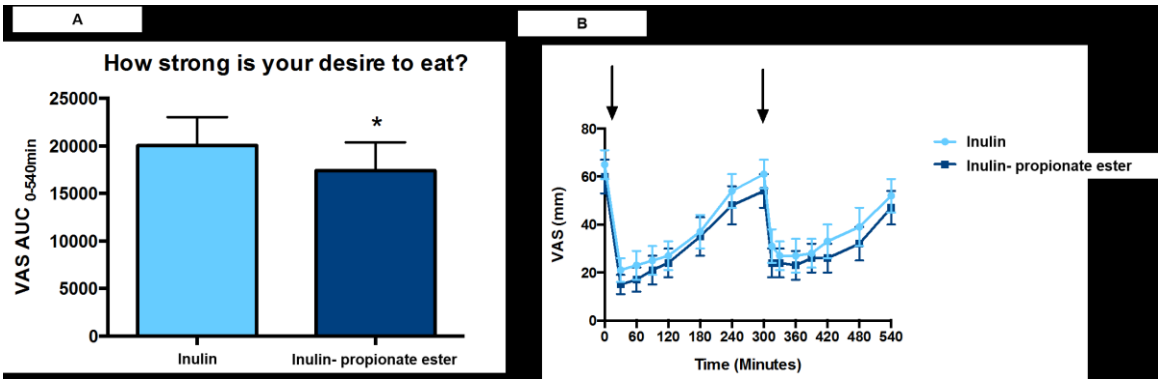


Figure 4.8: Inulin-propionate ester significantly decreases appetite.
A) Bar chart shows mean AUC from 0 to 540 mins for VAS "How strong is your desire to eat?" for all subjects given inulin-propionate ester or inulin. Error bars show SEM. (n=14) (p= 0.040168). B) Appetite response curves for the two different trials as measured by VAS. Arrows show breakfast and lunch.

4.6 Summary of results

In this chapter we have shown the effects of acutely supplementing humans with IPE on gastric emptying, appetite and satiety we found that:

- IPE significantly decreased appetite
- IPE significantly increased satiety
- IPE does not affect gastric emptying

4.7 Discussion

4.7.1 **Inulin-propionate ester does not affect gastric emptying**

We sought to understand the effect of IPE on gastric emptying in humans. We hypothesised that IPE would delay gastric emptying in this study because increasing colonic propionate should increase levels of the gut hormones GLP-1 and PYY as we found and described in chapter 3 and may reduce the time taken for gastric emptying. Delayed gastric emptying would cause the activation of the chemoreceptor and mechanoreceptors of the GI tract which would then stimulate central changes to reduce appetite and reduce food intake (Yamada and Alpers, 2009). However, in this study we actually found a 10g dose of IPE to raise colonic levels of propionate did not delay gastric emptying.

In our study, IPE or inulin were given with breakfast and then coincident with the peak in GLP-1 and PYY as described in chapter 3, ^{13}C-octanoic acid was provided with lunch to measure the time taken for gastric emptying. However, there was no significant differences between IPE and control on the time taken for gastric emptying in this study.

Our findings were in disagreement with several previous studies looking at the effect of SCFAs or the gut hormones on gastric emptying. Specifically, in a human study, volunteers fed bread containing sodium propionate showed a significantly delayed gastric emptying measured by plasma levels of paracetamol (Liljeberg and Bjorck, 1996). Approximately 50 mmol of propionate was given orally in this study whereas in our study approximately 103.5 mmol was delivered to the colon suggesting the method of administration of propionate is crucial to any effect on gastric emptying. However, some evidence suggests that increasing SCFAs in the colon will slow gastric emptying. Pigs given an ileal infusion of mixed SCFAs showed a dose-dependent inhibition of GI motility (Cuche and Malbert, 1999). In a follow up study they found that SCFAs slow gastric emptying and at the same time PYY was increased suggesting the delaying of gastric emptying was caused by increased PYY rather than SCFAs alone (Cuche et al., 2000). It is important to note that this effect on gastric emptying was a mixture of SCFAs, so acetate and butyrate may affect gastric emptying independently of propionate.

If gut hormones increased in this study as in chapter 3, we would have also predicted delayed gastric emptying with IPE. In a study conducted in humans, different doses of PYY were given by

infusion and they found PYY delayed gastric emptying compared to control (Savage et al., 1987). Savage had 7 volunteers who were given high PYY, low PYY or a Saline infusion. The average time to empty the stomach was delayed in subjects who had received high PYY. In the chapter 3, the PYY plasma levels with IPE were 53.3 pmol/L, which is lower than in this study where the peak was 86 pmol/L. In addition, in a further study in 9 healthy humans, doses of GLP-1 given by infusion, also dose-dependently delayed the time taken for gastric emptying (Nauck et al., 1997). The different doses in this study achieved GLP-1 plasma levels between 20 to 60 pmol/L, similar to the increases in GLP-1 with IPE in chapter 3, suggesting this physiologically relevant dose should have had an effect on gastric emptying.

4.7.2 **Inulin-Propionate ester significantly increases subjective ratings of appetite and decreases satiety.**

In this study, we have shown that increasing colonic propionate by using 10 g of IPE significantly decreases appetite and increases satiety for the first time in humans. Appetite for a meal was significantly reduced from 0 to 540 mins after consuming IPE compared to control. In addition, there was significant increases in satiety with IPE compared to control. Together, these data suggest that increasing colonic propionate with 10 g of IPE can reduce appetite and increase satiety before reaching the colon as well as after reaching the colon.

A small amount of evidence for the effect of SCFAs on appetite exists, but the majority of this has been conducted in animals. In a study in mice, activation of areas involved in the brain regulation of appetite was significantly increased with fermentable CHO over a long term period. These effects on brain activity involved in appetite were accompanied by increased levels of SCFAs suggesting a direct effect of SCFAs on appetite (Anastasovska et al., 2012). Additionally, mice given acetate show a reduced activation in central brain areas involved in appetite regulation (Frost et al., 2014). Furthermore, FCs have been linked to increasing SCFA levels, and in a human study where subjects received oligofructose over a period of 8 weeks subjective appetite was reduced significantly compared with control (Daud et al., 2014). Similarly, in another study, subjects received oligofructose over a period of two weeks and showed an increased satiety compared to control (Cani et al., 2005).

We anticipated IPE to increase levels of the gut hormones GLP-1 and PYY as we found and described in chapter 3. Both PYY and GLP-1 have been linked to an increased satiety and reduced appetite. Human infusion of GLP-1 significantly increases satiety (Flint et al., 1998).

4.7.3 **Limitations of this work**

We used the 13C-octanoic acid breath test to measure gastric emptying which has been identified as the most suitable, safest method to measure the gastric emptying of solid food (Delbende et al., 2000).In this study we found that using 10g of IPE to raise colonic levels of propionate significantly increased satiety and decreased appetite. However, this was not accompanied by delayed gastric emptying. It is important to note that the time of measuring gastric emptying is always an estimation, as the start of gastric emptying varies between individuals. To conclusively rule out an effect of IPE on gastric emptying it will be important to give IPE chronically, to non-healthy individuals and in larger sample sizes to confirm IPE does not affect gastric emptying.

4.7.4 **Final conclusions**

In this study we found that using 10g of IPE to raise the colonic levels of propionate significantly increased satiety and decreased appetite. However, this was not accompanied by delayed gastric emptying. As a delay in gastric emptying does not explain the decreased appetite and reduced energy intake (chapter 3), the next chapter will explore whether 10g of IPE can affect appetite through modulating energy expenditure and fat oxidation, as SCFA receptor activation has been implied in these mechanisms (Inoue et al., 2014).

Chapter 5 Exploring the effect of increasing colonic propionate on central regulation of appetite

5.1 Background

The central regulation of appetite was described in chapter 1. In addition, the relationship between SCFAs and the gut hormones PYY and GLP-1, and evidence of their involvement in appetite and energy regulation through modulation of the central nervous system from several studies was also described.

Appetite and energy homeostasis are strictly controlled in healthy individuals by a complex relationship between the gastrointestinal tract and the central nervous system. This is more commonly known as the gut-brain axis (Dockray, 1988). This complex relationship between the brain and the gut is bidirectional, with the gastrointestinal tract influencing brain control of satiety and vice versa. This constant bidirectional communication allows strict control of energy homeostasis in the brain, predominantly in the hypothalamus, brainstem and vagus nerve.

We are interested to understand the mechanism of increasing colonic propionate in central anticipatory food behaviour. In a long-term study over 6 months where participants received IPE every day, individuals in the IPE group showed reduced weight and adipose gain compared to the control group. However, no changes in the levels of the gut hormones GLP-1 and PYY were detected (Chambers et al., 2014). However, in chapter 3, acute supplementation of propionate significantly increased the plasma levels of the gut hormones GLP-1 and PYY whilst significantly reducing food intake. Taken together, this suggests differential effect of acute and long term supplementation on the gut hormones. It is clear that propionate has beneficial short and long term effects on food intake and weight modulation and this lead us to speculate that propionate may alter food intake through modulating brain control of appetite reward.

In order to understand activity in the brain and changes in appetite, the non-invasive technique of fMRI is used to measure activity in relevant brain areas under specific conditions. Levels in neural activity in response to treatments and in reaction to different tasks can be detected by the BOLD signal which detects blood flow, volume and oxygen consumption, with more activity in a brain

region showing a higher BOLD signal. When studying central regulation of appetite, BOLD signal in the hypothalamus, brainstem and other areas involved in appetite regulation can be mapped in response to non-food images or smells and to food images or smells. In addition, changes in regions of the brain implicated in response to food reward can also be analysed, for example, the striatum, amygdala and prefrontal cortex (Table 5.1). fMRI has been used to find out the areas in the brain that change in activation when food has been used as stimuli, it is likely the activation of these areas is important in controlling food intake (Van Vugt, 2010). These brain regions implicated in control of food intake and reward can be seen in Table 5.1. Several studies have used pictures of food as a stimulus to study these areas in the brain by using fMRI (Killgore et al., 2003, Beaver et al., 2006). By using control pictures and food pictures the food-induced BOLD activation can be calculated. Many factors have been shown to affect the BOLD response upon presentation of food pictures. Reward- responsiveness of food can determine the level of BOLD activation, for example, high calorie and palatable food is deemed more rewarding than low calorie and bland foods (Killgore et al., 2003, Beaver et al., 2006). In addition, chronic and acute nutritional state can affect BOLD activation; volunteers in the fasted state have higher activation than those in the fed state (Führer et al., 2008, Goldstone et al., 2009, LaBar et al., 2001, Siep et al., 2009). Differences in BOLD activation of appetite response areas of the brain are also found between obese and lean individuals (Rothemund et al., 2007, Stoeckel et al., 2008).

Table 5.1: Regions of the brain implicated in the non-homeostatic control of food intake and their identified roles.
Adapted from (Berthoud, 2006, Neary and Batterham, 2010).

Brain region	Abbreviation	Role	Projections to
Amygdala	AMY	Active in response to visual food stimuli or test food stimuli, also involved in memory, learning and emotion.	OFC, VS, DS, ACC, VTA, NAc, LH
Ventral striatum/ Nucleus Accumbens	VS/NAc	Roles in opioid and endocannabinoid signalling in reward. Activation increases with fasting and increased motivation.	VP, VTA
Orbitofrontal cortex	OFC	Associated with the taste and smell of food. Also involved in learning and behaviour.	AMY, INS, HIP
Ventral pallidum	VP	Roles in food pleasure and motivation.	OFC, PFC
Ventral Tegmental area	VTA	Associated with motivation and behaviour. Lots of dopaminergic neurons present in this area.	NAc, PFC, AMY, HIP
Anterior cingulate cortex	ACC	Responds to stimuli involved in changing behaviour. This usually occurs simultaneously with OFC.	AMY, NAc, Hypothalamus, INS, OFC
Dorsal Striatum (Caudate, Putamen)	DS	Associated with the motivation to eat.	DLPFC, PFC INS
Hippocampus	HIP	Associated with recall memories. Damage in this area can increase food intake.	NAc, AMY
Insula Cortex	INS	Associated with food reward and craving. Damage in this area reduces addiction	ACC, OFC, NAc, AMY
Pre frontal cortex	PFC	Associated with the control of feeding behaviours control and hedonism.	NAc, AMY, ACC
Dorsolateral prefrontal cortex.	DLPFC	Cognitively modulates feeding behaviours	NAc, AMY, ACC

The traditional view of appetite regulation highlights the importance of balanced anorexigenic and orexigenic signalling to maintain homeostasis. Any disruption in these signalling systems can lead to obesity by causing increased energy intake and weight gain. The gut hormones GLP-1 and PYY can modulate appetite by affecting anorexigenic and orexigenic signalling in the central nervous system. PYY and GLP-1 can reduce food intake by enhancing activity of POMC and CART neurons and reducing activity of NPY and AgRP neurons (Bewick, 2012). Furthermore, several studies in humans have shown that infusion of PYY and GLP-1 can reduce the BOLD signal in brain areas involved in food reward and energy homeostasis (De Silva et al., 2011). Modulating the brain response to food reward may be an effective target to reduce weight gain and appetite. In addition, the enteroendocrine L-cells can release PYY and GLP-1 (Tolhurst et al., 2012) which can influence activity in the brainstem, ARC and vagus nerve to also modulate energy homeostasis (De Silva et al., 2011).

As well as direct effects of GLP-1 and PYY on the central nervous system, SCFAs may have a direct effect on appetite regulation by affecting the central appetite response. In a study in rats given acetate, food intake was reduced, as well as, the signal intensity in the hypothalamus (Frost et al., 2014), however this study used manganese-enhanced MRI rather than fMRI. In addition, FC given to mice can regulate neuronal activity in appetite centres of the brain, likely through increasing levels of SCFAs in the colon (Anastasovska et al., 2012).

It is highly likely that there is a link between SCFAs and the gut hormones in order to affect central nervous system regulation of appetite, however little is known about the precise mechanisms underlying this. As demonstrated earlier, SCFAs can increase levels of the gut hormones PYY and GLP-1 and together these may modulate orexigenic and anorexigenic signalling to control energy intake. However, further research is required to understand the potential mechanisms behind this. In particular, the effect of increasing propionate to the colon using inulin-propionate ester on the human central nervous system needs to be understood.

5.2 Aims and Objectives

In this chapter we aim to investigate the effect of increasing levels of propionate to the human colon by using 10 g of inulin-propionate ester on:

- The BOLD signal in the Caudate, Nacc, OFC, Amygdala and Insula.
- Plasma levels of GLP-1, PYY, insulin, and glucose
- Subjective ratings of appetite, satiety and nausea
- Food intake

5.3 Hypotheses

I hypothesise that increasing amounts of propionate to the colon will:

- reduce the BOLD signal in the following brain areas implicated in food reward: Caudate, Nacc, OFC, Amygdala and Insula
- and reduce appetite and energy intake
- by increasing the gut hormones GLP-1 and PYY.

5.4 Methods

5.4.1 **Study Design**

This study was carried out in a single–blind, crossover manner, in two study visits, approximately a week apart. This study was conducted to compare 10g inulin-propionate ester to 10g inulin on brain responses to food stimuli. Subjects did not partake in strenuous exercise nor drink alcohol 24 hours before the study day and were asked to consume the same meal between 19:00 and 20:00 the evening before each study visit. They fasted overnight and arrived at Hammersmith hospital at 8:30am on each visit. Subjects were given chocolate milkshake and biscuits (574.5 kcal; 86.4g carbohydrate, 18.8g fat, 14.7g protein, 3.2g fibre) and inulin-propionate ester (10 g) in a standardised breakfast at the start of the study visit. A cannula was inserted into a forearm vein and baseline blood samples were collected at baseline and during the 300 min postprandial period (30, 60, 90, 120, 180, 240 and 300 min) to assess plasma concentrations of substrates and hormones. At 180 mins a snack was served to subjects – a cheese sandwich and biscuits (558 kcal; 62.3g carbohydrate, 24.9g fat, 21.7g protein, 2.8g fibre) After 300 min, volunteers had an fMRI assessment for 60 minutes. The fMRI was performed at 300 minutes because this coincided with

the peak in plasma levels of PYY and GLP-1 and also the time at which propionate was delivered to the colon (chapter 3). After the fMRI assessment, free access to a buffet meal was provided to volunteers to measure the food intake. Appetite and satiety was assessed by VAS every 60 min during the study visit and breath hydrogen concentration was measured at the same time. A schematic diagram showing the study design of the fMRI study can be seen in Figure **5.1**.

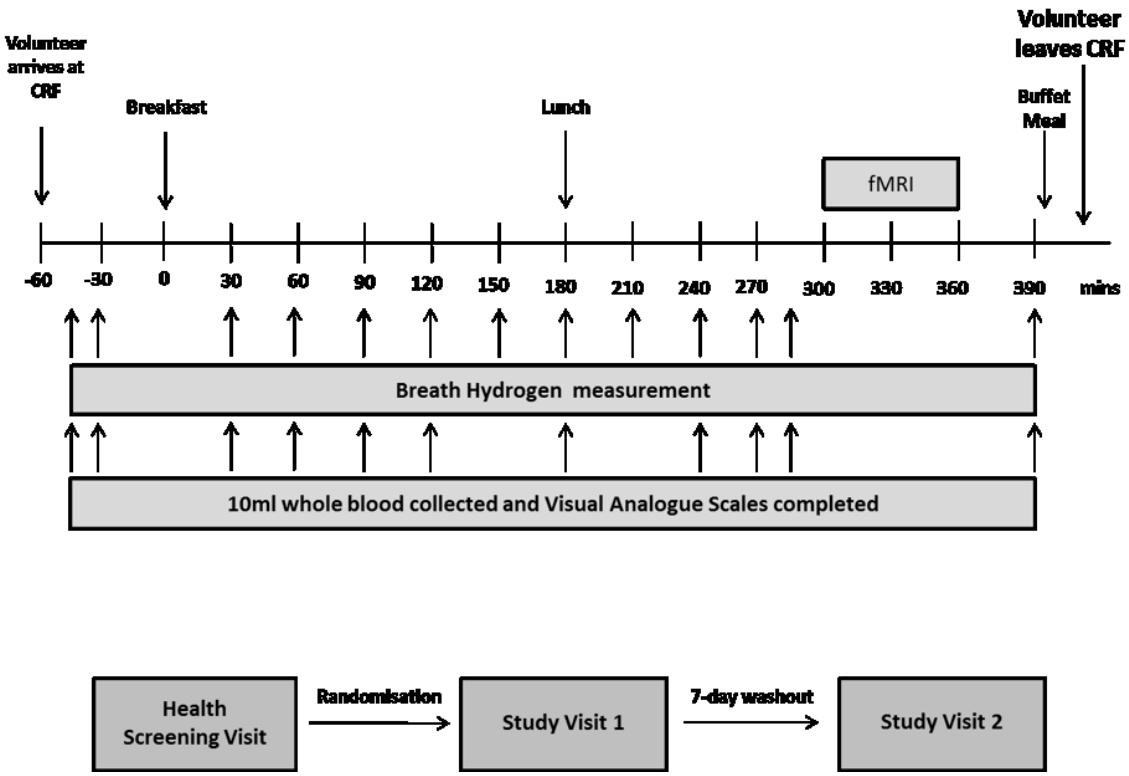


Figure 5.1: Schematic diagram showing the study design for the fMRI study.
In this study 20 healthy adult volunteers were recruited. This is a single blind randomised trial with 2 visits, a week apart. Subjects receive inulin-propionate ester or inulin with breakfast and then 3 hours later they receive lunch. Assessment of the gut hormones GLP-1 and PYY was made through blood tests and appetite was assessed by VAS as in previous investigations. 5h after breakfast volunteers have an fMRI scan to measure the level of Blood oxygen in appetite centres of the brain to identify if propionate reduces the signal compared to the control. After the fMRI scan, food intake is measured by giving them free access to food as in the first investigation.

5.4.2 **Power Analysis**

This is a pilot study in a new area and therefore a power calculation was not possible. However, similar studies using PYY and GLP-1 to investigate the appetite response in humans used 16 volunteers in a cross-over design (De Silva et al., 2011).

5.4.3 **Study Participants**

In this study 20 healthy male adult volunteers were recruited. Study participants were recruited via a public advertisement and volunteer database and selected according to the following criteria. The inclusion criteria were a body mass index (BMI) of 20 to 35 kg/m^2 and 18 to 65 years of age.

The exclusion criteria were: all men were right-handed, smoking, substance abuse, use of medications, a change in body weight > 3 kg in the previous 3 months, medical or psychiatric illness, female, gluten/lactose intolerance, vegan or vegetarianism, depression as assessed by Beck Depression Inventory II score >10 and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, thyroid function and liver function). All subjects provided informed, written consent prior to starting the study, which was approved by the Hammersmith and Queen Charlotte's Research Ethics Committee (08/H0707/99). The study was carried out in accordance with the Declaration of Helsinki. A flow diagram of recruitment to this study can be seen in Figure **5.2**.

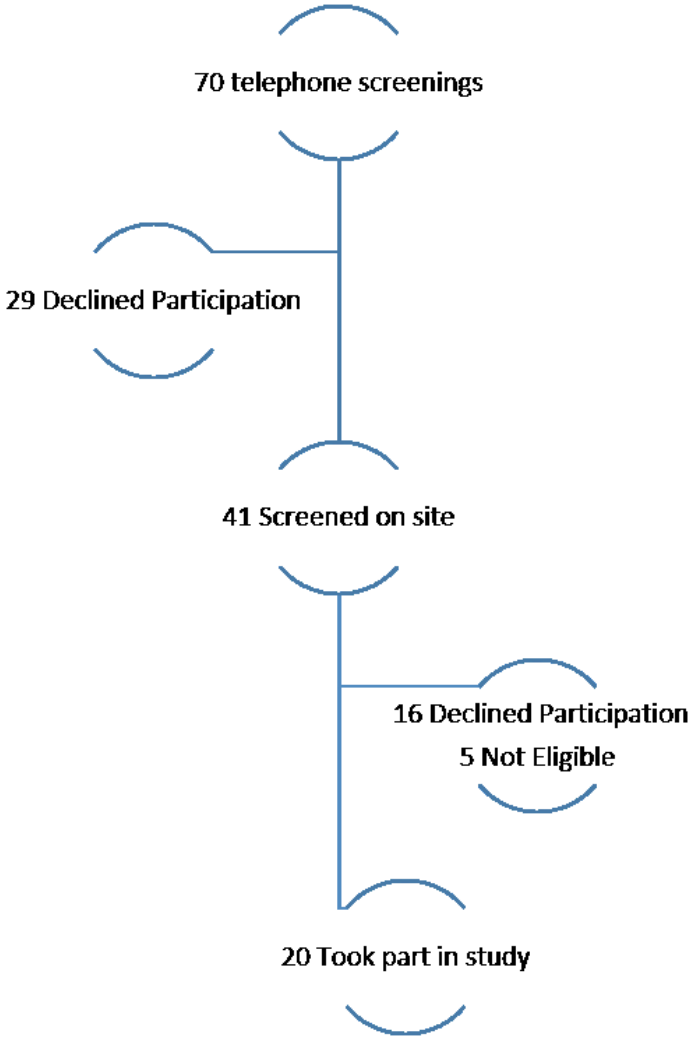


Figure 5.2: Schematic diagram showing the recruitment of participants to the central response study.
The number of telephone screenings, on site screenings, participants and exclusions throughout can be seen for this study.

5.4.4 **Measurement of central appetite response using fMRI**

fMRI scans were performed on the Philips 3.0 Tesla MRI scanner in the Clinical Imaging Facility at Hammersmith hospital. The fMRI scans identified any local changes in the ratio of oxygenated to deoxygenated haemoglobin which due to their varying paramagnetic properties alter the local BOLD signal in specific regions of brain activation implicated in food reward including the amygdala, caudate, insula, nucleus accumbens, orbitofrontal cortex and putamen which can be detected by magnetic resonance. In addition, anatomical T1-, T2- and DTI-weighted brain scans were collected to provide structural data on which to overlay the functional data. In the 1 hour scan subjects were asked to rest whilst doing no specific task, view a variety of different pictures (e.g. food, household objects, animals, blurred pictures as a baseline), and as a control undertake simple tests (e.g. viewing a 4Hz flashing checkerboard or fixation cross, pressing a button, reading, listening, speaking, recalling, thinking about words or numbers). Whilst viewing pictures subjects were asked to rate how 'appealing' the pictures were using a keypad held in their hands.

fMRI scanning protocol

All volunteers underwent an MRI scan as described before in these studies (Goldstone et al., 2014a, Scholtz et al., 2014) between 300 and 360 minutes of their study visit. First, pictures of animals were used for an initial practice followed by a resting state fMRI scan for 10 mins. At 320 mins subjects underwent a food picture paradigm followed by an auditory-motor-visual control (AMV) fMRI task at 350 mins. After this structural MRI brain scans including high-resolution T1-weighted scans for image registration and Whole-brain fMRI data with T2* weighted gradient-echo echoplanar imaging were collected.

***Food evaluation fMRI paradig*m**

During the food evaluation fMRI paradigm at 320 min. Subjects were presented with four types of colour photographs showed in a block design (all images displayed for 2500 ms and 6 pictures per block). The blocks were divided across 2 runs: (1) 60 high energy foods (e.g. chocolate and cakes), (2) 60 low energy foods (e.g. fish and vegetables), (3) 60 non-food related household objects (e.g. clothing and household items) and (4) 180 blurred images of the other pictures (as a low-level baseline) in blocks after every food or non-food block (Goldstone et al., 2014a, Scholtz et al., 2014).

As the images were presented in the scanner, volunteers were asked to rate how 'appealing' each picture was using a 5 button hand-held keypad (1=not at all appealing, 5=very appealing).

Auditory-Motor-Visual Control fMRI Paradigm.

The auditory-motor-visual task was used as a control to dismiss non-specific changes between visits in BOLD signal, as described before (Goldstone et al., 2014a, Scholtz et al., 2014). In this task, volunteers completed two of the following tasks at the same time: (1) listening to a story, (2) using their right index finger to tap every second, or (3) looking at a 4Hz colour blinking checkerboard.

Image Processing.

fMRI data was processed by using FEAT v6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl), including field map-based EPI unwarping, temporal derivative and motion parameters as co-variates in the general linear model, boundary based registration of EPI to high resolution structural space, and non-linear registration to standard space. Higher level analysis used a fixed effect model to combine the 2 runs to determine activation for the following comparisons: HE food to object and LE food to object. Similar analysis was performed for the single run AMV paradigm including the onsets of each task (auditory, motor and visual) to compare activation during performance of each task to when it was not being performed.

Whole Brain Analysis.

FEAT v6.00 was also used to analyse the whole brain data separately for the HE and LE food comparisons. A paired t-test to find out the specific regions with significant differences in BOLD signal between IPE and inulin control by using both cluster-wise correction family wise error (FWE) $Z>2.3$, $P<0.05$ and voxel-wise correction false discovery rate (FDR) $P<0.05$.

fMRI Regions of Interest.

In a previous separate cohort of 21 healthy human volunteers, functional ROIs were selected (Goldstone et al., 2014a). For HE or LE food compared to object: caudate, OFC, insula (anterior), amygdala and nucleus accumbens. For the control AMV task, the functional ROIs were the lingual gyrus for primary visual cortex (VIS); precentral gyrus for primary motor cortex (MOT); and superior temporal gyrus posterior division for secondary auditory cortex (AUD).

Comparison of fMRI Activation between Groups.

Within each region of interest (ROI) the average bilateral BOLD signal was determined for each individual volunteers for high energy (HE) and low energy (LE) comparisons at each visit in order to measure differences between IPE and inulin control. Similar analysis was carried out to compare activation in the related fROI between IPE and inulin in the AMV task.

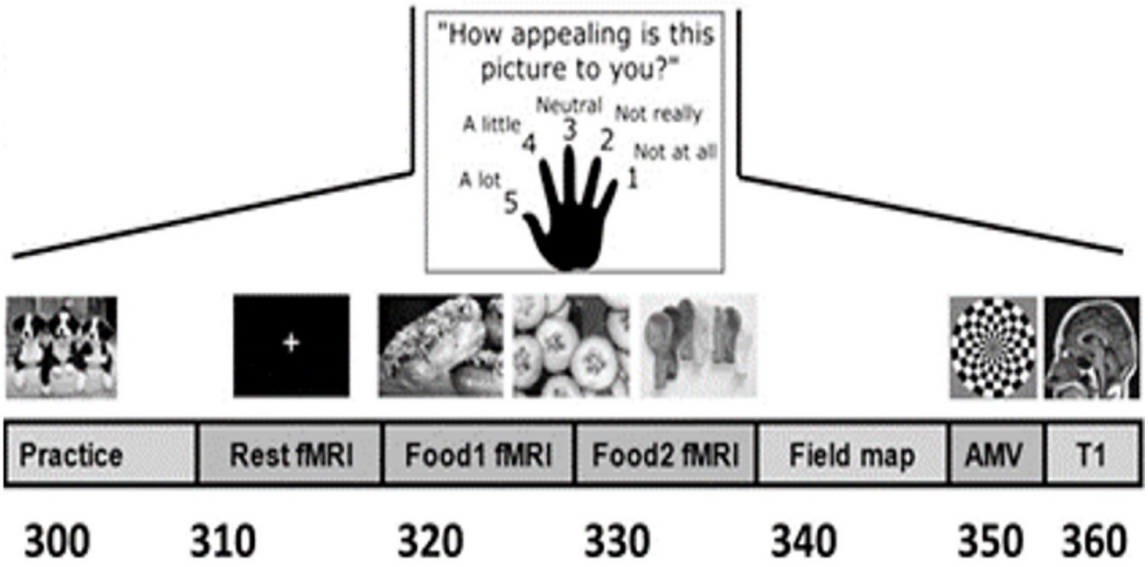


Figure 5.3: Schematic diagram of the fMRI scanning protocol.
The scan was performed from 300 to 360 mins. Subjects were first shown pictures of animals during a test run to acclimatise to the speed of the picture presentation and to use the 5 button hand-held keypad (1=not at all, 5=a lot). At 310 mins a resting fMRI scan was performed. At 320 mins subjects were presented with the food evaluation fMRI paradigm over two runs. The auditory-motor-visual fMRI task was performed at 350 mins and the scan was finished with a final T1 anatomical MRI scan

5.4.5 **Measurement of energy intake**

To investigate the effect of IPE or inulin on energy intake, free access to food via a buffet meal was provided 7 hours after the 10g of IPE or inulin was given. Subjects were told to eat until they felt comfortably full and the amount of food eaten was calculated by weighing the amount of food at the start and after the subject had eaten and calculating the difference.

5.4.6 **Intervention**

The IPE was developed by Dr Douglas Morrison from the University of Glasgow. The IPE is 3g of propionate bound to 7g of inulin via an ester linkage and delivers 2.36g of propionate directly to the colon (Chambers et al., 2014), as described in the methods chapter. The control in this study is 10g of inulin.

5.4.7 **Subjective ratings of appetite, satiety and nausea**

To investigate the effect of IPE or inulin on appetite, satiety and nausea, 100 mm VAS were used at several time points during the study visit as described in the methods chapter.

5.4.8 **Radioimmunoassays for GLP-1 and PYY**

To investigate the effect of IPE or inulin on the gut hormones GLP-1 and PYY blood samples were taken at several time points (-30,0, 30, 60, 90, 120, 180, 240, 300 and 360 min) after receiving a standardised breakfast with inulin control or IPE and collected as described in the methods chapter. Levels of GLP-1 and PYY were assessed by an in-house radioimmunoassay as described previously in the methods chapter.

5.4.9 **Radioimmunoassay for Insulin**

To investigate the effect of IPE or inulin on levels of insulin, blood samples were taken at several time points (-30, 0, 30, 60, 90, 120, 180, 240, 300 and 360 min) after receiving a standardised breakfast with inulin control or IPE and collected as described in the methods chapter. Levels of insulin in 50 μL serum were detected in the plasma by radioimmunoassay as described in methods chapter.

5.4.10 **Measurement of Glucose**

To investigate the effect of IPE or inulin on levels of glucose were measured using an automated clinical chemistry analyser - the Abbott Architect ci8200 analyser (Abbott Diagnostics, USA).

5.4.11 **Marker for fermentation of inulin**

Delivery of the IPE or inulin control to the gut was measured by using a breath hydrogen test as described in the methods chapter.

5.4.12 **Statistical Analysis**

fMRI data, food appeal and reaction times were analysed in a two-way repeated measures ANCOVA, including energy density of food pictures and treatment as within subject factors and visit order as a covariate. Breath hydrogen and food intake was analysed by paired t-test. Insulin, glucose, GLP-1, PYY, VAS were analysed by two-way ANOVA.

5.5 Results

5.5.1 **Study Participants**

In this study 20 healthy adult male volunteers were recruited. Characteristics of the volunteers are listed in Table 5.2.

Table 5.2: Characteristics of participants in the effect of inulin-propionate ester on central response study.

Participants' Characteristics	**Mean ± SD**
Age (years)	45.6 ± 2.6
Weight (kg)	78.9 ± 6.6
BMI (kg/m^2)	25.2 ± 2.2
Gender	20 men

5.5.2 **Demonstration of the time fermentation begins**

Figure **5.4** shows the response curve for the production of breath hydrogen. After 240 min breath hydrogen increased significantly from the baseline value ($p<0.05$) with both inulin-propionate ester and inulin. This significant increase in breath H_2 is a marker for inulin-propionate ester and inulin being delivered to the colon and fermented.

