The Effect of Fermentable Carbohydrate on Glucose Homeostasis and Weight Management in People at High-Risk of Developing Diabetes

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Subjects with prediabetes are at much higher risk of developing diabetes than healthy subjects. Weight loss helps reduce the risk of developing diabetes in these subjects, but long-term weight loss is difficult to achieve due to increases in appetite. Consumption of fermentable carbohydrate has been shown to reduce food intake and body weight, and also improve insulin sensitivity independent of weight loss. Therefore, fermentable carbohydrates may help prevent the development of diabetes in subjects with prediabetes via a two-pronged effect.

This thesis comprises three investigations which examine the effects of inulin compared to cellulose control on 1) appetite and food intake, 2) measures of glucose homeostasis and 3) a long-term randomised control trial examining the effect of inulin on weight loss maintenance. In each investigation, subjects take 30g/inulin a day following a 4-week dose-escalation period. In investigations 1 and 2 all subjects take both inulin and cellulose supplements for a 6-week period each, separated by a 4-week wash-out phase. Investigation 3 is a randomised control trial comprising a 9-week weight loss phase, during which participants aim to reach a 5% weight loss at 9 weeks, and a 9-week weight maintenance phase during which subjects are asked to maintain the weight they have lost.

In this body of work I demonstrate that inulin reduces appetite, food intake and weight in subjects with prediabetes. I also demonstrate that inulin was significantly associated with an increase in early insulin secretion and GLP-1, potentially due to an improvement in the incretin effect. Inulin also appears to improve insulin sensitivity in subjects with a specific
subtype of prediabetes only. Finally, I demonstrate that inulin supplementation results in significantly greater weight loss maintenance, alongside changes in body composition likely to be beneficial long-term.

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DECLARATION OF CONTRIBUTORS

All of the work described in this thesis was performed by the author. All collaboration and assistance is detailed below:

For measurement of GLP-1 and PYY by radioimmunoassay (RIA), assistance was provided by:

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Andrew Hogben
Dr Claire Fives
Dr Michelle Sleeth
Dr James Minion
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ABBREVIATIONS

AMPK AMP-activated Protein Kinase
AUC Area Under the Curve
BAT Brown Adipose Tissue
BMI Body Mass Index
CHO Carbohydrate
CVD Cardiovascular Disease
FCHO Fermentable carbohydrate
FM Fat Mass
FFM Free Fat Mass
FFA Free Fatty Acid
FFA2 GPR43 (G-Protein Coupled Receptor 43)
FFA3 (GPR43 (G-Protein Coupled Receptor 42)
FOS Fructo-oligosaccharides
GI Gastrointestinal
GLP-1 Glucagon-Like Peptide One
G6Pase Glucose 6-phosphatase
HbA1C Haemoglobin A1c
HOMA B% Homeostatic Model
Assessment (Beta cell)
HOMA IR Homeostatic Model Assessment (Insulin Resistance)
HSL Hormone Sensitive Lipase
IFG Impaired Fasting Glucose
IFG/IGT Impaired Fasting Glucose
Glucose/Impaired Glucose Tolerance
i-iFG isolated Impaired Fasting Glucose
i-IGT Impaired Glucose Tolerance
IGT Impaired Glucose Tolerance
IGR Impaired Glucose Regulation
IHCL Intrahepatocellular Lipid
IMCL Intramyocellular Lipid
IVGTT Intravenous Glucose Tolerance Test
MRI Magnetic Resonance Imaging
MRS Magnetic Resonance Spectroscopy
MTT Meal Tolerance Test
OFS Oligofructose
OGTT Oral Glucose Tolerance Test
PEPCK Phosphoenolpyruvate carboxykinase
PRO Protein
PYY Peptide YY
RS Resistant Starch
SAT Subcutaneous Adipose Tissue
SCFA Short Chain Fatty Acid
SEM Standard Error of the Mean
SD Standard Deviation
T2DM Type 2 Diabetes
UEA-IFG University of East Anglia- Impaired Fasting Glucose Study
UCP1 Uncoupling Protein 1
VAS Visual Analogue Scales
VAT Visceral Adipose Tissue
VLCD Very Low Calorie Diet
CHAPTER ONE:

GENERAL INTRODUCTION
1.0. TYPE 2 DIABETES

Diabetes (T2DM) is a chronic metabolic disease characterised by hyperglycaemia. T2DM accounts for ~ 90% of all cases of T2DM (1). T2DM increases the risk of CVD 5-8 fold; the risk of stroke 3-fold, the risk of transient ischaemic attack 6-fold and increases admission to hospital 5-fold (2). In the UK, T2DM is a leading cause of blindness, stroke, kidney failure and amputation. There are approximately 3M people with T2DM in the UK with a new case being diagnosed every 5 minutes (3). The NHS currently spends £1M an hour on T2DM (2,3), and this is expected to nearly double by 2040. Worldwide, mean fasting plasma glucose is increasing at a rate of 0.07mmol/L per decade (4), while the prevalence of T2DM is expected to reach 7.7% (438.7M people) by 2030 (4).

1.1. CAUSES

The non-modifiable risk factors for T2DM are age, ethnicity and having a first-degree relative with T2DM. Non-modifiable risk factors are: overweight and obesity and low levels of physical activity. Women who have previously had gestational diabetes and women who have polycystic ovarian syndrome are also at higher risk (5). While an increasing number of genetic variants proposed to increase T2DM risk have been found, lifestyle factors have a stronger predictive value for T2DM risk (6-9).

While T2DM is a multi-factorial disease, it is clear that the increase in obesity worldwide is driving the T2DM epidemic. Between 1980 and 2008, worldwide obesity prevalence increased from 4.8% to 9.8% and from 7.9% to 13.8% in men and women, respectively (10). BMI is one of the strongest predictors of T2DM incidence with the relative risk of T2DM increasing as BMI increases above 22kg/m² (11,12). T2DM risk increases linearly with BMI with only a 2% prevalence in people with a BMI <25 kg/m² rising to 13% in those with a BMI > 35 kg/m² (13). Each addition 1kg increase in weight increases the risk of T2DM by 4.5-9% (14).
1.2 PATHOPHYSIOLOGY

T2DM develops over years and decades and has been described as primarily due to consequences of chronic fuel surfeit in genetically or epigenetically-susceptible individuals (15). However, it must also be remembered that diabetes occurs in lean subjects (16), while it is possible to be obese and metabolically healthy (17), indicating that obesity alone is not sufficient for diabetes to develop. Moreover, diabetes presents at a younger age, at lower degrees of adiposity, and progresses more rapidly in different ethnic groups (18), factors which are important to consider in light of approaches to prevention. In all cases, the progressive loss of β-cell function, and eventual β-cell failure are central in the development of diabetes, and in many subjects this may be the primary metabolic defect (19,20). The mechanisms underlying both insulin secretion and insulin resistance are not well understood. It has been proposed that while non-susceptible individuals are able to partition energy into subcutaneous adipose tissue (SAT) (21), susceptible individuals store the calorie excess ectopically, in organs such as the liver, pancreas and heart (22-27). Interestingly, subjects with lipodystrophy have elevated ectopic fat despite their lean phenotype (28), indicating that some mechanisms underlying diabetes development may be shared by normal-weight and overweight individuals (12,16,29). There are many factors driving ectopic storage of fat including impaired expansion of SAT (23,30), insulin resistance and inappropriate inflammatory response, including elevated cytokines and reduced adiponectin (31,32). Furthermore, inflammatory cytokines including TNF-α and IL-6 have also been shown to impair insulin signalling in 3T3 cells (33). It is important to note that the loss of β-cell function is critical, not least because robust first-phase insulin secretion suppresses endogenous glucose production in the liver, in addition to driving the uptake of glucose into peripheral tissue (26). Figure 1.1 illustrates some of the underlying and physiological differences between overweight individuals resistant and susceptible to developing T2DM.
In addition, other mechanisms may be involved which exacerbate the increasing dysfunction: increased free fatty acids (FFA) released from adipocytes (34), reduced incretin effect (35); increased secretion of glucagon and hepatic sensitivity to glucagon (36,37); enhanced glucose reabsorption in the kidneys (38) and further inflammatory response (31). Different factors may contribute to different degrees in different individuals and the process is not completely understood.

What is clear is that the underlying molecular dysfunction eventually leads to impairments in insulin secretion and insulin resistance such that blood glucose begins to rise. In the Whitehall II study, a prospective longitudinal study following a cohort of 10,308 male and female civil servants in the UK, fasting and post-meal glucose concentrations increased gradually and modestly over the 12 years preceding diagnosis of T2DM (39), with a relatively sudden increase 2-6 years prior to diagnosis.
Importantly, however, some people develop diabetes via elevated fasting glucose, with normal post-load glucose, while others develop diabetes via increased post-load glucose but normal fasting glucose (40). However, by grouping all subjects together, the authors of the Whitehall II study masked what may have been disparate fasting and post-load glucose values in the “pre-diabetic” state.

1.3 PREDIABETES

Prediabetes is defined by glycaemic values higher than normal but not high enough to meet the T2DM threshold. The diagnostic criteria have changed over time and vary between institutions (Table 1.1). The WHO defines Impaired Fasting Glucose (IFG) at a fasting glucose value of 6.1-6.9 mmol/L and Impaired Glucose Tolerance (IGT) as a 2-hr plasma glucose following 75g of glucose of between 7.8-11.1 mmol/L (41) (42). NICE and Diabetes UK currently endorse the WHO definitions. The American Diabetes Association defines Impaired Fasting Glucose as a fasting glucose of 5.6-6.9 mmol/L (43). Prediabetes may also be defined by Hba1c of 5.7-6.4% (38-46 mmol/mol) (44) or 6.0-6.4% (42-46 mmol/mol) (41). IFG and IGT are commonly referred to as prediabetes.

<table>
<thead>
<tr>
<th>Prediabetic State</th>
<th>WHO (mmol/L)</th>
<th>ADA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFG</td>
<td>6.1-6.9</td>
<td>5.6-6.9</td>
</tr>
<tr>
<td>IGT</td>
<td>7.8-11.1</td>
<td>7.8-11.1</td>
</tr>
</tbody>
</table>

Table 1.1: Diagnostic criteria for prediabetes for WHO and ADA

1.3.1 RISK FACTORS

The risk factors for both IFG and IGT are similar to those of T2DM. Both states of prediabetes are associated with CVD risk factors including hypertension, dyslipidaemia, hyperinsulinaemia, microalbuminaemia and elevated inflammatory and haemostatic markers (45). Of equal if not greater concern, individuals with prediabetes have a significantly elevated conversion rate to frank T2DM (46).
1.3.2 CONVERSION TO TYPE 2 DIABETES

Individuals with any form of prediabetes have a higher risk of developing T2DM than those with normoglycaemia (46). The yearly conversion to T2DM is 6-9% (47-49) in people with i-IFG (Isolated IFG – elevated fasting glucose with normal postprandial glucose levels) and 4-6% (47-49) in people with i-IGT (Isolated IGT – normal fasting levels with elevated postprandial levels). Since IGT is more common than IGT, it is more sensitive, but less specific in identifying people who will develop diabetes. There are other limitations to consider when examining epidemiological data to establish risk of diabetes amongst the prediabetic states including methods used to measure glucose; whether >1 OGTTs were carried out, frequency of follow-up, and age, BMI and ethnicity of the subjects studied (45). However, at present, it appears that progression to diabetes from IGT is slightly higher than progression to diabetes from IFG (using WHO criteria). In subjects with combined IFG/IGT the rate of conversion is higher (16-19%) (47-49). The lifetime risk of developing T2DM is thought to be 70-90% in subjects with IGT (with or without IFG) (47,50-52). In prediabetic subjects mean plasma glucose increases by 0.4–1.2 mmol/L per year (39).

1.3.3 PREVELANCE OF PREDIABETES

The UK does not currently have a national screening program, but of 8696 participants in the LEADER study of primary care individuals aged 40–75 years without T2DM, approximately 18.5% were classified as having IGR according to HbA1c 6.0-6.4% (42-48 mmol/mol), and 44.9% using the lower HbA1c cut-off of 5.6-6.4% (39-48 mmol/mol) (53). The East Anglia Impaired Fasting Glucose (UEA-IFG) Study Group have reported a local prevalence of i-IFG of 4.5% and of T2DM of 2.2%, consistent with the finding in most populations that the prevalence of i-IFG is double that of T2DM (54). While there is no complete data on all the subtypes of prediabetes subtypes in the UK, the prevalences of i-IFG, i-IGT and IFG/IGT in different populations is shown in table 1.2.
<table>
<thead>
<tr>
<th>Study</th>
<th>IFG</th>
<th>IGT</th>
<th>IFG/IGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mauritius</td>
<td>13.9</td>
<td>4.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Pima</td>
<td>10.7</td>
<td>1.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Sweden</td>
<td>20.3</td>
<td>9.7</td>
<td>7.6</td>
</tr>
<tr>
<td>NHANES III*</td>
<td>11.0</td>
<td>4.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Australia</td>
<td>8.0</td>
<td>5.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>6.1</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>DECODE#</td>
<td>8.8</td>
<td>6.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 1.2: Prevalence (percentage) of prediabetic states in different adult study populations Taken from (Unwin et al 2002). * NHANES III (The National Health and Nutrition Examination Survey, 1988-1994, USA). # DECODE (European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe).

Roughly half the population with IFG also have IGT while approximately 30% of those with IGT also have IFG. However, within a population, the respective prevalence of IFG and IGT varies based on age, gender and ethnicity (45).

1.3.4 PATHOPHYSIOLOGY OF IFG AND IGT

Subjects with prediabetes have defects in insulin secretion and some form of tissue resistance and these abnormalities start before increases in glucose concentrations are detectable(15,55). IFG and IGT are considered distinct metabolic phenotypes and do not necessarily overlap. The relative contribution of insulin secretion and insulin resistance differs in IFG and IGT. Abdal-Ghani and colleagues have examined the relative contributions of insulin secretion, and hepatic, adipose tissue and skeletal muscle insulin resistance in prediabetic subjects (45,55-59). They have consistently found that subjects with i-IFG have impaired first phase insulin secretion and marked hepatic insulin resistance. Second phase insulin secretion is intact while peripheral insulin resistance appears normal. In subjects with i-IGT, both first and second phase insulin secretion are impaired and peripheral insulin resistance is marked. Hepatic insulin resistance is thought to be normal. Subjects with IFG/IGT have impaired first and second phase insulin secretion, and hepatic and peripheral
insulin resistance. These findings have been replicated by other researchers (45, 58-64). These distinct metabolic abnormalities will be considered in turn below.

1.3.4.1 INSULIN SECRETION

Insulin secretion from the pancreatic beta cell is biphasic. The first phase lasts approximately 10 minutes and is followed by a longer lasting second phase which reaches a plateau at about 2-3 hours (45, 65). Insulin secretion is determined by nutrient intake (45, 66, 67), the glucose-sensing capacity of the cell (45, 68), beta-cell mass (45, 69, 70), and the incretin effect (45, 71). Recent work also implicates central regulation of insulin secretion (45, 72). While an acute increase in free fatty acids, such as that typically seen following a meal, causes an increase in glucose-dependent insulin secretion, a sustained increase in FFA impairs insulin secretion (45, 73). It has been proposed that chronically elevated FFAs can cause lipotoxicity (45, 74), which is believed to reduce the glucose-sensing capacity of the cell and distort the usual insulin secretion profile. Lipotoxicity may also increase beta-cell apoptosis eventually leading to beta-cell failure (45, 75-77). The relative contribution of each of these factors to impaired insulin secretion in IFG and IGT are currently unknown.

1.3.4.2 INCRETIN EFFECT

The incretin effect is defined as the difference in insulin secretion when a given glucose load is given orally as compared to intravenously. This incretin effect is thought to account for 50-70% of the insulin secretion following oral consumption of carbohydrate (45, 78). The incretins include Glucagon-Like Peptide-1 (GLP-1) and Glucagon Inhibitory Peptide (GIP) and have a variety of physiological effects including enhancing glucose-stimulated insulin secretion, promoting β-cell growth and survival; inhibiting glucagon release from the α-cells of the pancreas, reducing gastric emptying, and reducing food intake (45, 78). While GLP-1 secretion is reduced in patients with frank
T2DM (45,79), the extent of defects in GIP and GLP-1 secretion or action in the prediabetic state is not clear (80,81).