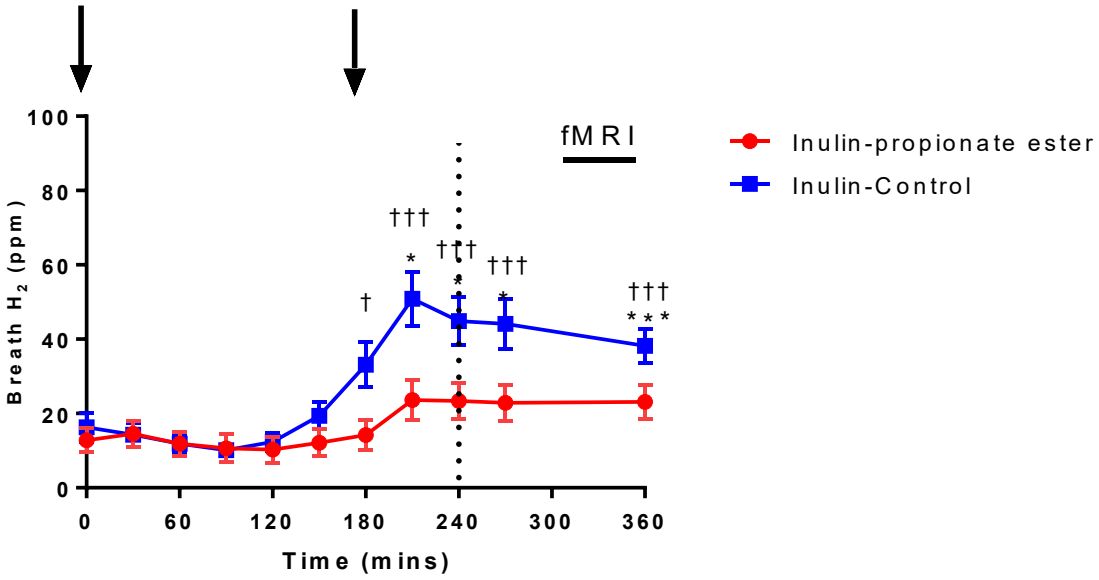


Figure 5.4: Breath hydrogen over time for subjects given inulin control or inulin-propionate ester.
At 240 min (dotted line) the breath hydrogen response in the propionate ester (red) and inulin (blue) trials identifies the arrival of the propionate or inulin at the colon. Breath hydrogen concentrations following inulin-control (†) or inulin-propionate ester (*) compared to baseline concentrations using paired samples t-tests (Calculations performed on normalised data): */† P<0.05, ***/††† P=0.005, n=20.

5.5.3 **Inulin-Propionate ester significantly reduces the BOLD signal in the caudate and in the Nucleus Accumbens**

20 healthy subjects were given 10 g of IPE or inulin control in this double-blind, randomised, crossover trial. BOLD signal after the IPE or inulin control was measured by fMRI 5 hours after the supplement was given. For the fMRI analysis, 2 subjects were removed due to severe motion in the scanner preventing an accurate analysis, leaving 18 subjects in the fMRI analysis. In a two-way repeated measures ANCOVA, including energy density (ED) of food pictures and treatment as within subject factors and visit order as a covariate, there was a significant ED x treatment interaction for BOLD signal in the caudate ($F(1,16)=8.86$, $P=0.009$, Bonferroni correction $P=0.045$ for multiple ROIs) and nucleus accumbens ($F(1,16)=10.81$, $P=0.005$, Bonferroni correction $P=0.040$) favouring HE foods, but not in the amygdala ($F(1,16)=1.65$, $P=0.22$), anterior insula ($F(1,16)=2.65$, $P=0.12$) or OFC ($F(1,16)=0.76$, $P=0.40$). We found that consumption of 10 g of inulin-propionate ester significantly reduced BOLD signal in the caudate in response to HE foods (effect size mean ± SEM [95% CI] -0.078 ± 0.032 [-0.147, -0.009], P=0.029), but not to LE foods (effect size -0.057 ± 0.037 [-0.134, 0.021], P=0.14) (Figure **5.5**., $p<0.05$). When healthy volunteers were given IPE the BOLD signal (%) was significantly lower (mean ± SD: 0.030 ± 0.121, P = 0.07)

compared to volunteers given the inulin control (0.118 ± 0.148) (Figure **5.6**, p<0.01**). Effect of treatment (p=0.43), effect of energy density (p=0.17), effect of treatment x ED p=0.005**.

In this ANCOVA analysis with visit order as a covariate, F indicates the test statistic, P indicates the significance value and the Bonferroni correction was used due to multiple testing of several brain regions to avoid false positives.

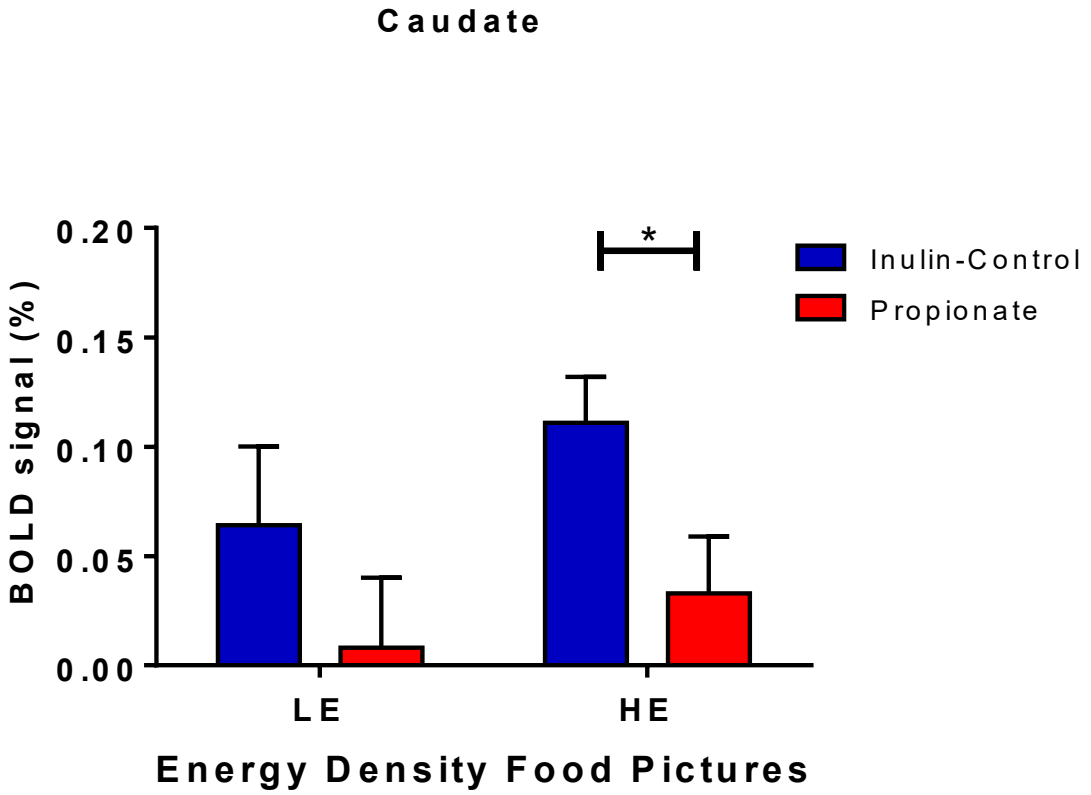


Figure 5.5: Effect of 10g Inulin-propionate ester vs. inulin (control) on the BOLD signal in the caudate.
Bar chart shows BOLD signal for subjects receiving inulin-propionate ester or control in response to pictures of High Energy or Low Energy foods (n = 18) (p= 0.045) Bars show mean ± SEM.

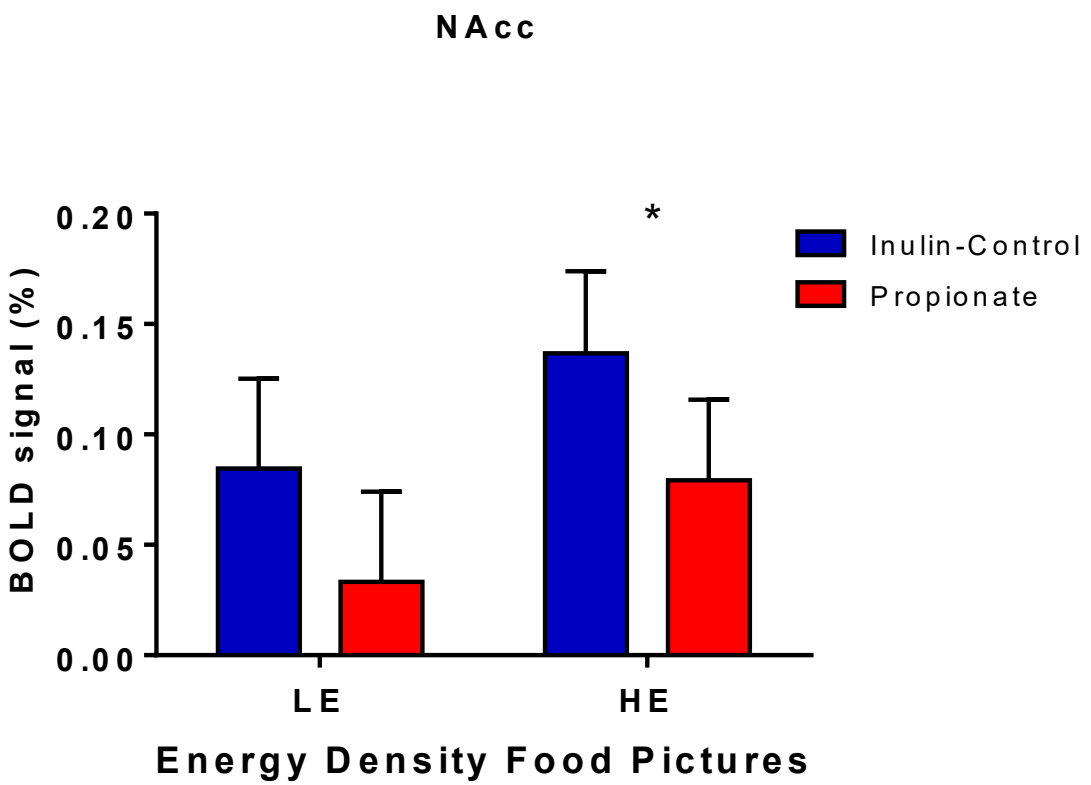


Figure 5.6: Effect of 10g inulin-propionate ester vs. inulin (control) on the BOLD signal in the Nucleus Accumbens (NAcc).
Bar chart shows BOLD signal for subjects receiving inulin-propionate ester or control in response to pictures of High Energy or Low Energy foods control (n = 18) (p= 0.04) Bars show mean ± SEM.

5.5.4 **Inulin-Propionate ester does not affect the BOLD signal in the amygdala, insula or OFC.**

No significant effects of inulin-propionate ester on BOLD signal were found in the amygdala ($F(1,16)=1.65$, $P=0.22$), anterior insula ($F(1,16)=2.65$, $P=0.12$) or OFC ($F(1,16)=0.76$, $P=0.40$).

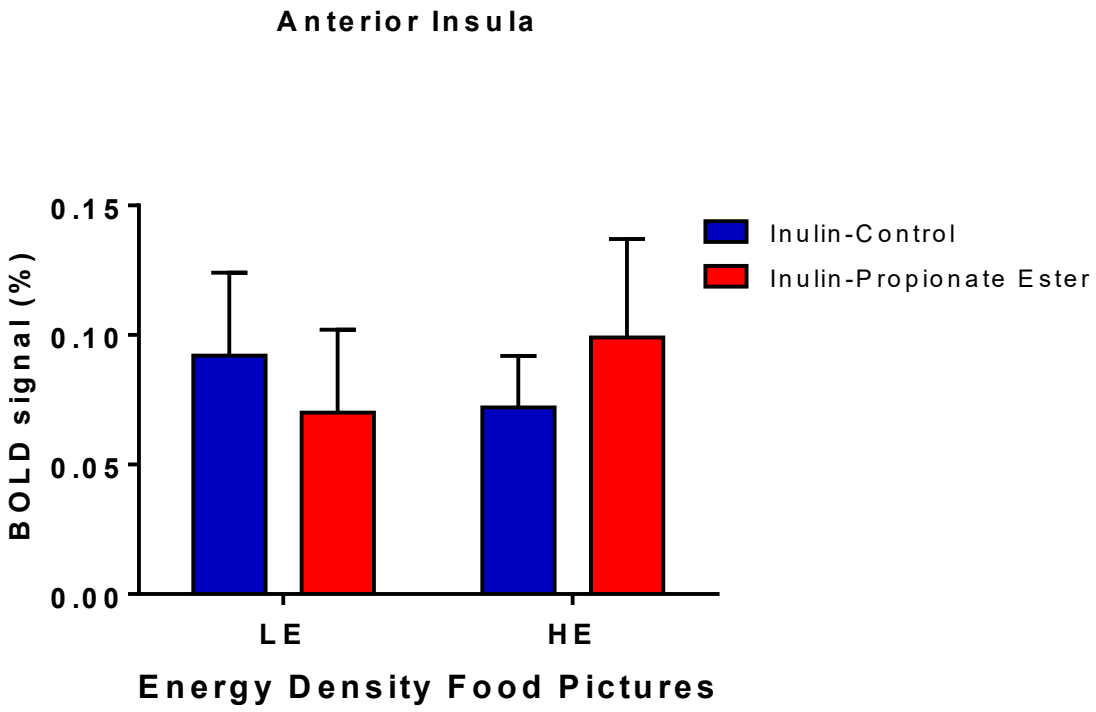


Figure 5.7: Inulin-Propionate ester does not affect the BOLD signal in the Insula.
Bar chart shows BOLD signal for subjects receiving inulin-propionate ester or control in response to pictures of High Energy or Low Energy foods control (n = 18) Bars show mean ± SEM. p= 0.12.

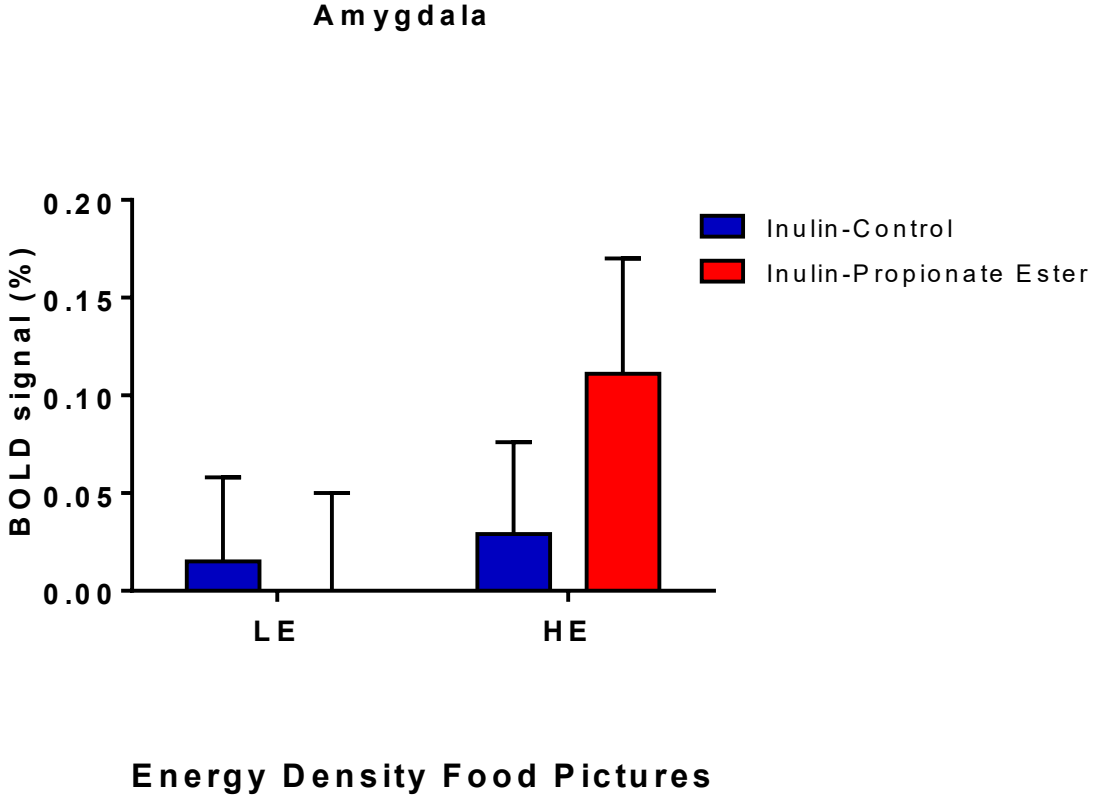


Figure 5.8: Inulin-Propionate ester does not affect the BOLD signal in the Amygdala.
Bar chart shows BOLD signal for subjects receiving inulin-propionate ester or control in response to pictures of High Energy or Low Energy foods control (n = 18) Bars show mean ± SEM. p= 0.22.

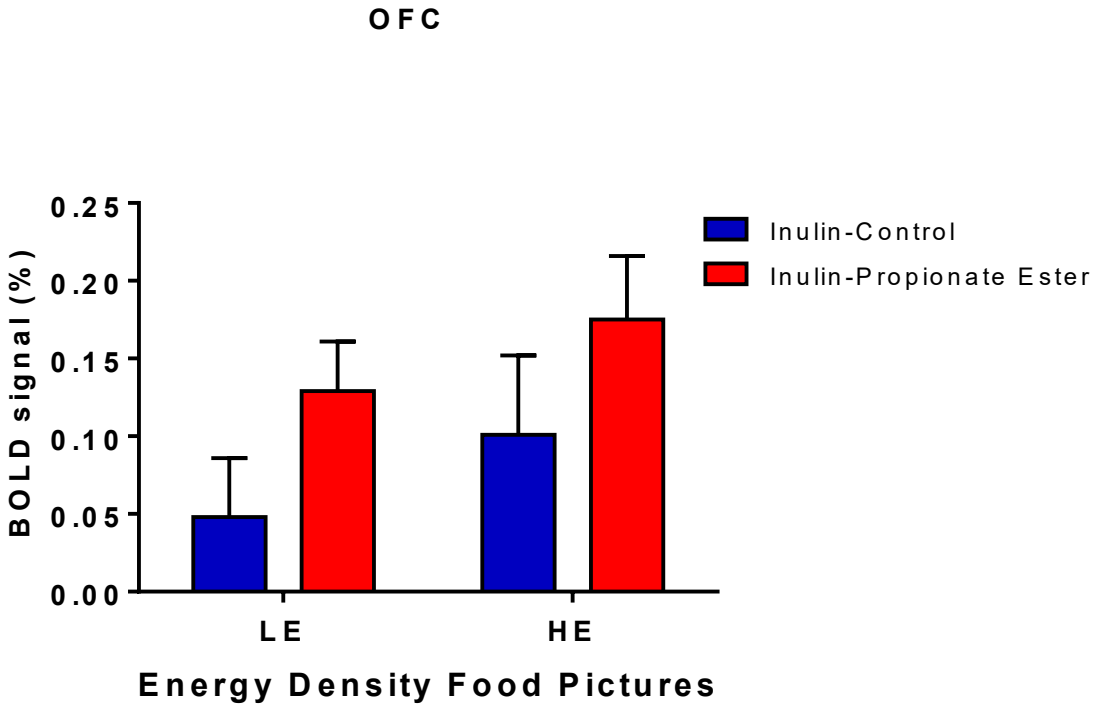


Figure 5.9: Inulin-Propionate ester does not affect the BOLD signal in the Orbitofrontal cortex.
Bar chart shows BOLD signal for subjects receiving inulin-propionate ester or control in response to pictures of High Energy or Low Energy foods control (n = 18) Bars show mean ± SEM. p= 0.40.

5.5.5 **The effect of inulin-propionate ester on food appeal rating and reaction time.**

There was a significant energy density x treatment interaction for food appeal ratings. This effect was larger for HE foods ($F(1,16)=5.50$, $P=0.032$, Figure **5.10**). After receiving IPE, foods were rated significantly less appealing compared to control ($F(1,16)=4.69$, $P=0.046$, Figure **5.10**).

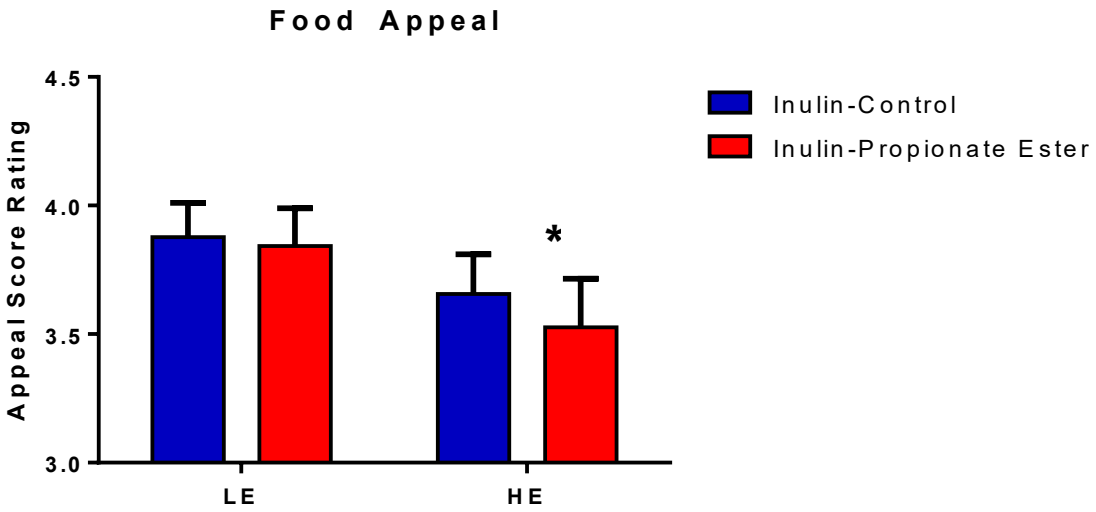


Figure 5.10: Inulin-Propionate ester significantly reduces the food appeal rating of food pictures.
Bar chart shows the appeal score rating for subjects receiving Inulin-propionate ester or inulin as control. (n = 16) Bars show mean ± SEM.

Independent of energy density of food pictures, IPE treatment significantly increased the reaction time to food pictures compared to control ($F(1,16)=13.82$, $P=0.002$, Figure **5.11**). Additionally, the reaction time for HE food pictures was significantly increased after receiving IPE ($F(1,16)=14.54$, $P=0.002$, Figure **5.11**).

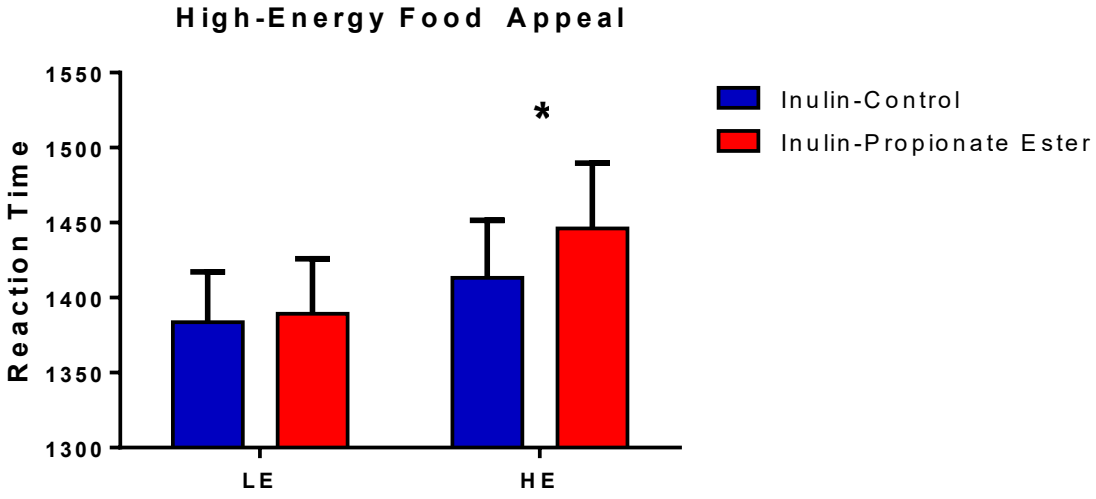


Figure 5.11: Inulin-Propionate ester significantly increases the reaction time to food pictures.
Bar chart shows the reaction time for subjects receiving Inulin-propionate ester or inulin as control. (n = 16) Bars show mean ± SEM.

5.5.6 **Inulin-Propionate ester significantly reduces food intake**

For the analysis of the food intake data, five volunteers were excluded because they ate all of the food provided to them in either one or both of the trials. Food intake after the IPE or inulin control was measured by the provision of free access to an unlimited buffet meal 5 hours after the supplement was given, and told to eat until comfortably full. Inulin-Propionate ester significantly reduced energy intake by 9.5 ± 5.3 % (mean ± SEM). The mean food intake with Inulin-propionate ester was 711.1 ± 79.9 Kcal, whilst food intake with inulin was 810.4 ± 83.4 Kcal.

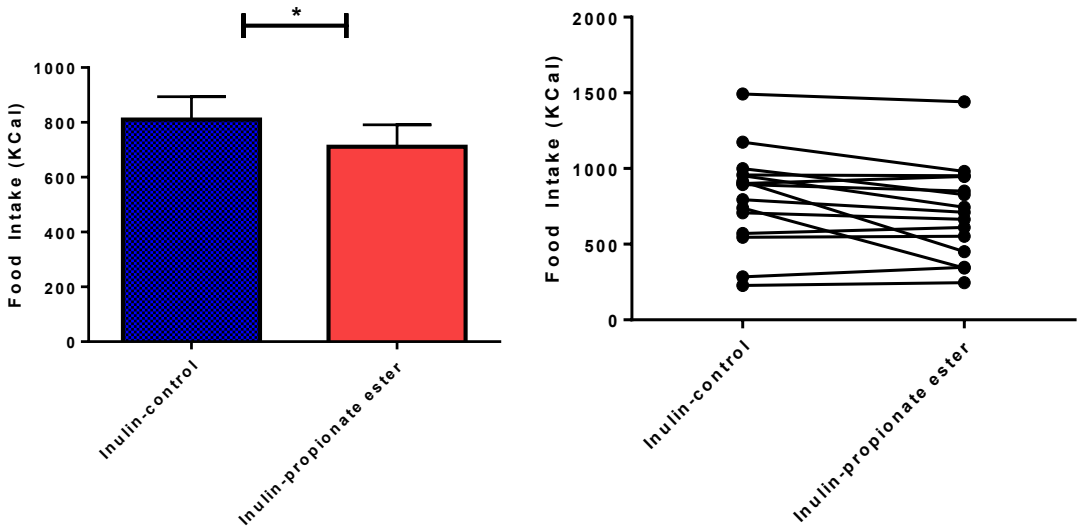


Figure 5.12: Inulin-Propionate ester significantly reduces food intake.
Bar chart shows food intake in Kcal for subjects receiving Inulin-propionate ester or control (n = 15) (p= 0.0302). Bars show mean ± SEM. Individual data is also shown for Inulin-control and inulin-propionate ester.

5.5.7 **Inulin-Propionate ester does not affect insulin levels**

We also sought to understand the effect of IPE on levels of insulin in humans. Blood samples were taken from the twenty volunteers every hour for 5 hours after 10 g of IPE or inulin control to detect levels of insulin. We found that consumption of 10 g of inulin-propionate ester does not affect levels of insulin. There was no significant difference in the mean ± SEM AUC $_{0\text{-}360\ min}$ for serum insulin, control was 11507 ± 1084 min x μU/L and with IPE was 11333 ± 879 min x μU/L (Figure **5.13**).

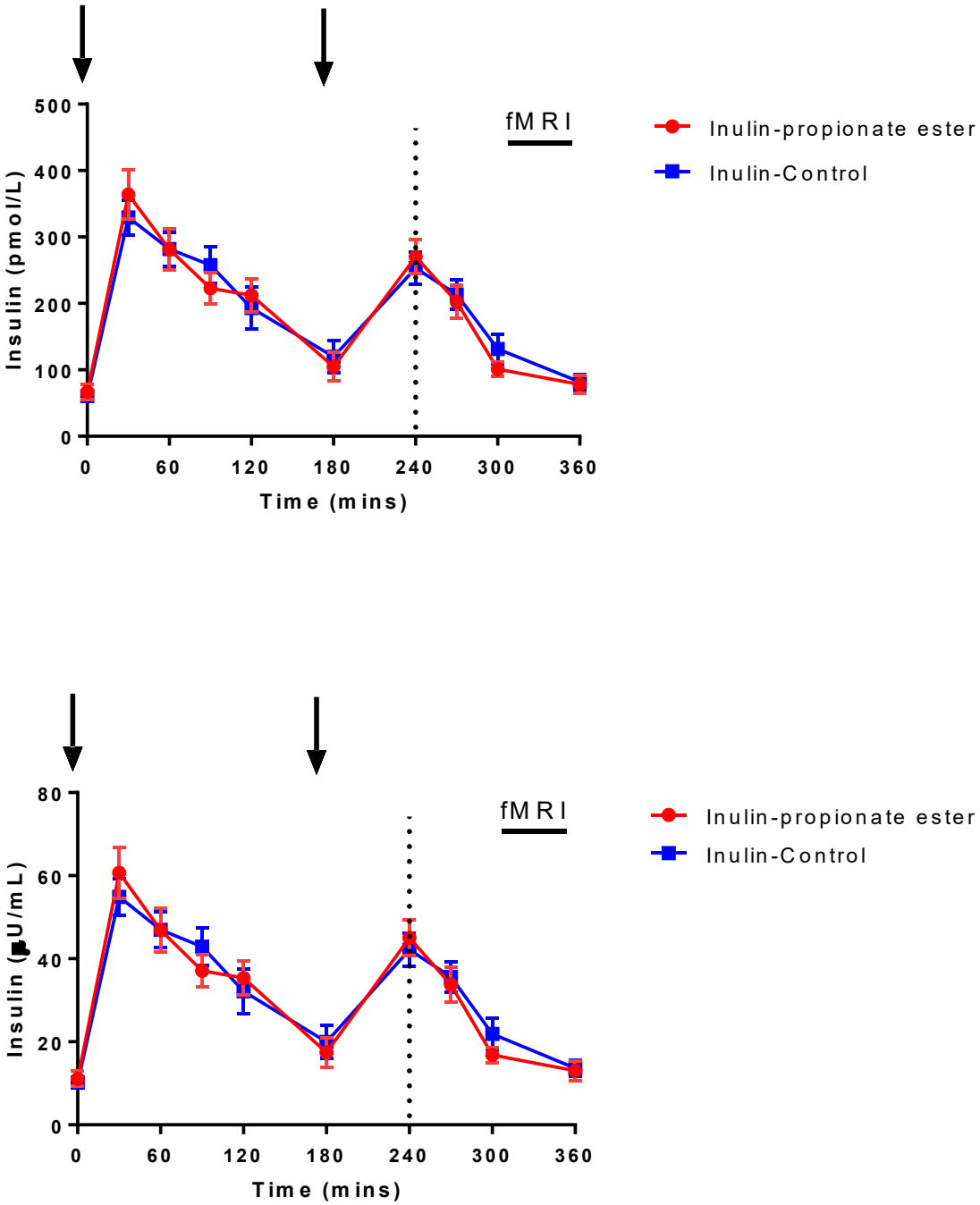


Figure 5.13: Inulin-Propionate ester does not affect insulin levels.
Line graph shows plasma levels of insulin (µU/mL) over time (n = 20) (p= 0.786). Arrows indicate the timings of the standardised breakfast (0 min) and snack (180 min). 10 g control or 10 g inulin-propionate ester were provided with breakfast at 0 min. Data show mean ± SEM (p>0.05). The dotted line shows when IPE and inulin were fermented in the colon demonstrated by increases in breath hydrogen. From 300 min until 360 min the fMRI scan was conducted.

5.5.8 **Inulin-Propionate ester does not affect glucose levels**

We also sought to understand the effect of IPE on plasma glucose levels in the volunteers. Again, blood samples were taken from the twenty volunteers every hour for 5 hours after 10 g of IPE or inulin control to detect levels of glucose. We found that consumption of 10 g of inulin-propionate

ester does not significantly affect levels of glucose. There was no significant difference in the mean ± SEM AUC $_{0\text{-}360\ min}$ for plasma glucose, control was 1955 ± 58 min x mmol/L and with IPE was 1971 ± 48 min x mmol/L (Figure **5.14**).

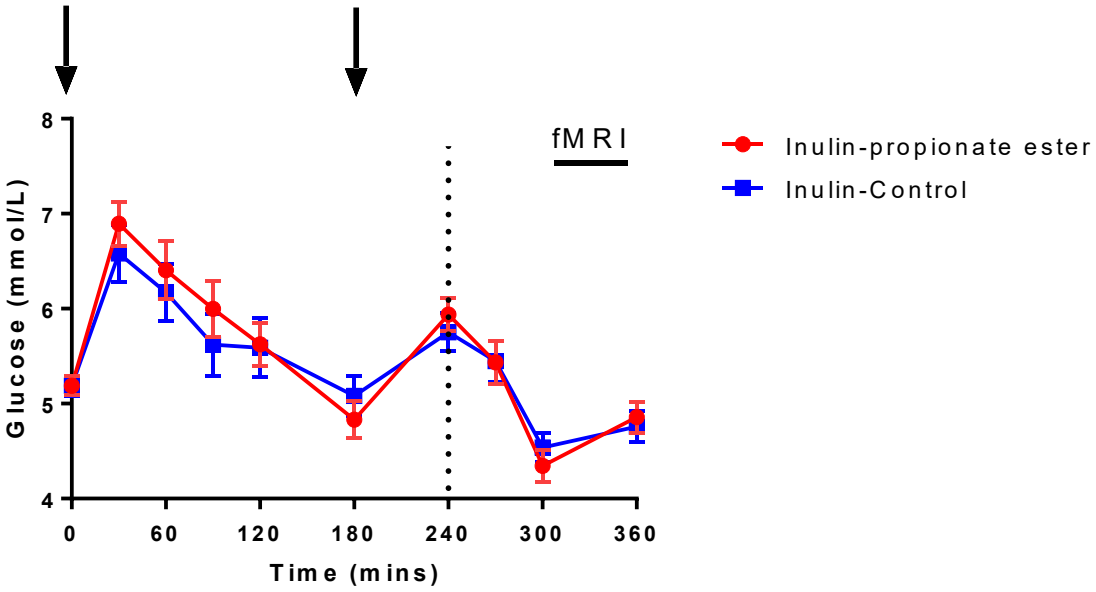


Figure 5.14: Inulin-Propionate ester does not affect glucose levels.
Line graph shows plasma levels of glucose (mmol/L) over time (n = 20). Arrows indicate the timings of the standardised breakfast (0 min) and snack (180 min). 10 g control or 10 g inulin-propionate ester were provided with breakfast at 0 min. Data show mean ± SD (p= 0.2576). The dotted line shows when IPE and inulin were fermented in the colon demonstrated by increases in breath hydrogen. From 300 min until 360 min the fMRI scan was conducted.

5.5.9 **Inulin-propionate ester does not affect GLP-1 and PYY levels**

We found that consumption of 10 g of propionate ester does not affect plasma levels of GLP-1 and PYY. Mean GLP-1 levels ± SEM for ΔAUC180-360min were 14588.0 ± 1011.2 min × pmol/L with control compared to 14428.5 ± 1135.4 min × pmol/L with the inulin-propionate ester (P>0.05) between 180-360 min and the AUC from 0-360 min was also not significant between treatments (Figure **5.15**). Mean PYY levels ± SEM for ΔAUC180-360min were 9833.2± 610.2 min × pmol/L with control compared to 9683.7 ± 683.0 min × pmol/L with the inulin-propionate ester (P>0.05) between 180-360 min and the AUC from 0-360 min was also not significant between treatments (Figure **5.16**).

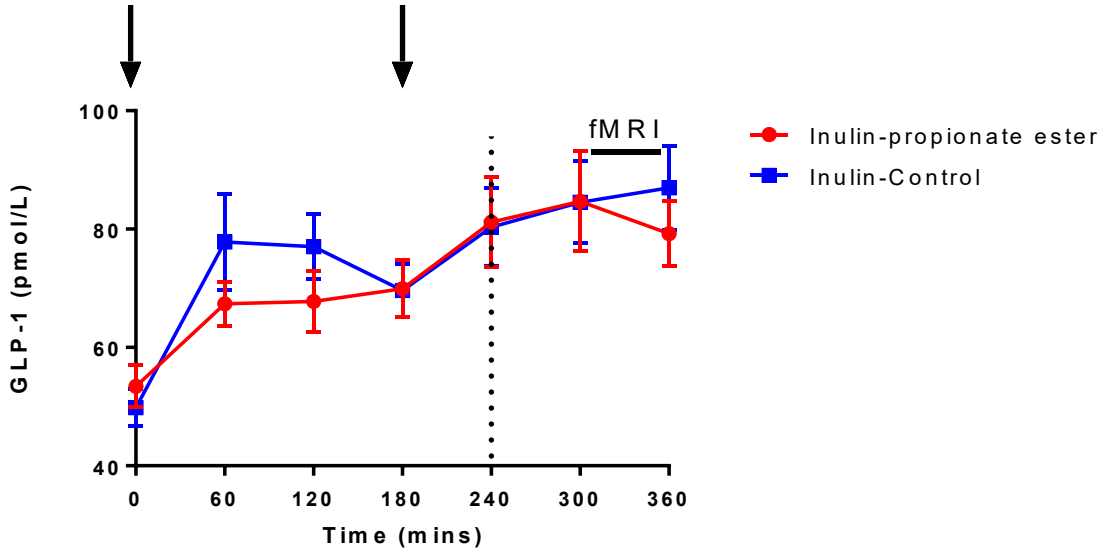


Figure 5.15: Effect of 10g IPE vs. inulin (control) on levels of GLP-1 in healthy humans. Line graph shows plasma levels of GLP-1 (pmol/L) over time (n = 20). Arrows indicate the timings of the standardised breakfast (0 min) and Snack (180 min). Red shows IPE, blue shows inulin control. (n = 20) (p= 0.1884). Bars show mean ± SEM. The dotted line shows when IPE and inulin were fermented in the colon demonstrated by increases in breath hydrogen. From 300 min until 360 min the fMRI scan was conducted.

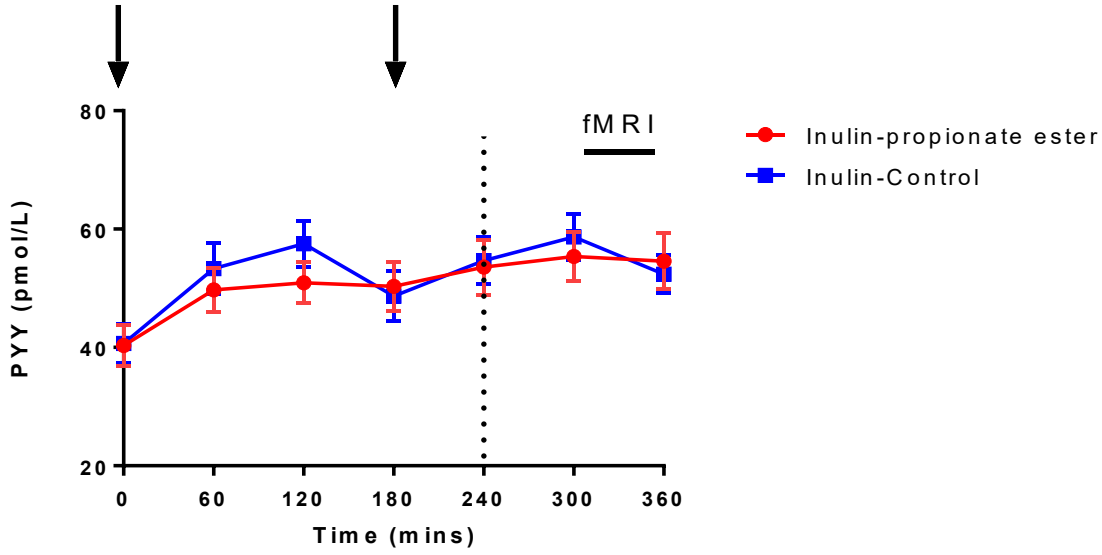


Figure 5.16: Effect of 10g IPE vs. inulin (control) on levels of PYY in healthy humans.
Line graph shows plasma levels of PYY (pmol/L) over time (n = 20). Arrows indicate the timings of the standardised breakfast (0min) and snack (180min). Red shows IPE, blue shows inulin control. (n = 20) (p= 0.2264). Bars show mean ± SEM

5.5.10 **Inulin-Propionate ester does not affect subjective ratings of appetite or satiety**

We found that consumption of 10 g of inulin-propionate ester does not affect levels of satiety and appetite as measured by responses to the questions – “How hungry do you feel right now?”, “Do you have a desire to eat?”, “How full Do You feel right now?”.

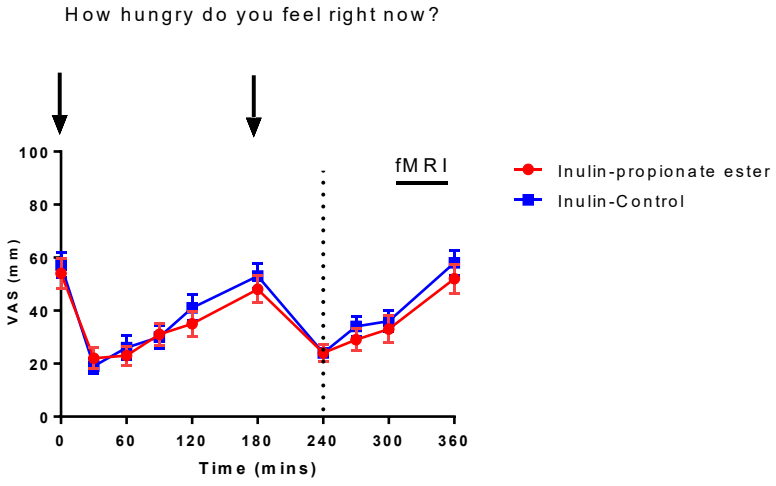


Figure 5.17: Increasing colonic propionate does not affect subjective ratings of appetite and satiety.
Graph shows hunger ratings made using 100 mm VAS with ends of the scale signposting extremes (e.g. 0 mm equalling not at all hungry and 100 mm being extremely hungry). Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as mean ± SEM (n=20). Blue is control, red is inulin-propionate ester (p=0.1909).

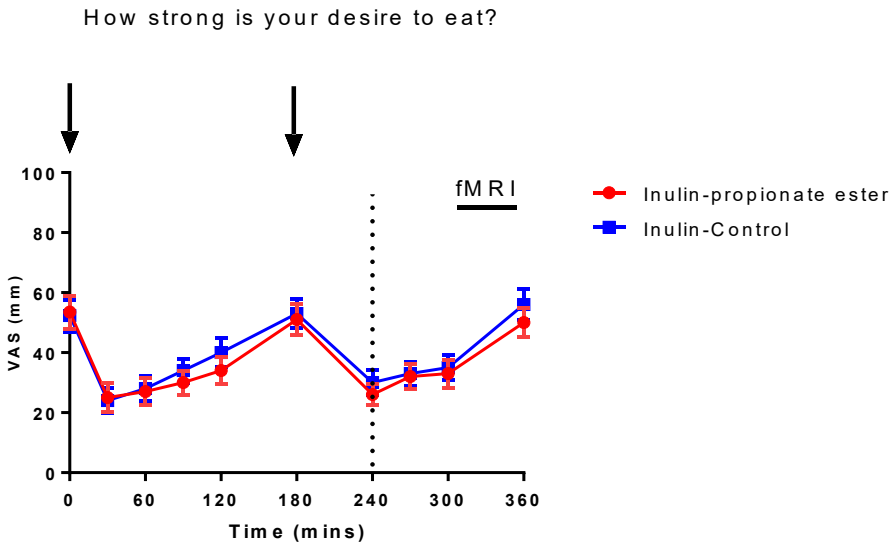


Figure 5.18: Increasing colonic propionate does not affect subjective ratings of appetite and satiety.
Graphs show responses to "How strong is your desire to eat?". Ratings were made using 100 mm visual analogue scales (VAS), with ends of the scale signposting extremes (e.g. 0 mm equalling not at all full and 100 mm being extremely hungry). Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as means ± SEM (n=20). Blue is control, red is inulin-propionate ester (p= 0.2522).

5.5.11 **Inulin-Propionate ester does not cause nausea**

We also sought to understand the effect of IPE on nausea in humans. VAS were used to measure nausea in the twenty volunteers every hour for 5 hours after 10 g of IPE or inulin control to detect any sickness associated with IPE. We found that consumption of 10 g of inulin-propionate ester does not affect levels of nausea in response to the question "do you feel sick?"

P= 0.8751

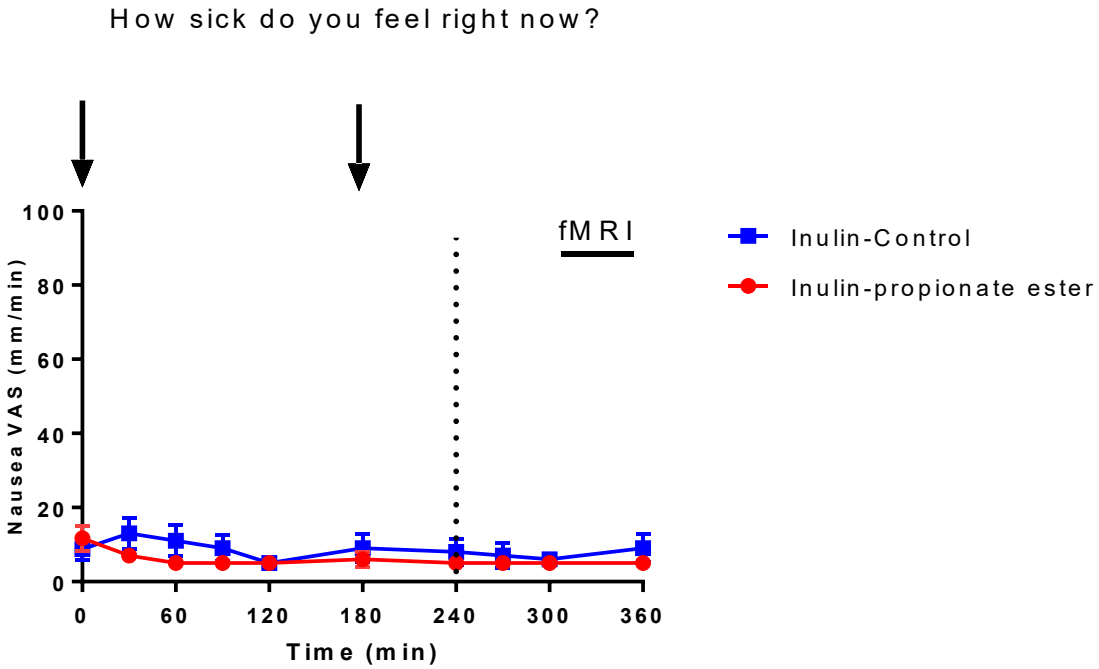


Figure 5.19: Increasing colonic propionate does not affect subjective nausea.
Graphs show responses to "How sick do you feel right now?". Ratings were made using 100 mm visual analogue scales (VAS), with ends of the scale signposting extremes (e.g. 0 mm equalling not at all full and 100 mm being extremely sick). Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as means ± SEM (n=20). Blue is control, red is inulin-propionate ester

5.6 Summary of Results

In this results chapter we have shown the effects of acutely supplementing humans with inulin-propionate ester on BOLD signal in food reward centres of the brain, food intake, levels of the gut hormones GLP-1 and PYY, nausea and insulin and glucose levels. We found that:

- Inulin-Propionate ester significantly decreased BOLD signal in the caudate and Nucleus Accumbens of the brain.
- Inulin-Propionate ester did not affect BOLD signal in the OFC, Amygdala and Insula.
- Inulin-Propionate ester does not affect plasma levels of PYY and GLP-1 in healthy humans in this study

- Inulin-Propionate ester does not affect subjective ratings of appetite or satiety in this study
- Inulin-Propionate ester significantly reduces energy intake.
- Inulin-Propionate ester does not significantly affect subjective ratings of nausea.
- Inulin-Propionate ester does not affect plasma levels of insulin and glucose.

5.7 Discussion

5.7.1 **Inulin-Propionate ester decreases the BOLD signal in the caudate and Nucleus Accumbens**

In this study we demonstrate for the first time in humans that increasing propionate in the colon by providing 10g of inulin-propionate ester with breakfast significantly decreases BOLD signal in the Caudate and Nucleus Accumbens, demonstrating for the first time that IPE can affect anticipatory food behaviour. This finding matched our original hypothesis that IPE would decrease the BOLD signal in regions of the brain involved in energy homeostasis, predominantly those involved in food reward.

Our findings extend from those of others in the field showing some of the effects of SCFAs on the central regulation of appetite and brain response to food reward. Rats given the SCFA - acetate showed reduced neuronal activity in the hypothalamus measured using manganese-enhanced MRI and reduced food intake (Frost et al., 2014). Furthermore, in a study in mice given a control or high fat diet with oligofructose or corn starch for 9 weeks, neuronal activity in the arcuate nucleus was significantly increased in the oligofructose-enriched diet compared to the group who received a corn starch diet (Anastasovska et al., 2012). In addition, this increased colonic propionate production suggests a reduced appeal of HE food pictures suggesting reduced wanting of food (Goldstone et al., 2014a, Finlayson et al., 2008). Originally, we postulated these alterations in brain appetite response to SCFAs may be a direct effect of the SCFAs or as a result of increases in the gut hormones GLP-1 and PYY. Indeed, we have shown that IPE increases GLP-1 and PYY in chapter 3 and several other studies have shown FC and SCFAs can increase the anorectic gut hormones PYY and GLP-1 (Cani et al., 2005, Delmée et al., 2006, Reimer et al., 2012, Cani et al., 2004, Tolhurst et al., 2012, Lin et al., 2012, Cherbut et al., 1998, Delzenne et al., 2002, Zhou et al., 2008, Keenan et al., 2006). However in this study, no changes in the gut hormones GLP-1 and PYY were observed, and the effects on the caudate and nucleus accumbens must be via other mechanisms.

Several studies have demonstrated that an increased intake of NDCs and increased production or delivery of SCFAs in the colon can reduce energy intake and alter body composition without altering levels of the gut hormones (Anastasovska et al., 2012a, So et al., 2007, Frost et al., 2014a). Therefore this opens the possibility that IPE acts by a different mechanism to alter the central response for appetite through altered BOLD levels in the caudate and nucleus accumbens as well

as the reduction in energy intake observed in this study. One way in which IPE may alter appetite could be through the hepatic portal vein. It is known that increasing propionate in the hepatic portal vein can decrease energy intake, so propionate may act here to alter anticipatory food behaviour (Anil and Forbes, 1980, Anil and Forbes, 1988). A second way in which increasing propionate colonic may alter neural activity in response to food reward is via vagal afferents. Particularly, stimulation of FFAR3 in the portal vein or in the gut may drive these changes in neural activity, without affecting GLP-1 and PYY (De Vadder et al., 2014, Lal et al., 2001, Kentish and Page, 2015). Finally, propionate may mediate these changes in the activity of the nucleus accumbens and the caudate through altering leptin signalling. Propionate can activate FFAR2 and FFAR3 in adipose tissue and stimulate the release of leptin (Xiong et al., 2004, Zaibi et al., 2010). Leptin can cross the blood brain barrier and increase anorexigenic signalling and decreased orexigenic signalling resulting in reduced appetite and reduced neural activity in response to food (Sahu, 2003). It is known that acetate can cross the blood brain barrier to directly affect the brain (Chambers et al., 2015) and there is also evidence that propionate can pass over the blood brain barrier (Conn et al., 1983) and it is highly likely that propionate may act by this or one of the mechanisms explained above to alter anticipatory food behaviour.

5.7.2 **Inulin-Propionate ester does not affect levels of PYY or GLP-1**

Unlike in chapter 3, PYY and GLP-1 levels were not altered in this study, despite inulin-propionate ester being given at the same time, and levels of PYY and GLP-1 being assessed over the same time period. However, a positive effect on energy intake and BOLD signal in appetite centres of the brain was observed suggesting that propionate acts via several mechanisms to alter energy homeostasis. In particular, evidence exists that an increased intake of NDCs and SCFAs in the colon can reduce energy intake and change the body composition without changing GLP-1 and PYY (Anastasovska et al., 2012a, So et al., 2007, Frost et al., 2014a). Further, as mentioned previously, effects of propionate on energy homeostasis may be mediated through altered hepatic portal vein activity (Anil and Forbes, 1980, Anil and Forbes, 1988) or through altering vagal innervation through stimulating FFAR3 in the portal vein or gut (De Vadder et al., 2014, Lal et al., 2001, Kentish and Page, 2015) or leptin signalling (Sahu, 2003). Despite PYY and GLP-1 increasing in the study presented in chapter 3, levels of PYY and GLP-1 were not significantly different between IPE and control in this study. It is plausible that the gut hormones did not increase because of stress levels associated with being in the fMRI scanner. To avoid this a dummy visit

should have been included prior to the study in order to familiarise the volunteers with the fMRI scanner and environment.

5.7.3 **Inulin-Propionate ester significantly reduces energy intake**

As we demonstrated in chapter 3, food intake is significantly reduced by increasing colonic propionate using IPE. This further corroborates our findings from this chapter, about the capacity of IPE to reduce energy intake. It is likely that these reductions in energy intake are mediated through the reported changes in brain activity in the caudate and nucleus accumbens, and not through effects on GLP-1 and PYY as postulated in chapter 3.

5.7.4 **Inulin-Propionate ester does not affect subjective ratings of appetite or satiety**

As we discussed in chapter 3 where you can be satiated but show no changes in subjective ratings of satiety or appetite, the same was observed in this study. There is, however, obviously a physiological effect on satiation by the IPE as observed by this significant reduction in energy intake and changes in BOLD activity. It could be that, appetite and satiety were unchanged despite increased satiation with IPE, or it could be that VAS to measure appetite and satiety may not be the most sensitive and reliable measurement of appetite.

5.7.5 **Inulin-Propionate ester does not induce nausea**

As in all previous chapters, we again show that acute doses of IPE do not cause nausea. Sodium propionate alone has previously been shown to be unpalatable and likely to nausea (Frost et al., 2003, Liljeberg et al., 1995). This suggests IPE overcomes these side effects and should be the preferred method to increase colonic propionate.

5.7.6 **Inulin-Propionate ester does not affect insulin or glucose levels**

In this study, acute supplementation of inulin-propionate ester did not alter plasma levels of glucose and insulin. As discussed in detail in chapter 3, this is further evidence which adds to a body of conflicting evidence of whether SCFAs do indeed alter glucose and insulin homeostasis. It is likely differences are dependent on whether propionate is given over a short or long period. The fact that acute propionate in this study does not affect glucose or insulin corroborates several other acute studies where propionate does not affect glucose or insulin (Wolever et al., 1989) (Laurent et al., 1995).

5.7.7 **Limitations of this work.**

It is important to discuss slight limitations associated with this study. Firstly, fMRI scanning has been reported to be often uncomfortable for scanning periods of 30 to 60 minutes or longer for several reasons (Szameitat et al., 2009). To overcome this, a familiarisation visit in the scanner would potentially have improved the study. In addition, sex differences in central processing of appetite have been reported (Del Parigi et al., 2002). In our study the effect of IPE on central anticipatory food behaviour was explored only in males so it is important to repeat this study in females to assess whether IPE has similar effects.

5.7.8 **Conclusions**

These results show for the first time in humans that increasing colonic propionate in the significantly decreases BOLD signal in the Caudate and Nucleus Accumbens, demonstrating for the first time the effects of propionate in humans on anticipatory food behaviour. In addition, increasing colonic propionate reduces food intake and does not induce nausea. No changes in levels of the gut hormones GLP-1 and PYY were detected, suggesting that propionate acts by alternative mechanisms to change neural activity involved in food reward. Further studies with propionate in humans should elucidate these mechanisms.

Chapter 6 Exploring the effect of increasing colonic propionate on Energy Expenditure

6.1 Introduction

We are interested in exploring the effects of increasing colonic levels of propionate on EE, following on from the findings of one long-term human study, where 10g of IPE was given daily for 24 weeks (Chambers et al., 2014). The IPE group were prevented from gaining weight compared to those given inulin but this was not associated with any changes in levels of the gut hormones, GLP-1 and PYY, and it could be that this prevention of weight gain was through altered energy expenditure, as SCFAs and EE have been linked in several previous studies (Gao et al., 2009, den Besten et al., 2015). EE is defined as the sum of the basal metabolic rate plus diet-induced thermogenesis and physical activity. EE can be calculated by formulae which use the respiratory quotient to provide an

estimate of the basal metabolic rate based on the quantity of the CO_2 eliminated divided by the quantity of O_2 consumed (Poehlman, 1989).

As described earlier, in one long-term human study, 10g of IPE was given daily for 24 weeks. The IPE group were prevented from gaining weight compared to those given inulin (Chambers et al., 2014). In addition, obese volunteers given acetate daily for 12 weeks showed a significantly decreased total body fat and visceral fat percentage at the end of the study (Kondo et al., 2009). These studies in humans identify that SCFAs beneficially prevent weight gain and can also promote fat loss. These studies did not look at the effects of SCFAs on EE but it is plausible that this long-term reduction in energy balance is through a chronic increase in energy expenditure, particularly due to previous findings in animal studies. Indeed, mice supplemented with propionate every day in their diet for 12 weeks, show a reduction in body weight alongside an increased oxygen consumption and energy expenditure (den Besten et al., 2015). In addition, recent evidence for this was found in a recent study in FFAR3 KO mice by Kimura et al., 2011. FFAR3 is highly expressed in sympathetic ganglia of the sympathetic nervous system, both *in vitro* and *in vivo* mice studies have shown that propionate can activate FFAR3 in the sympathetic nervous system (Kimura et al., 2011) and the activation of the sympathetic nervous system can increase energy expenditure (Messina et al., 2013). The activation of FFAR3 is also involved in increasing oxygen consumption in both a fed state and a starved state. WT mice show a significantly increased VO_2 in the fed state compared to a starved state. Additionally, WT mice show a significantly higher VO_2 in a fed state compared to FFAR3 KO mice in a fed state (Kimura et al., 2011). Furthermore, mice supplemented with propionate daily in their diet over a 12 week period, showed an increased oxygen consumption and energy expenditure (den Besten et al., 2015), suggesting a direct effect of propionate on EE. In addition to this, mice which lack FFAR3 have a lower energy expenditure and increased body fat content, further demonstrating a role for FFAR3 in regulating energy expenditure and fat oxidation, (Bellahcene et al., 2013), likely through increased sympathetic activation.

FFAR2 is also implicated in the control mechanisms of EE. FFAR2 knockout mice show decreased fat oxidation, and EE can be increased with the overexpression of FFAR2 in mice (Kimura et al., 2013). Further, obese mice given butyrate as a supplement showed increased adaptive thermogenesis, mitochondrial activity and fat oxidation, which further supports a role for SCFAs

affecting EE (Gao et al., 2009). Taken together this suggests a link between SCFAs, their receptors and EE.

To our knowledge, no studies have explored the effects of SCFAs directly on EE, however some evidence for the effects of fermentable carbohydrates on EE have has been demonstrated. Fermentable carbohydrates increase the rate of thermogenesis in humans; 10 healthy men given 30g of isomalt, of which, 7.3g was found to be fermented, show increased thermogenesis (Livesey, 2002). This is relevant as fermentable carbohydrates have been shown to increase levels of SCFAs in humans (Tarini and Wolever, 2010). It is likely that SCFAs released by increased fermentable carbohydrates may drive increases in EE and drive associated weight loss.

To explore the effects of SCFA on EE, a small pilot study in our own laboratory was conducted. 14 healthy volunteers were given 10g of IPE or control twice; the day before the test visit with a standard evening meal and 12 hours later with a standardised breakfast. EE was measured 12 hours later in a fasted state after the first dose of IPE but no significant effect on EE was found. However, 60 mins later after the second dose of IPE with a standardised breakfast a significantly increased EE in the IPE group compared to the control group was found (Figure **6.1**, $p<0.05$). Therefore, the effect of one dose of IPE to increase colonic propionate on EE in a fed state needs to be explored.

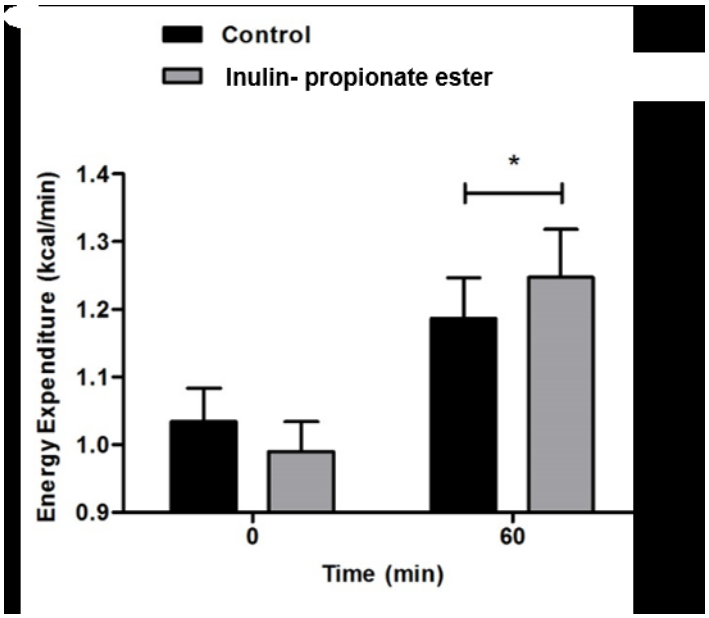


Figure 6.1: IPE significantly increases energy expenditure.
Bar chart shows EE (Kcal/min) for 14 volunteers who received two doses of 10g of IPE or Cellulose as control 12 hours apart. 0 mins shows fasted state 12 hours after 1 dose of IPE or control, 60 mins shows EE after second dose of IPE or inulin. *P<0.05, Mean ± SEM.

As well as SCFAs and SCFA receptors potentially having a role in EE, the gut hormones may also play a role. A few studies have been conducted which show that the gut hormones GLP-1 and PYY may affect EE. In humans, infusion of PYY_{1-36} and PYY_{3-36} increased EE, but this only approached significance (p=0.056). In this study, the plasma levels of PYY peaked after 60 min, and reached 214 ± 48 pmol/l for PYY_{1-36} and 190 ± 27 pmol/l for PYY_{3-36}. These peak plasma PYY levels are 10 times those what we found in chapter 3 where IPE was used to increase colonic propionate, suggesting these findings may be not physiologically relevant. Effects of PYY on EE are likely to be through increased sympathetic activity (Sloth et al., 2007). Acute infusion of GLP-1 in healthy volunteers resulted in increased EE and plasma GLP-1 levels peaked at ~100pmol/L (Tan et al., 2013). However, in another human study, infusion of GLP-1 decreased EE and CHO oxidation. In this study the GLP-1 infusion increased plasma GLP-1 levels to between 40 and 100 pmol/L. These plasma GLP-1 levels are similar to what we found in chapter 3 using IPE to increase colonic propionate, suggesting these findings to be more physiologically relevant (Flint et al., 2000). Similarly, in another study where humans were infused with more physiological levels of PYY, GLP-1 or PYY and GLP-1, no effects on EE were found (Schmidt et al., 2014). Therefore, the known effects of GLP-1 and PYY on EE and fat oxidation are still inconsistent and future studies need to address this.

The effect of using IPE to increase colonic levels of propionate on EE and fat oxidation in humans has not yet been reported, so the work in this chapter explores this. In addition, should IPE increase EE and fat oxidation, further work should be conducted to understand the mechanisms of this.

6.2 Hypotheses

I hypothesized that using IPE to increase levels of propionate in the colon would increase EE and fat oxidation in the postprandial period and reduce subjective appetite.

6.3 Aims and Objectives

In this chapter we sought to investigate the effect of increasing levels of propionate in the colon by providing subjects with a single dose of 10 g of Inulin-propionate ester with breakfast after being fasted overnight on:

- Energy expenditure, fat oxidation and carbohydrate oxidation
- Subjective appetite and satiety

6.4 Methods

6.4.1 **Study Design**

This study was carried out in a single–blind, crossover manner, with participants attending two study visits, a week apart. Subjects did not take part in any strenuous exercise or drink alcohol 24 hours before the study day and were asked to consume the same meal between 19:00 and 20:00 the evening before each study visit. Subjects fasted overnight and arrived at Hammersmith hospital at 8:30am on each visit. At 0 min, subjects were served a standardized breakfast (398 kcal; 71 g CHO, 8 g fat, 10 g protein) containing either 10 g of inulin-propionate ester, or 10 g cellulose control. At 180 min a standardised lunch (575 kcal; 86 g CHO, 19 g fat, 15 g protein and 3.2 g fibre) was provided. Indirect calorimetry was used to establish a baseline measure of O_2 consumption and CO_2 production (to be used to calculate EE, CHO and fat oxidation) and subsequently measured at 60, 120, 180, 240, and 300 mins. Subjective hunger, satiety, and nausea were monitored with the use of 100 mm VAS at 0, 30, 60, 120, 180, 210, 240, 270 and 300 mins. A schematic diagram of the EE study design can be seen in Figure **6.2**.

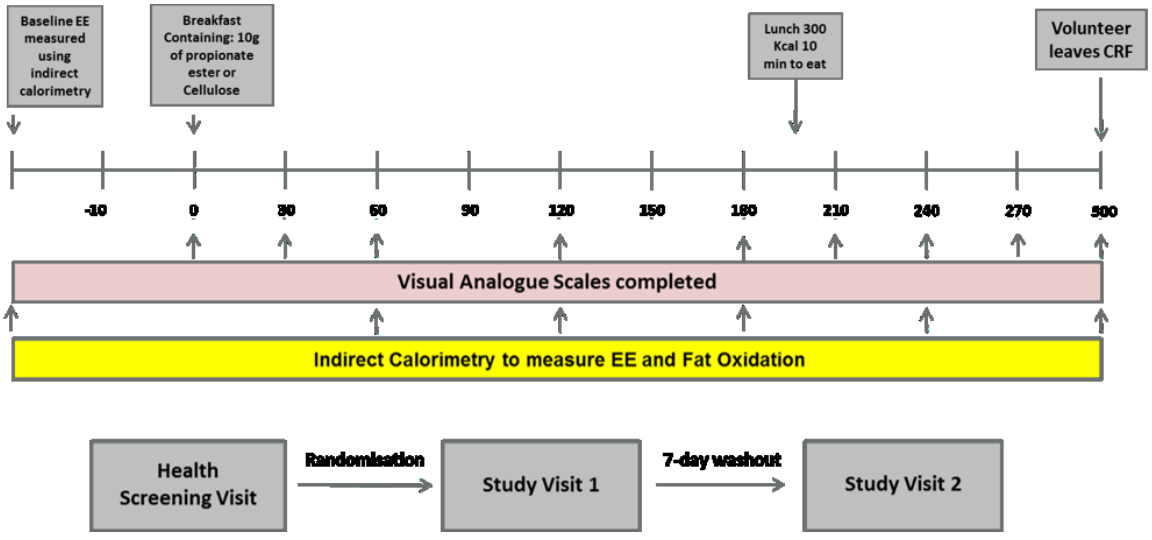


Figure 6.2: Schematic diagram showing the study design for EE study.
This study was conducted in 15 healthy volunteers. This was a single blind randomised trial with 2 visits, a week apart. Subjects received Inulin-propionate ester or cellulose with breakfast and then 3 hours later they received lunch. EE was measured using indirect calorimetry every 60 min for 5 hours to include the postprandial period after lunch at 3h. Appetite was assessed by visual analogue scales as in previous investigations.

6.4.2 **Power analysis**

This is a pilot study in a new area and therefore a power calculation was not possible. However, previous studies have reported altered substrate oxidation rates following dietary interventions of different glycaemic index profiles with 16 volunteers (Scazzina et al., 2011).

6.4.3 **Study participants**

In this study 15 healthy adult volunteers were recruited. The inclusion criteria were a BMI of 20 to 35 kg/m^2 and 21 to 65 years of age. The exclusion criteria were smoking, substance abuse, pregnancy, use of medications, a change in body weight > 5 kg in the previous 3 months, medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, thyroid function and liver function). All subjects provided informed, written consent prior to starting the study, which was approved by the Hammersmith and Queen Charlotte's Research Ethics Committee (08/H0707/99). The study was carried out in accordance with the Declaration of Helsinki. A flow diagram of recruitment to this study can be seen in Figure **6.3**.