1.3.4.3 INSULIN RESISTANCE

Insulin resistance is defined clinically as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population (82). The development of insulin resistance is multifactorial, many elements of which are not fully understood. A brief explanation is as follows: Surplus food energy is stored predominantly in the adipose tissue (45,83), and in metabolically-healthy individuals the adipose tissue is able to expand indefinitely to store this energy via a process of hyperplasia and to a lesser extent hypertrophy (84). Individuals at risk of T2DM are thought to have impaired adipose tissue expansion, which can lead to elevated concentrations of triglycerides and its associated metabolites in the circulation. For example, subcutaneous abdominal adipocytes are enlarged in first-degree relatives of people with T2DM, which in turn are negatively correlated with insulin sensitivity (85). Impaired lipolysis can exacerbate the existing pathology, leading to free-fatty acid (FFA) overflow, lipotoxicity and visceral and ectopic fat deposition. Experimentally increasing circulating FFA reliably induces insulin resistance, at least partly due to disrupted insulin signalling, while interventions which reduce FFA improve insulin resistance (86), (87). Visceral adipose tissue (VAT) is itself associated with insulin resistance and T2DM. This fat depot secretes an array of inflammatory adipokines, for example TNF-α, IL-6, and resistin, which are thought to worsen insulin sensitivity (88). VAT also has a higher rate of lipolysis than subcutaneous adipose tissue (SAT) (88). Critically, its anatomical position means that FFA from VAT can deposit directly into the liver, impairing the function of this organ (88). As mentioned previously, insulin resistance can also occur in lean subjects, including people with lipodystrophy and polycystic ovarian syndrome. Despite their lean phenotype, people with lipodystrophy and polycystic ovarian syndrome have increased ectopic fat (28,89), indicating that the location, not quantity of adipose tissue may be important. Similarly,
normal weight women with polycystic ovarian syndrome and insulin resistance also have elevated levels of TNF-α suggesting some of the mechanisms of insulin resistance, while not fully understood, may be shared in normal and overweight subjects(90).

1.3.4.4 HEPATIC INSULIN RESISTANCE

Insulin resistance can also occur in the liver, with elevated FFA also believed to be involved in the pathogenesis of hepatic insulin resistance (91). Glucose overfeeding also has a similar effect (92). Finally, certain adipokines which are increased in obesity are thought to impair hepatic insulin sensitivity(93).

1.4 THE ROLE OF WEIGHT LOSS

Since the consequences of chronic fuel surfeit, namely glucose and FFAs are involved in the development of metabolic dysfunction as described above, it follows that reducing or reversing the chronic fuel surfeit will lead to improvements in metabolic function. There is a wide body of evidence demonstrating that even modest weight loss normalises glycaemia, improves insulin secretion and decreases insulin sensitivity (94-96). A recent interventional trial in type 2 diabetic subjects demonstrated that 10 weeks’ of a 600kcal/day diet (a negative energy balance of approximately 1800kcal/25% of their energy requirements) leads to a significant reduction in triglyceride content of the lipid and pancreas as measured by Mass Spectroscopy, alongside normalisation of β-cell function (97). Six long-term clinical trials have demonstrated that in prediabetic subjects, lifestyle interventions are effective in reducing the development of T2DM by 66% (98-103). The trials were carried out in the United States(100), Finland(99), China(98), India(102), Japan(101) and Sweden(103) and utilised a lifestyle program incorporating dietary and/or physical activity goals aiming for a weight loss of 5-7%. While there were small differences between the trials – for example, the Finnish trial had a specific goal of 15g fibre per 1000kcal – a meta-
analysis of these studies concluded that weight-loss was the critical mediator of intervention success (104). While these studies demonstrated conclusively that lifestyle interventions are the cornerstone of T2DM prevention, their translation into primary care has not produced the same results where similar resources are not available. (105-109). In particular, the degree of weight loss did not meet the targets set in the original trials.

1.4.1 SUCCESSFUL WEIGHT LOSS MAINTENANCE

Successful weight loss maintenance has been defined as “individuals who intentionally lose at least 10% of their body weight and keep it off for at least one year” (110). Determining the prevalence of successful weight maintenance is difficult. Data from self-reported surveys suggest that the prevalence is around 10-20% (111-113), but self-reported data has well-documented limitations. In observation or clinical trials, the figure is 10-37% (114,115). However, clinical trial data are not necessarily applicable to free-living lifestyle change and many of these studies such as the Diabetes Prevention Programs were labour intensive to a degree not compatible with current resource limitations. For example, the trials included personal trainers, behavioural support, a $100 annual tool box fund to help motivate volunteers and frequent, long-term follow-up with a team of health specialists(100,109). Characteristics associated with “free-living” successful weight loss maintenance include high levels of physical activity, self-monitoring and self-restraint (111,116). However, data from national diet and physical activity surveys indicate these individuals are the exception to the norm (117).

Weight loss maintenance is particularly important as follow-up data from the European Diabetes Prevention RCT has shown that for every additional year prediabetic subjects who met their year one weight loss target maintain their weight loss, their cumulative survival free of diabetes increased significantly(118).

The continued increase in the prevalence of obesity over the last 3 decades has led researchers to examine the biochemical control of energy homeostasis.
1.5 HOMEOSTATIC OR PHYSIOLOGIC REGULATION OF APPETITE

Food intake is a complex process, particularly for humans, where factors including learned behaviours, cognitive factors, habits, social context, availability of food, and external sensory cues such as visual, smell, and taste inputs all affect the drive to eat (119,120). Observational data on descendants of famine sufferers during the Dutch winter famine and the Leningrad siege have demonstrated the so-called thrifty phenotype (121,122). That is, that environmental insult in utero or early in life promote a biological predisposition to obesity (123,124). Interestingly, these same early life, or multi-generational programming have also been linked to predisposition to diabetes (125-127), suggesting that the propensity to develop both obesity and diabetes may be linked.

Scientific advances in the last two decades have demonstrated the powerful role an array of appetite hormones play in driving food intake (128). These include the anorectic hormones leptin, PYY, GLP-1, oxyntomodulin, CCK, PP and the only known orexigenic hormone ghrelin (Table 1.3). In brief, it is thought that adiposity signals such as leptin and insulin trigger initial signals to the hypothalamus which ultimately results in a reduction in food intake (129), while the gut hormones PYY and GLP-1 are released post-prandially in response to nutrient intake and interact with areas of the hypothalamus to influence inter-prandial satiety and meal termination (43,130,131).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Secreted from</th>
<th>Effect on appetite</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYY (Peptide YY)</td>
<td>L cells in small intestine</td>
<td>↓ food intake</td>
</tr>
<tr>
<td>GLP-1 (Glucagon-Like Peptide 1)</td>
<td>L cells in small intestine</td>
<td>↓ food intake</td>
</tr>
<tr>
<td>Oxyntomodulin</td>
<td>L cells in small intestine</td>
<td>↓ food intake</td>
</tr>
<tr>
<td>PP (Pancreatic Polypeptide)</td>
<td>PP cells from islet of Langerhans</td>
<td>↓ food intake</td>
</tr>
<tr>
<td>CCK (Cholecystokinin)</td>
<td>CCK I cells in small intestine</td>
<td>↓ food intake</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>K cells in stomach</td>
<td>↑ food intake</td>
</tr>
<tr>
<td>Leptin</td>
<td>Adipocytes</td>
<td>↓ food intake</td>
</tr>
</tbody>
</table>

Table 1.3: Major hormones involved in regulation of appetite.
It is not known to what extent gut hormones have contributed to the increase in obesity prevalence over the last 30 years. Humans eat (and overeat) for a variety of reasons, as mentioned above. Nevertheless, it is known that there are alterations in the gut hormone profile of overweight and obese subjects (129-131). Therefore, whether or not gut hormones have a role in increasing the incidence of overweight and obesity, their altered concentrations in the overweight or obese state very likely play a role preventing an individual from losing weight.

### 1.5.1 PYY

PYY is a 36-amino acid peptide which has a number of biological effects in man, each of which leads to a reduction in food intake (131). PYY mRNA is found throughout the human gastrointestinal tract, with the highest concentrations found in the colon and rectum. PYY is secreted in response to food intake, at a concentration proportional to the energy content of the meal (132). There are different forms of PYY which are proposed to have different biological effects, including slowing gastric emptying, reducing gastric secretions and decreasing appetite via central effects (131). The central effects are complex, and have been proposed to be due to activation of POMC neurons in the arcuate nucleus of the hypothalamus; suppressive effects on NPY neurons, or a combination (131). PYY concentrations are lower in overweight than normal subjects (133), and have also been shown to be lower in type 2 diabetics and their normoglycaemic relatives (134,135). Peripheral administration of PYY at physiological concentration decreases appetite and food intake in normal and overweight subjects. In animal studies, chronic administration of PYY reduces food intake. Therefore PYY is considered a potential anti-obesity therapy (136-139).

### 1.5.2 GLP-1

GLP-1 is also a potent anorexigenic hormone, which like PYY is also released from L cells of the intestine in response to food intake (135) (140). GLP-1 is derived from the preproglucagon peptide by enzymatic cleavage. Acute administration of GLP-1 causes a reduction in hunger cravings and
food intake in overweight and obese subjects. (141-143), while long-term treatment with GLP-1 agonists causes sustained weight loss (144). There is emerging evidence that GLP-1 may also reduce the hedonic value of food (145). In addition, GLP-1 is a known incretin (146). In rodent models, a number of GLP-1 agonists have been shown to stimulate β-cell proliferation and reduce β-cell apoptosis (147). In human studies GLP-1 agonists inhibit glucagon release and reduce hepatic glucose output (148). Reduced circulating levels of GLP-1 have been observed in subjects with T2DM that may contribute to hyperglucagonaemia and increased hepatic glucose output, two important metabolic defects of T2DM. Subjects with IGT and T2DM have β-cells that respond poorly to small changes in glucose concentrations. In subjects with IGT but not T2DM this response can improve with low-doses of GLP-1 agonists, underlining the potential for a preventative approach (149). Importantly, it should be noted that in contrast to the effect of leptin secretion in overweight or obese subjects, both PYY and GLP-1 appear to retain their appetite suppressive effects (136).

1.6. HORMONAL ADAPTATIONS TO WEIGHT LOSS

Most studies have shown that weight-reduced individuals have an increased appetite when compared to obese-resistant or obese subjects. Tremblay et al found that fasting desire to eat and postprandial AUC for hunger were significantly increased after a 15-week weight loss intervention (150). However, they did not find any association between subjective appetite and reported or measured food intake. The same authors looked at a low-calorie (-2900kjoule/day) intervention on 59 overweight women. They found a significant association between the decrease in body fat mass and the change in appetite sensations suggesting that some measure of adiposity is related to the subsequent increased drive to eat (151). Supporting this hypothesis, plasma leptin levels and subjective measures of appetite (hunger, desire to eat and prospective food consumption) are significantly increased after 7 days of a moderate energy deficit (470kcal/day) (152). Casanueva et al studied 104 overweight subjects on an 8-week hypocaloric diet (-30% energy expenditure) over 32 weeks (153). They found that higher baseline leptin and lower ghrelin plasma levels predicted
weight regain after 8 weeks. In 13 obese subjects following a 6-month weight loss program, fasting leptin and ghrelin were significantly decreased and increased respectively. Moreover, the 17% loss of initial body weight was associated with a 24% increase tAUC of the 24-hour ghrelin profile (154). The recent DioGenes study found that a significant reduction in fasting insulin following a very low-calorie diet (VLCD) was significantly associated with a lower regain of weight (155). While it has been thought that reduced adiposity was the cue driving the increase in leptin, other studies have shown even acute or short-term energy reduction leads to an increase in food intake. Healthy subjects given a low-calorie breakfast (64Kcal) had significantly increased subjective appetite, and ate significantly more at an ad libitum test meal than subjects fed 500kcal (156). Even moderate calorie-restriction is associated with biological changes which increase the drive to eat and favour weight regain and that these changes persist at 6 and 12 weeks (141-143,151,157-159). More recent studies have demonstrated even longer-term persistence of such adaptations: A 10% weight loss on either a low-carbohydrate or low-fat diet over 8 weeks causes an increase in ghrelin, and reduction in PYY, changes which persist even at 12 months following initial weight loss (141-143,160). Functional MRI studies have also demonstrated increased activation in neuronal appetite centre in subjects following a 6-month weight loss program, a pattern of activation which is associated with increased appetite (161). Other authors have found that increased reactions to pictures of high-calorie foods predict short- and long-term weight maintenance (151,159,160,162). Taken together these data suggest that alterations in the body’s natural appetite hormones following weight loss cause a drive to eat which inevitably promotes weight regain. Interventions which can attenuate these biochemical alterations may help aid long-term weight loss maintenance.

1.6.1 PREVENTING OR AMELIORATING THE HORMONAL ADAPTATIONS TO WEIGHT LOSS

These gut hormones are potential obesity and T2DM therapies. However any treatment with PYY would require injections before each meal due to its rapid degradation in the stomach and circulation (141-143,146). GLP-1 agonists, while effective, are contraindicated for certain conditions
and can cause unpleasant side-effects (163). An alternative approach is using dietary interventions which increase the body’s endogenous production of these hormones.

1.7 FERMENTABLE CARBOHYDRATE

Fermentable carbohydrates (FCHO) are carbohydrates which are not digested and absorbed in the upper gastrointestinal tract. Various factors contribute to the digestibility of carbohydrates including the type of starch (amylose or amylopectin), rate of digestion, cooking or processing method and the fibre content (164). The glycosidic linkage is also a critical factor (165,166). Human α-amylase is able to hydrolyse the α-1-4 linkages found in starch, but cannot hydrolyse the β-1-4 linkage in lactose or fibres such as cellulose (164). Similarly, humans do not have the α-1-4 galacturonase required to digest pectin or the α-1-6 galactosidase needed to hydrolyse raffinose and stachyose (167). Instead these undigested carbohydrates pass intact to the colon where they are digested by resident gut bacteria (165). The amount of carbohydrate escaping digestion in the Western Diet is thought to be about 5-20% (168,169).

<table>
<thead>
<tr>
<th>Fibre Fraction</th>
<th>Examples</th>
<th>Physiological Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insoluble dietary fibre</td>
<td>Cellulose, some hemicelluloses, lignin</td>
<td>Bulking – increase stool weight and reduce transit time.</td>
</tr>
<tr>
<td>Resistant starch</td>
<td>RS1-physically enclosed starch, RS2 – raw starch granules, RS3- retrograded starch, RS4-chemically modified starch</td>
<td>Fermentable.</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Inulin, OFS, lactulose, oligosaccharides</td>
<td>Fermentable.</td>
</tr>
</tbody>
</table>

Table 1.4: Summary of the physiological effects of the fibre fractions.

The various fibre factions have long been known to have beneficial effects on human health via their commonly understood properties: bulking and viscosity. These effects are summarised in table 1.4.
However, the significance of the fermentability of fibres (and carbohydrates not classically considered as fibres) has only become evident in the last two decades (170,171). It is now clear that the products of colonic bacterial fermentation favourably influence health via multiple pathways. Carbohydrates such as inulin, which have are not known to have any function in the small bowel, allow for controlled examination of these beneficial effects (172).

1.7.1. INULIN

Inulins belong to a class of dietary carbohydrates called the fructans (Table 1.4) (166). Fructans are a diverse group of polymers of fructose molecules linked by mostly fructosyl-fructose (β-2-1) bonds, usually with a terminal glucose. The β-configuration of the anomeric C in the fructose molecule means that inulin-type fructans cannot be hydrolysed by human small intestine digestive enzymes (166). As described above, the undigested inulin passes intact to the colon where it is fermented by resident gut bacteria. The products of this fermentation are the short chain fatty acids (SCFA) (described in section 1.7.3 below) and the gases methane, carbon dioxide and hydrogen. The production of these gases explains the side-effects that can be caused by inulin (or any FCHO) consumption. These side-effects include flatulence, bloating and diarrhoea, which subside in most people after a period of 3-14 days’ adaptation (173,174).