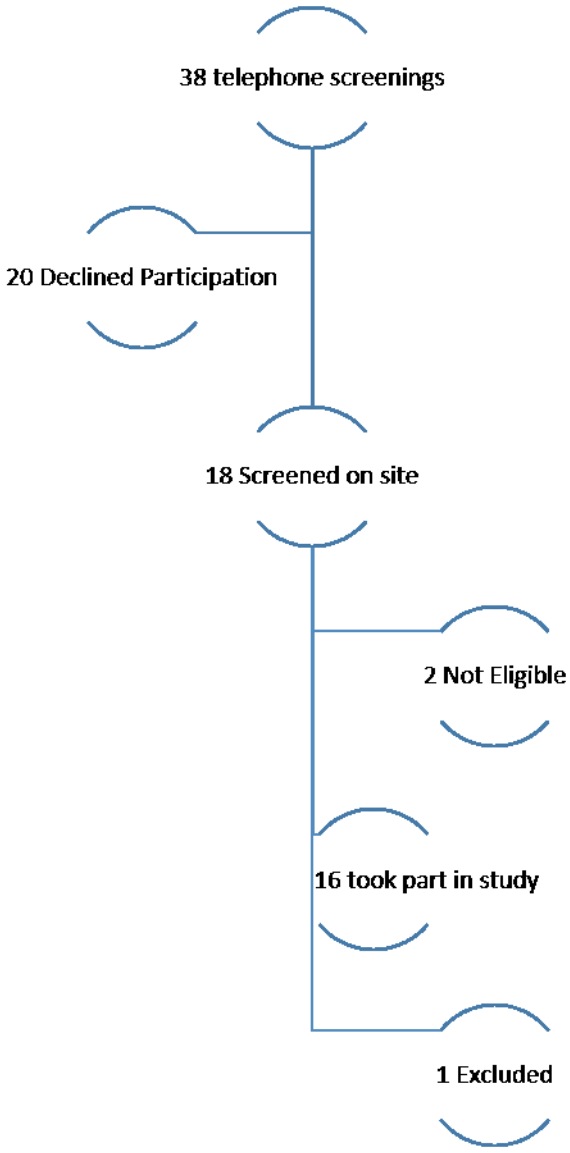


Figure 6.3: Schematic diagram showing the recruitment of participants to the energy expenditure study.
The number of telephone screenings, on site screenings, participants and exclusions throughout can be seen for this study.

6.4.4 **Intervention**

The IPE was developed by Dr Douglas Morrison from the University of Glasgow. The IPE is 3g of propionate bound to 7g of inulin via an ester linkage which aims to increase palatability compared to propionate alone as described in the methods chapter. 10g of IPE delivers 2.36 g of propionate to the colon after 0.49 g of losses in the intestine (Chambers et al., 2014). The control in this study was 10 g of cellulose.

6.4.5 **Assessment of appetite, satiety and nausea**

To investigate the effect of IPE or cellulose on appetite, satiety and nausea, 100 mm VAS were used at several time points during the study visit as described in the methods chapter.

6.4.6 **Indirect calorimetry**

This study looked at the effect of IPE on EE, CHO and fat oxidation using open loop indirect calorimetry (Gas Exchange Monitor; GEM Nutrition, Daresbury, U.K.) to measure O_2 consumption and CO_2 production to derive the respiratory quotient (RQ), EE, fat and CHO oxidation. Each participant was asked to lie in a semi-recumbent position under the comfortable, ventilated hood and canopy of the indirect calorimeter, and air flow through the hood is variable from 20 to 80 L/min for comfort. The open loop indirect calorimeter measures gas exchange volumes, respiratory quotient and energy expenditure by alternatively measuring O2 and CO_2 concentrations of inspired and expired air. Flow rate is continually measured to determine the dilution factor. Additionally, a standard disposable heat and moisture exchange filter in the air line removes particulates and prevents cross contamination. The calorimeter was calibrated before the start of each visit after allowing the machine to warm up for 20 min. Standard gas calibration of 1% CO_2 and 20% O_2 was used and the flow rate was manually set up at approximately 20 L/min. Resting O_2 consumption and CO_2 production were measured at baseline and at 60, 120, 180, 240, 300 mins. Once the CO_2 content of the air entering the chamber stabilised, measurements were taken for 20 min every 1 hour during the study for a duration of 5 hours.

6.4.7 **Analysis of indirect calorimetry data**

The indirect calorimeter provided direct readings of O_2 consumption (L/min) and CO_2 production (L/min) at each of the time points assessed in this study. These readings were then used to calculate the EE, fat and CHO oxidation and RQ. The resting energy expenditure (REE) at each time point was calculated using a formula which accounted for body weight and levels of O_2 consumption and CO_2 production and expressed in kilocalories burnt per minute per kg of body weight. REE was calculated using the Weir equation: REE = [3.9 (VO2) + 1.1 (VCO2)] 1.44 (Weir, 1949). RQ was calculated by (VO2)/(VCO2). Urea was collected to measure the protein oxidation. The fat and CHO oxidation at each time point was calculated using a formula based on levels of O_2 consumption and CO_2 production and urea and results were expressed for fat and CHO oxidation measured in grams of fat or carbohydrate burnt per minute, respectively. The formula to calculate CHO oxidation (g/min) was (4.55*VCO2) - (3.21*VO2) - (2.87*N) and to calculate fat oxidation (g/min) was. 1.67*(VO2-VCO2)-(1.92*N). All data were also calculated as a change from baseline.

The limits of detection for each study with indirect calorimetry were as follows; for RQ ~ 2.40%, EE ~9.45%, CHO oxidation ~14.55% and fat oxidation ~24.36%.

6.4.8 **Colonic delivery assessment**

As breath hydrogen was significantly increased from ~180 min in chapter 3 and chapter 4 marking the fermentation of the IPE it was also assumed that fermentation would occur at this time point in this study.

6.4.9 **Statistical Analysis**

Energy Expenditure, fat oxidation, CHO oxidation, RQ and area under the curve for VAS was analysed by paired t-test. Raw VAS data was analysed by two-way ANOVA.

6.5 Results

6.5.1 **Study participants**

Fifteen healthy volunteers were recruited to this study. The characteristics of the study participants can be seen in Table 6.1.

Table 6.1: Characteristics of participants in the effect of Inulin-propionate ester on energy expenditure and fat oxidation.

Participants' Characteristics	**Mean ± SD**
Age (years)	29.2 ± 2.6
Weight (kg)	63.5 ± 2.5
BMI (kg/m^2)	22.3 ± 0.6
Gender	6 men and 9 women

6.5.2 **10g of inulin-propionate ester does not affect EE in the postprandial period**

EE after IPE or cellulose control was measured every hour for 5 hours. Changes in EE at each time point were calculated as change (Kcal/kg/min) from baseline (0 mins), after an initial 30 minute equilibrium period with the calorimeter before the baseline value was recorded, mean EE values are also shown for reference. We found no significant difference between the control and IPE group in EE at any of the time points (Figure **6.4**, $p>0.05$). In addition, changes over time measured by AUC (0 to 180 min) were also not significant with IPE compared to control ($p>0.05$). Mean ± SEM EE for ΔAUC0-180min was 0.517 ± 0.15 min × Kcal/kg/min with control compared to 0.420 ± 0.107 min × Kcal/kg/min with IPE (Figure **6.4**, $p>0.05$). As breath hydrogen was significantly increased from ~180 min in chapter 3 and chapter 4 marking the fermentation of the IPE, EE was also assessed from 180 min. Mean ± SEM EE for ΔAUC180-300min was 0.502 ± 0.098 min × Kcal/kg/min with control compared to 0.445 ± 0.101 min × Kcal/kg/min with IPE (Figure **6.4**, $p>0.05$).

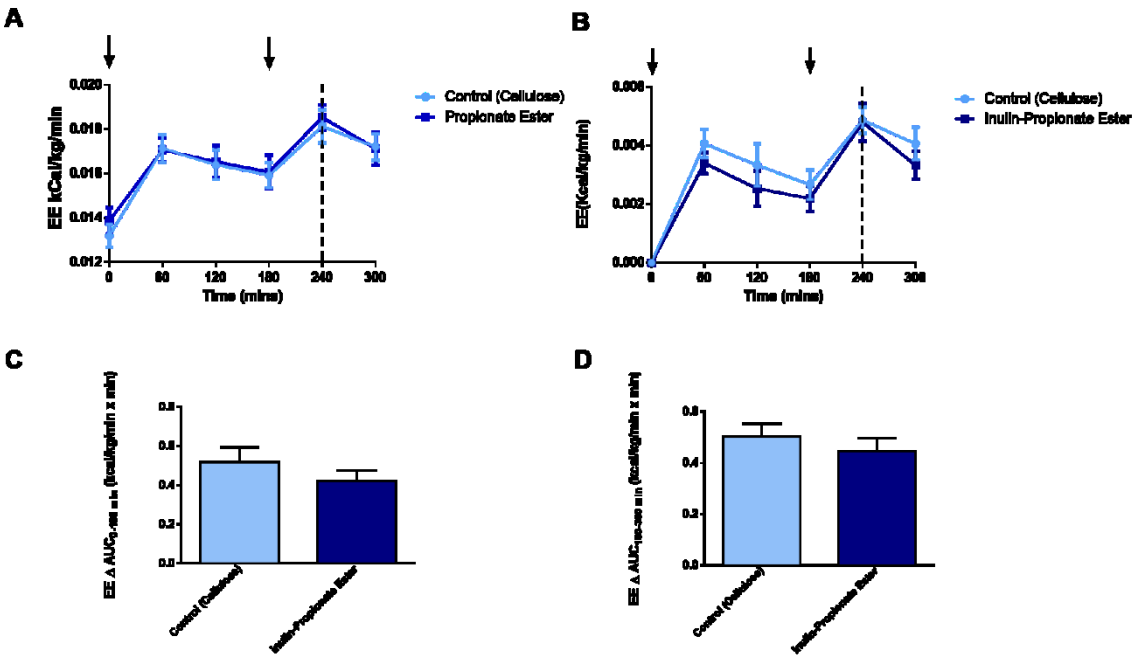


Figure 6.4: Effect of 10g Inulin-propionate ester vs. cellulose (control) on EE in healthy humans.
Line graphs show the mean EE (kCal/kg/min) over time for the control group and inulin-propionate ester group as (A) raw EE values and (B) as change from baseline every hour after the initial 10g supplement. Arrows mark 0 min where breakfast and supplement was served and at 180min when lunch was served, dashed line shows fermentation of IPE. Bar charts show (C) AUC 0-180 min (p= 0.1674) and (D) AUC 180-300 min (p= 0.2918) for EE for control and IPE.

6.5.3 **10g of inulin-propionate ester does not affect fat oxidation in the postprandial period**

Fat oxidation after IPE or cellulose control was measured every hour for 5 hours. Changes in fat oxidation at each time point were calculated as change (g/kg/min) from baseline (0 mins), after an initial 30 minute equilibrium period with the calorimeter before the baseline value was recorded. We found no significant difference (Figure **6.5**, p>0.05) between the control and inulin-propionate ester group in fat oxidation at any of the time points. In addition, changes over time measured by AUC (0 to 180 min and 180 to 300 min) were also not significant with IPE compared to control (Figure **6.5**, p>0.05). Mean ± SEM fat oxidation for ΔAUC0-180min was -2.248 ± 1.22 min × g/kg/min with control compared to -1.807 ± 0.844 min × g/kg/min with IPE (Figure **6.5**, p>0.05). Mean ± SEM fat oxidation for ΔAUC180-300min was -1.559 ± 0.701 min × g/kg/min with control compared to -0.915 ± 0.776 min × g/kg/min with IPE (Figure **6.5**, p>0.05).

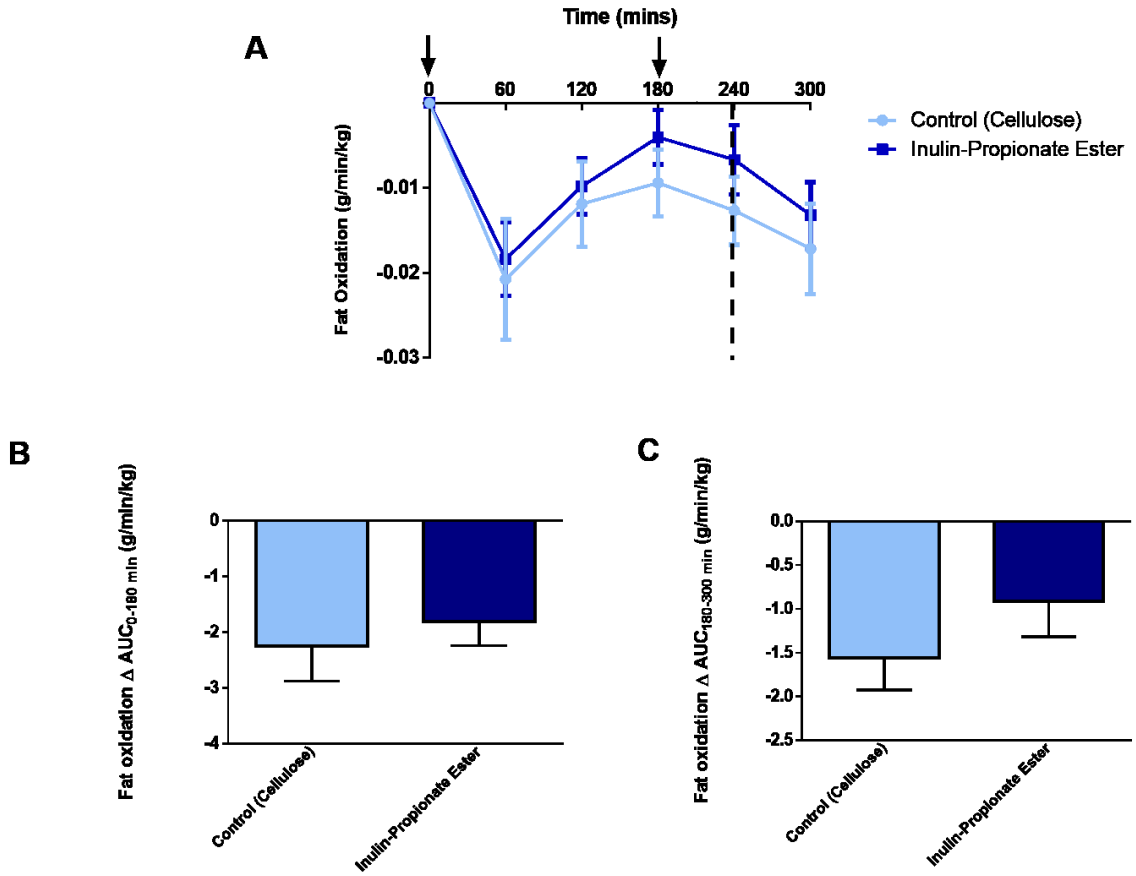


Figure 6.5: Effect of 10g Inulin-propionate ester vs. cellulose (control) on fat oxidation in healthy humans.
(A) Line graph shows the mean fat oxidation (g/kg/min) over time as change from baseline for the control group and inulin-propionate ester group every hour after the initial 10g supplement. Arrows mark 0 min where breakfast and supplement was served and at 180min when lunch was served. Dashed line marks fermentation of the IPE in the colon. Bar charts show (B) AUC 0-180 mins (p= 0.506) and (C) AUC 180- 300 mins (p= 0.172) for fat oxidation for control and IPE group. N=15, p>0.05.

6.5.4 **10g of inulin-propionate ester does not affect CHO oxidation in the postprandial period**

CHO oxidation after IPE or cellulose control was measured every hour for 5 hours. Changes in CHO oxidation at each time point were calculated as change (g/min) from baseline (0 mins), after an initial 30 minute equilibrium period with the calorimeter before the baseline value was recorded. We found no significant difference between the control and inulin-propionate ester group in CHO at any of the time points (Figure **6.6**, p>0.05). In addition, changes over time measured by AUC (0 to 180 min and 180 to 300 min) were also not significant with IPE compared to control (Figure **6.6**, p>0.05). Mean ± SEM CHO oxidation for ΔAUC0-180min was 14.32 ± 4.04 min × g/min with control compared to 11.50 ± 2.06 min × g/min with IPE (Figure **6.6**, p>0.05). Mean ± SEM CHO oxidation for ΔAUC180-300min was 12.21 ± 2.69 min × g/min with control compared to 9.77 ± 1.93 min × g/min with IPE (Figure **6.6**, p>0.05).

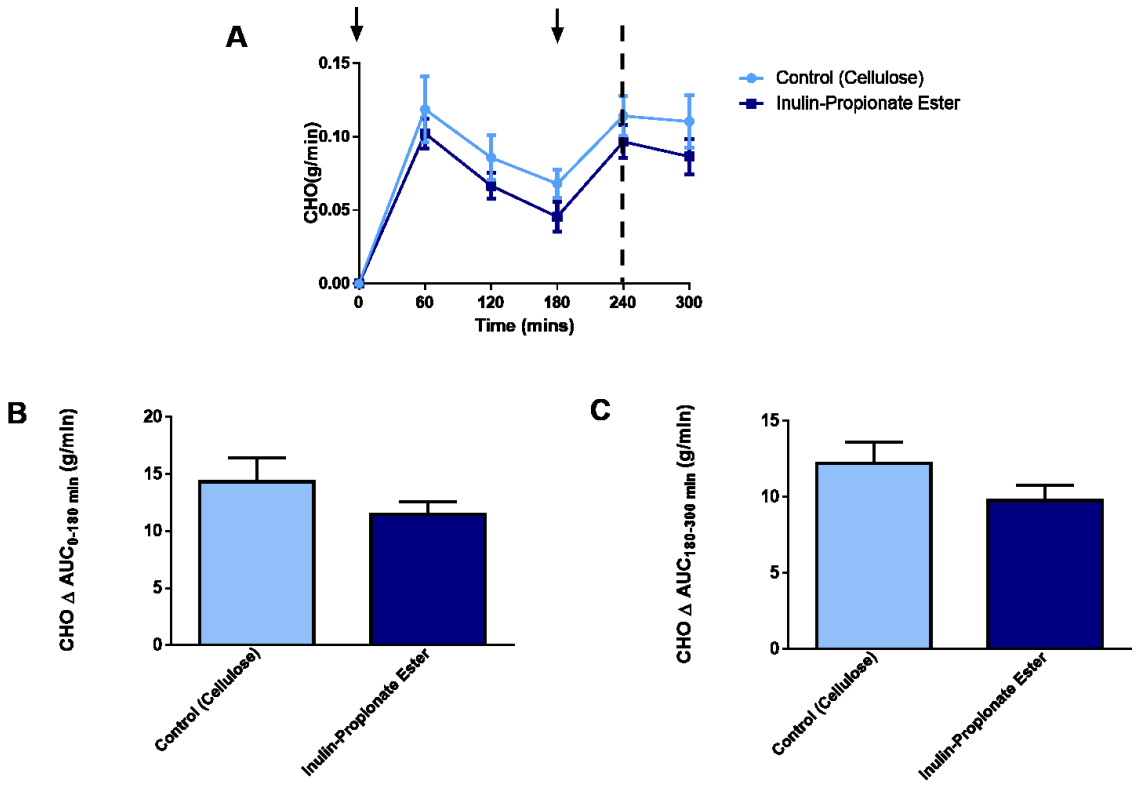


Figure 6.6 : Effect of 10g Inulin-propionate ester vs. cellulose (control) on CHO oxidation in healthy humans.

(A) Line graph shows the mean CHO oxidation (g/min) over time as change from baseline for the control group and inulin-propionate ester group every hour after the initial 10g supplement. Arrows mark 0 min where breakfast and supplement was served and at 180min when lunch was served. Dashed line marks fermentation of the IPE in the colon. Bar charts show (B) AUC 0-180 mins (p= 0.2583) and (C) AUC 180- 300 mins (p= 0.1398) for CHO oxidation for control and IPE group. N=15.

6.5.5 **10g of inulin-propionate ester does not affect RQ in the postprandial period**

RQ after IPE or cellulose control was measured every hour for 5 hours. Changes in RQ at each time point were calculated as change from baseline (0 mins), after an initial 30 minute equilibrium period with the calorimeter before the baseline value was recorded. We found no significant difference between the control and inulin-propionate ester group in RQ at any of the time points (Figure **6.7**, p>0.05).In addition, changes over time measured by AUC (0 to 180 min and 180 to 300 min) were also not significant with IPE compared to control (Figure **6.7**, p>0.05). Mean ± SEM RQ for ΔAUC0-180min was 9.89 ± 3.23 x min with control compared to 7.66 ± 2.33 x min with IPE (Figure **6.7**, p>0.05). Mean ± SEM RQ for ΔAUC180-300min was 7.52 ± 1.97 x min with control compared to 5.05 ± 2.09 x min with IPE (Figure **6.7**, p>0.05).

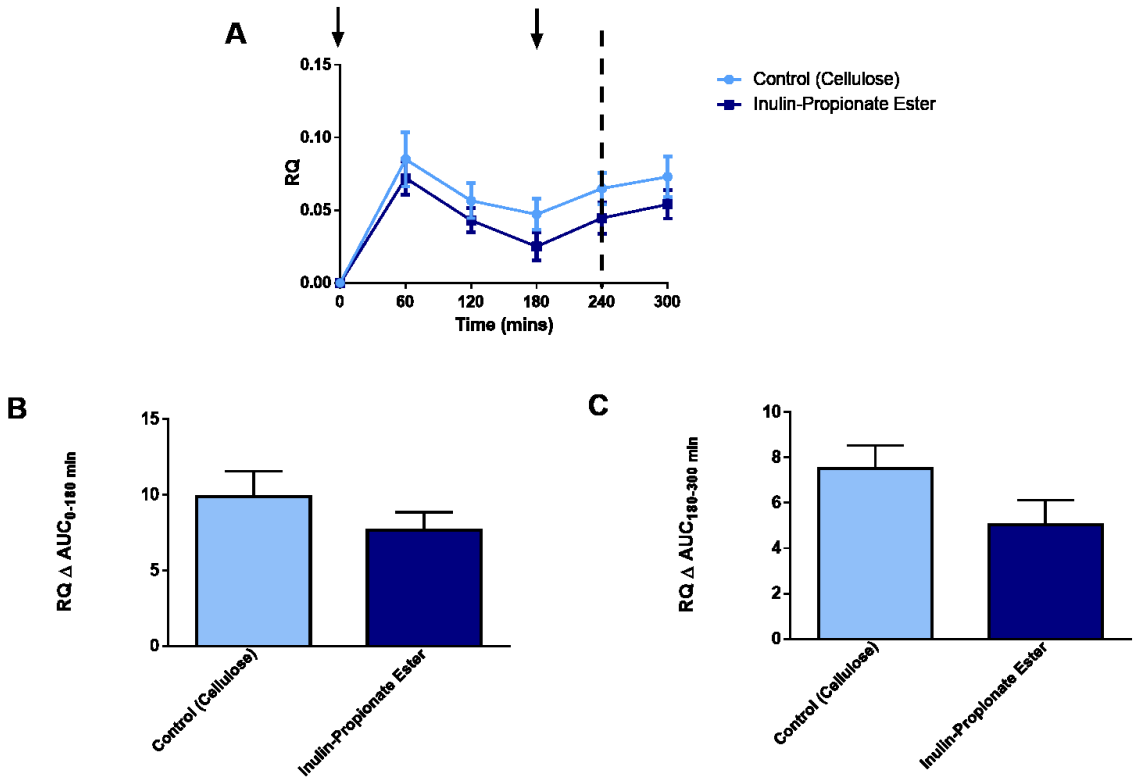


Figure 6.7: Effect of 10g Inulin-propionate ester vs. cellulose (control) on RQ in healthy humans.
(A) Line graph shows the mean RQ over time as change from baseline for the control group and inulin-propionate ester group every hour after the initial 10g supplement. Arrows mark 0 min where breakfast and supplement was served and at 180min when lunch was served. Dashed line marks fermentation of the IPE in the colon. Bar charts show (B) AUC 0-180 mins (p= 0.2839) and (C) AUC 180- 300 mins (p= 0.1033) for RQ for control and IPE group. N=15.

6.5.6 **10g of inulin-propionate ester significantly reduces appetite and increases satiety**

We sought to understand the effect of IPE on appetite in humans. VAS were used to measure satiety and appetite. We found that consumption of 10 g of inulin-propionate ester significantly reduced early levels of appetite as measured by responses to the questions – “How strong is your appetite for something sweet?” ($p<0.05$, Figure **6.8**).

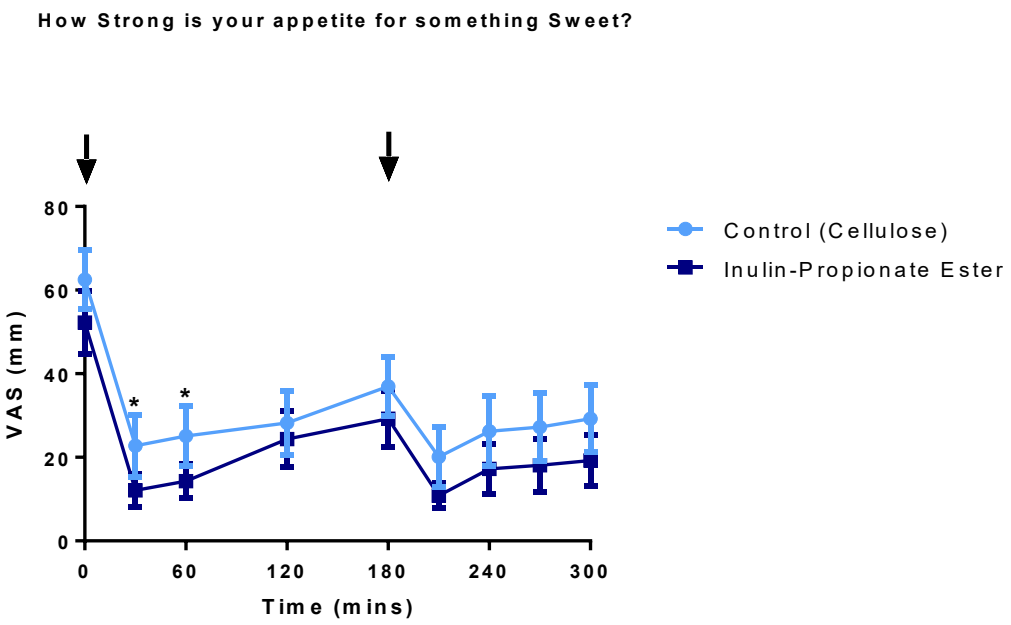


Figure 6.8: Increasing colonic propionate with 10g IPE reduces subjective ratings of appetite.
Figure shows appetite ratings were made using 100 mm VAS with ends of the scale signposting extremes (e.g. 0 mm equalling not at all hungry and 100 mm being extremely hungry). Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as mean ± SEM (n=15, p= 0.0001).

In addition, consumption of 10 g of inulin-propionate ester significantly reduced appetite as measured by responses to the question – "How strong is your appetite for a meal?". ($p<0.01$, Figure **6.9**).

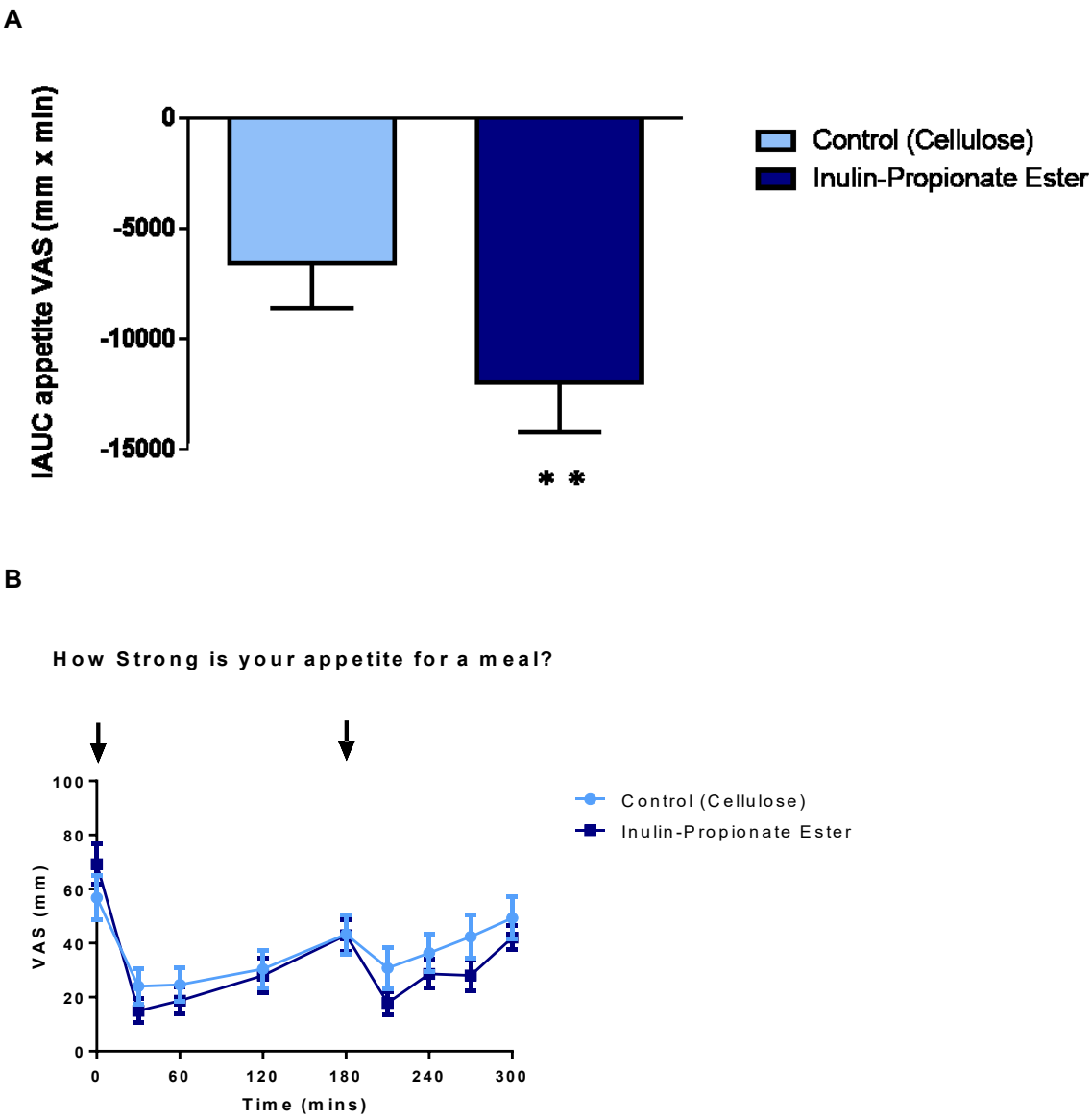


Figure 6.9: Inulin propionate ester significantly decreases appetite.
A) Bar chart shows mean iAUC0-300 mins for VAS "How strong is your appetite for a meal?" for all subjects given inulin-propionate ester or cellulose. Error bars show SEM. (n=15), p= 0.0092. B) Fullness response curves for the two different trials as measured by VAS. Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as means ± SEM (n=15).

However, consumption of 10 g of inulin-propionate ester did not affect responses to the question –

"How hungry do you feel right now?". ($p>0.05$, Figure **6.10**).

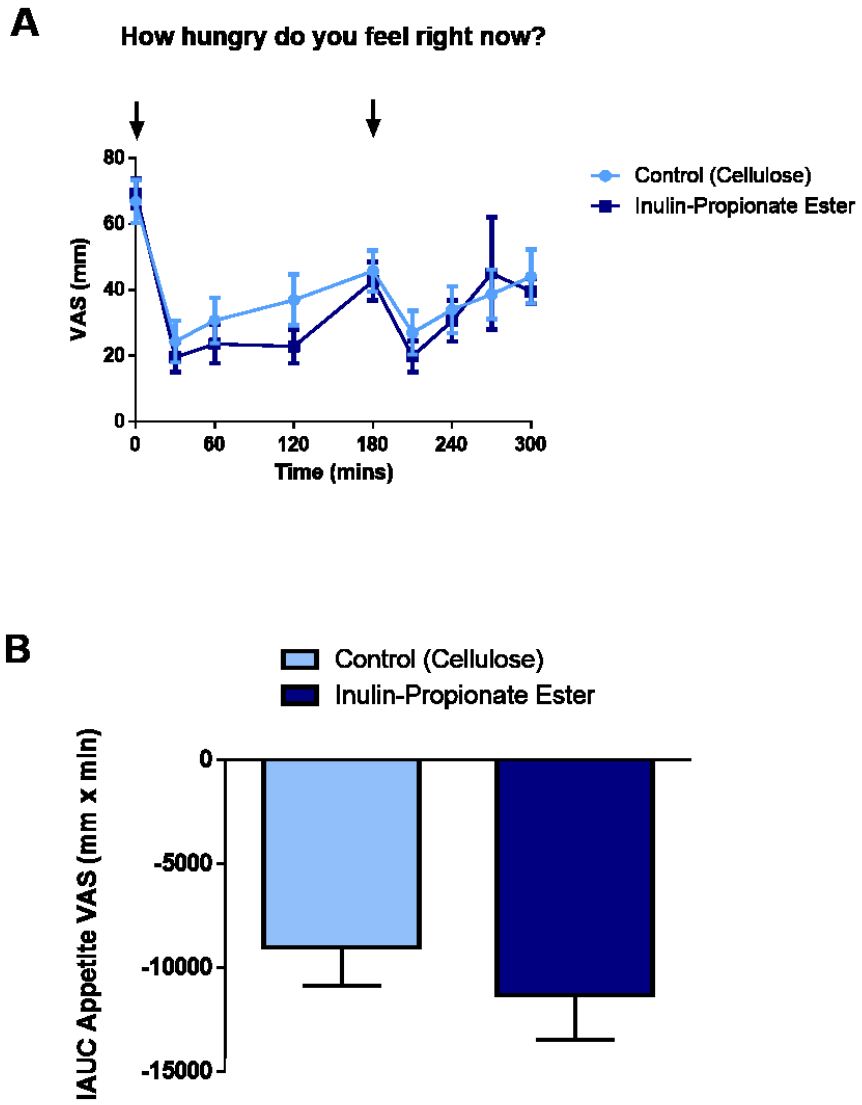


Figure 6.10: Inulin propionate ester does not affect appetite.
A) Fullness response curves for the two different trials as measured by VAS. Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as mean ± SEM (n=15)
B) Bar chart shows mean iAUC0-300 mins for VAS "How hungry do you feel right now?" for all subjects given inulin-propionate ester or cellulose. Error bars show SEM. (n=15), (p=0.0792)

In addition, effects of IPE on satiety were measured by responses to the question – "How strong is your desire to eat?". IPE significantly increased satiety when iAUC 0-300 min was measured ($p<0.05$, Figure **6.11**).

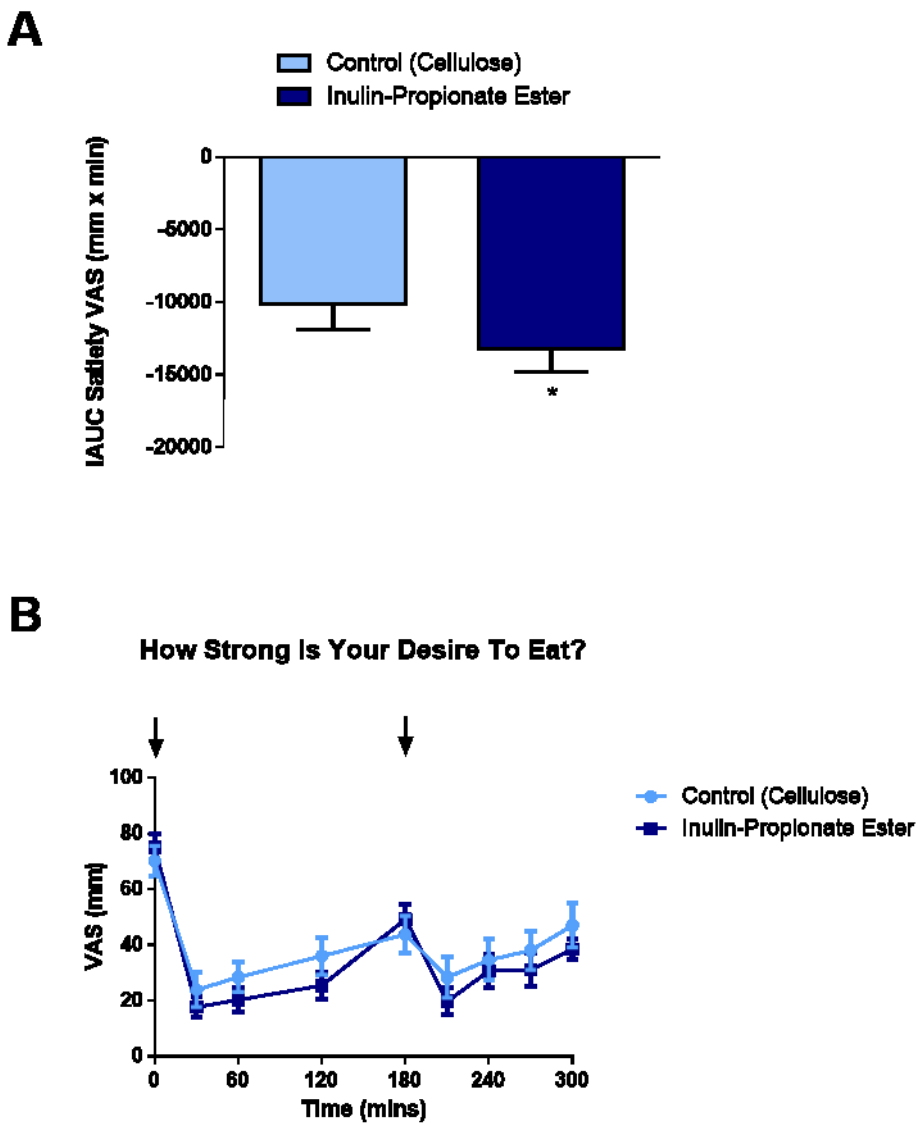


Figure 6.11: Inulin propionate ester significantly increases satiety.
A) Bar chart shows mean iAUC0-300 mins for VAS "How strong is your desire to eat?" for all subjects given inulin-propionate ester or cellulose. Error bars show SEM. (n=15), p= 0.0310. B) Satiety response curves for the two different trials as measured by VAS. Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as means ± SEM (n=15).

Effects of IPE on satiety measured by responses to the question – "How full do you feel right now?" did not show any significant changes. IPE did not affect satiety when iAUC 0-300 min was measured (p>0.05, Figure **6.12**).

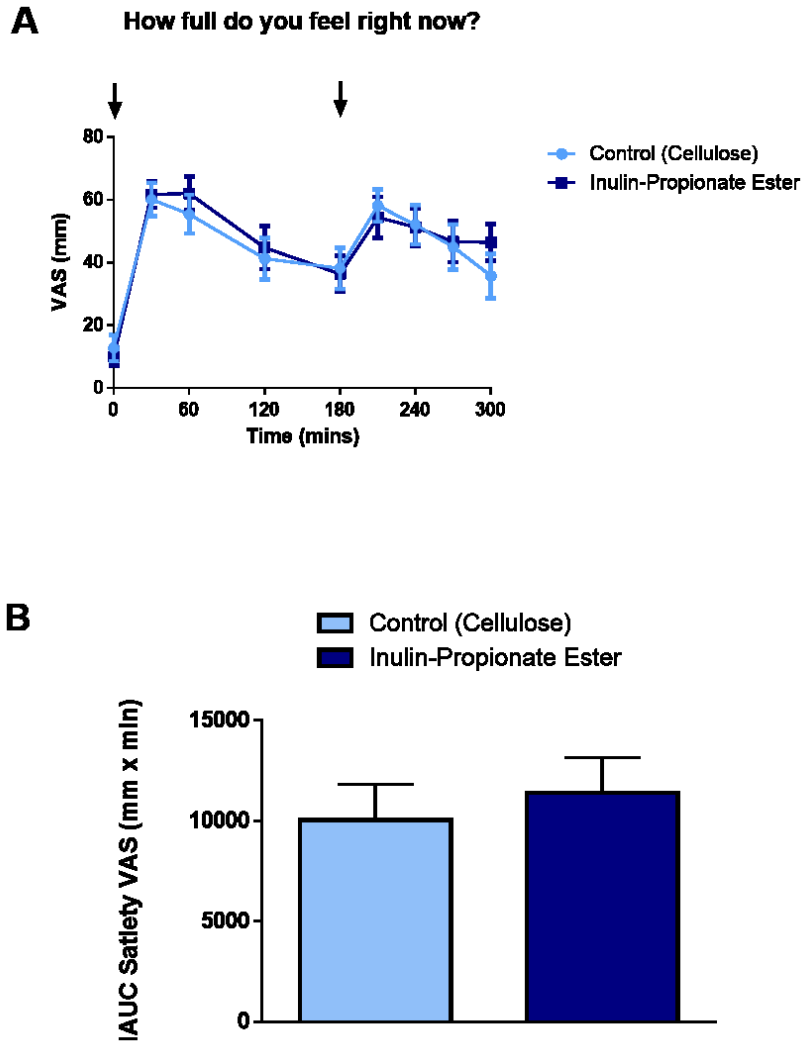


Figure 6.12: Inulin propionate ester does not affect satiety.
A) Satiety response curves for the two different trials as measured by VAS. Black arrows indicate standardized meals. 10 g inulin-propionate ester or 10 g control were provided with breakfast at 0 min. Data points are presented as mean ± SEM (n=15). B) Bar chart shows mean iAUC0-300 mins for VAS "How full do you feel right now?" for all subjects given inulin-propionate ester or cellulose. Error bars show SEM. (n=15), p=0.3111

6.6 Summary of Results

In this results chapter we have shown the effects of acutely supplementing humans with inulin-propionate ester to increase colonic levels of propionate on EE, fat and CHO oxidation and subjective appetite.

We found that:

- 10g of IPE does not affect EE in the postprandial period.
- 10g of IPE does not affect fat or CHO oxidation in the postprandial period.
- 10g of IPE does not affect oxygen consumption or carbon dioxide production (RQ).
- 10g of IPE significantly decreases subjective ratings of appetite and increases ratings of satiety.

6.7 Discussion

6.7.1 **10g of inulin-propionate ester does not affect EE in the postprandial period.**

This is the first study in humans looking at the effect of acute supplementation of IPE to raise colonic propionate levels on EE and fat and CHO oxidation. In this chapter we found no significant effect of one 10g dose of IPE on EE in the 5 hours after IPE supplementation after fermentation in the colon.

In a previous long-term study in our lab where volunteers consumed 10g of IPE every day for 24 weeks, significant effects on body weight were found which we speculated could be through changes in EE. Only one out of 25 volunteers (4%) from IPE group gained ≥3% of their baseline body weight and none gained ≥5% of their baseline body weight. This was compared to 6 out of 24 control volunteers (25%) who gained ≥3% of their baseline body weight and 4 (17%) who gained ≥5% of their baseline body weight (Chambers et al., 2014). In addition, in another previous study in our lab, in 14 healthy volunteers, where volunteers received 10g of IPE on day 1, and a further dose of 10g of IPE on day 2, effects on EE were found. In the fasted state there was no difference in energy expenditure, however, 60 min after consuming a standard breakfast, containing the second 10 g dose of inulin-propionate ester or cellulose, energy expenditure was 5% greater with IPE supplementation, when measured from 13 to 18 hours after the first IPE dose (Figure **6.1**). Similarly in mice supplemented with propionate every day in their diet for 12 weeks, an increased oxygen consumption and energy expenditure was found (den Besten et al., 2015). Together, these data suggest that IPE and increasing colonic propionate may have beneficial effects on EE, however these are likely only mediated greater than 5 hours after increasing colonic propionate or with more than one dose of inulin-propionate ester.

Evidence exists in animal studies suggesting a role for how propionate increases EE. A role for propionate activation of FFAR3 and increased EE has also been shown. WT mice have a significantly higher EE compared to FFAR3 KO mice in a fed state (Kimura et al., 2011). In addition, in another study in mice, supplementation with the SCFA butyrate every day significantly increases EE after 1 week in obese mice (Gao et al., 2009).

Together, our findings and those of others do not rule out a role of propionate in increasing EE to promote beneficial effects on body weight and energy homeostasis, however, it is likely that these

changes may be specific to obese individuals, long-term SCFA supplementation, or a more sustained and later response to SCFA supplementation on EE.

6.7.2 **10g of inulin-propionate ester does not affect fat oxidation in the postprandial period.**

This study is the first study in humans looking at the effect of acutely increasing propionate in the colon with IPE on fat oxidation. In this acute study we observed no changes in the levels of fat oxidation, 5 hours after supplementation with IPE after fermentation in the colon.

Previous studies have shown evidence for a role of SCFAs in fat oxidation (Kondo et al., 2009, Gao et al., 2009). However these effects may be specific to long-term supplementation or to individuals with obesity. Indeed obese patients supplemented every day for 12 weeks with acetate show significantly decreased total body fat and visceral fat percentage (Kondo et al., 2009) suggesting an increased fat oxidation with long term supplementation of SCFAs in obese individuals. Similarly, in an animal study, where obese mice were supplemented with butyrate in their diet, these show a significantly increased fat oxidation after 1 week in to the study obese mice (Gao et al., 2009).

Effects of propionate and other SCFAs on fat oxidation are likely mediated through changes in the activity of adipose tissue. The SCFA, acetate can activate FFAR2 in adipose tissue to reduce fat accumulation (Kimura et al., 2013). In addition, FFAR2 KO mice are obese suggesting that FFAR2 may mediate effects on fat oxidation and storage (Kimura et al., 2013). However, in humans the existence of FFAR2 and 3 in human adipose tissue are unclear. Le Poul et al, show expression of both FFAR2 and FFAR3 in human adipose tissue (Le Poul et al., 2003) whereas Hong et al., show that FFAR2 and 3 are not expressed in human adipose tissue (Hong et al., 2005).

Together, our findings and those of others do not rule out a role of propionate in increasing fat oxidation and changing adipose tissue activity to promote beneficial effects in body weight and energy homeostasis, however, it is likely that these changes may be specific to obese individuals, long-term SCFA supplementation, or a more sustained and later response to SCFA supplementation.

6.7.3 **10g of inulin-propionate ester does not affect CHO oxidation in the postprandial period.**

To our knowledge no previous studies in humans or animals have shown evidence for a role of SCFAs in CHO oxidation. We demonstrate for the first time in this study that in the 5 hour period after consumption of IPE and after fermentation in the colon, there is no effect on CHO oxidation compared to control in 15 healthy humans. This does not, however, rule out any long-term effects of propionate supplementation on CHO oxidation, changes in CHO oxidation after the 5 hour period which we measured CHO oxidation or to non-healthy individuals.

6.7.4 **Inulin-Propionate ester significantly reduces subjective ratings of appetite.**

In this study, we have shown that increasing colonic propionate by using 10 g of IPE significantly decreases appetite as demonstrated in chapter 4. Appetite for a meal was significantly reduced from 0 to 300 mins after consuming IPE and appetite for something sweet was significantly decreased at 30 min and 60 min with IPE compared to control. Together, these data suggest that increasing propionate with 10 g of IPE can reduce appetite before reaching the colon as well as after reaching the colon.

However, previously in this thesis, we have presented data with the same dose of IPE and measured appetite and satiety over the same time period and found no differences in subjective ratings of appetite, but did see increased satiation measured by a reduced food intake (chapter 3). These conflicting results we have across the several studies suggest very variable individual responses to IPE as well as how individuals complete the VAS to measure appetite.

A small amount of evidence for the effect of SCFAs on appetite exists, but to our knowledge there are no previous reports of the effect of propionate on appetite in humans. In a study in mice, activation of the ARC of the hypothalamus which is involved in the brain regulation of appetite was significantly increased with fermentable CHO over a long term period. These effects on brain activity involved in appetite were accompanied by increased levels of SCFAs suggesting a direct effect of SCFAs on appetite (Anastasovska et al., 2012). Similarly, mice given acetate show a reduced activation in central brain areas involved in appetite regulation (Frost et al., 2014). Additionally, FCs have been linked to increasing SCFA levels, and in a human study where subjects received oligofructose over a period of 8 weeks subjective appetite was reduced significantly compared with control (Daud et al., 2014).

6.7.5 **Limitations of this work**

In this study, we calculated a poor limit of detection for the indirect calorimeter between visits. The limit of detection for each study were as follows; for RQ ~ 2.40%, EE ~9.45%, CHO oxidation ~14.55% and fat oxidation ~24.36%. Therefore, with these poor limits of detection, any subtle small changes in EE, CHO, fat oxidation and RQ would not be identified. In order to detect any subtle but significant changes with IPE a large number of volunteers (approximately 200 to 300 volunteers with the above limits of detection) would be required to increase the power of this study or another method with a better limit of detection should be sought, for example, whole room calorimetry (Seale et al., 1991).

In addition, in hindsight, it could be possible that the study could have been designed better to measure EE, fat and CHO oxidation and RQ for a longer period of time after fermentation of IPE in the colon at around 240 mins. In this study, these were only measured up to 300 mins (only 60 mins after fermentation), and it is plausible that these effects could occur beyond this time point. Specifically in a pilot study in our own group, effects on EE were observed approximately 8 hours after the first dose of IPE was fermented in a fed state (Figure **6.1**).

6.7.6 **Final conclusions**

In this study there were no significant effects of raising colonic propionate by using a single dose of 10 g of IPE on EE, fat oxidation, CHO oxidation and RQ. This does not eliminate the possibility that EE, fat and CHO oxidation and RQ cannot be modulated by propionate but it is likely that these effects would occur with more than one dose of IPE, over a period of greater than 5 hours after the dose of IPE or are specific to non-healthy individuals. We did, however, show a significant effect of IPE on reducing appetite. Interestingly, this effect of IPE on appetite was detected before reaching the colon as well as after reaching the colon, suggesting an early effect of propionate on appetite and this will be explored further in a following chapter by using capsules which enable the release of propionate before the colon.

Chapter 7 Exploring the effect of the delivery of small intestinal propionate on appetite regulation

7.1 Introduction

Data presented already in this thesis has demonstrated an early effect of IPE on appetite and satiety well before the fermentation of IPE in the colon, leading us to explore the pre-colonic effect of propionate. Two previous studies have led us to explore the pre-colonic effect of propionate on appetite and satiety. Firstly, work using the ^{13}C isotope to explore how much of the bound propionate within 10g of IPE actually arrives to the colon alongside the rise in breath hydrogen to measure the colonic fermentation suggests ~25% is released before fermentation the colon. This means that ~75% of the bound propionate would have been released in the colon, so this ~25% released before the colon may be able to affect appetite and satiety. Precisely, 0.49g of the bound propionate in 10g of IPE is released before reaching the colon (Chambers et al., 2014). Additionally, results presented in chapter 4 (figure 4.6) demonstrate a significant increase in satiety with IPE in the first two hours after receiving the supplement. Subjective ratings of fullness were significantly increased between 0 and 120 min following ingestion of the IPE ($p<0.05$), suggesting that satiety may be affected by the bound propionate within this time. The AUC with IPE was 7264.29 ± 414.11mm, whilst the inulin AUC was 6122.14 ± 586.97mm (figure 4.7). The findings of both of these investigations combined leads us to speculate that the propionate released from the IPE before the colon, possibly in the stomach or small intestine, may be released in the bloodstream and able to affect appetite before even reaching the colon. Indeed, SCFAs receptors FFAR2/3 are found in the small intestine (Kaemmerer et al., 2010, Tazoe et al., 2009) and SCFAs are absorbed in the small intestine (Schmitt et al., 1976). Furthermore, in another study, healthy humans, that were fed 0.5g of propionate with a meal showed increased satiety 45 minutes after the meal, well before the propionate would be able to affect appetite through colonic L-cells (Liljeberg et al., 1995). It is possible that this early effect on appetite may be caused by the direct release of propionate to the bloodstream affecting upon the sympathetic nervous system before the colon (Kimura et al., 2011). Increased sympathetic activity has been suggested to increase satiety (Harthoorn and Dransfield, 2008). Alternatively, increasing propionate levels in the hepatic portal vein can decrease food intake and propionate may act here to affect appetite (Anil and Forbes, 1980, Anil and Forbes, 1988) or may act by targeting vagal afferents (Lal et al., 2001). Indeed, stimulation of the FFAR3 in

vagal afferents of the gut or in the hepatic portal vein may modulate appetite (Kentish and Page, 2015).

Previous evidence suggesting an effect of SCFAs on glucose tolerance in humans is unclear. Several studies show that FFAR2/3 are found in the beta cells of the pancreas, suggesting that SCFAs binding to these receptors could modulate insulin secretion (Priyadarshini and Layden, 2015, Priyadarshini et al., 2015). Additionally, acetate applied to beta cells stimulates insulin release *in vitro* (Priyadarshini et al., 2015). Data from humans is somewhat unclear. Acute infusion of mixtures of SCFAs show no effect on glucose or insulin levels in one study in humans (Wolever et al., 1989). Similarly, in another acute human study, infusions of acetate or propionate alone, or together, show no effect on fasting glucose or insulin levels (Laurent et al., 1995). However, in conflict with this, some studies in humans show that SCFAs may have an effect on glucose and insulin. For example, supplementation with propionate has been shown to significantly lower glucose levels in humans (Todesco et al., 1991). In addition, another human study shows significant reductions in fasting glucose levels with propionate (Venter et al., 1990). Any effects of propionate or other SCFAs on glucose and insulin, may be mediated through improved beta-cell function, which has been demonstrated previously in mice (den Besten et al., 2015, Lin et al., 2012) and rats (Khan and Jena, 2014). In addition, increasing levels of fermentable carbohydrates can affect insulin sensitivity and glucose tolerance (Robertson et al., 2005, Parnell and Reimer, 2009) and this may be due to increases in levels of SCFAs (Tarini and Wolever, 2010). Specifically, humans given 30g of resistant starch every day for 4 weeks showed increased insulin sensitivity compared to placebo (Robertson et al., 2005). In addition, in obese subjects 21g of oligofructose per day over 12 weeks significantly decreased insulin and glucose levels compared to control (Parnell and Reimer, 2009). However, in another long-term study where increasing doses of 15g, 25g, 35g, 45g and 55g of oligofructose were given weekly, no effect on insulin or glucose levels was found (Pedersen et al., 2013). Similarly, in obese subjects given 30g of oligofructose over 6 weeks showed no effect on insulin or glucose levels compared to control (Daud et al., 2014). In addition, the role of FFAR2 in obesity mediated insulin resistance has been demonstrated in mice (McNelis et al., 2015).

Whether propionate can affect appetite and satiety and glucose and insulin levels before reaching the colon are currently not understood and need to be explored. The work presented in this chapter aims to address this question. In this chapter, the effects of propionate on appetite will be explored

using the pharmacological supplementation of encapsulated sodium propionate which will enable the effects of propionate before reaching the colon to be explored, specifically these capsules breakdown in the small intestine, allowing any effects of propionate in the small intestine to be investigated.

7.2 Hypotheses

We hypothesised that providing subjects with different doses of encapsulated sodium propionate (1g, 2g, 3g and 5g) which enables absorption of propionate from the gut before the colon; specifically in the small intestine, would dose-dependently reduce subjective appetite and increase subjective satiety and may alter glucose tolerance and insulin sensitivity after consumption.

7.3 Aims and Objectives

In this chapter we sought to investigate the effect of delivery of propionate to the small intestine on:

- Subjective appetite, satiety, and nausea
- Plasma levels of insulin, and glucose

by providing subjects with capsules containing ~0.5g sodium chloride (placebo) or 1g, 2g, 3g and 5g sodium propionate.

7.4 Methods

7.4.1 **Intervention: design of enteric coated capsules for the delivery of propionate to the small intestine**

The supplementation of propionate in this study is provided in a hydroxypropylmethyl cellulose capsule. This is prevented from gastric digestion and delivered directly to the small intestine because of its specific enteric polymer coat - Eudragit® L100-55 which was used as it dissolves in

the small intestine after 60 minutes (personal communication, Quay Pharma, UK). The control in this study was ~0.5g encapsulated sodium chloride (placebo).

7.4.2 **Study Design**

This study was carried out in a single–blind, crossover manner, in five study visits, each a minimum of a week apart. This study was to compare ~0.5g encapsulated sodium chloride (placebo) to 1, 2, 3 and 5g of encapsulated sodium propionate on appetite and satiety, and glucose and insulin levels. Subjects did not partake in strenuous exercise nor drink alcohol 24 hours before the study day and were asked to consume the same meal between 19:00 and 20:00 the evening before each study visit. They fasted overnight and arrived at Hammersmith hospital at 8:30am on each visit. A cannula was inserted into a forearm vein and baseline -30 and -15 min blood samples were collected before participants swallowed capsules containing either sodium chloride (placebo control) or 1, 2, 3 or 5g of sodium propionate. This was immediately followed by consumption of a 250 ml drink containing 75g of glucose together with the capsules of propionate. Postprandial blood samples were taken at 0, 10, 20, 30, 60, 90, 120, 180 mins to assess plasma concentrations of insulin and glucose. Appetite and satiety were assessed by VAS every 30 min during the study visit. A schematic diagram showing the study design for the small intestinal propionate study can be seen in Figure **7.1**.

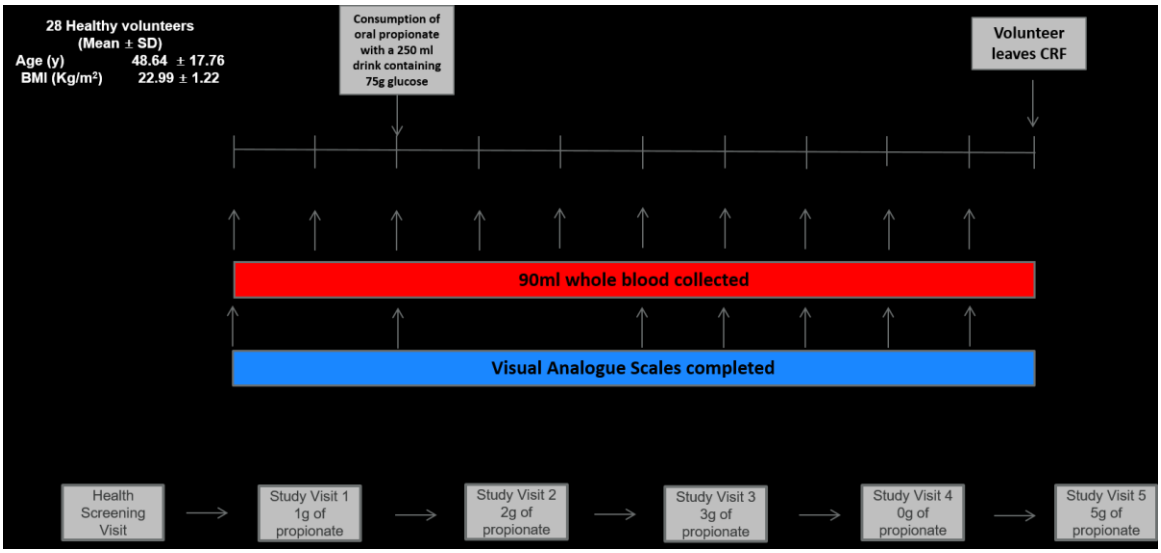


Figure 7.1: Schematic diagram showing the study design for the small intestinal propionate study.
This study was conducted in 28 healthy volunteers. This was a single blind randomised trial with 5 visits, a week apart. Subjects received 1g, 2g, 3g, 0g and 5g of propionate. Assessment of glucose and insulin was made through blood tests and appetite and satiety were assessed by VAS as in previous investigations.

7.4.3 **Power analysis**

This is a pilot study in a new area and therefore a power calculation was not possible. However, in chapter 4 using IPE to raise colonic propionate and explore its effects on appetite and satiety, 14 volunteers were studied in a cross-over design and showed significant decreases in appetite and increases in satiety well before the propionate was delivered to the colon.

7.4.4 **Study participants**

In this study 28 healthy adult volunteers were recruited. The inclusion criteria were a BMI of 20 to 25 kg/m^2 and 18 to 70 years of age with normal fasting blood glucose (below 5.5 mmol/l and HbA1C less than 5.7%). The exclusion criteria were type I or type II diabetes, smoking, substance abuse, pregnancy, use of medications, a change in body weight > 5 kg in the previous 3 months, medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, thyroid function and liver function). All subjects provided informed, written consent prior to starting the study, which was approved by the West London Research Ethics Committee (12/LO/1769). The study was carried out in accordance with the Declaration of Helsinki. A flow diagram of recruitment to this study can be seen in Figure **7.2**.

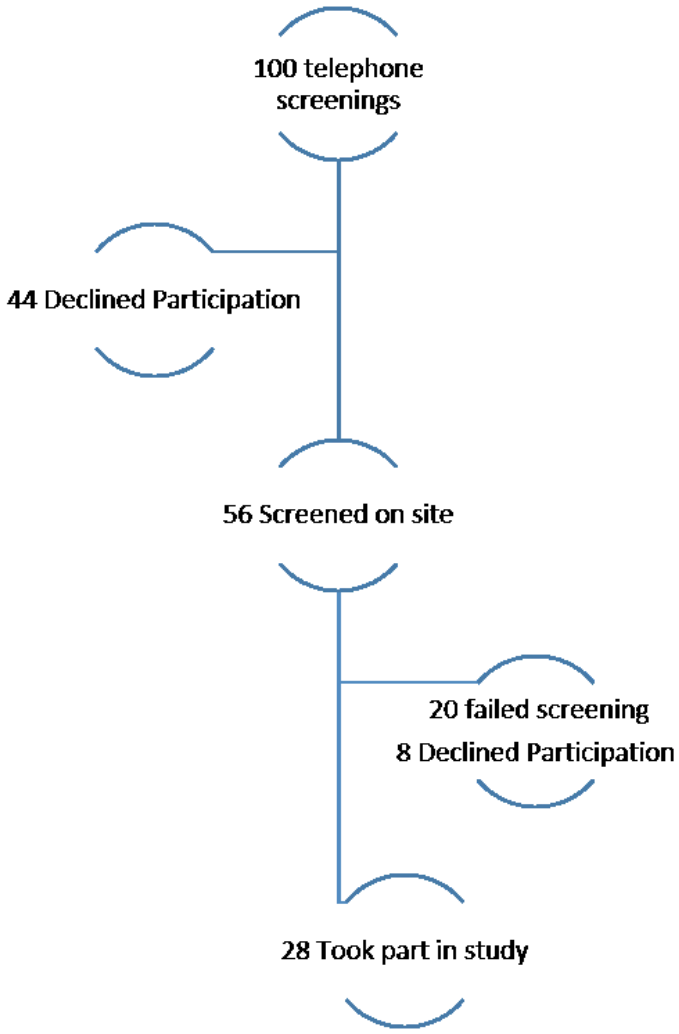


Figure 7.2: Schematic diagram showing the recruitment of participants to the encapsulated propionate study.
The number of telephone screenings, on site screenings, participants and exclusions throughout can be seen for this study.

7.4.5 **Subjective Ratings of Appetite, Satiety and Nausea**

To investigate the effect of encapsulated sodium propionate 1g, 2g, 3g and 5g compared to ~0.5g sodium chloride (placebo) on appetite, satiety and nausea, 100 mm VAS were used at several time points during the study visit as described in the methods chapter.

7.4.6 **Measurement of glucose and insulin**

Levels of insulin were assessed by radioimmunoassay and levels of glucose were measured by an automated clinical chemistry analyser as described previously in chapter 2.

7.4.7 **Statistical Analysis**

Insulin, glucose and VAS were analysed by repeated measures one-way ANOVA.

7.5 Results

7.5.1 **Study Participants**

28 healthy volunteers were recruited to this study. The characteristics of the study participants can be seen in Table 7.1.

Table 7.1: Characteristics of participants in the pre-colonic effect of propionate study

Participants' Characteristics	**Mean ± SD**
Age (years)	48.6 ± 17.8
Weight (kg)	66.7 ± 9.9
BMI (kg/m^2)	23.0 ± 1.2
Gender	13 men and 15 woman

7.5.2 **Encapsulated sodium propionate does not affect appetite.**

2, 3 and 5 g of encapsulated propionate do not significantly affect appetite after consumption of the capsule compared to control (~ 0.5g sodium chloride). This can be seen in Figure **7.3** and Figure **7.4**, p>0.05. We were unable to measure the effects of 1g of sodium propionate on appetite and appetite was only assessed in 11 volunteers because of missing data.

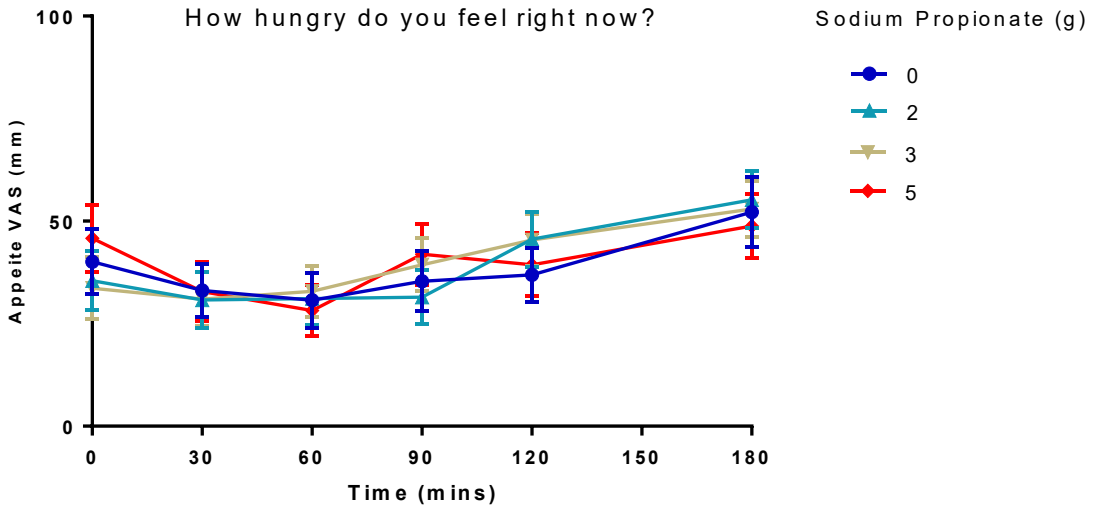


Figure 7.3: 2, 3 and 5 g of sodium propionate do not affect appetite compared to control. Line graph shows response to VAS "how hungry do you feel right now?" over 0 to 180 mins (n = 11). Subjects were provided with 2, 3 and 5 g of encapsulated sodium propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM (p= 0.0604).

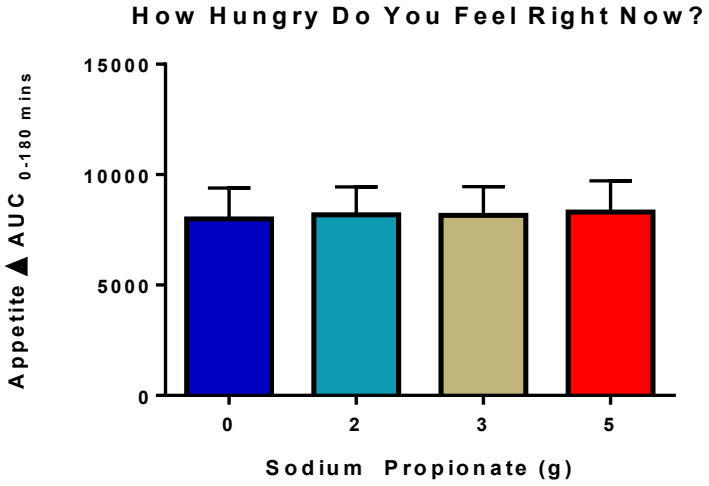


Figure 7.4: 2, 3 and 5 g of sodium propionate do not affect appetite compared to control. Bar graph shows AUC 0-180 mins in response to VAS "how hungry do you feel right now?" (n = 11). Subjects were provided with 2, 3 and 5 g of encapsulated sodium propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM (p= 0.9239).