Inulins are found in >36,000 plant species, though the highest concentrations are found in tuberous roots such as chicory, leek, garlic, and onion(166). Inulin is a storage carbohydrate which comprises about 70-80% of root dry weight in such foods. OFS is a sub-group of inulin and is the term used for short-chain inulins with a degree of polymerisation (DP) of 2-10. Inulin has a DP of 2-60. The estimated intake of inulin and OFS is estimated to be 3-12g in Europe, and 1-4g in the United States (172). It is also increasingly being used as a food ingredient due to its favourable physicochemical properties, including surfactant character, gel formation (depending upon chain length of the inulin), smooth mouth-feel and bland or mildly sweet taste (175). Industrially, the majority of inulin and OFS
is produced by the use of physical separation techniques and partial enzymatic hydrolysis of chicory (Cichorium intybus), though Jerusalem artichoke (Helianthus tuberosus) is also sometimes used (176). Synthetic fructans have been classified as a novel food as per EU Regulation on Novel Foods and Novel Food Ingredients 258/97(177).

1.7.2. GUT MICROBIOTA

The human GI tract contains trillions of microorganisms, thousands of bacterial phylotypes, with a collective genome more than 100-fold larger than the human genome. While considerable biodiversity exists and is influenced by many factors, including age, gender, diet, drugs and host genotype, a small number of phyla dominate (178). The diversity of the gut microbiota and in particular the products of microbial activity are closely linked to health and disease(170,171).

Gut microbiota can synthesise a variety of glycosidases which can digest and extract energy from the food entering the colon which is resistant to digestion to human α-amylase (179). The products of this fermentation include short chain fatty acids (SCFA) such as acetate, butyrate and citrate, the gases carbon dioxide hydrogen and methane and water. Both hydrogen and methane are produced in man and can be measured as a non-specific proxy of fermentation. Since only 30-60% of humans in Western countries produce methane, hydrogen is the most commonly used measure (180).

1.7.3. SHORT CHAIN FATTY ACIDS

SCFA are fatty acids with carbon chain length of 1-6. The principal SCFA produced by fermentation in the gut are acetate, propionate and butyrate, produced at a molar ratio of approximately 60:25:15, and account for about 83% of all SCFA (181). In humans consuming 80g soluble fibre per day, up to 300-800mmol/L of SCFA can be produced. Butyrate and propionate are primarily metabolised by the colonocytes and liver and therefore their circulating concentrations are low: 1-3 uM for butyrate and 4-5uM for propionate. Acetate enters the circulation in higher concentrations: 100-150 mM. The exact molar quantities of SCFA depend upon the diversity of the gut microbiota and the substrate.
entering the colon (182). SCFA are believed to have a variety of beneficial effects on health and metabolism.

1.7.3.1. SCFA AS SIGNALLING MOLECULES
SCFAs act as ligands for the recently discovered orphan G-protein coupled receptors GPR41 (FFA3) and GPR43 (FFA2). FFA3 is preferentially activated by propionate followed by butyrate and acetate, while FFA3 is activated with equal specificity by all 3 SCFAs (55,183).

1.7.3.2 SCFA AND GUT-DERIVED PEPTIDES
Both FFA2 and 3 are found on the L cells of the distal ileum and colon, where they are colocalised with PYY and preproglucagon-expressing cells in both humans and rodents (183). Infusions of rectal or intravenous acetate significantly increase PYY and GLP-1 in healthy human subjects (184). In FFA2 or FFA3 knockout mice, SCFA-induced GLP-1 secretion is attenuated, with no attendant change in glucose profiles in euglycaemic individuals (185).

1.7.3.3 SCFA AND ADIPOCYTE DIFFERENTIATION
In humans, FFA2 and 3 are expressed in subcutaneous and omental fat (186). Propionate and acetate increase lipid accumulation and adipocyte differentiation in 3T3-L1 cells. The same authors found that propionate also increases the expression of FFA2 and PPARγ2, a known adipocyte differentiation factor (187). Plasma free fatty acid levels are suppressed by acetate infusion in wild-type and ob/ob obese mice (188). However, knockdown of FFA2 abolishes this effect (188). In addition, high-fat feeding has been shown to increase FFA2 expression in adipose tissue in murine model of obesity (189). Encouragingly, feeding with FCHO attenuates the overexpression, an effect which correlates with reduced adiposity. However, extrapolating from animal to human has limitations as there is no evidence of increased FFA2 expression in primary preadipocytes isolated from omental adipose tissue taken from overweight adults (190).
1.7.3.4. SCFA AND THE PANCREAS

While SCFA are known to indirectly regulate insulin secretion via increases in GLP-1, the recent discovery of FFA2 in the murine beta-cell line MIN6 and isolated mouse islets suggests that SCFA may also have a direct role (191). However, what little work has been done in this area has produced conflicting results (192-194). Therefore at the present time, the direct effect of SCFA on insulin secretion is unknown.

Figure 1.3. Schematic of the multiple effects of SCFA in the colon, liver, muscle, adipose tissue and pancreas. Taken from the role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism, De Besten G et al.

1.7.3.6 SCFA AND CARBOHYDRATE AND LIPID METABOLISM

SCFAs are involved in the regulation of fatty acid synthesis, oxidation, and lipolysis(195) (Figure 1.3). This is considered especially critical, given the significance of disordered lipid and carbohydrate metabolism in the development of T2DM. SCFAs promote fatty acid oxidation, and inhibit de novo lipogenesis and lipolysis via multiple effects, including increasing AMPK activity in liver and muscle(195,196), increasing expression of uncoupling protein 1 (UCP1) and PGC1α in brown adipose
tissue (BAT) and suppression of hormone sensitive lipase (HSL) in adipose tissue. The net result is a reduction in circulating FFA, and improved glucose homeostasis.

The role of SCFA in the liver is less clear. Propionate increases glucose use and decreases glucose production in isolated rat hepatocytes, potentially via reduced expression of the gluconeogenic glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). However, gastric infusion of acetate, propionate or acetate+propionate had no effect on blood glucose, plasma insulin concentrations or hepatic glucose production in healthy volunteers in the fasting state.

1.7.4 FERMENTABLE CARBOHYDRATE AND SCFA PRODUCTION

Consumption of FCHO has been demonstrated to significantly increase SCFA in rodents and humans. It is important to note that the measurement of circulating SCFAs in humans is extremely challenging. The majority of butyrate is metabolised by the colonocytes (see below), while propionate is taken up by the liver. Therefore, in circulating plasma, acetate is usually the only elevated SCFA. For example, acute consumption of high RS (59.1g +4.7) versus low-RS (5.2 +0.4g per day) results in significant increases in breath hydrogen, serum acetate but only a trend towards increased butyrate (p = 0.087).

Since FCHO increases the production of SCFA, and SCFA have multiple beneficial effects on food intake, body weight and glucose homeostasis, numerous studies have been carried out to determine whether FCHO consumption alone can replicate these effects.

1.8. EFFECT OF FERMENTABLE CARBOHYDRATE ON SUBJECTIVE APPETITE AND APPETITE HORMONES

In a single-blinded randomised crossover trial, Cani et al found that 16g/day OFS reduced subjective hunger, desire to eat and prospective food intake while increasing fullness. Both self-reported and recorded food intake were also significantly reduced with total energy intake 5% lower during the OFS treatment compared to control. However, the effect of FCHO on appetite hormones and
appetite have not been consistent, and consuming a high-enough concentration seems to be key. While an acute dose of lactitol - a fermentable sugar alcohol - resulted in significant decreases in PYY and GLP-1 in rats, it was only able to attenuate the postprandial decrease of PYY in humans, and showed no effect on GLP-1 (207). The authors attributed the discrepancy to the higher dose given to the rats: 100g/kg body weight compared to a maximum dose given to the humans of 20g. Similarly, 16g/day OFS significantly decreases energy intake and increases the AUC of PYY and GLP-1 when compared to 10g/day (208) in mildly overweight subjects. However the same dose taken for 3 months was not effective in obese women (209). 3-months’ supplementation of 5-15g of either inulin or a high-viscosity polysaccharide in overweight and obese men and women resulted in a beneficial effect of the fibres in women only (210). The authors suggested this might be due to the lower dose per kg body mass given to the men. Similarly, Reimer et al found that 10g per day of the same high-viscosity polysaccharide was only effective in increasing PYY in adults with a BMI <23kg/m² (211). Finally, in a dose-escalation study, Frost et al found that a minimum of 30g inulin was required to cause an increase in PYY (174). The duration of supplementation for substances which are less fermentable may also be important with Wolever suggesting up to a year’s consumption of wheat fibre may be necessary to see increases in GLP-1(212).

FCHO are not believed to have acute effects, as fibres such as inulin are not known to affect gastric emptying, or viscosity of the lumen contents. As multiple researchers have demonstrated, it is the products of fermentation which appear to mediate the effects of FCHO on both appetite regulation and glucose homeostasis (213). An evening meal of indigestible boiled barley kernels significantly increases breath hydrogen, fasting and postprandial GLP-1, and reduces perceived hunger 10.5-16 hours after the meal, demonstrating the delayed effects of FCHO (214).

The critical test for any purported satiety-inducing substance is that its effects last long-term. 12 weeks’ of 21g daily OFS significantly lowers the AUC for ghrelin and significantly increases PYY concentrations in overweight (BMI >25mg/kg²) healthy subjects (215). The AUCs for PYY and ghrelin also significantly correlated with self-reported caloric intake. 14 weeks’ supplementation with OFS
alongside a weight-loss program significantly decreased weight, and increased satiety as measured by visual analogue scales (VAS), and PYY and GLP-1 concentrations at 60 minutes post-meal. However, this study had no control group (216). Finally, a year-long study of OFS supplementation in 97 adolescents showed a significantly smaller BMI increase, and significantly smaller increases in fat-mass in the experimental group. Promisingly, there was a trend towards maintenance of these differences one year after supplementation was stopped(217).

Recent work in rodents suggest that FCHO consumption is associated with maintenance of lean tissue, lowered insulin levels and reduced fat deposition in subcutaneous adipose tissue during weight loss and even during regain of weight, indicating that remodelling of tissue and improvements in metabolic profile may be long-term (218). This is supported by Parnell and Reimer’s data showing that OFS promotes loss of fat mass (FM) and maintenance of free fat mass (FFM)(173). More recent work in rodents has also suggested that consumption of FCHO may affect known appetite centres in the hypothalamus (219). Increased activation in the Arcuate Nucleus has been shown following 9 week’s supplementation with FCHO in rats(220).

1.9. EFFECT OF FERMENTABLE CARBOHYDRATE ON GLUCOSE HOMEOSTASIS

In addition to reducing appetite and food intake, there is also evidence that consumption of FCHO improves glucose homeostasis.

The addition of 40g resistant starch (RS) (digestible starch content matched to control) to the diet of insulin-resistant subjects for 12 weeks resulted in a significant increase in peripheral insulin sensitivity(221). The RS supplement was not given on the morning of the clamp studies, indicating the improvements in insulin sensitivity were not due to acute effects. The paper did not present data on glucose. 15g or 30g RS given the day before an IVGTT significantly improves insulin sensitivity in men but not in women (222). The same group also examined the effect of short-term consumption of 60g RS over 24 hours(223). All measurements were carried out the day after the 24-hour high-RS diet. They found a significant decrease in post-prandial glucose and insulin, and an increase in
postprandial insulin sensitivity when compared to control. Umpleby et al gave 40g of RS to 15 overweight insulin-resistant subjects for 8 weeks (224). Following a hyperinsulinaemic-euglycaemic clamp study with glucose tracers, a meal tolerance test (MTT) with arteriovenous sampling across the forearm muscle and a subcutaneous adipose tissue biopsy, they found that RS significantly improves peripheral insulin resistance, reduces fasting glucose concentrations and HOMA-IR. The subjects in this study had fasting glucose values of 5.2 mmol/L. It is unclear whether RS would reduce glucose in those with significantly elevated hepatic insulin resistance.

More recently Bodinham et al have shown that 4 weeks’ supplementation with RS significantly increases first-phase insulin secretion in healthy subjects (225). While the authors did not find any effect on insulin sensitivity, which is in contrast to three previously published findings by this group, the authors state the lack of effect on insulin sensitivity may be due to the short time frame of the study and that the improvement in first-phase insulin secretion may be an early step in this improvement of insulin sensitivity. Juntunen et al (226) had previously demonstrated a 9% increase in insulin secretion following 8 weeks consumption of rye bread compared to control (wheat bread). However, there were other significant differences between the rye and control groups: the rye group also consumed more protein and less fat than the control group. They also consumed significantly higher quantities of both soluble and insoluble fibre which makes attributing the improvements to rye bread alone troublesome (227). Therefore further studies are required to confirm the findings by Bodinham et al.

There is therefore a large body of evidence demonstrating the effect of FCHO on improving insulin sensitivity. However, it is not clear whether this translates into a reduction in fasting or postprandial glucose. The majority of studies found no effect on glucose in either healthy or diabetic subjects. 24 weeks’ of 10.6g/day short-chain (sc)-FOS reduced post-prandial insulin concentrations but had no effect on glucose or lipid metabolism in 30 overweight men and women with mild hypercholesterolaemia (228). However, there was some variability in the fasting glucose concentrations of the interventional group (5.44 ± 1.00 mmol/L) indicating that some subjects had
IFG. These subjects may have marked hepatic insulin resistance and thus may not respond the same way as normoglycaemic subjects. 3 weeks supplementation with 20g inulin given in the form of low-fat ice cream significantly reduced fasting triglycerides but had no effect on glucose during a follow-up OGTT(229). Finally, 15g fructooligosaccharides for 20 days had no effect on glucose levels in diabetic subjects (230). However, 17 of these subjects were already taking glucose-lowering medications, which may have increased the biological noise and potentially masked any effect the FCHO may have had. They also measured blood glucose using finger-prick capillary measurements which have known limitations, and different glucose meters were used across different sites.

Cani and Delzenne measured fasting and post-prandial glucose concentrations following 2 weeks’ supplementation of OFS, and found that the reduction in postprandial glucose significantly correlated with breath hydrogen levels(216). Robertson et al found a significant reduction in fasting glucose following 8 weeks’ supplementation with 40g RS, from 5.2 ± 0.11 mmol/L to 5.0 ± 0.1 mmol/L (mean ± SEM) (P = 0.017)(224). However this is only one of two studies (out of 13) to have found a glucose-lowering effect. The second study measured the effects of 14 days’ administration of 8g/day OFS and found a significant (8%) reduction in post-prandial glucose in subjects with T2DM (231). However, there was no control group in this study, nor any information on food intake making it difficult to determine whether the effects were due to other dietary factors.

Consistent with the second-meal effect of FCHO on appetite, there is also temporal delay in the effect of FCHO supplementation on glucose homeostasis further implicating the lower gut in mediating the effects of FCHO. In the study by Vantine above, RS had no effect on glucose homeostasis during the first meal (breakfast), but did improve glucose homeostasis following the lunch(232). Insulin sensitivity is improved 12 hours after a dose of RS is given.
FERMENTABLE CARBOHYDRATE IN PEOPLE WITH PREDIABETES

While studies have examined the effect of fermentable carbohydrate in healthy or insulin-resistant normoglycaemic subjects, no studies have yet examined the effect of inulin supplementation on food intake and glucose homeostasis in subjects with prediabetes. It has been reported that GLP-1 and GIP secretion is increased in IFG (233). A potential explanation for increased GIP and GLP-1 secretion may be an attempt to counteract resistance at the β-cell (234). However, this is speculation and other studies have not replicated these findings (235,236). PYY secretion may also be impaired in subjects with prediabetes since postprandial PYY levels are reduced in normoglycaemic first-degree relatives on type 2 diabetics (135). Finally, while FCHO have been shown to improve early insulin secretion in healthy subjects, people with prediabetes already have impaired first-phase insulin secretion.

In summary, subjects with prediabetes are at high-risk of diabetes, and weight loss appears to be effective at preventing the development of diabetes. FCHO appears to suppress hunger and food intake via its effects on appetite hormones, and may therefore have a role in promoting long-term weight loss maintenance, which would be beneficial to prediabetic subjects. In addition, FCHO has been shown to improve glucose homeostasis in healthy subjects and in subjects with the metabolic syndrome.