7.5.3 **Encapsulated sodium propionate does not affect satiety.**

2, 3 and 5 g of encapsulated sodium propionate do not significantly affect satiety after consumption of the capsule compared to control (~0.5g sodium chloride). This can be seen in Figure **7.5** and Figure **7.6**, p>0.05. We were unable to measure the effects of 1g of sodium propionate on satiety and satiety was only assessed in 11 volunteers because of missing data.

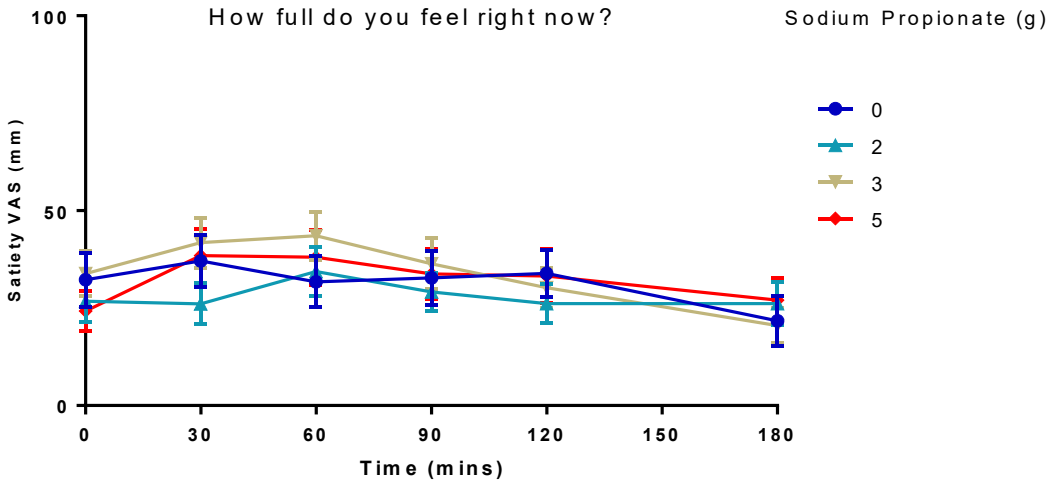


Figure 7.5: 2, 3 and 5 g of sodium propionate do not affect satiety compared to control. Line graph shows response to VAS "how full do you feel right now?" over 0 to 180 mins (n = 11). Subjects were provided with 2, 3 and 5 g of encapsulated sodium propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM (p= 0.0822).

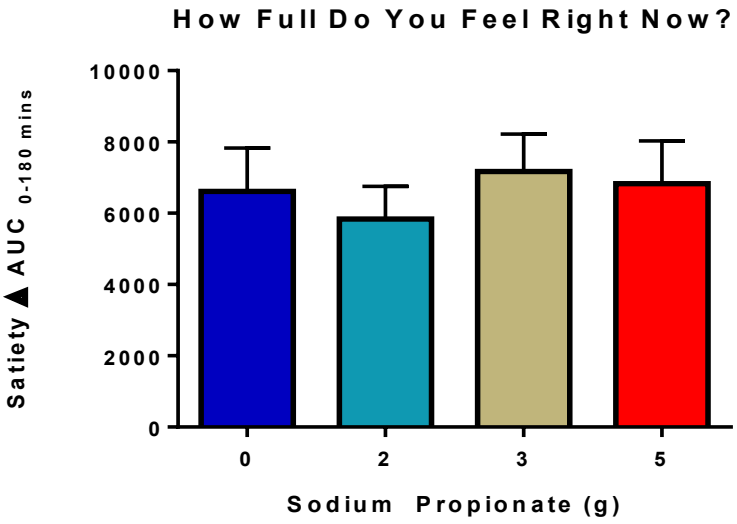


Figure 7.6: 2, 3 and 5 g of sodium propionate do not affect satiety compared to control. Bar graph shows AUC 0-180mins in response to VAS "how full do you feel right now?" (n = 11). Subjects were provided with 2, 3 and 5 g of encapsulated sodium propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM (p= 0.1184).

7.5.4 **Encapsulated sodium propionate does not affect insulin levels**

1, 2, 3 and 5 g of encapsulated sodium propionate do not significantly affect insulin levels between 0 and 180 minutes after consumption of the capsule compared to control (~0.5 g sodium chloride). This can be seen in Figure **7.7**; dose effect (sphericity assumed), $p>0.05$, dose x time effect (sphericity assumed), $p>0.05$. The mean AUC for insulin from 0 to 30 mins and for 0 to 180 mins was also not significant with any of the doses of sodium propionate compared to control (~0.5g sodium chloride) (Figure **7.8**, $p>0.05$).

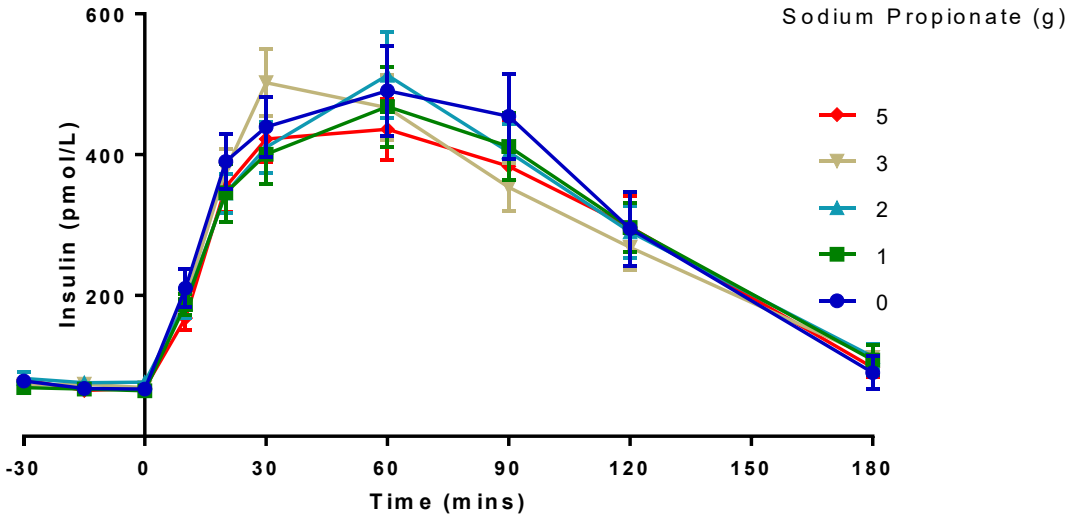
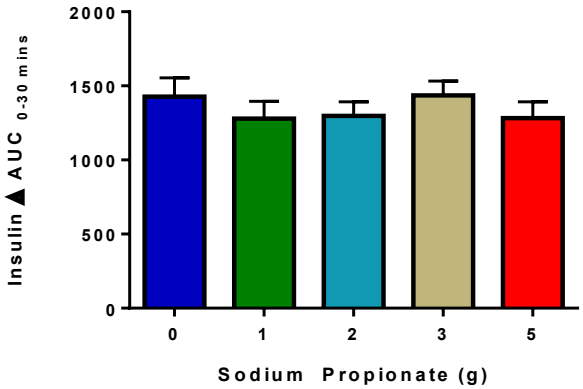


Figure 7.7: 1, 2, 3 and 5 g of sodium propionate do not affect insulin levels compared to control. Line graph shows plasma levels of insulin (pomol/L) over time (n = 28). Subjects were provided with 1, 2, 3 and 5 g of encapsulated propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM ($p>0.05$).



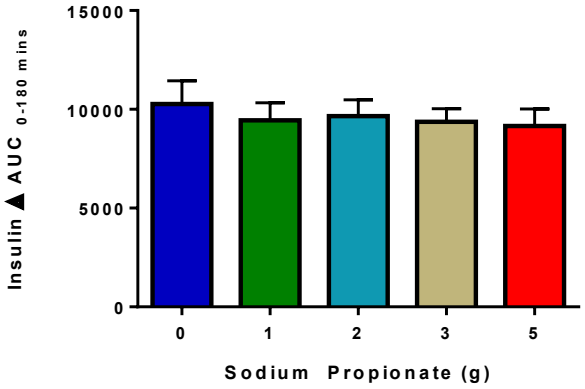


Figure 7.8: 1, 2, 3 and 5 g of sodium propionate do not affect insulin levels compared to control.
Bar charts show mean ∆ Insulin AUC 0-30min and mean ∆ Insulin AUC 0-180min for subjects receiving 1, 2, 3 and 5 g of encapsulated propionate or ~0.5g sodium chloride (placebo) control at 0 mins (n = 28) (p>0.05). Bars show mean ± SEM. P=0.5636. 0-30AUC p=0.3781

7.5.5 **Encapsulated sodium propionate does not affect glucose levels**

1, 2, 3 and 5 g of encapsulated sodium propionate do not significantly affect glucose levels between 0 and 180 minutes after consumption of the capsule compared to control (~0.5g sodium chloride). This can be seen in Figure **7.9**, dose effect (sphericity assumed), p>0.05, dose x time effect (sphericity assumed), p>0.05. The mean AUC for glucose from 0 to 30 min and for 0 to 180 mins were also not significant with any of the doses of sodium propionate compared to control (~0.5g sodium chloride) Figure **7.10**, p>0.05).

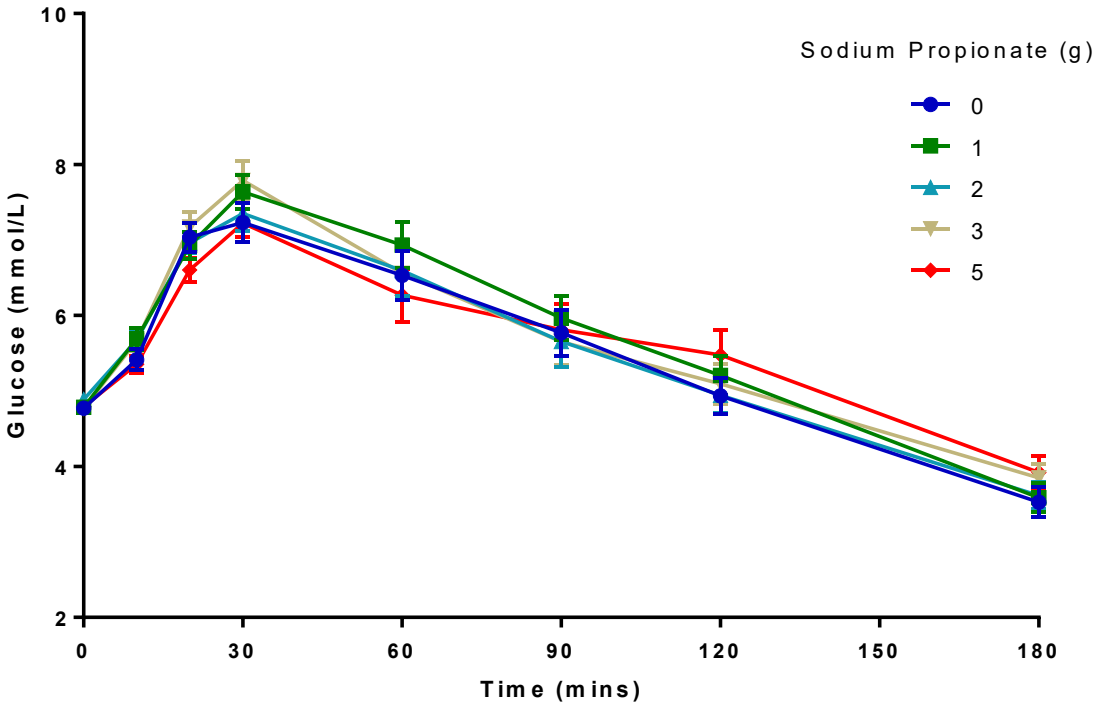
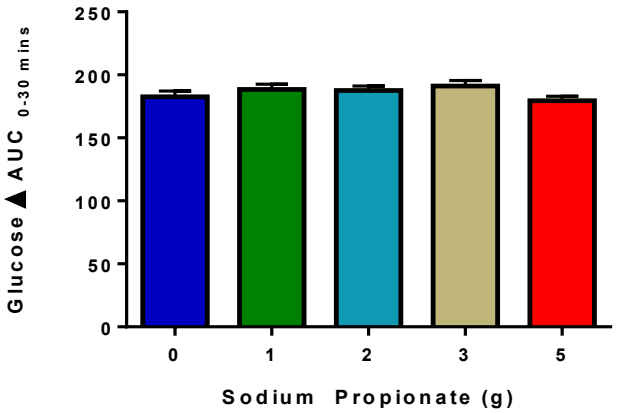


Figure 7.9: 1, 2, 3 and 5 g of sodium propionate do not affect glucose levels compared to control.
Line graph shows plasma levels of glucose (mmol/L) over time (n = 28). Subjects were provided with 1, 2, 3 and 5 g of encapsulated propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM (p= 0.3913).



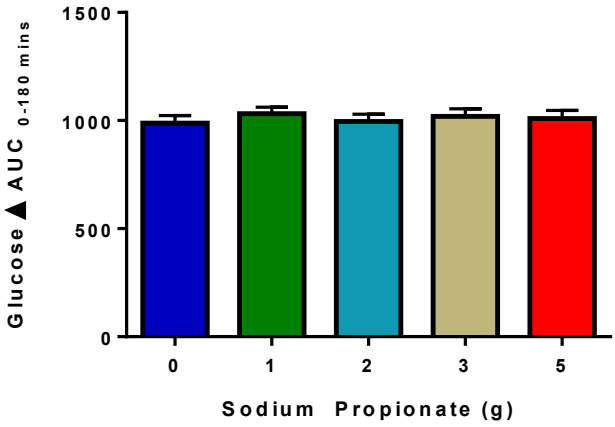


Figure 7.10: 1, 2, 3 and 5 g of sodium propionate do not affect glucose levels compared to control.
Bar charts show mean ∆ glucose AUC 0-30min and mean ∆ glucose AUC 0-180min for subjects receiving 1, 2, 3 and 5 g of encapsulated propionate or ~0.5g sodium chloride (placebo) control at 0 mins (n = 28) (p>0.05). Bars show mean ± SEM.
0-30 RM ANOVA p=0.0271, post-hoc analysis p>0.05. 0-180 p=0.4813

7.5.6 **Encapsulated sodium propionate causes nausea**

Statistical analysis by repeated-measures ANOVA suggests a dose-dependent significant effect of sodium propionate on nausea ($p<0.05$), and post-hoc linear trend analysis confirms that sodium propionate dose-dependently increases nausea ($p<0.05$). This can be seen in Figure **7.11** and Figure **7.12**, $p<0.05$. We were unable to measure the effects of 1g of sodium propionate on nausea and nausea was only assessed in 11 volunteers because of missing data.

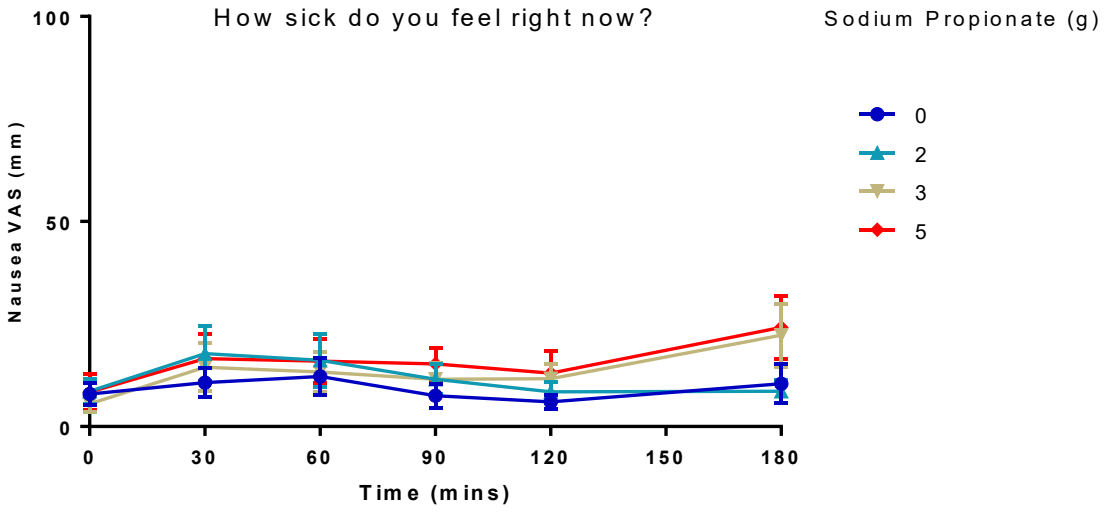


Figure 7.11: 2, 3 and 5 g of sodium propionate cause nausea compared to control.
Line graph shows response to VAS "how sick do you feel right now?" over 0 to 180 mins (n = 11). Subjects were provided with 2, 3 and 5 g of encapsulated sodium propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM.

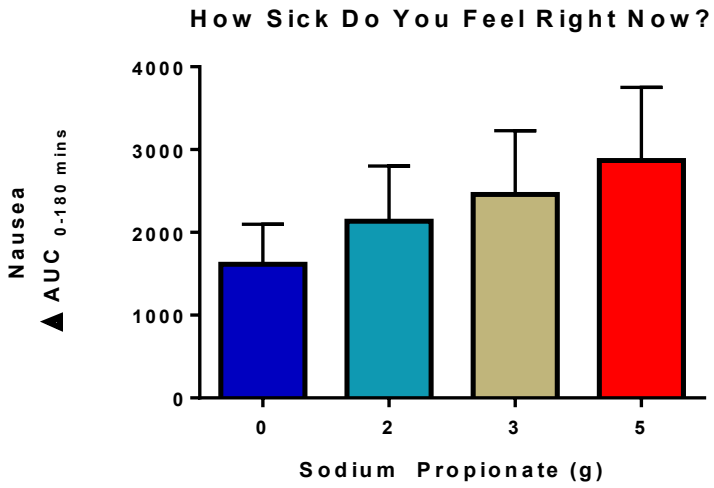


Figure 7.12: 2, 3 and 5 g of sodium propionate do not affect nausea compared to control. Bar graph shows AUC 0-180mins in response to VAS "how sick do you feel right now?" (n = 11). Subjects were provided with 2, 3 and 5 g of encapsulated sodium propionate or ~0.5g sodium chloride (placebo) control at 0 mins. Data show mean ± SEM (p=0.0391).

7.6 Summary of Results

In this final results chapter we have shown the effects of acutely supplementing humans with encapsulated sodium propionate to deliver propionate to the small intestine on appetite, satiety, and plasma insulin and glucose levels**.** We found that a single dose of 1, 2, 3 and 5g of encapsulated sodium propionate delivered to the small intestine:

- does not significantly affect subjective ratings of appetite and satiety
- does not significantly affect insulin levels
- does not significantly affect glucose levels
- but significantly increases subjective ratings of nausea.

7.7 Discussion

7.7.1 **Sodium propionate delivered to the small intestine does not affect appetite and satiety**

Two previous studies conducted in our laboratory lead us to investigate the pre-colonic effect of propionate on satiety and appetite. However in this study, we have shown that propionate released in the small intestine does not affect appetite and satiety, and it is likely that the early effects of propionate on satiety and appetite observed in previous studies occur elsewhere in the GI tract. The first study demonstrated that approximately ~25% of IPE has been released before the colon. This 25% of IPE may have an effect on appetite and satiety, as approximately 0.49g of propionate has been released before arriving to the colon (Chambers et al., 2014). In addition, in chapter 4, we found that IPE significantly increased satiety and decreased appetite within 2 hours after receiving the single dose of 10g of IPE compared to the inulin control. This suggests that satiety and appetite may be affected by the ~25% (0.49g) of propionate released before the colon. These two findings lead us to hypothesise that the 25% of IPE released before colon, maybe in the stomach or small intestine, may be released to the bloodstream and have an effect on satiety and appetite before reaching the colon. To corroborate this, in another human study, volunteers given 0.5g of sodium propionate showed significantly increased satiety within 45 min before the propionate arrived to the colon (Liljeberg et al., 1995). It is likely this effect may be caused by a direct effect of propionate when released to the bloodstream able to affect upon the sympathetic nervous system before arriving to the colon (Kimura et al., 2011, Harthoorn and Dransfield, 2008). Propionate may also affect appetite before the colon but not in the small intestine via the hepatic portal vein (Anil and Forbes, 1980, Anil and Forbes, 1988) or by via vagal afferents (Lal et al., 2001). Propionate stimulating FFAR3 before the small intestine or in the hepatic portal vein may modulate appetite (Kentish and Page, 2015).

7.7.2 **Sodium propionate delivered to the small intestine causes nausea**

In this study, encapsulated propionate to enable delivery to the small intestine significantly increases subjective ratings of nausea. This confirms previous findings of when sodium propionate has previously been shown to be unpalatable and likely to cause nausea when delivered orally (Frost et al., 2003, Liljeberg et al., 1995). Therefore delivering propionate to the colon using IPE is likely to be a better delivery method as this does not induce nausea.

7.7.3 **Sodium propionate delivered to the small intestine does not affect plasma levels of insulin and glucose**

In this study none of the doses of encapsulated propionate affected plasma levels of insulin and glucose. These findings are in agreement with other acute studies where no effects on insulin or glucose are observed with SCFAs. In 6 healthy volunteers, given infusions of mixed doses of SCFAs (90mmol acetate + 30 mmol propionate or 180mmol acetate + 60 mmol propionate), no significant effect on either insulin or glucose levels was found (Wolever et al., 1989). In addition, in another study, 6 healthy volunteers were given 36mmol of acetate or 12mmol of propionate or 36mmol of acetate + 12mmol of propionate or control over a 3 h period, and again no significant effect on fasting glucose or insulin was detected (Laurent et al., 1995). In another acute study, obese subjects were given one dose of acetate and again, this did not affect glucose or insulin levels in a fed state (Freeland and Wolever, 2010). The significant increase in nausea observed with encapsulated propionate may have confounded seeing any effect on glucose tolerance or insulin sensitivity in this study. Venter, 1990 found increased glucose tolerance and insulin sensitivity using encapsulated sodium propionate with long-term supplementation of 7.5g sodium propionate, a higher dose than what was used in this study. The authors, however, did not assess nausea in this investigation.

However, this does not mean that propionate or the other SCFAs do not affect insulin or glucose tolerance. It is more likely that a long term supplementation of SCFAs is required to induce the change in the plasma levels of glucose and insulin. In one human study, oral sodium propionate (9.9g in white bread) or control was given for 1 week and resulted in significantly reduced glucose levels (Todesco et al., 1991). In addition, in another human study, 7.5g sodium propionate or placebo in capsules were given every day for 7 weeks to 20 women, a significantly reduced effect on insulin sensitivity and fasting blood glucose was demonstrated (Venter et al., 1990). Similarly, in a study in mice, where they were given acetate, butyrate or propionate in their diets for 12 weeks, they showed an increased glucose tolerance and insulin sensitivity, likely attributed to an improved β-cell function (den Besten et al., 2015). Similarly, a single dose of butyrate or propionate (400mg/kg) given to WT mice fasted overnight significantly improved beta-cell function (Lin et al., 2012). Furthermore, diabetic rats given butyrate (500 mg/kg/day) for 3 weeks showed increased beta cell function and proliferation and reduced beta cell apoptosis (Khan and Jena, 2014).

Increasing consumption of NDCs has also been shown to increase levels of SCFAs (Tarini and Wolever, 2010) and can also affect glucose tolerance and insulin sensitivity. In one chronic human study, 10 healthy volunteers were given 30g of resistant starch or control each day for 4 weeks. After receiving resistant starch a significant improvement in insulin sensitivity was detected (Robertson et al., 2005). In addition, in another human study, 48 overweight or obese individuals were given 21g of oligofructose or control every day for 12 weeks. This chronic supplementation of NDCs significantly reduced insulin and glucose levels (Parnell and Reimer, 2009). However, healthy human volunteers, in a long term study over 12 weeks were given increasing doses of 15g, 25g, 35g, 45g and 55g of oligofructose every week. They found no significant differences in the plasma levels of glucose and insulin compared to control (Pedersen et al., 2013). Finally, in another human study, 22 overweight or obese individuals were given 30g of oilgofructose or 30g of cellulose (control) for 6 weeks, and no significant effect on insulin and glucose levels was observed (Daud et al., 2014).

The conflicting results between the chronic and acute supplementation of SCFAs on glucose and insulin may be dependent on long-term physiological changes with chronic supplementation. In addition, the effects of SCFAs on insulin and glucose may be dependent on whether an individual is of a normal or obese weight. SCFAs may encourage long-term insulin sensitivity via enhanced adipose tissue function via improved adipogenesis and decreased intracellular lipolysis. Furthermore, SCFAs may alter insulin and glucose function via long-term modulation of lipogenesis and gluconeogenesis in the liver (Canfora et al., 2015).

Our study has shown that encapsulated sodium propionate delivered to the small intestine does not have an effect on insulin and glucose levels. However, ours and findings of others contribute to a body of conflicting results of whether SCFAs do have an effect on insulin and glucose homeostasis, and whether these are dependent on body mass or chronic supplementation. In addition, we have shown that encapsulated propionate which enables delivery to the small intestine does not affect satiety, appetite but does cause nausea. It is clear more research needs to be conducted to explore the use of SCFAs to regulate insulin and glucose and in particular short and long term SCFA effects on glucose tolerance and insulin sensitivity need to be understood.

7.7.4 **Limitations of this work.**

To our knowledge there are no limitations to this study. However, VAS data for 17 subjects for control, 2g, 3g and 5g of encapsulated sodium propionate was lost and all VAS data for 1g of encapsulated sodium propionate was lost, which is not acceptable when performing human research. The data for 1g of encapsulated propionate delivered to the small intestine would have been the most comparable to the 0.64g of propionate lost before the colon when using IPE (Chambers et al., 2014) which had previously reduced subjective appetite (chapter 4). However, as there was no effect of higher doses of encapsulated sodium propionate on appetite this could be extrapolated to suggest the missing data for 1g of encapsulated sodium propionate would have also not been significant.

7.7.5 **Final conclusions**

In this study, early effects on appetite and satiety observed with 10g of IPE in previous chapters are not seen when sodium propionate is delivered to the small intestine suggesting that these observed early effects of propionate on satiety and appetite occur before reaching the small intestine. In addition, different acute doses of encapsulated sodium propionate delivered to the small intestine do not affect glucose and insulin levels but do induce nausea indicating IPE is the preferred delivery method of propionate.

Chapter 8 Discussion

The results from all experimental chapters are discussed in detail at the end of each chapter. In this final discussion the results are brought together to explore the original hypothesis that increasing colonic propionate would reduce food intake in healthy humans and other questions which we have answered whilst exploring this original hypothesis.

8.1 Effect of increasing colonic propionate on satiation

For the first time in humans we have shown that acutely increasing colonic propionate by delivering 10 g of IPE significantly decreased food intake. This is the first time individual delivery of propionate to the human colon using oral methods has been shown to affect satiation.

Our results corroborate those of others in the same field which have shown that SCFAs have an effect on energy intake. *In vivo* in rats, acetate given intraperitoneally significantly reduced food intake (Frost et al., 2014). Furthermore, NDCs have been shown to affect energy intake, and increased levels of SCFA production are associated with increased NDC consumption (Tarini and Wolever, 2010). Long-term consumption of NDCs leads to a significantly reduced food intake (Cani et al., 2005), likely through NDCs driving increases in SCFAs (Tarini and Wolever, 2010).

Our results which found that 10g of IPE to raise colonic propionate can decrease food intake adds to growing evidence that increasing the levels of SCFAs (and more specifically propionate) in the colon may be beneficial to modulate energy intake. However, it still needs to be understood if this will work as a long-term approach, and also whether this will be useful for individuals with obesity.

The mechanism by which propionate reduces energy intake was explored in this thesis. We previously speculated that it could be through increases in PYY and GLP-1 (Psichas et al., 2015), and found increases in PYY and GLP-1 whilst using IPE to raise colonic propionate, and this was concomitant with the reduced energy intake. Instead, we found reductions in brain activity in the nucleus accumbens and caudate - areas involved in anticipatory food behaviour alongside reduced food appeal ratings and reaction times to food, suggesting propionate reduces food anticipatory behaviour. It is likely that this reduced food anticipatory behaviour driven by propionate reduces overall energy intake.

8.2 Effect of increasing levels of propionate in the colon and small intestine on appetite and satiety

We found that 10g of IPE to increase colonic propionate significantly decreased appetite and increased satiety in our group of healthy volunteers. These increases in satiety and reductions in appetite occurred when propionate had been delivered to the colon, but also before reaching the colon, suggesting that IPE can modulate appetite and satiety before and after reaching the colon.

Our findings corroborate a small amount of previous evidence exists showing that SCFAs or increasing SCFAs using NDCs has an effect on appetite. NDC have been linked to increases in SCFAs in the colon, alongside significant reductions in appetite in humans (Daud et al., 2014) and significant increases in satiety (Cani et al., 2005). In addition, findings exploring NDCs in animals have linked SCFAs to reducing appetite. Mice given a fermentable CHO over a chronic period showed reductions in brain activity in areas of the brain involved in appetite regulation. These effects on brain activity were shown alongside significantly increased levels of SCFAs, which suggest a direct effect of SCFAs on appetite (Anastasovska et al., 2012). Furthermore, in mice given acetate a reduction in the activation of brain areas involved in appetite regulation was observed (Frost et al., 2014).

However, we also showed that using IPE to raise colonic propionate sometimes does not affect appetite or satiety over the same period of time despite finding an increased satiation measured by a reduced food intake. These conflicting results highlight there may be a very variable individual response to IPE in the colon on appetite and satiety and also variability in how individuals answer the VAS questions to measure subjective appetite and satiety.

As we observed early pre-colonic effects of IPE on satiety and appetite and know that ~25% of bound propionate is released from the IPE before reaching the colon, alongside preventing weight gain but not concomitant with changes in GLP-1 and PYY (Chambers et al., 2014), we also explored pre-colonic effects of propionate on appetite and satiety. Specifically, the sodium propionate was targeted to the small intestine using capsules which breakdown only in the small intestine. In this study, we found that encapsulated sodium propionate targeted to the small intestine does not affect

subjective ratings of appetite and satiety, suggesting that propionate does not exert effects on appetite through the small intestine.

Overall, we found that propionate released in the colon can modulate subjective appetite and satiety responses, albeit not consistently. Propionate delivered to the small intestine, even in high doses, cannot modulate appetite and satiety responses. It must be noted that subjective ratings using VAS vary considerably between individuals and further work is required to establish the mechanisms of how propionate can affect appetite and satiety in the colon and upstream in the GI tract.

8.3 Effect of increasing colonic propionate on GLP-1 and PYY levels

For the first time in humans we have demonstrated that 10g of IPE, designed to raise colonic propionate levels, significantly increases the plasma levels of PYY and GLP-1 between 4 and 8 hours after ingesting the supplement. We had hypothesised that increasing colonic propionate would significantly increase levels of GLP-1 and PYY, as adding propionate to colonic cell cultures has previously been shown to increase GLP-1 (Tolhurst et al., 2012) and propionate can significantly increase PYY and GLP-1 *in vivo* in rats through activation of FFAR2 (Psichas et al., 2015).

Similarly, effects of propionate on GLP-1 and PYY levels were also predicted because of the relationship between FCs and SCFAs (Tarini and Wolever, 2010). Humans acutely consuming high doses of FCs show significant increases in PYY and propionate (Nilsson et al., 2013). Others have also observed these significant increases in PYY with FCs (Pedersen et al., 2013). Increased levels of PYY are also observed with long-term consumption of FCs in obese individuals (Parnell and Reimer, 2009) and in type II diabetics (Freeland et al., 2010).

However, we have also demonstrated that using IPE to raise colonic levels of propionate sometimes does not affect levels of GLP-1 and PYY. This suggests that one acute dose to increase colonic propionate does not consistently affect the levels of the gut hormones, GLP-1 and PYY, and also may be highly dependent on an individual volunteer's diet, gut microbiota and normal levels of PYY and GLP-1. Others have shown that FCs do not significantly affect levels of GLP-1 and PYY. FCs given acutely, do raise PYY, but not GLP-1 (Nilsson et al., 2013, Pedersen et al., 2013). Chronic provision of FCs significantly increases levels of acetate but does not change levels of PYY or GLP-

1 (Daud et al., 2014). In hyperinsulinaemic subjects, increases in GLP-1 alongside increases in acetate and butyrate were observed after 12 months, but no changes in levels of propionate were observed, therefore suggesting that other SCFAs may be required for significant effects on the gut hormones PYY and GLP-1 (Freeland et al., 2010). Alternatively, effects of SCFAs on increasing levels of PYY and GLP-1 may be due to long-term modulation of the gut microbiota which is not seen with acute supplementation with propionate (David et al., 2014, Cani et al., 2009). It is also possible that we did not see consistent increases in PYY and GLP-1 due to the possible stress associated with being in the fMRI scanner in this particular study. Stress has been linked to suppressed gut hormone levels and could be the reason why increases were not seen in this particular study, despite being observed in the food intake study (chapter 3). We could have controlled for this by offering a dummy visit prior to the study visit in order to familiarise the volunteers with the fMRI scanner and hopefully reduce any stress which could confound the gut hormone results (Muehlhan et al., 2011, Kiessl and Laessle, 2016)

The acute supplementation of propionate and its effects on GLP-1 and PYY still remain unclear. Evidence presented in this thesis demonstrates that using IPE to increase colonic propionate can significantly increase GLP-1 and PYY, but on other occasions no effects are seen on these gut hormones. Taking this, and evidence from others together, suggests that the effects of increasing colonic propionate on GLP-1 and PYY may be dependent on BMI, the state of the gut microbiota, individual variability in levels of FFAR2/3 and differences between acute and chronic supplementation.