Therefore, in this body of work we investigated the effect of 30g of inulin supplementation on appetite, food intake, glucose homeostasis and weight loss maintenance in subjects with IFG, IGT and IFG/IGT.
1.11. HYPOTHESIS

Increased intake of dietary fermentable carbohydrate will lead to a reduction in risk of developing diabetes in high risk individuals via a reduction in weight and improvements in glucose homeostasis independent of weight loss.

1.11.1. OBJECTIVES

To assess the effect of fermentable carbohydrate in subjects with prediabetes on:

1. Appetite regulation and food intake
2. Glucose homeostasis and β-cell function
3. Weight loss and weight loss maintenance.
CHAPTER TWO:

METHODS
2.0 METHODS

2.1 STUDY DESIGN

This study comprises 3 investigations. Investigations 1 and 2 are both double-blind randomised crossover trials. Crossover trials were used for the first two investigations to reduce inter-person variability and to allow a smaller sample size. Investigation 1 aims to describe the physiology and mechanisms behind appetite regulation and food intake and investigation 2 aims to examine the physiology and mechanisms behind glucose homeostasis. Investigation 3 is a longer-term randomised control trial (RCT) to examine the effect of inulin supplementation of weight loss and weight-loss maintenance.

2.2 METHODS COMMON TO ALL INVESTIGATIONS

2.2.1 ETHICAL APPROVALS

The three studies were approved by the North West 1 Research Ethics Committee (10/H0717/32) and the Imperial College Healthcare NHS Trust Joint Research Office.

2.2.2 RECRUITMENT

The same recruitment strategy was used for all investigations. Participants were recruited via invitation letters sent out to potentially eligible individuals from a variety of sources. These sources include: people who have previously participated in research at Hammersmith Hospital and women who have previously attended Gestational Diabetes Clinics at Hammersmith and St Mary’s Hospitals. We also used the Diabetes and Primary Care Research Networks who are able to search multiple GP Practice databases for participants who fit the defined criteria. Finally we also used posters and advertisements in the local and national press.

Following telephone screening to ensure potential eligibility based on the criteria below, interested participants were sent a Participant Information Sheet and an invitation to attend a medical screening. During the medical screening at the Sir John McMichael/Wellcome Trust Clinical
Investigation Unit (CIU) subjects underwent an Oral Glucose Tolerance Test (OGTT) and blood tests to assess full blood count, lipid profile, liver function, thyroid function and HbA1c. Participants were then contacted with the results of their screening and given a start date for the study.

2.2.3 INCLUSION AND EXCLUSION CRITERIA

**Inclusion Criteria**

- **Age:** 18-75
- **BMI:** $>25$ and $\leq 35$ kg/m$^2$
- **OGTT:** indicative of IGT for investigations 1 and 3; Isolated IGT for investigation 2.
- **Stable body weight:** no change in body weight $>$3kg over 3 months.
- **Non-smoker**
- **No history of endocrine disease**
- **No history of gastrointestinal disease**

**Exclusion Criteria**

- **Use of antibiotic:** less than three months prior to participation in the study
- **Participation in other research studies:** in the previous three months
- **Blood donation:** less than three months before participation in study
- **Anaemia**
- **Pregnancy or breastfeeding**
- **Substance abuse**
2.2.4 SUPPLEMENTATION

The supplements used in each of the investigations are inulin and cellulose. The inulin used in this study is Synergy1, a 1/1 mixture of long chain and short chain fractions of inulin. Inulin is manufactured from chicory root and can be hydrolysed into a long-chain fraction degree of polymerisation (DP) (ranging from 10 to 65, average 25) which is slowly fermentable or a short-chain fraction oligofructose (DP ranging from 3 to 8, average 4) which is more rapidly fermented. Synergy1 is a mixture of both fractions which represent 90 to 94% of total weight. Glucose and fructose represent 4-6% and sucrose 2-4% respectively of the remainder. The energy content of the inulin is approximately 13.0kJ/g.

The cellulose is Vitacell L600 which is a powdered cellulose made by mechanical micropulverizing of purified cellulose. Cellulose is a linear chain of several hundred to over ten thousand β-(1-4) linked D-glucose units and was selected because it is the least fermentable of the dietary fibres.

The supplements were packed into identical packages with either A or B on the side. The investigator was blinded to the packaging.

2.2.5 LENGTH OF INVESTIGATIONS

Investigations 1 and 2 were each 16 weeks long, comprising 2 consecutive periods of 6 weeks duration separated by a 4 week wash out period. All subjects took both supplements.

Investigation 3 was 18 weeks long. All subjects received only one fibre supplement to take for the entire 18 weeks.

2.2.6 RANDOMISATION AND INCREMENT DOSING

For all investigations randomisation was stratified by age and gender. For investigations 1 and 2 participants were randomised to take one fibre first. Participants took each fibre for a total of 6 weeks, attending a study day on the day preceding the first dose of fibre, and on the final day of the supplementation period. The dose of fibre was increased from 10g to 30g over 4 weeks to minimise
side-effects. For investigation 3 participants underwent the same incremental dosing over 4 weeks as in investigation 1 and 2, but then proceeded to take 30g a day of their randomised fibre supplement over the next 14 weeks.

The supplementation was not given on the morning of each follow-up visit in investigation 1, but it was given the morning of the follow-up visits in investigations 2 and 3.

2.2.7 BREATH HYDROGEN

In all studies breath hydrogen was taken as a measure of colonic fermentation. Subjects were asked to hold their breath for 15 seconds before breathing into a Bedford Gastrolyzer. Breath hydrogen is given in ppm.

2.3 STUDY DAYS

All study days took part in Clinical Investigation Unit (CIU) at Hammersmith Hospital. For each of the visits, subjects were instructed to avoid alcohol, excessive exercise and caffeine in the 24 hour period prior to the study day. All subjects were weighed on each visit. Following arrival and after undergoing checks to ensure the participants felt well; that there were no changes to their medication and that they were still happy to proceed with the study, a cannula was be inserted into the forearm to collect blood samples based on the schedules for each investigation in appendix A. During each study session, participants were asked to minimize physical activity. Water was allowed *ad libitum*. Other aspects of the study days differed as described below:

2.3.1 INVESTIGATION ONE

2.3.1.1 SCHEDULE OF STUDY DAY

Participants were asked to fast for 10 hour prior to their 9am start time. An overview of the study design is seen in figure 2.1. Breakfast was served at 0 minutes, lunch at 240 minutes and an *ad libitum* meal at 440 minutes. The supplementation was not given with the breakfast on the follow-up
visit. The foods given at breakfast and lunch are shown in Appendix B. Visual Analogue Scales (VAS) were completed by the participants at each blood sample to assess subjective appetite. Blood samples were taken at frequent tie-points as per the schedule in appendix A. See appendix C for full list of VAS questions asked.

The serving of food given at lunch and dinner was calculated according to the participant’s individual energy requirements, based on age, weight, height and sex in order to avoid over-feeding the participants using the Schofield equations (237). An activity factor of 17% (Bed-bound: mobile/sitting) was used. Differences in macronutrient composition were minimised. Breakfast comprised a choice of Rice Krispies or Cornflakes with semi-skimmed milk with orange juice. Lunch comprised a cheese sandwich, crisps, kit-kat and a yoghurt. Each participant was given identical serving sizes on each visit. The ad libitum buffet meal was weighed before and after being served to the participant and the difference calculated. Whole day energy and macronutrient intake was determined using Dietplan6. The nutritional content of the available ad libitum meals are shown in Appendix B. For each 7-day period prior to a study day, participants were asked complete VAS for side-effects and appetite assessment, and a 3-day food diary.

![Figure 2.1: Schematic of Study Design for Investigation 1](image)

### 2.3.1.2 VISUAL ANALOGUE SCALES

Visual Analogue Scales (VAS) were used in investigation 1 and 3 to determine subjective sensations of appetite, and also to examine factors which could potentially confound feelings of hunger or
fullness, such as thirst and anxiety. VAS are 10cm long scales with “not at all” at 0 and “completely” at 100. The subject completing the VAS indicates on this 10cm how they feel at that particular time. The appetite questions relate to slightly different facets of the desire to eat and have been extensively validated previously.(238-240) The appetite questions used in this study were “How hungry are you right now?” (general hunger), “How much can you eat?” (prospective food consumption), “How pleasant would it be to eat right now?” (desire to eat) and “How full do you feel right now?” (a feeling of fullness in the stomach). A full list is shown in appendix C.

2.3.1.3 AD LIBITUM MEAL TEST

An ad libitum meal test was used at 440 minutes in investigation 1 and at 180 minutes in investigation 3 to get an alternative measure of prospective energy intake. Subjects were offered a choice of 3 meals, tomato and mozzarella pasta bake, spaghetti bolognaise and macaroni cheese. See appendix B for energy and macronutrient content. The meal was weighed before serving, participants were given up to 20 minutes to eat the meal, and the remainder was weighed.

2.3.1.4 DIET DIARIES

A copy of the 3-day food diary is in Appendix D. A 3-day diary was selected to strike a balance between participant burden and validity of energy and macronutrient intake data (241). Food diaries were sent to each participant at least 7 days prior their study day. Participants were requested to complete 2 week days and 1 weekend day. On return to the investigators, the diary was reviewed by a dietitian and further questions and probing used if there were any omissions or insufficient detail in the food diaries. The diaries were then analysed by two dietitians independently, with any discrepancies between the analyses discussed and amended based on mutual agreement. Where insufficient information on portion size was lacking, the average portion size from the Food Standard Agency Portion Size book were used(242). Average daily energy and macronutrient intake were calculated using Dietplan6.
2.3.2 INVESTIGATION TWO

2.3.2.1 SCHEDULE OF STUDY DAY

Participants were asked to fast for 12 hour prior to their 9am start time. An overview of the study design is seen in figure 2.2. Participants underwent a Meal Tolerance Test (MTT) comprising an Ensure Plus and a Kellogg’s Nutrigrain cereal bar given at 0 minutes. Nutritional content of the meal is shown in appendix B. Participants were instructed to consume the Ensure and cereal bar within 4 minutes and were observed by the investigator to ensure compliance with this instruction. Breath hydrogen was taken at 220 minutes. The supplementation was mixed with the Ensure shake on the follow-up visit of each study arm.

The decision to use a MTT was made after careful consideration. Measuring insulin secretion can be done via intravenous glucose tolerance test (IVGTT), the hyperglycaemic clamp and oral glucose tolerance test or a liquid or mixed meal tests. Each of these methods has advantages and limitations. For example, first-phase insulin secretion – defined as the initial burst of insulin released in the first 5–10 minutes after the β-cell is exposed to a rapid increase in glucose(243) – can be measured using the IVGTT and hyperglycaemic clamp. However, neither of these methods takes into account the incretin effect, which can be responsible for up to 70% of glucose-dependent insulin release(244). The well-defined first-phase does not occur when glucose is given orally. However, it is important to note that if glucose is infused intravenously at a more gradual rate, then the neatly defined first and second phases are also not apparent, and there is a smoother line of insulin secretion similar to that seen when glucose is delivered orally(245). This smoother rise over the first 30 minutes is termed the early phase of insulin secretion. (245,246). In this study, given the importance of the incretin effect in type 2 diabetes, and that FCHO are postulated to exert their effects via increases in GLP-1, it was decided that the use of a glucose tolerance test would be most suitable. The choice of a meal versus an oral glucose tolerance test was made due to the practicalities of the study day. Subjects would be fasting for 12 hours, and would then have over 4 hours of blood sampling. The MTT that was given provided the subjects with 450kcal, which was felt
to be sufficient energy content to keep the subjects satisfied and comfortable for the duration of the study day.

2.3.2.2 CONTINUOUS GLUCOSE MONITORING

Subjects were asked to wear a Medtronic iPro Continuous Glucose Recorder for the 5 days following their baseline visit and the 5 days preceding their follow-up visit to determine their free-living glucose excursions. This monitor comprises a light-weight memory storage which attaches to a sensor. Using a spring-loaded device, the sensor is placed in the subcutaneous layer of tissue of the abdomen where it measures glucose concentrations in interstitial fluid are every 5 minutes.

Subjects were asked to take a minimum of 3 capillary blood measurements per day by using the finger prick method and were provided with a glucose meter and strips in order to do this. The capillary measurements are required in order to calibrate the sensor.

The subjects returned the iPro monitor and the dates, time and concentrations of capillary measurements to the researcher. This data was uploaded onto an internet-based platform which uses a computer-driven algorithm to relate capillary glucose measurements to subcutaneous interstitial fluid glucose measurements. This print-out data gives the calculated values for blood measurements every five minutes.

The recorded data were analysed for mean glucose and glycaemic variability measures including Mean Amplitude Glycaemic Excursions (MAGE), Continuous Overlapping Net Glycaemic Action (CONGA), Mean of Daily Differences (MODD) and risk indices such as Low Blood Glucose Index (LBGI), High Blood Glucose Index (HBGI) and average daily risk ratio (ADRR). Descriptions of each of these variables are found in Appendix E.
2.3.3 INVESTIGATION THREE

This investigation was an 18 week-long study. The first 9 weeks was the weight loss phase, during which every subject attended 4 dietary counselling sessions to help them reach their target of 5% weight loss by the week 9 study day. Weeks 9-18 was the weight maintenance phase, during which subjects were asked to maintain the weight that they had lost. During weeks 9-18, subjects received minimal contact from the researcher. See figure 2.3 for outline of the study.

2.3.3.1 SCHEDULE OF STUDY DAY

Participants were asked to fast for 10 hour prior to their 9am start time. An overview of the study design is seen in figure 2.3. Two fasting blood samples were taken. An Ensure Plus was given at 0 minutes followed by 180 minutes of blood sampling. Following removal of the cannula an ad libitum meal test was served. Visual Analogue Scales (VAS) were completed by the participants at each blood sample to assess subjective appetite. A breath hydrogen measure was taken at 160 minutes.

2.3.3.2 BIOIMPEDANCE

On each study day visit, body composition analysis was done using a BC-418 Segmental Body Composition Analyzer.
2.3.3.3 MRI SCAN

Whole body anatomical MR scanning was performed on eligible subjects to determine total and regional fat volumes, and magnetic resonance spectroscopy (MRS) performed to measure lipid content in the internal organs, such as liver (IHCL) and muscles (IMCL), such as soleus and tibialis. Following a change into hospital clothes, participants had their weight, height, waist and hip circumference recorded. Subjects were asked to lie supine or prone in the scanner for commencement of MRI scanning.

Rapid T1-weighted magnetic resonance images were obtained using a 1.5T Phillips Achiva scanner (Phillips, Best, the Netherlands). Images were analyzed using SliceOmatic (Tomovision, Montreal, Quebec, Canada). The total and regional volumes were measured and 1H MR spectra were also obtained from the liver, pancreas, soleus-intramyocellular (IMCL) and tibialis.

2.3.3.4 DIETARY COUNSELLING SESSIONS

The participants attended 4 dietary counselling sessions during the 9 week active weight loss period. The first took place within 7 days of their baseline appetite study day, the second at 2 weeks, the third at 4 weeks and the fourth at 6 weeks. Each participant was given individualised advice on diet and exercise goals towards meeting a 5% weight loss target. Participants were weighed at each visit.

Figure 2.3: Schematic of Study Design for Investigation 3
2.4 POWER CALCULATIONS

2.4.1 INVESTIGATION ONE

We estimated a sample minimum sample of 21 was required. This was based on the paper by Cani et al (206) where total energy intake decreased by 6% with a SD of 8%, and a power of 90%, alpha: 5%. For clarification, the power calculation was based on the delta change between groups following a crossover trial, ie, the same design used in this investigation. The study was powered to examine effects on food intake, not on insulin sensitivity. Although we tried to keep drop-outs to a minimum we aimed to recruit 30.

2.4.2. INVESTIGATION TWO

We estimated a minimum number of 10 volunteers was required. This was based on data from Robertson et al (247) where a supplement of resistant starch resulted in an 18 unit increase in insulin sensitivity, with an SD of 15 between groups at a power of 90%. We tried to minimise drop-outs but to cover this we aimed to recruit 15 volunteers.