8.4 Effect of increasing colonic propionate and small intestinal propionate on nausea

Previous studies have shown that sodium propionate is unpalatable and likely to cause nausea (Liljeberg et al., 1995, Frost et al., 2003). We have shown in all our studies with IPE that acutely increasing colonic propionate using 10g of IPE does not cause nausea. However, when using encapsulated sodium propionate to deliver to the small intestine, nausea was significantly increased. This suggests that using IPE to deliver propionate overcomes these side effects and should be a good method for increasing propionate in the colon.

8.5 Effect of increasing levels of propionate in the colon and small intestine on glucose and insulin

In this thesis, the effects of 10g of IPE to raise colonic propionate on plasma levels of insulin and glucose were explored, but no effects were observed. In addition, encapsulated sodium propionate to deliver propionate to the small intestine did not have an effect on glucose or insulin levels. These findings corroborate other similar studies which have shown that acute propionate or SCFA supplementation do not alter glucose or insulin levels compared to controls in healthy individuals (Wolever et al., 1989, Laurent et al., 1995) and obese individuals (Freeland and Wolever, 2010). Taken together, these findings suggest that acute supplementation of both healthy and obese individuals with SCFAs does not affect glucose and insulin levels.

However, others have shown a role for SCFAs, and specifically propionate in affecting glucose tolerance or insulin sensitivity, but it seems that a more chronic provision of SCFAs is needed to encourage these effects. For example, chronic oral provision of propionate significantly lowered glucose levels in healthy individuals (Todesco et al., 1991) and similarly decreased blood glucose levels and increased insulin sensitivity in another group of healthy individuals (Venter et al., 1990). Furthermore, mechanisms for these improvements in glucose tolerance and insulin sensitivity with SCFAs have been demonstrated in mice. Mice given chronic supplementation of acetate, butyrate or propionate showed improvements in glucose tolerance and insulin sensitivity, which is likely through improvements in β-cell function (den Besten et al., 2015).

Overall, our findings and those of others do not allow clear conclusions to be made about whether SCFAs can affect plasma levels of glucose and insulin. It is likely that any SCFA effects on glucose and insulin are dependent on chronic and sustained supplementation which enable β-cell function to improve (den Besten et al., 2015). Additionally, the method of SCFA administration or body mass may be critical to their effects. SCFAs may enhance insulin sensitivity through long-term changes in adipose tissue function via increased adipogenesis and reduced intracellular lipolysis. Lastly, SCFAs may affect glucose and insulin function through sustained modulation of lipogenesis and gluconeogenesis in the liver (Canfora et al., 2015). It is clear that further work is required to establish the effects of SCFAs on glucose tolerance and insulin sensitivity in humans.

8.6 Effect of increasing colonic propionate on gastric emptying

We also explored the effect of increasing colonic levels of propionate by using IPE on gastric emptying, and this could be a plausible mechanism by which energy intake is reduced and appetite is reduced. We hypothesised that IPE would delay gastric emptying because increasing propionate in the colon would increase the gut hormones GLP-1, and these hormones have been previously shown to delay gastric emptying (Wisen and Hellstrom, 1995, Savage et al., 1987, Hellström et al., 2006, Little et al., 2006). However, we found that raising colonic propionate does not affect the time taken for gastric emptying.

Our findings contrast with other previous studies looking at the effect of SCFAs on gastric emptying. Healthy humans given acute oral propionate showed a significant delay in gastric emptying measured by plasma levels of paracetamol (Liljeberg and Bjorck, 1996). However, in this study approximately 50 mmol of propionate was provided orally whereas in our study approximately 103.5 mmol of propionate was delivered directly to the colon. This suggests the method of administration of propionate is critical to its effect on gastric emptying. However, previous evidence also exists that suggests that increasing SCFAs levels in the colon can slow gastric emptying. Pigs were infused with a mixture of SCFAs acetate, propionate and butyrate and this caused a significant dose-dependent inhibition of gastrointestinal motility (Cuche and Malbert, 1999), but this was later shown alongside increases in PYY suggesting the main reason for delayed gastric emptying with SCFAs is the increased levels of PYY rather than a direct effect of SCFAs (Cuche et al., 2000). Additionally, in these studies in pigs, the effects on gastric emptying were through a colonic infusion of a mixture of SCFAs, and it could be that butyrate and acetate may affect gastric emptying independently of propionate.

In our study, levels of PYY and GLP-1 were not measured but would have been predicted to increase as we have shown that raising colonic propionate can increase PYY and GLP-1 in humans. It is likely any effects of propionate on gastric emptying are likely mediated by changes in levels of gut hormones but may also occur through other mechanisms. High dose PYY infusion in humans delays gastric emptying (Savage et al., 1987) and caused plasma PYY levels to peak at 86 pmol/L, whereas we found raising colonic propionate produces peak plasma levels of PYY of 53.3 pmol/L, which is lower than infusing PYY. In addition, infusion of GLP-1 in humans also delayed gastric emptying (Nauck et al., 1997) driving peak plasma levels of GLP-1 of 20 to 60 pmol/L, which is

similar to what we found when raising colonic propionate with IPE. We speculate that if IPE had significantly increased GLP-1 in this study, this physiologically relevant dose should have had an effect on gastric emptying.

We found no effect of raising colonic propionate on gastric emptying, and effects on food intake and appetite through raised colonic propionate are likely not through changes in gastric emptying. Effects on gastric emptying with SCFAs are likely administration-dependent, e.g. orally or direct to the colon, a specific effect of acetate or butyrate on gastric emptying but not propionate or it could be the effect of SCFAs on gastric emptying are mediated through significant increases in GLP-1 and PYY, which may not have occurred in this study, or an alternative mechanism.

8.7 Effect of increasing colonic propionate on energy expenditure and fat and CHO oxidation

We also explored whether reduced energy intake with colonic propionate were mediated by changes in EE or fat and CHO oxidation. This is the first time, to our knowledge, that the effect of acutely increasing propionate in the colon by using IPE on energy expenditure and fat and CHO oxidation has been explored. However, we found no significant on EE or fat or CHO oxidation after 10g of IPE in the 5 hour period afterwards in which they were measured.

In a long-term study over a period of 24 weeks conducted in our laboratory a significant effect on body weight was found which we speculated may be through increases in EE. Subjects receiving IPE to raise colonic propionate showed a significantly reduced weight gain compared to those receiving control but this was not through changes in PYY and GLP-1 (Chambers et al., 2014). Our findings that increasing colonic propionate does not affect EE are in disagreement with others. Mice given long-term propionate supplementation show an increased energy expenditure and oxygen consumption (den Besten et al., 2015). In humans, fermentable carbohydrates have been shown to increase thermogenesis, (Livesey, 2002) likely as a result of increased SCFAs (Tarini and Wolever, 2010).

Raising colonic propionate did not affect fat oxidation in our study. This is again, in disagreement with other studies in mice and man which have shown that SCFAs can affect fat oxidation (Kondo

et al., 2009, Gao et al., 2009). However, these effects may be specific to obese individuals or as a result of long-term supplementation. For example, obese individuals given chronic provision of acetate showed significantly decreased total adiposity and visceral adiposity (Kondo et al., 2009). Similarly, obese mice given butyrate showed a reduced adiposity after 1 week of supplementation (Gao et al., 2009). The effects of raising colonic propionate on fat oxidation are likely to be dependent on long-term supplementation. When IPE to raise colonic propionate was given long-term and it prevented body weight gain, a reduced intra-abdominal adipose tissue gain, internal adipose tissue gain was observed and the ratio of internal adipose tissue to subcutaneous adipose tissue was also reduced (Chambers et al., 2014).

Evidence from others suggest that propionate likely affects EE and fat oxidation by activating FFAR3 and FFAR2 and through activation of the sympathetic nervous system (Kimura et al., 2011, Bellahcene et al., 2013, Kimura et al., 2013). However, in humans whether activation of FFAR2/3 can modulate fat accumulation is unclear as FFAR2/3 has been shown to be not expressed in human adipose tissue (Hong et al., 2005), whilst others have shown that FFAR2/3 are present in adipose tissue in humans (Le Poul et al., 2003)

Our findings do not rule out an effect of propionate to improve energy expenditure, fat oxidation and body weight, however, these effects may be highly dependent on one or several other variables. For example, an individual being obese, more doses of SCFAs being required (chronic supplementation), individual variability in FFAR2/3 expression, an effect which occurs more than 5 hours after the supplement or an effect of the other SCFAs acetate and butyrate having a more important role in mediating EE and fat oxidation than propionate.

8.8 Effect of increasing colonic propionate on brain control of anticipatory food behaviour

We have shown for the first time, to our knowledge, in humans that increasing propionate in the colon by feeding 10g of inulin-propionate ester significantly reduced BOLD signal in the caudate and nucleus accumbens, whilst also reducing food appeal scores and reaction times suggesting raising colonic propionate significantly reduces anticipatory food behaviour (Goldstone et al.,

2014b, Finlayson et al., 2008). This reduction in anticipatory food behaviour likely drives the reductions in energy intake observed when colonic propionate is raised.

Our findings support others in the field that have shown the effects of SCFAs on areas of the brain involved in food reward. In rats given the SCFA acetate, significantly reduced neuronal activity in the hypothalamus alongside a reduced food intake was found (Frost et al., 2014). Mice given long-term FCs show significantly increased neuronal activity in the arcuate nucleus (Anastasovska et al., 2012).

The effect of inulin-propionate ester on BOLD levels in the caudate and nucleus accumbens as well as in food intake was not as an effect of changes in PYY and GLP-1. One of the possible ways how inulin-propionate ester affects appetite may be via the hepatic portal vein. Indeed, when propionate is increased in the hepatic portal vein energy intake is also reduced (Anil and Forbes, 1980, Anil and Forbes, 1988). IPE may also change neural activity in food reward areas via vagal afferents through SCFA activation of FFAR3 in the portal vein or in the gut may act and effect the in neural activity without changing the levels of PYY and GLP-1 (De Vadder et al., 2014, Lal et al., 2001, Kentish and Page, 2015). Furthermore, propionate may act by changing activity of the nucleus accumbens and the caudate via leptin signalling. Indeed, propionate can activate the SCFAs FFAR3 and FFAR2 in adipose tissue and release leptin (Xiong et al., 2004, Zaibi et al., 2010). Leptin may increase anorexigenic signalling and decreased orexigenic signalling which can reduce the appetite and affect neural activity in response to food (Sahu, 2003). There is also evidence that acetate - another SCFA - can access the brain directly via crossing the blood brain barrier (Chambers et al., 2015). There is also evidence that propionate can pass over the blood brain barrier (Conn et al., 1983) and it is highly likely that propionate may act by this or one of the mechanisms explained above.

Demonstrating that raising colonic propionate significantly reduces anticipatory food behaviour furthers our understanding of how IPE can be used to raise colonic propionate and reduce food intake and possibly drive weight loss to improve overall human health.

8.9 Future directions

The work presented in this thesis acts as a foundation of the capability of supplementing man with IPE on energy homeostasis. As with all findings, further work will increase our knowledge of the effects of this treatment as well its efficacy in disease. The following suggestions provide reasonable investigations which would further our knowledge of the effects of increasing colonic levels of propionate.

8.9.1 **Providing IPE in solid foods rather than as a supplement**

In this thesis, we have shown that providing an acute dose of 10g of IPE to raise colonic levels of propionate can significantly reduce acute food intake and increase subjective ratings of satiety and reduce subjective ratings of appetite. In addition, 10g of IPE can significantly increase levels of the anorectic gut hormones, PYY and GLP-1 and also reduce anticipatory food behaviour as measured by fMRI, food appeal ratings and reaction times. The 10g of IPE in these studies was provided as a powder mixed into a milkshake, however, in order to make the IPE more appealing and marketable it would be desirable to provide IPE already in a solid food rather than as a supplementary powder for greater convenience. For example, mixed in a ready-to-go juice or milkshake, or in a cereal bar or muffin. Firstly, we need to explore whether providing IPE in a more convenient manner rather than as a supplement is possible and remains palatable without inducing nausea. In addition, we need to ensure that providing IPE in solid food will show similar effects on food intake, the gut hormones; PYY and GLP-1, and on satiety and appetite.

8.9.2 **Increase the amount of propionate that can be delivered to the colon by IPE**

In the studies presented in this thesis, 10g of IPE delivers amounts of propionate to the colon that are approximately 2.5 fold higher than normal (Chambers et al., 2014). It would be interesting to ascertain whether we can increase the amount of propionate bound to inulin, and whether we can increase the amounts of IPE delivered to the colon. For example; deliver 20g of IPE in one dose to increase colonic levels of propionate to 5 times higher than normal. It would be important to establish whether these much higher colonic levels of propionate can reduce food intake even more and also increase satiety further. In addition, it is possible that these higher increases in colonic propionate would increase levels of the gut hormones; PYY and GLP-1 to greater amounts than which we found in our studies which may then be high enough to modulate gastric emptying and also reduce food intake further. The highest plasma levels of PYY in this thesis were detected in

chapter 3, where PYY levels peaked at approximately 50 pmol/L. Infusions of PYY which effectively raise plasma levels of PYY to 87 pmol/L can delay gastric emptying in humans (Savage et al., 1987). In addition, infusion of PYY can acutely decrease food intake by 33% when plasma levels of PYY reach 43.5 pmol/L (Batterham et al., 2002). If greater amounts of propionate can be bound to inulin via esterification it would be interesting to see whether this can make acute doses of IPE more effective by increasing GLP-1 and PYY to these higher than physiological levels which may improve energy intake, satiety and delay gastric emptying.

8.9.3 **Investigate the effects of IPE in obese individuals**

After determining whether IPE is effective in solid foods and whether we can increase the amounts of propionate to the colon, it would then be important to determine its effect in obese individuals. A long-term study should be conducted in obese individuals with IPE provided in these new forms every day. In a long-term study in normal individuals given 10g of IPE over a 6 month period were prevented from gaining weight (Chambers et al., 2014), so it could be predicted that long-term provision of IPE may induce weight loss in obese individuals. Furthermore, obese people are at a high risk of developing type II diabetes and showing a reduced insulin sensitivity (Klein et al., 2004). SCFAs can improve insulin sensitivity in obese mice (Gao et al., 2009). In addition, fermentable carbohydrates can increase levels of SCFAs in humans (Tarini and Wolever, 2010) and can improve insulin sensitivity and glucose tolerance after chronic provision in obese individuals (Parnell and Reimer, 2009), suggesting these effects may be seen in obese individuals provided with IPE in a long-term study. Finally, in chapter 6, 10g of IPE reduces anticipatory food behaviour in normal individuals, as detected by fMRI, food appeal ratings and reaction times. Importantly, several studies have shown an increased anticipatory food behaviour in obese compared to lean individuals (Stice et al., 2009). It is highly likely providing IPE in a long term study of obese individuals could significantly reduce anticipatory food behaviour and may prove to be an effective therapy for weight loss.

8.10 Final conclusions

In the data presented in this thesis we have shown for the first time in humans that one dose of 10g of IPE to acutely increase colonic levels of propionate significantly decreases energy intake, increases PYY and GLP-1 levels, increases subjective satiety, and decreases subjective appetite. In addition, we have demonstrated this reduced energy intake is through reduced central

anticipatory food behaviour. Importantly delivering propionate to the colon using IPE does not cause nausea.

As obesity is a worldwide epidemic, can induce several other chronic diseases and health issues, whilst also causing a huge economical and financial burden (Kelly et al., 2008 and World health organization 2014) it is important to develop new strategies to combat obesity. Data presented in this thesis has shown several beneficial effects of acutely raising colonic propionate on energy homeostasis and may provide a novel means in which to target obesity. IPE is a 100% natural product, without any known side effects which make it a sensible method to continue to explore as an anti-obesity agent.

Appendix

Publications arising from this thesis:

Byrne, C. S., Chambers, E. S., **Alhabeeb, H.,** Chhina, N., Morrison, D. J., Preston, T., Tedford, C., Fitzpatrick, J., Irani., C., Busza, A., Garcia-Perez, I., Fountana, S., Holmes, E., Goldstone, A. P., Frost, G. S. 2016. Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. *The American Journal of Clinical Nutrition (Submitted)*

Poster presentations arising from this thesis:

Alhabeeb, H., Chambers, E. S., Frost, G. S., Morrison, D. J., Preston, T. 2013. Inulin propionate ester increases satiety and decreases appetite but does not affect gastric emptying in healthy humans. *The Nutrition Society Winter Meeting, December 2013*

References

ABBOTT, C. R., MONTEIRO, M., SMALL, C. J., SAJEDI, A., SMITH, K. L., PARKINSON, J. R. C., GHATEI, M. A. & BLOOM, S. R. 2005. The inhibitory effects of peripheral administration of peptide YY3–36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal–brainstem–hypothalamic pathway. *Brain Research,* 1044**,** 127-131.

ADRIAN, T. E. 1985. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology,* 89**,** 1070-1077.

AL-LAHHAM, S. H., PEPPELENBOSCH, M. P., ROELOFSEN, H., VONK, R. J. & VENEMA, K. 2010. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids,* 1801**,** 1175-1183.

ALLIROT, X., SAULAIS, L., DISSE, E., ROTH, H., CAZAL, C. & LAVILLE, M. 2012. Validation of a buffet meal design in an experimental restaurant. *Appetite,* 58**,** 889-897.

ANASTASOVSKA, J., ARORA, T., SANCHEZ CANON, G. J., PARKINSON, J. R., TOUHY, K., GIBSON, G. R., NADKARNI, N. A., SO, P. W., GOLDSTONE, A. P., THOMAS, E. L., HANKIR, M. K., VAN LOO, J., MODI, N., BELL, J. D. & FROST, G. 2012. Fermentable carbohydrate alters hypothalamic neuronal activity and protects against the obesogenic environment. *Obesity (Silver Spring),* 20**,** 1016-23.

ANIL, M. & FORBES, J. 1980. Feeding in sheep during intraportal infusions of short-chain fatty acids and the effect of liver denervation. *The Journal of physiology,* 298**,** 407-414.

ANIL, M. & FORBES, J. 1988. The roles of hepatic nerves in the reduction of food intake as a consequence of intraportal sodium propionate administration in sheep. *Quarterly Journal of Experimental Physiology,* 73**,** 539-546.

ARCHER, B. J., JOHNSON, S. K., DEVEREUX, H. M. & BAXTER, A. L. 2004. Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in ment. *British Journal of Nutrition,* 91**,** 591-599.

BALSIGER, B. M., LUQUE DE LEON, E. & SARR, M. G. 1997. Surgical treatment of obesity: who is an appropriate candidate? *Mayo Clin Proc,* 72**,** 551-7; quiz 558.

BATTERHAM, R. L., COHEN, M. A., ELLIS, S. M., LE ROUX, C. W., WITHERS, D. J., FROST, G. S., GHATEI, M. A. & BLOOM, S. R. 2003. Inhibition of Food Intake in Obese Subjects by Peptide YY3–36. *New England Journal of Medicine,* 349**,** 941-948.

BATTERHAM, R. L., COWLEY, M. A., SMALL, C. J., HERZOG, H., COHEN, M. A., DAKIN, C. L., WREN, A. M., BRYNES, A. E., LOW, M. J., GHATEI, M. A., CONE, R. D. & BLOOM, S. R. 2002. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature,* 418**,** 650-654.

BEAVER, J. D., LAWRENCE, A. D., VAN DITZHUIJZEN, J., DAVIS, M. H., WOODS, A. & CALDER, A. J. 2006. Individual Differences in Reward Drive Predict Neural Responses to Images of Food. *The Journal of Neuroscience,* 26**,** 5160-5166.

BELLAHCENE, M., O'DOWD, J. F., WARGENT, E. T., ZAIBI, M. S., HISLOP, D. C., NGALA, R. A., SMITH, D. M., CAWTHORNE, M. A., STOCKER, C. J. & ARCH, J. R. 2013. Male mice that lack the G-protein-coupled receptor GPR41 have low energy expenditure and increased body fat content. *Br J Nutr,* 109**,** 1755-64.

BERTHOUD, H.-R. 2006. Homeostatic and Non-homeostatic Pathways Involved in the Control of Food Intake and Energy Balance. *Obesity,* 14**,** 197S-200S.

BERTHOUD, H.-R., KRESSEL, M., RAYBOULD, H. & NEUHUBER, W. 1995. Vagal sensors in the rat duodenal mucosa: distribution and structure as revealed by in vivo DiI-tracing. *Anatomy and Embryology,* 191**,** 203-212.

BEWICK, G. A. 2012. Bowels control brain: gut hormones and obesity. *Biochem Med (Zagreb),* 22**,** 283-97.

BILLINGTON, C. J., BRIGGS, J. E., GRACE, M. & LEVINE, A. S. 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. *Am J Physiol,* 260**,** R321-7.

BLACKBURN, T. H. & HUNGATE, R. E. 1963. Succinic acid turnover and propionate production in the bovine rumen. *Appl Microbiol,* 11**,** 132-5.

BLOMQVIST, A. G. & HERZOG, H. 1997. Y-receptor subtypes—how many more? *Trends in Neurosciences,* 20**,** 294-298.

BOND, J. H., JR., LEVITT, M. D. & PRENTISS, R. 1975. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H2) measurements. *J Lab Clin Med,* 85**,** 546-55.

BRIEFEL, R. R. & JOHNSON, C. L. 2004. Secular trends in dietary intake in the United States. *Annual Review of Nutrition,* 24**,** 401-431.

BROBERGER, C., JOHANSEN, J., JOHANSSON, C., SCHALLING, M. & HÖKFELT, T. 1998. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proceedings of the National Academy of Sciences of the United States of America,* 95**,** 15043-15048.

BROWN, A. J., GOLDSWORTHY, S. M., BARNES, A. A., EILERT, M. M., TCHEANG, L., DANIELS, D., MUIR, A. I., WIGGLESWORTH, M. J., KINGHORN, I., FRASER, N. J., PIKE, N. B., STRUM, J. C., STEPLEWSKI, K. M., MURDOCK, P. R., HOLDER, J. C., MARSHALL, F. H., SZEKERES, P. G., WILSON, S., IGNAR, D. M., FOORD, S. M., WISE, A. & DOWELL, S. J. 2003. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem,* 278**,** 11312-9.

BUCHWALD, H., AVIDOR, Y., BRAUNWALD, E. & ET AL. 2004. Bariatric surgery: A systematic review and meta-analysis. *JAMA,* 292**,** 1724-1737.

CALBET, J. A. L. & MACLEAN, D. A. 1997. Role of caloric content on gastric emptying in humans. *Journal of Physiology-London,* 498**,** 553-559.

CALLE, E. E., RODRIGUEZ, C., WALKER-THURMOND, K. & THUN, M. J. 2003. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med,* 348**,** 1625-38.

CANFORA, E. E., JOCKEN, J. W. & BLAAK, E. E. 2015. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol,* 11**,** 577-591.

CANI, P. D., DEWEVER, C. & DELZENNE, N. M. 2004. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr,* 92**,** 521-6.

CANI, P. D., JOLY, E., HORSMANS, Y. & DELZENNE, N. M. 2005. Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr,* 60**,** 567-572.

CANI, P. D., LECOURT, E., DEWULF, E. M., SOHET, F. M., PACHIKIAN, B. D., NASLAIN, D., DE BACKER, F., NEYRINCK, A. M. & DELZENNE, N. M. 2009. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *The American Journal of Clinical Nutrition,* 90**,** 1236-1243.

CHAMBERS, E. S., MORRISON, D. J. & FROST, G. 2015. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? *Proceedings of the Nutrition Society,* 74**,** 328-336.

CHAMBERS, E. S., VIARDOT, A., PSICHAS, A., MORRISON, D. J., MURPHY, K. G., ZAC-VARGHESE, S. E., MACDOUGALL, K., PRESTON, T., TEDFORD, C., FINLAYSON, G.

S., BLUNDELL, J. E., BELL, J. D., THOMAS, E. L., MT-ISA, S., ASHBY, D., GIBSON, G. R., KOLIDA, S., DHILLO, W. S., BLOOM, S. R., MORLEY, W., CLEGG, S. & FROST, G. 2014. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut*.

CHERBUT, C. 2003. Motor effects of short-chain fatty acids and lactate in the gastrointestinal tract. *Proc Nutr Soc,* 62**,** 95-9.

CHERBUT, C., FERRIER, L., ROZÉ, C., ANINI, Y., BLOTTIÈRE, H., LECANNU, G. & GALMICHE, J.-P. 1998. *Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat*.

CHO, I., YAMANISHI, S., COX, L., METHE, B. A., ZAVADIL, J., LI, K., GAO, Z., MAHANA, D., RAJU, K., TEITLER, I., LI, H., ALEKSEYENKO, A. V. & BLASER, M. J. 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature,* 488**,** 621-6.

CHOQUET, H. & MEYRE, D. 2011. Genetics of Obesity: What have we Learned? *Current Genomics,* 12**,** 169-179.

CONN, A. R., FELL, D. I. & STEELE, R. D. 1983. Characterization of alpha-keto acid transport across blood-brain barrier in rats. *Am J Physiol,* 245**,** E253-60.

CRAWFORD, D., JEFFERY, R. W. & FRENCH, S. A. 2000. Can anyone successfully control their weight? Findings of a three year community-based study of men and women. *International Journal of Obesity,* 24**,** 1107-1110.

CUCHE, G., CUBER, J. C. & MALBERT, C. H. 2000. Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway. *American Journal of Physiology - Gastrointestinal and Liver Physiology,* 279**,** G925-G930.

CUCHE, G. & MALBERT, C. H. 1999. Ileal short-chain fatty acids inhibit transpyloric flow in pigs. *Scand J Gastroenterol,* 34**,** 149-55.

CUMMINGS, J. H. 1981. Short Chain Fatty-Acids in the Human-Colon. *Gut,* 22**,** 763-779.

CUMMINGS, J. H., POMARE, E. W., BRANCH, W. J., NAYLOR, C. P. & MACFARLANE, G. T. 1987. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut,* 28**,** 1221-7.

DARZI, J., FROST, G. S., MONTASER, R., YAP, J. & ROBERTSON, M. D. 2014. Influence of the tolerability of vinegar as an oral source of short-chain fatty acids on appetite control and food intake. *Int J Obes,* 38**,** 675-681.

DARZI, J., FROST, G. S. & ROBERTSON, M. D. 2011. Do SCFA have a role in appetite regulation? *Proceedings of the Nutrition Society,* 70**,** 119-128.

DARZI, J., FROST, G. S. & ROBERTSON, M. D. 2012. Effects of a novel propionate-rich sourdough bread on appetite and food intake. *Eur J Clin Nutr,* 66**,** 789-94.

DAUD, N. M., ISMAIL, N. A., THOMAS, E. L., FITZPATRICK, J. A., BELL, J. D., SWANN, J. R., COSTABILE, A., CHILDS, C. E., PEDERSEN, C., GOLDSTONE, A. P. & FROST, G. S. 2014. The impact of oligofructose on stimulation of gut hormones, appetite regulation and adiposity. *Obesity,* 22**,** 1430-1438.

DAVID, L. A., MAURICE, C. F., CARMODY, R. N., GOOTENBERG, D. B., BUTTON, J. E., WOLFE, B. E., LING, A. V., DEVLIN, A. S., VARMA, Y., FISCHBACH, M. A., BIDDINGER, S. B., DUTTON, R. J. & TURNBAUGH, P. J. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature,* 505**,** 559-563.

DE SILVA, A. & BLOOM, S. R. 2012. Gut Hormones and Appetite Control: A Focus on PYY and GLP-1 as Therapeutic Targets in Obesity. *Gut and Liver,* 6**,** 10-20.

DE SILVA, A., SALEM, V., LONG, C. J., MAKWANA, A., NEWBOULD, R. D., RABINER, E. A., GHATEI, M. A., BLOOM, S. R., MATTHEWS, P. M., BEAVER, J. D. & DHILLO, W. S. 2011. The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. *Cell Metab,* 14**,** 700-6.

DE VADDER, F., KOVATCHEVA-DATCHARY, P., GONCALVES, D., VINERA, J., ZITOUN, C., DUCHAMPT, A., BÄCKHED, F. & MITHIEUX, G. 2014. Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. *Cell*.

DE WIT, L., LUPPINO, F., VAN STRATEN, A., PENNINX, B., ZITMAN, F. & CUIJPERS, P. 2010. Depression and obesity: A meta-analysis of community-based studies. *Psychiatry Research,* 178**,** 230-235.

DE SILVA, A., SALEM, V., LONG, CHRISTOPHER J., MAKWANA, A., NEWBOULD, REXFORD D., RABINER, EUGENII A., GHATEI, MOHAMMAD A., BLOOM, STEPHEN R., MATTHEWS, PAUL M., BEAVER, JOHN D. & DHILLO, WALJIT S. 2011. The Gut Hormones PYY(3-36) and GLP-1(7-36 amide) Reduce Food Intake and Modulate Brain Activity in Appetite Centers in Humans. *Cell Metabolism,* 14**,** 700-706.

DEGEN, L., OESCH, S., CASANOVA, M., GRAF, S., KETTERER, S., DREWE, J. & BEGLINGER, C. 2005. Effect of Peptide YY3–36 on Food Intake in Humans. *Gastroenterology,* 129**,** 1430-1436.

DEL PARIGI, A., CHEN, K., GAUTIER, J.-F., SALBE, A. D., PRATLEY, R. E., RAVUSSIN, E., REIMAN, E. M. & TATARANNI, P. A. 2002. Sex differences in the human brain's response to hunger and satiation. *The American Journal of Clinical Nutrition,* 75**,** 1017-1022.

DELBENDE, B., PERRI, F., COUTURIER, O., LEODOLTER, A., MAUGER, P., BRIDGI, B., BIZAIS, Y., DES VARANNES, S. B., ANDRIULLI, A. & GALMICHE, J. P. 2000. 13C-octanoic acid breath test for gastric emptying measurement. *Eur J Gastroenterol Hepatol,* 12**,** 85-91.

DELMÉE, E., CANI, P. D., GUAL, G., KNAUF, C., BURCELIN, R., MATON, N. & DELZENNE, N. M. 2006. Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. *Life Sciences,* 79**,** 1007-1013.

DELZENNE, N. M., DAUBIOUL, C., NEYRINCK, A., LASA, M. & TAPER, H. S. 2002. Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. *Br J Nutr,* 87 Suppl 2**,** S255-9.

DEN BESTEN, G., BLEEKER, A., GERDING, A., VAN EUNEN, K., HAVINGA, R., VAN DIJK, T. H., OOSTERVEER, M. H., JONKER, J. W., GROEN, A. K., REIJNGOUD, D.-J. & BAKKER, B. M. 2015. Short-Chain Fatty Acids Protect Against High-Fat Diet–Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes,* 64**,** 2398-2408.

DEN BESTEN, G., VAN EUNEN, K., GROEN, A. K., VENEMA, K., REIJNGOUD, D.-J. & BAKKER, B. M. 2013a. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research,* 54**,** 2325-2340.

DEN BESTEN, G., VAN EUNEN, K., GROEN, A. K., VENEMA, K., REIJNGOUD, D. J. & BAKKER, B. M. 2013b. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research,* 54**,** 2325-2340.

DILLON, J. S., TANIZAWA, Y., WHEELER, M. B., LENG, X. H., LIGON, B. B., RABIN, D. U., YOO-WARREN, H., PERMUTT, M. A. & A E BOYD, R. 1993. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. *Endocrinology,* 133**,** 1907-1910.

DOCKRAY, G. J. 1988. The G.L. Brown lecture. Regulatory peptides and the neuroendocrinology of gut-brain relations. *Q J Exp Physiol,* 73**,** 703-27.

ELIAS, C. F., LEE, C., KELLY, J., ASCHKENASI, C., AHIMA, R. S., COUCEYRO, P. R., KUHAR, M. J., SAPER, C. B. & ELMQUIST, J. K. 1998. Leptin Activates Hypothalamic CART Neurons Projecting to the Spinal Cord. *Neuron,* 21**,** 1375-1385.

FINLAYSON, G., KING, N. & BLUNDELL, J. 2008. The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control. *Appetite,* 50**,** 120-127.

FLINT, A., RABEN, A., ASTRUP, A. & HOLST, J. J. 1998. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *Journal of Clinical Investigation,* 101**,** 515-520.

FLINT, A., RABEN, A., REHFELD, J. F., HOLST, J. J. & ASTRUP, A. 2000. The effect of glucagon-like peptide-1 on energy expenditure and substrate metabolism in humans. *International Journal of Obesity,* 24**,** 288-298.

FLINT, H. J., SCOTT, K. P., LOUIS, P. & DUNCAN, S. H. 2012. The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology,* 9**,** 577-589.

FREELAND, K. R., WILSON, C. & WOLEVER, T. M. S. 2010. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. *British Journal of Nutrition,* 103**,** 82-90.

FREELAND, K. R. & WOLEVER, T. M. S. 2010. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-α. *British Journal of Nutrition,* 103**,** 460-466.

FROST, G., SLEETH, M. L., SAHURI-ARISOYLU, M., LIZARBE, B., CERDAN, S., BRODY, L., ANASTASOVSKA, J., GHOURAB, S., HANKIR, M., ZHANG, S., CARLING, D., SWANN, J. R., GIBSON, G., VIARDOT, A., MORRISON, D., LOUISE THOMAS, E. & BELL, J. D. 2014. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun,* 5.

FROST, G. S., BRYNES, A. E., DHILLO, W. S., BLOOM, S. R. & MCBURNEY, M. I. 2003. The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. *Eur J Clin Nutr,* 57**,** 293-298.

FÜHRER, D., ZYSSET, S. & STUMVOLL, M. 2008. Brain Activity in Hunger and Satiety: An Exploratory Visually Stimulated fMRI Study. *Obesity,* 16**,** 945-950.

GAO, Z., YIN, J., ZHANG, J., WARD, R. E., MARTIN, R. J., LEFEVRE, M., CEFALU, W. T. & YE, J. 2009. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes,* 58**,** 1509-17.

GHOOS, Y. F., MAES, B. D., GEYPENS, B. J., MYS, G., HIELE, M. I., RUTGEERTS, P. J. & VANTRAPPEN, G. 1993. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology,* 104**,** 1640-7.

GOLDSTONE, A. P., PRECHTL, C. G., SCHOLTZ, S., MIRAS, A. D., CHHINA, N., DURIGHEL, G., DELIRAN, S. S., BECKMANN, C., GHATEI, M. A. & ASHBY, D. R. 2014a. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. *The American journal of clinical nutrition,* 99**,** 1319-1330.

GOLDSTONE, A. P., PRECHTL, C. G., SCHOLTZ, S., MIRAS, A. D., CHHINA, N., DURIGHEL, G., DELIRAN, S. S., BECKMANN, C., GHATEI, M. A., ASHBY, D. R., WALDMAN, A. D., GAYLINN, B. D., THORNER, M. O., FROST, G. S., BLOOM, S. R. & BELL, J. D. 2014b. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. *Am J Clin Nutr,* 99**,** 1319-30.

GOLDSTONE, A. P., PRECHTL DE HERNANDEZ, C. G., BEAVER, J. D., MUHAMMED, K., CROESE, C., BELL, G., DURIGHEL, G., HUGHES, E., WALDMAN, A. D., FROST, G. & BELL, J. D. 2009. Fasting biases brain reward systems towards high-calorie foods. *European Journal of Neuroscience,* 30**,** 1625-1635.

GROOMS, K. N., OMMERBORN, M. J., PHAM, D. Q., DJOUSSE, L. & CLARK, C. R. 2013. Dietary Fiber Intake and Cardiometabolic Risks among US Adults, NHANES 1999–2010. *The American journal of medicine,* 126**,** 10.1016/j.amjmed.2013.07.023.

HABIB, A. M., RICHARDS, P., ROGERS, G. J., REIMANN, F. & GRIBBLE, F. M. 2013. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. *Diabetologia,* 56**,** 1413-1416.

HARTHOORN, L. F. & DRANSFIELD, E. 2008. Periprandial changes of the sympathetic–parasympathetic balance related to perceived satiety in humans. *European Journal of Applied Physiology,* 102**,** 601-608.

HEATON, K. W. 1973. Food Fiber as an Obstacle to Energy-Intake. *Lancet,* 2**,** 1418-1421.

HELLSTRÖM, P. M., GRYBÄCK, P. & JACOBSSON, H. 2006. The physiology of gastric emptying. *Best Practice & Research Clinical Anaesthesiology,* 20**,** 397-407.

HOLST, J. J. 2007. The physiology of glucagon-like peptide 1. *Physiol Rev,* 87**,** 1409-39.

HONG, Y. H., NISHIMURA, Y., HISHIKAWA, D., TSUZUKI, H., MIYAHARA, H., GOTOH, C., CHOI, K. C., FENG, D. D., CHEN, C., LEE, H. G., KATOH, K., ROH, S. G. & SASAKI, S. 2005. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology,* 146**,** 5092-9.

HOWARTH, N. C., SALTZMAN, E. & ROBERTS, S. B. 2001. Dietary fiber and weight regulation. *Nutr Rev,* 59**,** 129-39.

I˙MERYÜZ, N., YEĞEN, B. Ç., BOZKURT, A., COŞKUN, T., VILLANUEVA-PEÑACARRILLO, M. L. & ULUSOY, N. B. 1997. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *American Journal of Physiology - Gastrointestinal and Liver Physiology,* 273**,** G920-G927.

INOUE, D., TSUJIMOTO, G. & KIMURA, I. 2014. Regulation of Energy Homeostasis by GPR41. *Front Endocrinol (Lausanne),* 5**,** 81.

JENKINS, D. J. & JENKINS, A. L. 1985. Dietary fiber and the glycemic response. *Proc Soc Exp Biol Med,* 180**,** 422-31.

JOHNS, D. J., HARTMANN-BOYCE, J., JEBB, S. A. & AVEYARD, P. 2014. Diet or Exercise Interventions vs Combined Behavioral Weight Management Programs: A Systematic Review and Meta-Analysis of Direct Comparisons. *Journal of the Academy of Nutrition and Dietetics,* 114**,** 1557-1568.

JORGENSEN, J. R., CLAUSEN, M. R. & MORTENSEN, P. B. 1997. Oxidation of short and medium chain C2-C8 fatty acids in Sprague-Dawley rat colonocytes. *Gut,* 40**,** 400-5.

KAEMMERER, E., PLUM, P., KLAUS, C., WEISKIRCHEN, R., LIEDTKE, C., ADOLF, M., SCHIPPERS, A., WAGNER, N., REINARTZ, A. & GASSLER, N. 2010. Fatty acid binding receptors in intestinal physiology and pathophysiology. *World Journal of Gastrointestinal Pathophysiology,* 1**,** 147-153.

KAJI, I., KARAKI, S., TANAKA, R. & KUWAHARA, A. 2011. Density distribution of free fatty acid receptor 2 (FFA2)-expressing and GLP-1-producing enteroendocrine L cells in human and rat lower intestine, and increased cell numbers after ingestion of fructo-oligosaccharide. *J Mol Histol,* 42**,** 27-38.

KARAKI, S., MITSUI, R., HAYASHI, H., KATO, I., SUGIYA, H., IWANAGA, T., FURNESS, J. B. & KUWAHARA, A. 2006. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell and Tissue Research,* 324**,** 353-360.

KARAKI, S., TAZOE, H., HAYASHI, H., KASHIWABARA, H., TOOYAMA, K., SUZUKI, Y. & KUWAHARA, A. 2008. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol,* 39**,** 135-42.

KEENAN, M. J., ZHOU, J., MCCUTCHEON, K. L., RAGGIO, A. M., BATEMAN, H. G., TODD, E., JONES, C. K., TULLEY, R. T., MELTON, S., MARTIN, R. J. & HEGSTED, M. 2006. Effects

of Resistant Starch, A Non-digestible Fermentable Fiber, on Reducing Body Fat. *Obesity,* 14**,** 1523-1534.

KENTISH, S. J. & PAGE, A. J. 2015. The role of gastrointestinal vagal afferent fibres in obesity. *The Journal of physiology,* 593**,** 775-786.

KHAN, S. & JENA, G. B. 2014. Protective role of sodium butyrate, a HDAC inhibitor on beta-cell proliferation, function and glucose homeostasis through modulation of p38/ERK MAPK and apoptotic pathways: Study in juvenile diabetic rat. *Chemico-Biological Interactions,* 213**,** 1-12.

KIEFFER, T. J., MCINTOSH, C. H. & PEDERSON, R. A. 1995. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology,* 136**,** 3585-3596.

KIESSL, G. R. & LAESSLE, R. G. 2016. Stress inhibits PYY secretion in obese and normal weight women. *Eat Weight Disord,* 21**,** 245-9.

KILLGORE, W. D. S., YOUNG, A. D., FEMIA, L. A., BOGORODZKI, P., ROGOWSKA, J. & YURGELUN-TODD, D. A. 2003. Cortical and limbic activation during viewing of high- versus low-calorie foods. *NeuroImage,* 19**,** 1381-1394.

KIMURA, I., INOUE, D., MAEDA, T., HARA, T., ICHIMURA, A., MIYAUCHI, S., KOBAYASHI, M., HIRASAWA, A. & TSUJIMOTO, G. 2011. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A,* 108**,** 8030-5.

KIMURA, I., OZAWA, K., INOUE, D., IMAMURA, T., KIMURA, K., MAEDA, T., TERASAWA, K., KASHIHARA, D., HIRANO, K., TANI, T., TAKAHASHI, T., MIYAUCHI, S., SHIOI, G., INOUE, H. & TSUJIMOTO, G. 2013. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun,* 4**,** 1829.

KLEIN, S., SHEARD, N. F., PI-SUNYER, X., DALY, A., WYLIE-ROSETT, J., KULKARNI, K. & CLARK, N. G. 2004. Weight Management Through Lifestyle Modification for the Prevention and Management of Type 2 Diabetes: Rationale and Strategies: A statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. *Diabetes Care,* 27**,** 2067-2073.

KONDO, T., KISHI, M., FUSHIMI, T., UGAJIN, S. & KAGA, T. 2009. Vinegar intake reduces body weight, body fat mass, and serum triglyceride levels in obese Japanese subjects. *Biosci Biotechnol Biochem,* 73**,** 1837-43.

KREYMANN, B., GHATEI, M. A., WILLIAMS, G. & BLOOM, S. R. 1987. GLUCAGON-LIKE PEPTIDE-1 7-36: A PHYSIOLOGICAL INCRETIN IN MAN. *The Lancet,* 330**,** 1300-1304.

KRISTENSEN, P., JUDGE, M. E., THIM, L., RIBEL, U., CHRISTJANSEN, K. N., WULFF, B. S., CLAUSEN, J. T., JENSEN, P. B., MADSEN, O. D., VRANG, N., LARSEN, P. J. & HASTRUP, S. 1998. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature,* 393**,** 72-76.

KROMHOUT, D., BLOEMBERG, B., SEIDELL, J. C., NISSINEN, A. & MENOTTI, A. 2001. Physical activity and dietary fiber determine population body fat levels: the Seven Countries Study. *Int J Obes Relat Metab Disord,* 25**,** 301-6.

LABAR, K. S., GITELMAN, D. R., PARRISH, T. B., KIM, Y.-H., NOBRE, A. C. & MESULAM, M. M. 2001. Hunger selectively modulates corticolimbic activation to food stimuli in humans. *Behavioral Neuroscience,* 115**,** 493-500.

LAL, S., KIRKUP, A. J., BRUNSDEN, A. M., THOMPSON, D. G. & GRUNDY, D. 2001. Vagal afferent responses to fatty acids of different chain length in the rat. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 281**,** G907-G915.

LAMBERT, P. D., COUCEYRO, P. R., MCGIRR, K. M., DALL VECHIA, S. E., SMITH, Y. & KUHAR, M. J. 1998. CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse,* 29**,** 293-8.

LAURENT, C., SIMONEAU, C., MARKS, L., BRASCHI, S., CHAMP, M., CHARBONNEL, B. & KREMPF, M. 1995. Effect of acetate and propionate on fasting hepatic glucose production in humans. *Eur J Clin Nutr,* 49**,** 484-91.

LE BLAY, G., MICHEL, C., BLOTTIERE, H. M. & CHERBUT, C. 1999. Enhancement of butyrate production in the rat caecocolonic tract by long-term ingestion of resistant potato starch. *Br J Nutr,* 82**,** 419-26.

LE POUL, E., LOISON, C., STRUYF, S., SPRINGAEL, J. Y., LANNOY, V., DECOBECQ, M. E., BREZILLON, S., DUPRIEZ, V., VASSART, G., VAN DAMME, J., PARMENTIER, M. & DETHEUX, M. 2003. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *Journal of Biological Chemistry,* 278**,** 25481-25489.

LENARD, N. R. & BERTHOUD, H.-R. 2008. Central and Peripheral Regulation of Food Intake and Physical Activity: Pathways and Genes. *Obesity (Silver Spring, Md.),* 16**,** S11-S22.

LEVRAT, M. A., REMESY, C. & DEMIGNE, C. 1991. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. *J Nutr,* 121**,** 1730-7.

LILJEBERG, H. G. M. & BJORCK, I. M. E. 1996. Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: Studies in humans and rats using test products with added organic acids or an organic salt. *American Journal of Clinical Nutrition,* 64**,** 886-893.

LILJEBERG, H. G. M., LONNER, C. H. & BJORCK, I. M. E. 1995. Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *Journal of Nutrition,* 125**,** 1503-1511.

LIN, H. V., FRASSETTO, A., KOWALIK JR, E. J., NAWROCKI, A. R., LU, M. M., KOSINSKI, J. R., HUBERT, J. A., SZETO, D., YAO, X., FORREST, G. & MARSH, D. J. 2012. Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. *PLoS ONE,* 7**,** e35240.

LITTLE, T. J., PILICHIEWICZ, A. N., RUSSO, A., PHILLIPS, L., JONES, K. L., NAUCK, M. A., WISHART, J., HOROWITZ, M. & FEINLE-BISSET, C. 2006. Effects of Intravenous Glucagon-Like Peptide-1 on Gastric Emptying and Intragastric Distribution in Healthy Subjects: Relationships with Postprandial Glycemic and Insulinemic Responses. *The Journal of Clinical Endocrinology & Metabolism,* 91**,** 1916-1923.

LIU, S. M., WILLETT, W. C., MANSON, J. E., HU, F. B., ROSNER, B. & COLDITZ, G. 2003. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *American Journal of Clinical Nutrition,* 78**,** 920-927.

LIVESEY, G. 2002. Thermogenesis associated with fermentable carbohydrate in humans, validity of indirect calorimetry, and implications of dietary thermogenesis for energy requirements, food energy and body weight. *Int J Obes Relat Metab Disord,* 26**,** 1553-69.

LOIS, K. & KUMAR, S. 2009. Obesity and diabetes. *Endocrinología y Nutrición,* 56, Supplement 4**,** 38-42.

LUDWIG, D. S., PEREIRA, M. A., KROENKE, C. H., HILNER, J. E., VAN HORN, L., SLATTERY, M. L. & JACOBS, D. R., JR. 1999. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA,* 282**,** 1539-46.

MACFARLANE, S. & MACFARLANE, G. T. 2003. Regulation of short-chain fatty acid production. *Proc Nutr Soc,* 62**,** 67-72.

MCGAVIGAN, A. K. & MURPHY, K. G. 2012. Gut hormones: the future of obesity treatment? *British Journal of Clinical Pharmacology,* 74**,** 911-919.

MCGILL, H. C., MCMAHAN, C. A., HERDERICK, E. E., ZIESKE, A. W., MALCOM, G. T., TRACY, R. E., STRONG, J. P. & GROUP, F. T. P. D. O. A. I. Y. R. 2002. Obesity Accelerates the Progression of Coronary Atherosclerosis in Young Men. *Circulation,* 105**,** 2712-2718.

MCNELIS, J. C., LEE, Y. S., MAYORAL, R., VAN DER KANT, R., JOHNSON, A. M. F., WOLLAM, J. & OLEFSKY, J. M. 2015. GPR43 Potentiates β-Cell Function in Obesity. *Diabetes,* 64**,** 3203-3217.

MCPHERSON, K., MARSH, T. & BROWN, M. 2007. Foresight Tackling Obesities: Future Choices – Modelling Future Trends in Obesity and the Impact on Health. 2nd Edition ed.

MESSINA, G., DE LUCA, V., VIGGIANO, A., ASCIONE, A., IANNACCONE, T., CHIEFFI, S. & MONDA, M. 2013. Autonomic nervous system in the control of energy balance and body weight: personal contributions. *Neurol Res Int,* 2013**,** 639280.

MILLER, T. L. & WOLIN, M. J. 1996. Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Appl Environ Microbiol,* 62**,** 1589-92.

MITCHELL, A. B., COLE, J. W., MCARDLE, P. F., CHENG, Y. C., RYAN, K. A., SPARKS, M. J., MITCHELL, B. D. & KITTNER, S. J. 2015. Obesity increases risk of ischemic stroke in young adults. *Stroke,* 46**,** 1690-2.

MUEHLHAN, M., LUEKEN, U., WITTCHEN, H. U. & KIRSCHBAUM, C. 2011. The scanner as a stressor: evidence from subjective and neuroendocrine stress parameters in the time course of a functional magnetic resonance imaging session. *Int J Psychophysiol,* 79**,** 118-26.

NAUCK, M. A., NIEDEREICHHOLZ, U., ETTLER, R., HOLST, J. J., ØRSKOV, C., RITZEL, R. & SCHMIEGEL, W. H. 1997. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *American Journal of Physiology - Endocrinology and Metabolism,* 273**,** E981-E988.

NEARY, M. T. & BATTERHAM, R. L. 2010. Gaining New Insights into Food Reward with Functional Neuroimaging. *Frontiers in Eating and Weight Regulation,* 63**,** 152-163.

NELSON, L. H. & TUCKER, L. A. 1996. Diet composition related to body fat in a multivariate study of 203 men. *J Am Diet Assoc,* 96**,** 771-7.

NILSSON, A., JOHANSSON, E., EKSTRÖM, L. & BJÖRCK, I. 2013. Effects of a Brown Beans Evening Meal on Metabolic Risk Markers and Appetite Regulating Hormones at a Subsequent Standardized Breakfast: A Randomized Cross-Over Study. *PLoS ONE,* 8**,** e59985.

NILSSON, N. E., KOTARSKY, K., OWMAN, C. & OLDE, B. 2003. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem Biophys Res Commun,* 303**,** 1047-52.

OFFICE OF DISEASE PREVENTION AND HEALTH PROMOTION. 2008. *2008 Physical Activity Guidelines for Americans* [Online]. http://www.health.gov/paguidelines/guidelines/. [Accessed 3rd July 2015].

PARNELL, J. A. & REIMER, R. A. 2009. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *The American Journal of Clinical Nutrition,* 89**,** 1751-1759.

PAYNTER, M. J. & ELSDEN, S. R. 1970. Mechanism of propionate formation by Selenomonas ruminantium, a rumen micro-organism. *J Gen Microbiol,* 61**,** 1-7.

PEDERSEN, C., LEFEVRE, S., PETERS, V., PATTERSON, M., GHATEI, M. A., MORGAN, L. M. & FROST, G. S. 2013. Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. A dose-escalation study. *Appetite,* 66**,** 44-53.

PLOURDE, B., SARRAZIN, J. F., NAULT, I. & POIRIER, P. 2014. Sudden cardiac death and obesity. *Expert Rev Cardiovasc Ther,* 12**,** 1099-110.

POEHLMAN, E. T. 1989. A review: exercise and its influence on resting energy metabolism in man. *Medicine and science in sports and exercise,* 21**,** 515-525.

PONTIROLI, A. E. & MORABITO, A. 2011. Long-term prevention of mortality in morbid obesity through bariatric surgery. a systematic review and meta-analysis of trials performed with gastric banding and gastric bypass. *Ann Surg,* 253**,** 484-7.

PRIYADARSHINI, M. & LAYDEN, B. T. 2015. FFAR3 modulates insulin secretion and global gene expression in mouse islets. *Islets***,** e1045182.

PRIYADARSHINI, M., VILLA, S. R., FULLER, M., WICKSTEED, B., MACKAY, C. R., ALQUIER, T., POITOUT, V., MANCEBO, H., MIRMIRA, R. G., GILCHRIST, A. & LAYDEN, B. T. 2015. An Acetate-Specific GPCR, FFAR2, Regulates Insulin Secretion. *Molecular Endocrinology,* 29**,** 1055-1066.

PSICHAS, A., SLEETH, M. L., MURPHY, K. G., BROOKS, L., BEWICK, G. A., HANYALOGLU, A. C., GHATEI, M. A., BLOOM, S. R. & FROST, G. 2015. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *International Journal of Obesity,* 39**,** 424-429.

PUBLIC HEALTH ENGLAND. 2007. *Obesity and Health* [Online]. http://www.noo.org.uk/NOO_about_obesity/obesity_and_health. [Accessed July 3rd 2015].

PUCCI, A. & FINER, N. 2015. New medications for treatment of obesity: metabolic and cardiovascular effects. *Can J Cardiol,* 31**,** 142-52.

QUIGLEY, E. M. M. 2011. Microflora Modulation of Motility. *Journal of Neurogastroenterology and Motility,* 17**,** 140-147.

RE, R. N. 2009. Obesity-related hypertension. *Ochsner J,* 9**,** 133-6.

REIMER, R. A., MAURER, A. D., ELLER, L. K., HALLAM, M. C., SHAYKHUTDINOV, R., VOGEL, H. J. & WELJIE, A. M. 2012. Satiety Hormone and Metabolomic Response to an Intermittent High Energy Diet Differs in Rats Consuming Long-Term Diets High in Protein or Prebiotic Fiber. *Journal of Proteome Research,* 11**,** 4065-4074.

ROBERTSON, M. D., BICKERTON, A. S., DENNIS, A. L., VIDAL, H. & FRAYN, K. N. 2005. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *The American Journal of Clinical Nutrition,* 82**,** 559-567.

ROBERTSON, M. D., CURRIE, J. M., MORGAN, L. M., JEWELL, D. P. & FRAYN, K. N. 2003. Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects. *Diabetologia,* 46**,** 659-665.

ROTH, C. L., ENRIORI, P. J., HARZ, K., WOELFLE, J., COWLEY, M. A. & REINEHR, T. 2005. Peptide YY Is a Regulator of Energy Homeostasis in Obese Children before and after Weight Loss. *The Journal of Clinical Endocrinology & Metabolism,* 90**,** 6386-6391.

ROTHEMUND, Y., PREUSCHHOF, C., BOHNER, G., BAUKNECHT, H.-C., KLINGEBIEL, R., FLOR, H. & KLAPP, B. F. 2007. Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. *NeuroImage,* 37**,** 410-421.

ROTHMAN, R. B. & BAUMANN, M. H. 2009. Serotonergic Drugs and Valvular Heart Disease. *Expert opinion on drug safety,* 8**,** 317-329.

SAHU, A. 2003. Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. *Frontiers in Neuroendocrinology,* 24**,** 225-253.

SAMUEL, B. S., SHAITO, A., MOTOIKE, T., REY, F. E., BACKHED, F., MANCHESTER, J. K., HAMMER, R. E., WILLIAMS, S. C., CROWLEY, J., YANAGISAWA, M. & GORDON, J. I. 2008. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 16767-16772.

SAVAGE, A. P., ADRIAN, T. E., CAROLAN, G., CHATTERJEE, V. K. & BLOOM, S. R. 1987. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut,* 28**,** 166-170.

SCAZZINA, F., DEL RIO, D., BENINI, L., MELEGARI, C., PELLEGRINI, N., MARCAZZAN, E. & BRIGHENTI, F. 2011. The effect of breakfasts varying in glycemic index and glycemic load on dietary induced thermogenesis and respiratory quotient. *Nutrition, Metabolism and Cardiovascular Diseases,* 21**,** 121-125.

SCHICK, R. R., ZIMMERMANN, J. P., WALDE, T. V. & SCHUSDZIARRA, V. 2003. Glucagon-like peptide 1-(7–36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 284**,** R1427-R1435.

SCHMIDT, J. B., GREGERSEN, N. T., PEDERSEN, S. D., ARENTOFT, J. L., RITZ, C., SCHWARTZ, T. W., HOLST, J. J., ASTRUP, A. & SJÖDIN, A. 2014. Effects of PYY3-36 and GLP-1 on energy intake, energy expenditure and appetite in overweight men. *American Journal of Physiology - Endocrinology and Metabolism*.

SCHMITT, M. G., JR., SOERGEL, K. H. & WOOD, C. M. 1976. Absorption of short chain fatty acids from the human jejunum. *Gastroenterology,* 70**,** 211-5.

SCHOLTZ, S., MIRAS, A. D., CHHINA, N., PRECHTL, C. G., SLEETH, M. L., DAUD, N. M., ISMAIL, N. A., DURIGHEL, G., AHMED, A. R., OLBERS, T., VINCENT, R. P., ALAGHBAND-ZADEH, J., GHATEI, M. A., WALDMAN, A. D., FROST, G. S., BELL, J. D., LE ROUX, C. W. & GOLDSTONE, A. P. 2014. Obese patients after gastric bypass surgery have lower brain-hedonic responses to food than after gastric banding. *Gut,* 63**,** 891-902.

SCIENTIFIC ADVISORY COMMITTEE ON NUTRITION 2014. Draft Carbohydrates and Health report.

SEALE, J. L., RUMPLER, W. V. & MOE, P. W. 1991. Description of a direct-indirect room-sized calorimeter. *Am J Physiol,* 260**,** E306-20.

SIEP, N., ROEFS, A., ROEBROECK, A., HAVERMANS, R., BONTE, M. L. & JANSEN, A. 2009. Hunger is the best spice: An fMRI study of the effects of attention, hunger and calorie content on food reward processing in the amygdala and orbitofrontal cortex. *Behavioural Brain Research,* 198**,** 149-158.

SJOSTROM, L., LINDROOS, A. K., PELTONEN, M., TORGERSON, J., BOUCHARD, C., CARLSSON, B., DAHLGREN, S., LARSSON, B., NARBRO, K., SJOSTROM, C. D., SULLIVAN, M., WEDEL, H. & SWEDISH OBESE SUBJECTS STUDY SCIENTIFIC, G. 2004. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med,* 351**,** 2683-93.

SLAVIN, J. & GREEN, H. 2007. Dietary fibre and satiety. *Nutrition Bulletin,* 32**,** 32-42.

SLAVIN, J. L. 1987. Dietary fiber: classification, chemical analyses, and food sources. *J Am Diet Assoc,* 87**,** 1164-71.

SLEETH, M. L., THOMPSON, E. L., FORD, H. E., ZAC-VARGHESE, S. E. & FROST, G. 2010. Free fatty acid receptor 2 and nutrient sensing: a proposed role for fibre, fermentable carbohydrates and short-chain fatty acids in appetite regulation. *Nutr Res Rev,* 23**,** 135-45.

SLOTH, B., HOLST, J. J., FLINT, A., GREGERSEN, N. T. & ASTRUP, A. 2007. *Effects of PYY1–36 and PYY3–36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects*.

SMALL, C. J., KIM, M. S., STANLEY, S. A., MITCHELL, J. R. D., MURPHY, K., MORGAN, D. G. A., GHATEI, M. A. & BLOOM, S. R. 2001. Effects of Chronic Central Nervous System Administration of Agouti-Related Protein in Pair-Fed Animals. *Diabetes,* 50**,** 248-254.

SNYDER, E. M., CARR, R. D., DEACON, C. F. & JOHNSON, B. D. 2008. Overnight hypoxic exposure and glucagon-like peptide-1 and leptin levels in humans. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme,* 33**,** 929-935.

STICE, E., SPOOR, S., NG, J. & ZALD, D. H. 2009. Relation of Obesity to Consummatory and Anticipatory Food Reward. *Physiology & behavior,* 97**,** 551-560.

STOECKEL, L. E., WELLER, R. E., COOK III, E. W., TWIEG, D. B., KNOWLTON, R. C. & COX, J. E. 2008. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *NeuroImage,* 41**,** 636-647.

SZAMEITAT, A. J., SHEN, S. & STERR, A. 2009. The functional magnetic resonance imaging (fMRI) procedure as experienced by healthy participants and stroke patients – A pilot study. *BMC Medical Imaging,* 9**,** 1-11.

TAN, T. M., FIELD, B. C. T., MCCULLOUGH, K. A., TROKE, R. C., CHAMBERS, E. S., SALEM, V., GONZALEZ MAFFE, J., BAYNES, K. C. R., DE SILVA, A., VIARDOT, A., ALSAFI, A., FROST, G. S., GHATEI, M. A. & BLOOM, S. R. 2013. Coadministration of Glucagon-Like Peptide-1 During Glucagon Infusion in Humans Results in Increased Energy Expenditure and Amelioration of Hyperglycemia. *Diabetes,* 62**,** 1131-1138.

TARINI, J. & WOLEVER, T. M. 2010. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Appl Physiol Nutr Metab,* 35**,** 9-16.

TAZOE, H., OTOMO, Y., KARAKI, S.-I., KATO, I., FUKAMI, Y., TERASAKI, M. & KUWAHARA, A. 2009. Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomedical Research,* 30**,** 149-156.

TODESCO, T., RAO, A. V., BOSELLO, O. & JENKINS, D. J. 1991. Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. *The American Journal of Clinical Nutrition,* 54**,** 860-5.

TOLHURST, G., HEFFRON, H., LAM, Y. S., PARKER, H. E., HABIB, A. M., DIAKOGIANNAKI, E., CAMERON, J., GROSSE, J., REIMANN, F. & GRIBBLE, F. M. 2012. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes,* 61**,** 364-71.

TSUJII, S. & BRAY, G. A. 1989. Acetylation alters the feeding response to MSH and beta-endorphin. *Brain Research Bulletin,* 23**,** 165-169.

TUCKER, L. A. & THOMAS, K. S. 2009. Increasing total fiber intake reduces risk of weight and fat gains in women. *J Nutr,* 139**,** 576-81.

TUOMILEHTO, J., LINDSTRÖM, J., ERIKSSON, J. G., VALLE, T. T., HÄMÄLÄINEN, H., ILANNE-PARIKKA, P., KEINÄNEN-KIUKAANNIEMI, S., LAAKSO, M., LOUHERANTA, A., RASTAS, M., SALMINEN, V., AUNOLA, S., CEPAITIS, Z., MOLTCHANOV, V., HAKUMÄKI, M., MANNELIN, M., MARTIKKALA, V., SUNDVALL, J. & UUSITUPA, M. 2001. Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. *New England Journal of Medicine,* 344**,** 1343-1350.

VAN VUGT, D. A. 2010. Brain imaging studies of appetite in the context of obesity and the menstrual cycle. *Human Reproduction Update,* 16**,** 276-292.

VAN WYMELBEKE, V., LOUIS-SYLVESTRE, J. & FANTINO, M. 2001. Substrate oxidation and control of food intake in men after a fat-substitute meal compared with meals supplemented with an isoenergetic load of carbohydrate, long-chain triacylglycerols, or medium-chain triacylglycerols. *The American Journal of Clinical Nutrition,* 74**,** 620-630.

VENTER, C. S., VORSTER, H. H. & CUMMINGS, J. H. 1990. Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. *Am J Gastroenterol,* 85**,** 549-53.

VERBEKE, K. 2009. Will the 13C-octanoic acid breath test ever replace scintigraphy as the gold standard to assess gastric emptying? *Neurogastroenterology & Motility,* 21**,** 1013-1016.

VERDICH, C., FLINT, A., GUTZWILLER, J.-P., NÄSLUND, E., BEGLINGER, C., HELLSTRÖM, P. M., LONG, S. J., MORGAN, L. M., HOLST, J. J. & ASTRUP, A. 2001. A Meta-Analysis of the Effect of Glucagon-Like Peptide-1 (7–36) Amide on Ad Libitum Energy Intake in Humans. *The Journal of Clinical Endocrinology & Metabolism,* 86**,** 4382-4389.

VIJAY-KUMAR, M., AITKEN, J. D., CARVALHO, F. A., CULLENDER, T. C., MWANGI, S., SRINIVASAN, S., SITARAMAN, S. V., KNIGHT, R., LEY, R. E. & GEWIRTZ, A. T. 2010. Metabolic Syndrome and Altered Gut Microbiota in Mice Lacking Toll-Like Receptor 5. *Science,* 328**,** 228-231.

WEIR, J. B. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol,* 109**,** 1-9.

WEISS, E. C., GALUSKA, D. A., KETTEL KHAN, L., GILLESPIE, C. & SERDULA, M. K. 2007. Weight regain in U.S. adults who experienced substantial weight loss, 1999-2002. *Am J Prev Med,* 33**,** 34-40.

WEN, J., PHILLIPS, S. F., SARR, M. G., KOST, L. J. & HOLST, J. J. 1995. PYY and GLP-1 contribute to feedback inhibition from the canine ileum and colon. *American Journal of Physiology - Gastrointestinal and Liver Physiology,* 269**,** G945-G952.

WISEN, O. & HELLSTROM, P. M. 1995. Gastrointestinal motility in obesity. *J Intern Med,* 237**,** 411-8.

WOLEVER, T. M., BRIGHENTI, F., ROYALL, D., JENKINS, A. L. & JENKINS, D. J. 1989. Effect of rectal infusion of short chain fatty acids in human subjects. *Am J Gastroenterol,* 84**,** 1027-33.

WOLEVER, T. M., VUKSAN, V., ESHUIS, H., SPADAFORA, P., PETERSON, R. D., CHAO, E. S., STOREY, M. L. & JENKINS, D. J. 1991. Effect of method of administration of psyllium on glycemic response and carbohydrate digestibility. *J Am Coll Nutr,* 10**,** 364-71.

WOLIN, K. Y., CARSON, K. & COLDITZ, G. A. 2010. Obesity and Cancer. *The Oncologist,* 15**,** 556-565.

WOOD, W. G., WACHTER, C. & SCRIBA, P. C. 1981. Experiences Using Chloramine-T and 1,3,4,6-Tetrachloro-3-Alpha,6-Alpha-Diphenylglycoluril (Iodogen) for Radioiodination of Materials for Radioimmunoassay. *Journal of Clinical Chemistry and Clinical Biochemistry,* 19**,** 1051-1056.

WORLD HEALTH ORGANIZATION. 2015. *Obesity and overweight Fact sheet N°311* [Online]. http://www.who.int/mediacentre/factsheets/fs311/en/. [Accessed 3rd July 2015].

XIONG, Y., MIYAMOTO, N., SHIBATA, K., VALASEK, M. A., MOTOIKE, T., KEDZIERSKI, R. M. & YANAGISAWA, M. 2004. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proceedings of the National Academy of Sciences of the United States of America,* 101**,** 1045-1050.

YAMADA, T. & ALPERS, D. H. 2009. *Textbook of gastroenterology,* Chichester, West Sussex ; Hoboken, NJ, Blackwell Pub.

YEO, G. S. H., FAROOQI, I. S., AMINIAN, S., HALSALL, D. J., STANHOPE, R. G. & O'RAHILLY, S. 1998. A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet,* 20**,** 111-112.

YOUNG, A. A. 2012. Brainstem sensing of meal-related signals in energy homeostasis. *Neuropharmacology,* 63**,** 31-45.

ZAIBI, M. S., STOCKER, C. J., O'DOWD, J., DAVIES, A., BELLAHCENE, M., CAWTHORNE, M. A., BROWN, A. J. H., SMITH, D. M. & ARCH, J. R. S. 2010. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Letters,* 584**,** 2381-2386.

ZHANG, H., DIBAISE, J. K., ZUCCOLO, A., KUDRNA, D., BRAIDOTTI, M., YU, Y., PARAMESWARAN, P., CROWELL, M. D., WING, R., RITTMANN, B. E. & KRAJMALNIK-BROWN, R. 2009. Human gut microbiota in obesity and after gastric bypass. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 2365-2370.

ZHOU, J., MARTIN, R. J., TULLEY, R. T., RAGGIO, A. M., MCCUTCHEON, K. L., SHEN, L., DANNA, S. C., TRIPATHY, S., HEGSTED, M. & KEENAN, M. J. 2008. Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. *American Journal of Physiology - Endocrinology and Metabolism,* 295**,** E1160-E1166.