2.4.3 INVESTIGATION THREE

The power calculation has been taken from the Parnell et al (173) paper which estimated a weight loss of 2.0 kg and an SD of 2.0 kg based on 0.8 power to detect a significant difference \( P < 0.05, 2 \) sided), a minimum of 32 subjects were needed; 16 additional subjects were added to account for dropouts.

2.5 OUTCOME MEASURES

2.5.1 INVESTIGATION 1

Primary outcome: Energy intake from 3-day food diary.

Secondary outcome: Energy intake from *ad-libitum* meal, subjective appetite scores from VAS, anorectic hormones: GLP-1, PYY, insulin, glucose.
2.5.2 INVESTIGATION 2

Primary outcome: Glucose tolerance during the Meal Tolerance Test.

Secondary outcome: Insulin resistance and β-cell function as calculated using HOMA2. Measures of glycaemic variability. The insulinogenic index, disposition index and Matsuda index will also be calculated as further measures of glucose homeostasis.

2.5.3 INVESTIGATION 3

Primary outcomes: Maintenance of weight loss at 18 weeks as a percentage of weight loss at 9 weeks.

Secondary outcome: Weight loss at 9 weeks. Body composition as assessed by MRI. Food intake as assessed by *ad libitum* meal. Subjective appetite as assessed by VAS at 9 and 18 weeks.

2.6 BIOCHEMICAL PARAMETERS

2.6.1 SAMPLE COLLECTION

Blood for glucose analysis were collected into fluoride oxalate tubes whilst blood for FFA and insulin samples was transferred to serum gel separator tubes containing gel clotting activator Vacutte®. Blood for GLP-1 was transferred to lithium heparin containing tubes while blood for C Peptide was collected in potassium EDTA containing tubes, both with aprotonin (Trasylol, Bayer, Newbury, UK) (200 µL/7.5 ml blood) added. The collected blood samples were spun at 4000 g at 4°C for 10 mins, separated into plasma and stored at -20°C until analysed.

2.6.2 SAMPLE ANALYSIS

Plasma glucose was measured in the Department of Clinical Biochemistry, Hammersmith Hospital, using an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK). Glucose assay sensitivity was 0.3 mmol/L with an intra-assay CV of 1%. Concentrations of plasma insulin and C-
peptide were measured by RIA with commercially available kits (Millipore; Watford, UK). The sensitivity and intra-assay CV for insulin and C Peptide were 7.5 pmol and 0.1 ng/mL, and 3.3% and <10%. Concentrations of plasma free fatty acids (FFA/NEFA) were determined using an enzymatic-colorimetric method (NEFA kit, Wako Chemicals, GmBH).

GLP-1 and PYY were quantified using in-house radioimmunoassay. These assay is described in detail below. The sensitivity and intra-assay CV of the GLP-1 and PYY assays were 7.5 pmol/l and 2.5 pmol/l and 3.3% and 5.8% respectively.

2.6.2.1 IN HOUSE GLP-1 RIA

GLP-1-like immunoreactivity was measured using an in-house radioimmunoassay. The antibody was produced in rabbits against GLP-1 coupled to bovine serum albumin. The antibody cross reacts with all amidated forms of GLP-1 but did not cross react with glycine reacted forms (GLP-1 (1-37) and GLP-1 (7-37)) or any other known pancreatic or gastrointestinal peptide. $^{125}$I-GLP-1 was prepared by the iodogen method and purified by HPLC. The specific activity of the $^{125}$I-GLP-1 label was 48 Bq/fmol. The assay was performed in a total volume of 0.7ml of 0.06 M sodium barbitone buffer (pH 8) containing 0.3% BSA. Sample volume was 100 μL. The assay was incubated for three days at 4°C before separation of the free and antibody label by charcoal absorption.

2.6.2.2 IN HOUSE PYY RIA

Total PYY-like immunoreactivity was measured with a specific and sensitive in-house radioimmunoassay. The antiserum (Y21) was produced in rabbits against synthetic porcine PYY coupled to bovine serum albumin by glutaraldehyde and used at a final dilution of 1:50,000. This antibody cross-reacts fully with both PYY3-36 and PYY1-36, but not with pancreatic polypeptide, neuropeptide Y, or other known gastrointestinal hormones. The $^{125}$I PYY was prepared by the iodogen method and purified by high pressure liquid chromatography (HPLC). The specific activity of the $^{125}$I PYY label was 54 Bq/fmol. The assay was performed in total volume of 0.7 ml of 0.06 M
phosphate buffer PH 7.2 containing 0.3% bovine serum albumin (BSA). The assay was incubated for three days at 4°C before separation of the free and antibody bound label by sheep anti-rabbit antibody.

2.7 STATISTICAL ANALYSIS

Biochemical and experimental measures will be presented as mean and SEMs unless otherwise stated. Variables were tested for normality (Kolmogorov-Smirnov and Shapiro-Wilks tests). Residuals were explored by histograms, Q-Q plots and box plots and tested for normality. Non-parametric data were compared by using Mann-Whitney test. The postprandial response curves for PY, GLP-1, glucose, insulin and FFA were calculated as AUC, calculated using the trapezoid rule. Within-group differences were calculated using a paired t-test. The delta change between inulin and cellulose groups was compared using independent t-test. A P value of 0.05 was considered significant. The statistical analyses were carried out using GraphPad Prism 5 version 5.03. (GraphPad Software, San Diego CA, USA).
CHAPTER THREE:

EFFECT OF FERMENTABLE CARBOHYDRATE ON APPETITE AND FOOD INTAKE IN SUBJECTS WITH PREDIABETES
3.0 INVESTIGATION 1

This investigation was a double-blind crossover trial which aimed to examine the effect of 6 weeks’ supplementation with inulin on measures of appetite and food intake, as assessed via visual analogue scales, an *ad libitum* meal test, a 3-day food diary and the appetite hormones GLP-1 and PYY and changes in weight.

3.1 RECRUITMENT DATA

25 subjects were recruited to the study. 20 completed both arms of the study, 1 person completed the inulin arm only and there were 4 drop-outs. Unfortunately, the 21st participant was 2 weeks away from their final visit when they became very unwell (not related to the trial) and dropped out. It was intended that 21 people would finish the trial. 1 person dropped out due to side effects from the inulin, and 3 people dropped out due to time constraints.

Participant characteristics are shown in table 3.1. There were no differences between the groups for any of the characteristics. While the participants who were randomised to take the cellulose first had higher fasting and post-prandial glucose, the nature of the crossover design meant that all subjects acted as their own controls – each subject took both the inulin and the cellulose. There were no differences in response to the intervention between those who took inulin first or second (data not shown).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inulin</th>
<th>Cellulose</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M:F)</td>
<td>5:4</td>
<td>8:4</td>
<td>0.6251</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>64.4 ± 8.4</td>
<td>62.2 ± 9.1</td>
<td>0.5642</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.5 ± 17.6</td>
<td>86.8 ± 13.8</td>
<td>0.8587</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.4 ± 3.1</td>
<td>29.7 ± 2.5</td>
<td>0.8042</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.8 ± 0.3</td>
<td>6.1 ± 0.5</td>
<td>0.1048</td>
</tr>
<tr>
<td>2hPG (mmol/L)</td>
<td>6.9 ± 2.3</td>
<td>8.1 ± 2.2</td>
<td>0.2304</td>
</tr>
</tbody>
</table>

Table 3.1: Baseline demographic and biochemical data for the groups randomised to receive inulin or cellulose first.
3.2 FOOD INTAKE AND APPETITE DATA

3.2.1 ENERGY INTAKE UNDER FREE-LIVING CONDITIONS

Subjects taking insulin consumed significantly less energy (Pre: 2032.94 ± 476.13 kcal, Post: 1759.83 ± 476.99, N = 21, P = 0.001), carbohydrate (Pre: 202.93 ± 46.04 g, Post: 174.09 ± 49.84 g, N = 21, P = 0.011), and fat (Pre: 82.04 ± 24.56, Post: 63.31 ± 17.47, N = 21, P = 0.001) when compared to baseline. There was also a non-significant reduction in sugar intake (Pre: 72.81 ± 31.66 g, Post: 60.26 ± 26.63 g, N = 21, P = 0.06). There was a significant increase in carbohydrate intake when taking the cellulose control (P = 0.048). Independent t-tests comparing the delta change in measures of food intake of the inulin and cellulose groups found significant differences between groups for energy intake (P = 0.003), total carbohydrate (P = 0.001) and fat (P = 0.003) (Table 3.2). There were no differences in baseline values for food intake (Data not shown). Calories from alcohol were not different at baseline or between groups (Table 3.2), and the reduction in energy intake was still significant (P = 0.001) after calories from alcohol were removed from the calculation.

<table>
<thead>
<tr>
<th></th>
<th>Inulin</th>
<th>Cellulose</th>
<th>P Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Difference</td>
<td>Pre</td>
</tr>
<tr>
<td>Kcal/d</td>
<td>2032.9±476.1</td>
<td>1759.8±476.99</td>
<td>-273.1±39.3*</td>
<td>1943.5±537.1</td>
</tr>
<tr>
<td>CHO/d (g)</td>
<td>202.9±46.0</td>
<td>174.1±49.84</td>
<td>-28.8±24.6*</td>
<td>183.7±57.0</td>
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<tr>
<td>Protein/d (g)</td>
<td>74.7±17.8</td>
<td>73.1±23.86</td>
<td>-1.6±20.0</td>
<td>78.3±34.3</td>
</tr>
<tr>
<td>Fat/d (g)</td>
<td>82.0±24.6</td>
<td>63.3±17.47</td>
<td>-18.7±16.7*</td>
<td>77.7±28.0</td>
</tr>
<tr>
<td>Sugar/d (g)</td>
<td>72.8±21.7</td>
<td>60.3±26.63</td>
<td>-12.6±26.4</td>
<td>67.7±20.9</td>
</tr>
<tr>
<td>Fibre/d (g)</td>
<td>18.8±6.3</td>
<td>17.0±9.44</td>
<td>-1.7±7.4</td>
<td>16.7±6.0</td>
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<tr>
<td>Alcohol (g)</td>
<td>24.3±7.9</td>
<td>25.7±32.7</td>
<td>-1.4±29.1</td>
<td>28.2±30.6</td>
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<tr>
<td>Meal (g)</td>
<td>486.6±204.7</td>
<td>384.3±212.80</td>
<td>-102.2±158.8*</td>
<td>482.2±237.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.9±14.1</td>
<td>84.3±13.4</td>
<td>-0.5±1.2</td>
<td>85.6±13.9</td>
</tr>
</tbody>
</table>

Table 3.2: Food intake values and body weight pre- and post-inulin and cellulose interventions. *Post-intervention value significantly different from pre-intervention value (within group difference) P<0.05. #Delta change for inulin group significantly different to delta change for cellulose group (between group difference), P<0.05. N = 20.
While the absolute changes in carbohydrate and fat intake did alter, the percentage of calories from each did not change (Figure 3.2). (Percentage of calories from carbohydrate and fat, pre-inulin: 40.61 ± 1.73% and 36.60 ± 1.13%; post-inulin: 39.99 ± 1.97% and 33.75 ± 1.61%, P = 0.582 and P = 0.128 respectively). There was a non-significant increase in calories from protein. (Pre inulin: 15.28 ± 0.65%, post inulin: 16.61 ± 0.93% (P = 0.055).

Figure 3.1a Percentage of kilocalories from each of the macronutrients pre- and post-inulin supplementation. There were no significant differences in kcal intake from carbohydrate (P = 0.5828), fat (P = 0.1168) or protein (P = 0.055), N = 21. Figure 3.1b: Delta change in percentage of kilocalories from carbohydrate, fat and protein following inulin (I) and cellulose (C) supplementation. There were no significant differences in the delta change for percentage kcal from carbohydrate (P = 0.2664), fat (P = 0.071) or protein (P = 0.7229) N = 21.

3.2.2 ENERGY INTAKE AT TEST MEAL

Subjects consumed significantly less of the test meal at the follow-up visit when compared to the baseline visit (Pre: 486.57 ± 269.69g, Post: 384.33 ± 212.80g, N = 21, P = 0.008) but the delta change was not significantly different between groups (-102.2 ± 34.66g vs -35.58 ± 28.47g, P = 0.15) (Table 3.2, Figure 3.2). This equates to 127.5 ± 44.83kcal and 44.83 ± 35.87kcal less following inulin and cellulose supplementation respectively.

Figure 3.2: Difference in food intake at the test meal for inulin and cellulose. There was no difference in food intake between the groups (P = 0.104), N = 21.
3.3 WEIGHT

Subjects lost weight when taking the inulin supplement (-0.52 ± 0.27kg, P = 0.072), and gained weight when taking the cellulose supplement (0.63 ± 0.41kg, P = 0.141) (Figure 3.3a). The delta change was significantly different between groups (P = 0.025, Figure 3.3b). There were no differences between the groups at baseline (data not shown), and there was no effect of treatment order on weight change (data not shown).

![Figure 3.3a: Body weight pre- and post-inulin and cellulose supplementation. 3b: Delta change in body weight following inulin and cellulose supplementation. Subjects lost significantly more weight when taking inulin compared with control (P = 0.0251). N = 21.]

3.4 SUBJECTIVE ASSESSMENT OF APPETITE

Inulin supplementation had no effect on subjective appetite scores as measured using Visual Analogue Scales (VAS). On the following pages are the time course and tAUC data for all 4 subjective appetite questions: 1) How hungry are you right now (Figure 3.4.1), 2) How pleasant would it be to eat right now (Figure 3.4.2), 3) How much could you eat right now (Figure 3.4.3) and 4) How full do you feel right now (Figure 3.4.4). The VAS score is shown on the y axis and is shown in centimetres. There was no effect on any of the 3 subjective assessments on fasting measures of appetite or tAUC for any of the appetite questions.
3.4.1 How hungry do you feel right now?

Fig 3.4.1: Time course data for pre and post subjective hunger feelings for inulin (a) and cellulose (b). Breakfast was served at 0 mins, and lunch was served at 240 minutes. Values are means and vertical bars are SEM. Inulin: (N = 21); Cellulose (N = 20). The VAS (Visual Analogue Scale) score is on the Y axis and measured in cm.

Figure 3.4.1 Delta change for tAUC hunger (c) and fasting hunger (d) are shown. Inulin: (N = 21); Cellulose (N = 20). Values are means and vertical bars are SEM. There were no significant differences in tAUC hunger scores (P = 0.57) or fasting hunger scores (P = 0.2).

There were no significant differences in tAUC for hunger scores either within (P = 0.61) or between groups (P = 0.57). There were no significant difference for fasting hunger scores when compared to baseline (P = 0.86) or when compared to cellulose supplementation (P = 0.2).

3.4.2 How pleasant would it be to eat right now?

There were no significant differences in tAUC for pleasant to eat scores either within (P = 0.65) or between groups (P = 0.21). There were no significant difference for fasting pleasant to eat scores within (P = 0.2) or between groups (P = 0.13).
Figure 3.4.2: Time course data for pre and post subjective pleasant to eat sensations for inulin (a) and cellulose (b). Breakfast was served at 0 mins, and lunch was served at 240 minutes. Values are means and vertical bars are SEM. Inulin: (N = 21); Cellulose (N = 20). The VAS (Visual Analogue Scale) score is on the Y axis and measured in cm.

Figure 3.4.2: Delta change for tAUC (c) and fasting pleasant to eat (d) are shown. Inulin: (N = 21); Cellulose (N = 20). There were no significant differences in tAUC for pleasant to eat scores (P = 0.21). There were no significant difference for fasting pleasant to eat scores within (P = 0.2) or fasting pleasant to eat scores (P = 0.13).

3.4.3. How much do you think you can eat right now? (Prospective Food Consumption)

Figure 3.4.3: Time course data for pre and post prospective food consumption feelings for inulin (a) and cellulose (b). Breakfast was served at 0 mins, and lunch was served at 240 minutes. Values are means and vertical bars are SEM. The VAS (Visual Analogue Scale) score is on the Y axis and measured in cm.
Figure 3.4.3: Delta change for tAUC (c) and fasting prospective food consumption (d) are also shown. Inulin: (N = 21); Cellulose (N = 20). There were no significant differences in tAUC (P = 0.42). There was a borderline significant difference in fasting prospective food consumption scores between groups (P = 0.053).

There were no significant differences in tAUC for prospective food consumption either within (P = 0.20) or between groups (P = 0.42). There was no difference in fasting prospective food consumption following inulin supplementation when comparing pre and post (P = 0.51). There was a borderline significant difference in fasting prospective food consumption scores between groups (P = 0.053).

3.4.4 How full do you feel right now?

Figure 3.4.4: Time course data for pre and post fullness for inulin (a) and cellulose (b). Breakfast was served at 0 mins, and lunch was served at 240 minutes. Values are means and vertical bars are SEM. The VAS (Visual Analogue Scale) score is on the Y axis and measured in cm.

There were no significant differences in tAUC for fullness scores either within (P = 0.187) or between groups (P = 0.45). There were no significant difference for fasting fullness scores either within (P =0.489) or between groups (P = 0.987).
Figure 3.4.4: Time course data for pre and post fullness for inulin (a) and cellulose (b). Breakfast was served at 0 mins, and lunch was served at 240 minutes. Values are means and vertical bars are SEM. Delta change for tAUC(c) and fasting fullness (d) are also shown. Inulin: (N = 21); Cellulose (N = 20). There were no significant differences in fullness tAUC scores (P = 0.45) or fasting fullness scores (P = 0.987).

3.5 APPETITE SENSATIONS IN A FREE-LIVING ENVIRONMENT

There were no differences in appetite, hunger or fullness sensations at home for the inulin group or when the delta change was compared between groups. (Within group differences for Hunger: P = 0.145, Fullness: P = 0.3 and Hunger between meals: P = 0.179). Between group differences: Hunger: P = 0.487, Fullness: P = 0.137 and Hunger between meals: P = 0.6427 (Figure 3.5).

Figure 3.5: Difference in hunger (a), fullness (b) and hunger between meals (c) scores at home for inulin and cellulose. Values are means and vertical bars are SEM. Inulin: (N = 21); Cellulose (N = 20). There were no significant differences in any measure of appetite at home.
3.6 SIDE EFFECTS

There were no differences in FCHO-related side effects such as flatulence, bloating and diarrhoea during the appetite study day. There were no differences pre- and post-inulin supplementation for bloating \( tAUC \) (\( P = 0.087 \)), \( tAUC \) discomfort (\( P = 0.163 \)), \( tAUC \) flatulence (\( P = 0.412 \)), \( tAUC \) diarrhoea (\( P = 0.416 \)) \( tAUC \) or sickness (\( P = 0.6016 \)). There were no differences between the groups for bloating \( tAUC \) (\( P = 0.08 \)), \( tAUC \) discomfort (\( P = 0.39 \)), \( tAUC \) flatulence (\( P = 0.8 \)), \( tAUC \) diarrhoea (\( P = 0.444 \)) and \( tAUC \) sickness (\( P = 0.816 \)) (Table 3.3).

In a free-living environment there were significant increases in bloating within but not between groups (\( P = 0.005; P = 0.275 \)). There were significant increases in flatulence within and between groups (\( P = 0.006; 0.0218 \)). There were no differences in diarrhoea within or between groups (\( P = 0.3255, P = 0.163 \)) (Table 3.3).

<table>
<thead>
<tr>
<th>Sensation</th>
<th>Inulin (n=20)</th>
<th>Control (n=20)</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Appetite Study Day</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bloating (tAUC)</td>
<td>512.7 ± 82.85</td>
<td>768.4 ± 149.5</td>
<td>536.5 ± 98.35</td>
<td>456.6 ± 115.6</td>
</tr>
<tr>
<td>Discomfort (tAUC)</td>
<td>283.6 ± 60.28</td>
<td>407.8 ± 109.9</td>
<td>332.4 ± 83.12</td>
<td>307.4 ± 105.7</td>
</tr>
<tr>
<td>Flatulence (tAUC)</td>
<td>397.5 ± 89.13</td>
<td>494.8 ± 115.6</td>
<td>370.1 ± 93.49</td>
<td>384.6 ± 112.3</td>
</tr>
<tr>
<td>Diarrhoea (tAUC)</td>
<td>324.6 ± 123.9</td>
<td>213.5 ± 70.41</td>
<td>284.8 ± 87.12</td>
<td>223.1 ± 65.54</td>
</tr>
<tr>
<td>Sickness (tAUC)</td>
<td>340.1 ± 73.80</td>
<td>307.5 ± 78.96</td>
<td>364.5 ± 95.83</td>
<td>267.2 ± 83.37</td>
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<td>At home</td>
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<tr>
<td>Bloating (cm)*</td>
<td>1.44 ± 0.36</td>
<td>3.64 ± 0.59</td>
<td>1.79 ± 0.44</td>
<td>2.87 ± 0.75</td>
</tr>
<tr>
<td>Flatulence (cm)*</td>
<td>1.49 ± 0.47</td>
<td>4.04 ± 0.91</td>
<td>1.68 ± 0.42</td>
<td>2.15 ± 0.62</td>
</tr>
<tr>
<td>Diarrhoea (cm)*</td>
<td>0.83 ± 0.43</td>
<td>1.45 ± 0.58</td>
<td>0.88 ± 0.43</td>
<td>1.02 ± 0.51</td>
</tr>
</tbody>
</table>

Table 3.3: Appetite and side-effect scores pre and post inulin and cellulose consumption. *Post inulin value significantly higher than pre-inulin value (within group difference) \( p<0.05 \). **Difference pre-post inulin significantly greater than difference pre-post cellulose (between group difference). \( P<0.05 \).
3.7 BREATH HYDROGEN

Breath hydrogen was significantly increased following the inulin supplementation (Pre: 5.45 ± 1.6ppm, Post: 13.4 ± 3.08ppm, P = 0.0379), and the difference was significant between groups (8.83 ± 1.82ppm vs 3.27 ± 1.70ppm, P = 0.013)(Figure 3.6). There was considerable variation in breath hydrogen measures: At baseline 3 subjects had a breath hydrogen measure of 0ppm, while one subject had a pre-intervention breath hydrogen of 24ppm. 2 subjects of those with 0ppm at baseline had no increase in breath hydrogen post-inulin supplementation, one subject had an increase to 1ppm and one had an increase to 7ppm.

![Figure 3.6: Delta change in breath hydrogen concentration for inulin and cellulose interventions. Significant difference in delta change in breath hydrogen (P = 0.013) (N = 18).](image)

3.8 BIOCHEMICAL DATA

3.8.1 PLASMA GLUCOSE

The time course data, delta change for tAUC, fasting glucose and peak glucose are shown in figure 3.7a-d. There were no significant differences in tAUC (within group difference: P = 0.7226, between group differences P = 0.3669). There were also no differences in fasting glucose within group (Pre: 6.05 ± 0.09mmol/L, Post: 6.03 ± 0.11mmol/L, N = 18, P = 0.89), between group difference: (0.02 ± 0.14 mmol/L vs 0.045 ± 0.16mmol/L, N = 18, P = 0.82) or peak glucose either within group (Inulin Pre: 8.68 ± 0.42mmol/L, Post: 8.76 ± 0.27mmol/L, N = 18, P = 0.48) or between groups (0.22 ± 0.36mmol/L vs 0.51 ± 0.56mmol/L, N = 18, P = 0.27).
Figure 3.7: Time course data for pre and post venous plasma glucose concentrations for inulin (A) and cellulose (B). Values are means and vertical bars are SEM. Breakfast was served at 0 minutes and lunch was served at 240 minutes. Inulin N = 18, cellulose N = 16.

Figure 3.7c: Delta change for tAUC, and d: fasting plasma glucose also shown following inulin and cellulose supplementation. Inulin: (N = 18); Cellulose (N = 16). Values are means and vertical bars are SEM. There were no significant differences between groups.

3.8.2 PLASMA INSULIN

The time course data is shown in figure 3.8. Unfortunately due to haemolysis of samples, there were only 18 pairs of samples for analysis for inulin and 13 pairs for cellulose. There were no significant differences in insulin tAUC (within group difference: P = 0.9006, between group differences P = 0.512). Fasting insulin decreased following the inulin supplementation (Pre: 25.30 ± 2.12μU/ml, Post: 20.35 ± 1.54μU/ml, P = 0.032), and the difference was significant between groups (-5.25 ± 2.23 μU/ml vs 1.52 ± 1.50 μU/ml, P = 0.0257). There was no significant increase in peak insulin following the inulin supplementation from baseline (P = 0.0653) and no difference between groups. (P = 0.6277) (Figure 3.8c and d).
Figure 3.8: Time course data for pre and post venous plasma insulin concentrations for inulin (a) and cellulose (b). Values are means and vertical bars are SEM. Breakfast was served at 0 minutes and lunch at 240 minutes.

Figure 3.8c: Delta change in tAUC and d: delta change in fasting insulin following inulin and cellulose supplementation. Inulin: (N = 18); Cellulose (N = 13). Values are means and vertical bars are SEM. Fasting insulin decreased both within (P = 0.032), and between groups (P = 0.0257).

3.8.3 HOMA-IR

HOMA-IR (a measure of insulin resistance in the fasting state) was calculated using the HOMA-IR2 calculator by the Oxford Diabetes Trial Unit(248). There was a significant reduction in HOMA-IR following inulin supplementation (Pre: 6.32 ± 0.94, Post: 4.42 ± 0.45, N=12, P = 0.0252), and the difference was significant between groups (-1.29 ± 0.52 vs 0.40 ± 0.48, N=12, P = 0.0303)(Figure 3.9).
3.8.4 GLP-1

The time course data for GLP-1 is shown in figure 3.10. There were no differences in tAUC either within (P= 0.4309) or between groups (P = 0.9623). There were no differences in fasting GLP-1 within group (Pre: 8.77 ± 1.60pmol/L, Post: 9.81 ± 2.02pmol/L, P = 0.1338), but there was no effect of inulin on fasting GLP-1 between the groups (1.03 ± 0.66pmol/L vs -0.35 ± 0.31pmol/L, P = 0.0698). Subjects had lower baseline fasting and tAUC GLP-1 concentrations prior to starting the cellulose supplementation but were not significantly different (P = 0.389, P = 0.263).

Figure 3.10: Time course data for pre and post GLP-1 concentrations for inulin (a) and cellulose (b). Inulin: (N = 21); Cellulose (N = 20). Values are means and vertical bars are SEM. Breakfast was served at 0 minutes and lunch at 240 minutes.

Figure 3.10c: Delta change for tAUC, and d: fasting GLP-1 following inulin and cellulose supplementation. There was no difference in tAUC between groups (P = 0.9623). There was a no effect on fasting GLP-1 between the groups (1.03 ± 0.66pmol/L vs -0.35 ± 0.31pmol/L, P = 0.0698). Inulin: (N = 21); Cellulose (N = 20). Values are means and vertical bars are SEM.
### 3.8.5 PYY

The time course data for PYY are shown in figure 3.11. Although it appears that PYY concentrations post-inulin supplementation were lower than pre-intervention values, the tAUC was not significantly different \((P = 0.324)\). Examining only the post-prandial PYY concentrations \((0-180, 280-440 \text{ min}, \text{AUC})\) made no difference to the significance \((P = 0.458)\). There was also no difference in the delta change in tAUC between groups \((P = 0.5352)\). There were no differences in fasting PYY within \((9.31 \pm 0.99 \text{ pmol/L vs } 12.33 \pm 2.39 \text{ pmol/L}, N = 21, P = 0.2426)\) or between groups \((2.43 \pm 2.12 \text{ pmol/L}, N = 21 \text{ vs } -0.71 \pm 2.02 \text{ pmol/L}, N = 20, P = 0.291)\).

**Figure 3.11:** Time course data for pre- and post-PYY concentrations for inulin (a) and cellulose (b). Values are means and vertical bars are SEM. Breakfast was served at 0 minutes and lunch at 240 minutes.

**Figure 3.11:** Delta change for tAUC, and d: fasting PYY following inulin and cellulose supplementation. There was no difference in tAUC between groups. Inulin: \(N = 21\); Cellulose \(N = 20\).
3.9 DISCUSSION

In this investigation, I have demonstrated:

- Significant reduction in food intake according to the 3-day food diary
- Significant within group reduction in food intake at the *ad libitum* meal
- Significant between group difference in body weight
- Significant reduction in fasting insulin and HOMA-IR.

3.9.1 FOOD INTAKE

In this mechanistic investigation, inulin significantly reduced energy intake by a mean 274kcal/day (13%), leading to a significant reduction in weight at the end of the supplementation period when compared to control. There was no effect on subjective or biochemical measures of appetite.

Food intake was assessed by 3-day food diary and *ad libitum* meal. Self-reported food intake has known limitations, including the propensity for subjects to under-report, particularly in those with a BMI > 25kg/m² (249). The percentage of subjects who underreported was 19% across the four visits (data not shown). However, the magnitude of reported calorie deficit between the pre-inulin and post-inulin groups is supported by the magnitude of weight loss recorded. A daily deficit of 273kcal would equal ~3800kcal over 14 days. Based on a commonly accepted estimation that one pound of fat has as an energy value of 3500kcal (250), the expected mean weight loss would be 1.086 pounds of weight, or 0.49kg. This corresponds well to the weight loss of 0.51kg seen in the inulin group.

The reliability of the self-reported energy intake can also be assessed by comparing the dietary changes to similar studies. While exact comparisons are not possible due to differences in methodology, such as dose and duration of intervention, the energy deficit in this study (13%) was comparable to similar studies. 16g OFS for 14 days was associated with a 5% reduction in energy intake (206), while 21g OFS for 12 weeks was associated with a 300-500kcal (29%) deficit (173).
The reduction in calories was due to absolute reductions in fat (22%) and carbohydrate (6.7%), with no changes in absolute protein intake. In the Parnell and Reimer study cited above, they found significant reductions in food intake from each of the macronutrients, with a 40% reduction in fat intake, 27% reduction in carbohydrate intake, and a 24% reduction in protein intake (173). However, the reduction in protein in that study may be due to the higher protein intake at baseline of 94.6g compared to the 74.7g in this study (173).

The reduction in self-reported energy intake is also supported by data from the ad libitum meal test carried out on the clinical research unit, where subjects taking inulin consumed significantly less at the post-inulin visit compared to the pre-inulin visit. Critically, the inulin supplementation was not given the morning of the study day, and therefore the meal test was undertaken at approximately 18-20 hours since the last dose of inulin. This provides further evidence that the effects of inulin on food intake are not immediate. The fact that breath hydrogen levels - which were taken at 410 minutes in the protocol (approximately 4.15pm) - were also elevated at the same time as the ad libitum meal test was given supports the hypothesis that the products of fermentation play a role in the reduction of food intake.

While the within group difference of inulin on ad libitum food intake was significant, the delta change in food intake was not significant between inulin and the control group. This may be due to the inulin supplement – which would be expected to have second meal effects - not being given on the day. While the time of peak fermentation depends upon the quantity and type of carbohydrate entering the colon and intra-individual differences (205, 251), peak hydrogen levels with inulin normally occur at 3-4 hours post-ingestion (unpublished data from the Nutrition and Dietetics Research Group). It may therefore be the case that while fermentation activity was elevated at the time of the meal test, it had declined from its peak, meaning the appetite suppressing effects were not optimal. As such this may mean that to maintain appetite suppressing effects, inulin may need to be taken frequently and daily.
It should also be noted that while the *ad libitum* meal test is designed to be an objective measure of appetite, there were concerns during the study that having this test at the end of a long protocol may have affected its ability to detect changes in appetite. All of the subjects knew that following the meal test they would be allowed to go home, and it may have been possible that some subjects ate less at certain visits because they wanted to leave. In particular, 2 subjects on follow-up visits (one following the inulin and one following the cellulose supplementation) had indicated that they were anxious to leave as they had plans for the evening, and both subjects ate less at this visit when compared to their baseline visit. However, when these data was removed from the analysis, the result did not change.

### 3.9.2 SUBJECTIVE APPETITE ASSESSMENT

#### 3.9.2.1 SUBJECTIVE APPETITE ASSESSMENT DURING THE STUDY DAY

Appetite can be assessed by objective or proxy measures of appetite, i.e., an *ad libitum* meal test or by subjective measures of appetite by using Visual Analogue Scales (VAS). In this investigation, while there was an effect on *ad libitum* food intake, there was no effect on subjective measures of appetite at any point during the study. There may be a number of reasons for this.

Bodinham *et al.* had suggested that the lack of effect on subjective appetite seen was due to inadvertently overfeeding the participants during the study day (252). Therefore, in this investigation the protocol was specifically designed to underfeed the participants based on their estimated energy requirements and the physical activity factor used was for bed-bound hospitalised subjects. However, this may still have inadvertently overfed the participants as many reported that the lunch was too big. In this study, the participants were given 256.4-407.1 kcal for the breakfast and 469.5-589.0 kcal for lunch. This was due to data from NDND suggesting 20% of Britons never eat breakfast, while 48% skip breakfast at least 4 times a week (253). Only half of the participants in this study ate
breakfast habitually. Therefore it was decided to feed the participants only a small amount for
breakfast, with more calories taken at lunch. However, it is likely that the dietary habits of the
subjects in this study were so diverse that no protocol would have satisfied all of their requirements.
Future studies should consider the breakfast question and also consider reducing the energy intake
taken during the day.

Secondly, hunger, appetite and the desire to eat are complex, multi-factorial concepts, and are
notoriously difficult to interpret and describe (238). For example, it is well known that people eat
when they are not hungry and don’t always want to eat when they are hungry. In investigations 1
and 3, it was noted that some individuals had contradictory responses to some of the appetite
questions. For example, where someone had indicated that they were very hungry, could eat a lot
and had a great desire to eat, they had also put that they were very full. Similarly, some people had
scores which indicated that it would be extremely pleasant to eat and they could eat a lot, but the
hunger score indicated that they were not at all hungry. These observations indicate the difficulty in
interpreting feelings of hunger.

Potentially, these responses could also represent errors on the part of the volunteer. In this
investigation, the subjects were asked to complete 13 sets of 10 VAS questions, over 440 minutes.
The majority of validation studies on VAS have been of a duration up to 4.5 hours (238,239),
compared to 7.5 hours in this study. Questionnaire fatigue is therefore a possible explanation for the
contradictory findings. However, there were there was as many contradictory responses in the
morning as there were in the afternoon making this unlikely.

Nevertheless, the overall time course data for each of the appetite questions matched the pattern
expected, ie higher hunger and lower fullness scores before meals, a suppression of hunger and
increased fullness straight after meals, with a gradual return to the fasting profile. Therefore it
appears that overall, the questions were well understood.
Finally, even modest and short-term reductions in food intake can cause an increase in subjective appetite scores (156). Therefore, while there were no significant reductions in appetite scores, nor any increase in fullness scores, the lack of changes may actually be interpreted as an amelioration of weight-loss associated increase in appetite.

### 3.9.2.2 FREE-LIVING APPETITE SENSATIONS

In this investigation, participants also filled in subjective appetite assessments for the 7 days preceding their study visit. There was no significant effect on appetite found. As above, appetite is an extremely complex sensation, and the effect of a dietary compound such as inulin may be extremely subtle. Some authors have argued that while assessment of appetite in free-living conditions has several advantages, such as greater external validity, it also has substantial limitations, including poor compliance and reduced sensitivity (254). Since appetite can be affected by multiple confounders, a well-designed environment in which these confounders can be tightly controlled is the most suitable for disentangling the effects of a food component on appetite (254). While the at-home side-effects questions did elicit a significant result; this is likely due to sensations such as bloating having far fewer contributors.

### 3.9.3 APPETITE HORMONES: GLP-1 AND PYY

The effect of FCHO on appetite is proposed to act via signals produced by bacterial fermentation, specifically via an increase in SCFA acting via the FFA2/3 receptor is associated with an increase in GLP-1 and PYY. In this investigation, despite a significant reduction in food intake, and significant weight loss following inulin supplementation, there were no differences in either PYY or GLP-1.
Weight loss is known to lead to reductions in appetite hormones. Even modest weight loss of 1.5kg (similar in magnitude to the weight loss in this investigation) significantly decreases 24-hr PYY concentrations (255). In this context, the results from investigation could be interpreted as an amelioration of weight-induced reductions in PYY and GLP-1.

The lack of change in GLP-1 and PYY secretion may also be partly due to the composition of the meal provided. Both GLP-1 and PYY are known to be released in proportion to calories consumed (131,256). In this investigation, the mean energy content of the breakfast was 293kcal. While lunch provided a greater number of calories, FCHO is known to act via a second meal effect. Since the supplement was not provided on the morning of the study day in investigation 1, the lunch was the “third” meal post supplementation, and therefore fermentation – and its products - may have declined from their peak.

In addition, while PYY has been shown to increase in healthy subjects following FCHO supplementation, this is the first study to examine this question in prediabetic subjects. First degree relatives of subjects with type 2 diabetes had reduced post-prandial secretion of PYY(135). The subjects examined had normal glycaemia and plasma insulin levels, leading the authors to suggest that the blunted PYY may be a primary defect. It may therefore be the case that the small but significant effect of FCHO is not able to overcome the reduced PYY secretion in prediabetic subjects.

3.9.4 GLUCOSE AND INSULIN

Inulin had no effect on glucose but did significantly decrease fasting insulin and HOMA-IR. However, since the inulin subjects also consumed significantly less energy, carbohydrate and fat compared to control, and also lost significantly more weight, it is not clear what contribution these factors made to the improvements in insulin and HOMA-IR. These will be examined more closely in the next chapter.
3.9.5 COMPLIANCE AND SIDE-EFFECTS

In order to assess compliance in this study, a breath hydrogen measure was taken on each study day, and subjects were asked to return any unused sachets. Unfortunately, less than half of subjects did this. Of these, 8 had said that there were some packets that they threw away while they were at work or out during the day. Therefore, in this investigation, assessment of the degree of compliance cannot be done with any confidence based on the sachets being returned. However, since the supplement was not given on the morning of the study day, the measure of breath hydrogen taken at 420 minutes during the study day provides some confidence that subjects were taking the supplementation.

Inulin, like all dietary fibres can cause side-effects such as flatulence, bloating and diarrhoea when added into the diet. Therefore, in this study, the dose was increased by 10g every 2 weeks to a maximum dose of 30g per day. Nevertheless, despite this dose-escalation method, one person dropped out due to side-effects from the inulin. In addition, the side-effect scores completed at home demonstrate significant increases in bloating and flatulence during inulin supplementation. It may therefore be the case that tolerance to high-levels of fermentable carbohydrates may take longer than 2 weeks. Parnell and Reimer observed that complaints of side-effects peaked at the 4-week mark and declined during the subsequent weeks of their 12-week trial(173).

3.10 CONCLUSION

In conclusion, this investigation supports previous findings that inulin supplementation decreases food intake and body weight. Inulin also appears to decrease insulin resistance. The effects of inulin on glucose homeostasis will be explored in the next chapter.
CHAPTER FOUR:
THE EFFECT OF FERMENTABLE CARBOHYDRATE ON GLUCOSE HOMEOSTASIS IN SUBJECTS WITH PREDIABETES
4.0 INVESTIGATION 2

This investigation was a double-blind crossover study aimed to examine the effect of 6 weeks’ supplementation with inulin on measures of glucose homeostasis using a Meal Tolerance Test and Continuous Glucose Monitoring.

4.1 RECRUITMENT DATA

In total 17 subjects were recruited to the study all with IGT with or without IFG/IGT. The study had originally intended to recruit only 15, but due to failures with the Continuous Glucose Monitor, it was decided to recruit an addition 2 people to complete only the Glycaemic Variability part of the study. Of the 17 subjects, two dropped out due to side effects from the cellulose. One of these subjects had already completed the inulin arm. One person dropped out due to ill health and one dropped out following the inulin arm due to personal reasons. Therefore 10 people completed both arms of the study, while an additional 2 people completed either the inulin or cellulose arms only.

Participant characteristics are shown in table 4.1. There was a significant difference in weight (P = 0.022) but not BMI (P = 0.307) between the group starting inulin first and the group starting cellulose first.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inulin</th>
<th>Cellulose</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M:F)</td>
<td>3:5</td>
<td>5:4</td>
<td>0.488</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58.43 ± 15.83</td>
<td>61.13 ± 10.43</td>
<td>0.546</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.13 ± 9.47</td>
<td>92.09 ± 11.74</td>
<td>0.022</td>
</tr>
<tr>
<td>BMI</td>
<td>29.42 ± 1.34</td>
<td>31.45 ± 4.08</td>
<td>0.307</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.3 ± 0.32</td>
<td>5.65 ± 0.72</td>
<td>0.175</td>
</tr>
<tr>
<td>2hPG (mmol/L)</td>
<td>9.17 ± 0.97</td>
<td>8.71 ± 0.89</td>
<td>0.425</td>
</tr>
</tbody>
</table>

Table 4.1: Baseline demographic and biochemical data for the groups randomised to receive inulin or cellulose first. Results shown are mean ± SD.
4.2 GLUCOSE

4.2.1 GLUCOSE CONCENTRATIONS FROM MEAL TOLERANCE TEST

The time-course data for glucose are shown in figure 4.1.

Figure 4.1: Time course data for venous plasma glucose pre and post inulin (a) and cellulose (b) supplementation. (Inulin, N = 12), Cellulose (N = 11). Values are means and vertical bars are SEM. The meal tolerance test was served at 0 minutes.

There was a significant increase in postprandial plasma glucose AUC (ppAUC) (P=0.05) following inulin supplementation. The between group delta change was not different (P=0.281). Individual time-points were also analysed to determine the degree of difference. At 90 minutes, the mean increase in glucose concentration following inulin supplementation was 0.33 ± 0.72 mmol/L (Paired t-test, P = 0.5349). At 120 minutes, the mean increase was -0.295 ± 0.56 mmol/L (Paired t-test, P = 0.2614).

Figure 4.1c Pre and post values inulin and cellulose for ppAUC plasma glucose and 4.1d) delta change of glucose ppAUC between inulin (N=12) and cellulose (N=11) groups. There was a significant increase in the post-prandial plasma glucose AUC following inulin supplementation (P=0.0477). The delta change in ppAUC was not significant between groups.
There were no differences in fasting glucose either within (Pre: 5.53 ± 0.20mmol/L, Post: 5.63 ± 0.18, 
P = 0.278) or between groups (0.11 ± 0.09 ± 0.16 ± 0.12, P = 0.734).

Figure 4.1e: Pre and post inulin and cellulose values for fasting plasma glucose and 4.1f) delta change of fasting plasma glucose between inulin (N = 12) and cellulose (N = 11) groups. There were no differences within or between groups. Values are means and vertical bars are SEM.

4.2.2 CONTINUOUS GLUCOSE MONITORING

Unfortunately due to problems with the Medtronic iPro Continuous Glucose Monitoring system, only 7 complete sets of data were collected, with an addition 2 subjects having complete data for the inulin arm only.

<table>
<thead>
<tr>
<th>GV Measure</th>
<th>Inulin</th>
<th>Cellulose</th>
<th>P Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Mean (mmol/L)</td>
<td>5.70 ± 0.19</td>
<td>5.84 ± 0.33</td>
<td>5.58 ± 0.25</td>
<td>5.40 ± 0.12</td>
</tr>
<tr>
<td>SD</td>
<td>0.94 ± 0.13</td>
<td>0.93 ± 0.24</td>
<td>0.85 ± 0.15</td>
<td>0.73 ± 0.10</td>
</tr>
<tr>
<td>MAGE</td>
<td>2.33 ± 0.75</td>
<td>2.52 ± 0.73</td>
<td>2.63 ± 0.91</td>
<td>2.46 ± 0.48</td>
</tr>
<tr>
<td>CONGA</td>
<td>5.00 ± 0.21</td>
<td>5.28 ± 0.32</td>
<td>5.03 ± 0.23</td>
<td>4.80 ± 0.09</td>
</tr>
<tr>
<td>HBGI</td>
<td>0.70 ± 0.33</td>
<td>1.25 ± 0.63</td>
<td>0.37 ± 0.17</td>
<td>0.21 ± 0.11</td>
</tr>
<tr>
<td>LBGI</td>
<td>1.93 ± 0.43</td>
<td>2.09 ± 0.73</td>
<td>2.53 ± 0.68</td>
<td>2.07 ± 0.37</td>
</tr>
</tbody>
</table>

Table 4.2: Differences in measures of glycaemic variability (GV). There were no differences in any of the methods used. SD= standard deviation; MAGE: mean amplitude of glucose excursions; CONGA: continuous overlapping net glycaemic action; HBGI: High Blood Glucose Index; LBGI: Low Blood Glucose Index.

There were no differences in mean glucose concentration (Pre: 5.70 ± 0.19 mmol/L, Post: 5.84 ± 
0.33, P = 0.6708), SD (Pre: 0.94 ± 0.13, Post: 0.93 ± 0.24, P = 0.9583), CONGA (Pre: 5.00 ± 0.21, Post:
5.28 ± 0.32, P = 0.4621), LBGI (Pre: 1.93 ± 0.43, Post: 2.09 ± 0.73, P = 0.8149), HBGI (Pre: 0.70 ± 0.33, Post: 1.25 ± 0.63, P = 0.2602), and MAGE (Pre: 2.33 ± 0.75, Post: 2.52 ± 0.73, P = 0.8228) following inulin supplementation (Table 4.2). There were no significant differences between groups (Table 4.2).

### 4.3 INSULIN

The time-course data for insulin are shown in figure 4.4a and b.

**Figure 4.4** Time course data for venous plasma insulin pre and post inulin (a) and cellulose (b) supplementation. (Inulin, N = 12), Cellulose (N = 11). Values are means and vertical bars are SEM.

There were no significant differences for ppAUC either within or between groups (Figures 4.4c and d).

**Figure 4.4c:** Values for ppAUC plasma insulin pre and post inulin and cellulose supplementation; 4.4d: delta change of insulin ppAUC between inulin (N=12) and cellulose (N=11) groups. There were no differences in ppAUC insulin either within or between groups. Values are means and vertical bars are SEM.

Fasting insulin was calculated for each subject using a mean of 3 values. Where subjects had only 2 samples due to haemolysis or lipaemia of plasma, a mean of the two samples was taken. Inulin had
no effect on fasting insulin (Pre: 32.35 ± 5.74μU/ml, Post: 33.30 ± 5.341 μU/ml (P = 0.7069), and the delta changes between groups were not different (0.95 ± 2.47 vs -1.89 ± 3.19, P = 0.489) (Figure 4.4e and 4.4f).

There were no significant differences in fasting insulin at baseline prior to randomisation (Data not shown. P= 0.9276).

4.4 MEASURES OF INULIN SENSITIVITY

4.4.1 HOMA-IR

There were no differences in HOMA-IR following inulin supplementation either (Pre: 4.02 ± 0.67
Post: 4.16 ± 0.62, N=12, P = 0.6958) within or between group (0.14 ± 0.30 vs -0.23 ± 0.39, N=12, P = 0.7028) (Figure 4.5).
4.5 MEASURES OF INSULIN SECRETION

4.5.1 HOMA-B%

There were no differences in HOMA-B% either within (Pre: 198.8 ± 27.53, Post: 216.1 ± 33.67, N = 11, P 0.6953) or between groups (17.28 ± 32.51 vs -39.23 ± 18.47, N=13, P = 0.1303) (Figure 4.6).

Figure 4.6a: Pre and post values inulin and cellulose for HOMA B%; 4.6b: Delta change of HOMA B% (B) between inulin (N=12) and cellulose (N=11) groups. There were no differences either within or between groups. Values are means and vertical bars are SEM.

4.5.2 INSULINOGENIC INDEX

There were no differences in the Insulinogenic Index following inulin supplementation (Pre: 34.07 ± 6.84; Post: 35.80 ± 7.38, N=12, P = 0.4776) or when compared to control (1.73 ± 2.35 vs 6.35 ± 9.06 N=12, P = 0.21) (Figure 4.7).

Figure 4.7a: Pre and post values inulin and cellulose for Insulinogenic Index; 4.7b: Delta change of Insulinogenic Index between inulin (N=12) and cellulose (N=11) groups. There were no differences in insulinogenic indices either within or between groups. Values are means and vertical bars are SEM.
4.5.3 DISPOSITION INDEX

There were no differences in the Disposition Index following inulin supplementation (Pre: 183.0 ± 37.94, Post: 173.9 ± 39.61, N = 21, P = 0.7408) or when compared to control (-9.13 ± 26.92 vs -38.70 ± 92.79, P = 0.7537) (Figure 4.8).

Figure 4.8a: Pre and post values inulin and cellulose for Disposition Index 4.8b: Delta change of Disposition Index between inulin (N=12) and cellulose (N=11) groups. There were no differences in disposition indices either within or between groups. Values are means and vertical bars are SEM.

4.7 GLP-1

There were no differences in tAUC for plasma GLP-1 following inulin supplementation (within group difference: P = 0.5294, between group difference: P = 0.4223)(Figure 4.9c).

Figure 4.9: Time course data for plasma total GLP-1 concentrations for inulin (a) and cellulose (b) supplementation. The MTT was served at 0 mins. Values are means and vertical bars are SEM.
However, GLP-1 secretion was also significantly increased in the early post-prandial period (0-60 min AUC) (P = 0.0018) (Figure 4.9d). The difference in 0-60 AUC was not significant between the groups (P = 0.7373 respectively) but due to missing data there were only 4 paired data sets in the control group for the 0-60 minute period.

4.7 Free Fatty Acids

Inulin supplementation did not affect FFA concentrations when compared to baseline, but the delta change in tAUC was significantly different when compared to the control (P = 0.0465). The fasting FFA concentration was not different when compared to baseline (Pre: 0.60 ± 0.07mmol/L, 0.60 ± 0.075 N=12, P = 0.9833) nor was the delta change different when compared to the control (0.001 ± 0.06mmol/L N=12 vs 0.006mmol/L ± 0.06, N = 12) (Figure 4.10).
4.8 WEIGHT

Subjects lost weight when taking the inulin supplement, but there were no significant differences between the groups (Pre: 83.05 ± 3.41kg, Post: 82.78 ± 3.36kg, P = 0.057) (Figure 4.11a). The between group difference was not significant (-0.26 ± 0.12kg vs -0.03 ± 0.10kg, P = 0.175) (Figure 4.11b). Weight loss was not consistent within the group, with 3 people gaining weight (from 0.1-0.3kg), one person remaining the same weight and the remainder losing weight (0.1-0.9kg).

Figures 4.11a: Weight pre and post inulin and cellulose supplementation. There was a borderline significant within-group difference following inulin supplementation (Pre: 83.05 ± 3.41kg, Post: 82.78 ± 3.36kg, P = 0.057) 4.11b: Delta change in body weight following inulin and cellulose supplementation. The between group difference was not significant. Values are means and vertical bars are SEM.
4.9 BREATH HYDROGEN

Breath hydrogen significantly increased after both inulin (Pre: 3.47 ± 0.95ppm, Post: 20.79 ± 3.65ppm, N = 13, P = 0.0003). There was also a non-significant rise following cellulose supplementation (3.67 ± 0.63ppm vs 5.90 ± 1.40ppm, N=12, P = 0.1468). The delta change between groups was significant (17.36 ± 3.57ppm, N=14 vs 2.39 ± 0.96ppm, N=13, P = 0.0006)(Figure 4.12).

4.10 DISCUSSION

In this investigation I have demonstrated:

- Significant increase in \( (0-30\text{min}) \) AUC insulin
- Significant increase in \( (0-60\text{min}) \) AUC GLP-1
- Significant post-prandial glucose concentrations

4.10.1 INSULIN SENSITIVITY

Based on results from previous studies which have found that either acute or chronic consumption of FCHO improves insulin sensitivity, it was expected in these investigations that insulin sensitivity
would improve in all prediabetic subjects. However, there was no effect of inulin on fasting insulin concentrations or on insulin resistance in this investigation. This is in contrast to the findings in investigation 1, where fasting insulin and HOMA IR improved.

Insulin resistance is a highly complex process and has been proposed to involved ectopic fat deposition, alterations in lipid intermediates; alterations in adipokines including a reduction in adiponectin and increases in the so-called inflammatory compounds TNF-α, IL-6 and IL-10(257). However, why a chronic fuel surfeit leads to hepatic insulin resistance in some subjects (ie, those with i-IFG) and to peripheral insulin resistance in other subjects (ie, those with IGT) is unknown.

Chronically elevated free fatty acids (FFAs) reliably induce insulin resistance, and interventions which reduce circulating FFAs result in improved sensitivity to the actions of insulin(86,87,91). Potentially, reductions of FFA of differing magnitude may affect insulin resistance in difference tissues. Alberti et al have demonstrated that modest reductions in FFA improve hepatic but not peripheral insulin sensitivity measured by hyperinsulinaemic-euglycaemic clamp(258). Furthermore, hepatic glucose production is highly synchronized with FFA levels in the circadian cycle (Clore et al., 1989) and T2DM subjects have elevated plasma FFA levels that are correlated with increased overnight hepatic glucose output (Miles et al., 2003; Taskinen et al., 1989) indicating that FFAs may be more closely related to hepatic as opposed to peripheral carbohydrate metabolism. Other in vivo experiments have supported this hypothesis. While whole-body glucose oxidation increases following experimental reductions in FFA concentrations, pyruvate dehydrogenase, the regulatory enzyme for glucose oxidation does not increase in skeletal muscle tissue, suggesting the increase in glucose oxidation occurs in tissue other than muscle tissue (259). Similarly, phosphofructokinase, the rate-limiting enzyme of glycolysis does not increased in skeletal muscle following experimental reduction of FFA concentrations(259). The subjects in investigation 2 had IGT with or without elevating fasting glucose, whereas 7 subjects in investigation 1 had IFG. Subjects with i-IFG have marked hepatic but
normal peripheral resistance, while subjects with IGT have peripheral insulin resistance (55,59,60,62,63). This may partly explain the discrepancy in the results on insulin resistance.

Other researchers who found a reduction in hepatic glucose output following FCHO-consumption have also recorded a reduction in FFA concentrations. Thorburn et al studied the effect of an evening barley based-meal on measures of glucose homeostasis the following morning. The AUC for FFA was reduced by 22%, and hepatic glucose production was decreased by 30% following a glucose tolerance test the following morning (260). Lou et al gave 20g day of short-chain-fructooligosaccharides to 12 healthy volunteers and found a significant reduction in hepatic glucose output, and a trend towards reduction of FFA (261). Both these studies used labelled glucose techniques and studied a healthy population. Finally, another study using the same isotope technique and study design as Thorburn et al’s found that acute consumption of a barley evening meal had no effect on FFA or endogenous glucose production, but did significantly increase peripheral glucose uptake following the next day’s breakfast in healthy volunteers. (262). Therefore FFA may play a role in reducing hepatic glucose output.

However, there were no differences in FFA suppression in this investigation between the group in which insulin resistance improved and the group in which insulin resistance did not improve, nor does this hypothesis explain why other researchers found significant improvements in peripheral insulin-stimulated glucose uptake alongside reductions in FFA following FCHO supplementation. Umbleby et al used a sophisticated protocol including a two-step hyperinsulinaemic-euglycaemic clamp and a MTT with arterio-venous sampling to demonstrate a significant insulin-stimulated uptake of glucose into the forearm and decreased release of FFA from adipose tissue (263).

Potentially there may be differences in the pathophysiology of peripheral insulin resistance between subjects with the metabolic syndrome and subjects with i-IGT and IFG/IGT (ie, those in this investigation). For example subjects with i-IGT have much higher concentrations of FFAs compared to subjects with metabolic syndrome and i-IFG(264-266). FCHO’s apparent FFA-reducing properties
may not be sufficient to reduce the much elevated FFA levels in these subjects, and therefore has no effects on peripheral insulin resistance in subjects with i-IGT. This is supported by the fact that short-chain fructooligosaccharides had no effect on peripheral insulin sensitivity in subjects whose fasting FFA s were 2-fold higher (267) than in Robertson’s study (247). Furthermore, while Lou et al had carried out a study in healthy subjects demonstrating an effect on FFA and hepatic glucose output as described above, they did not reproduce these effects in an identical protocol in subjects with type 2 diabetes (268). FFA levels were higher in the diabetic subjects than the healthy subjects and did not reduce following sc-FOS supplementation (269). Finally, in the study by Alberti et al which found that modest reductions in FFA improved hepatic but not insulin-sensitivity, the authors noted that in the most insulin sensitive subject, they did see an increase in insulin-dependent glucose uptake in the forearm muscle, suggesting background differences in tissue-specific insulin sensitivity can modify the effects of interventions (270).

However, there is strong evidence that other factors also play a role in hepatic glucose metabolism, including direct effects of SCFA and GLP-1.

In Lou et al’s study which found a reduction in FFA and HGP, they also observed a significant reduction in the acetate:propionate ratio. Propionate has previously been shown to reduce fasting glucose levels in humans subjects when delivered orally (271,272). If infused rectally in humans, it does not have the same effect (273), suggesting that delivery of propionate to the liver (via the portal vein) is necessary for its glucose-lowering effects. Work in rat hepatocytes has demonstrated that administration of propionate or acetate significantly reduces gluconeogenesis while increasing glycolysis (274). It is therefore possible that the fermentation of fibres which tend to produce relatively more propionate to butyrate may affect hepatic glucose homeostasis. Interestingly, in the studies by Priebe in humans, butyrate but not propionate was significantly increased. However, in Robertson’s study examining the effect of RS on subjects with metabolic syndrome, they found increases in glucose uptake into the periphery, alongside significant increases in acetate and
propionate AUC following 4 weeks’ treatment (263). Therefore, it is unlikely that differences in the relative production of SCFA explain the different effects of inulin supplementation between investigation 1 and 2. Nevertheless, the question of whether fermentation of different carbohydrates produce different SCFA profiles is an area in need of further exploration.

Furthermore, more reliable measures of in vivo SCFA dynamics would also help to illuminate the dynamic effects of SCFA on tissue-specific metabolism.

In conclusion, inulin had no effect on insulin resistance in this investigation. The differences may potentially be due to baseline metabolic differences between the subjects, but this question needs to be examined further.

4.10.2 INSULIN SECRETION

In this investigation, inulin significantly increased early phase insulin release. Bodinham et al have previously found an increase in first-phase insulin secretion using IVGTTs in healthy subjects (225) while Juntunen found an increase in early insulin secretion following 8 weeks’ consumption of rye bread in healthy post-menopausal women (226). However, since the loss of first phase insulin secretion is considered a fundamental defect in the development of diabetes, it is promising that this improvement also occurs in subjects with prediabetes.

The increase in GLP-1 following inulin supplementation suggests that a more pronounced incretin response is likely to be partly responsible for this improved insulin secretion. Critically, GLP-1 only promotes insulin secretion in the presence of glucose (ie, when it is required), therefore sparing the β-cell from over-secretion. Other researchers have also identified other potential mechanisms by which FCHO may improve insulin secretion. Fat deposition in the pancreas is associated with impaired insulin secretion (275), while interventions which reduce pancreatic fat improve insulin secretion (276).
The SCFA are known to act as signalling molecules, and the recent identification of FFA2 receptors on β-cells raises the possibility that the SCFA may also directly increase first-phase insulin secretion (277). However, this has yet to be conclusively demonstrated in vivo.

Since the rise in GLP-1 occurs between 0-60 minutes, the rise in insulin secretion it cannot be due to an acute effect of the inulin. In this investigation, the inulin supplement was given at 0 minutes on the study day. Inulin usually reaches the large bowel 2-4 hours (278,279) following consumption, and peak fermentation occurs about 3-4 hours (data from the Nutrition and Dietetic Research Group). FCHO has been shown to promote L cell differentiation in the proximal colon in mice (280) and increase preproglucagon mRNA in the colon (281). Therefore, the rise in GLP-1 seen on the study morning likely reflects the nutrients entering the colon and the increased capacity of the L cell to secrete GLP-1.

The loss of early-phase insulin secretion is considered a critical defect in the development of diabetes. This early burst of insulin is essential in suppressing glucagon and EGP and promoting the uptake of glucose into tissues. This early phase of insulin secretion not only limits the rise in blood glucose concentration, experimental data suggest that the larger the insulin excursion, the longer the effect on glucose homeostasis (282). In a metabolically healthy subject, insulin is low during fasting and peaks following meals, returning quickly to fasting levels once euglycaemia is restored. The usual pattern is a rapid increase, then a decrease back to baseline (283). This ensures hyperglycaemia is avoided, but that the capacity of the beta cell is preserved (284). It is therefore interesting that in this study, inulin appeared to reduce fasting insulin, indicating improvement in insulin sensitivity, but also significantly increased the early phase of insulin secretion, suggesting a return to the normal insulin secretory pattern (283). Potentially, this may spare the beta-cell long-term, reducing the risk of diabetes developing (284).
4.10.3 GLUCOSE CONCENTRATIONS

A surprising finding in this body of work is that inulin, despite measurable effects on insulin sensitivity, does not reduce the concentration of glucose in the circulation. In investigation 2 there was a small but significant increase in post-prandial blood glucose. However, data from the continuous glucose monitors (CGM) demonstrate no change between the groups either during the day or at night. As discussed in the introduction, results from other researchers regarding the effects of FCHO on glucose homeostasis are conflicting.

The degree of insulin resistance and existing glucose dysfunction may partly explain the discrepancy in results. In this study, $\int_{0-120} T{AUC}$ for insulin was nearly twice that found in Cani et al’s study which reported a significant reduction in postprandial glucose (216). Therefore, it could tentatively be suggested that in prediabetic subjects, improved insulin sensitivity allows a reduction in insulin secretion without deterioration in plasma glucose control, which could potentially be an insulin sparing effect. Supporting this view, Luo et al (261) found that 20 days’ of 15g FOS significantly reduced basal hepatic insulin production, but that the plasma glucose concentration was not affected. Although the subjects used in that study were young (mean age: 24 years, BMI 21kg/m$^2$) their fasting plasma glucose was higher than would be expected (5.4mmol). Potentially, they may also have been hypersecreting insulin and thus a similar sparing effect could be proposed.

Different methodology may also explain differences between the studies. For example, there is a significant uptake of glucose into peripheral tissue following 4 weeks’ 30g/day resistant starch (RS) treatment using a euglycaemic-hyperinsulinaemic clamp (263), but 4 weeks treatment of 40g/day RS had no effect on glucose uptake during an IVGTT (225). Both these studies were carried out in a similar study population. IVGTTs are a better method at measuring changes in insulin secretion, but have less utility at measuring insulin sensitivity (285). Additionally hepatic glucose uptake is greater with oral compared with intravenous glucose (57), meaning that studies using IVGTTs are less likely to identify changes in hepatic insulin sensitivity. In this investigation, MTTs were used. MTTs can be
considered the most physiological of methods, and therefore in a real-life setting, 30g inulin does not reduce glucose concentrations in subjects with prediabetes.

The type, dose and duration of the FCHO given in previous studies varies immensely, with some doses given acutely (the night before), others range from 2 weeks to 12 weeks. Robertson et al suggest that FCHO improves different aspects of glucose homeostasis in a temporal fashion, potentially with improvements in insulin secretion preceding changes in insulin sensitivity (225). Alberti et al also support this view by suggesting in subjects with elevated FFA, normalisation of FFA metabolism precedes changes in glucose uptake (270). Finally, some authors have suggested that the length of the saccharide chain may influence the location of fermentation (286), with longer-chain inulin being fermented in the distal colon, and short-chain fructooligosaccharides and RS in the proximal colon (287). However, the extent to which chain length affects metabolic parameters has not been established.

It had been intended that the 24 hour glucose profiles measured by using CGM would provide valuable information regarding free-living fasting and post-prandial glucose levels. However, there was no measurable effect of inulin supplementation on any measure of glucose concentration or glucose variability in this investigation. This may suggest that the effects seen on post-prandial glucose following the MTT were a type I error.

### 4.10.4 LIMITATIONS

Since subjects taking inulin lost weight, it is not possible to be 100% confident that the beneficial effects seen in this study on GLP-1 and insulin secretion were due solely to the effects of inulin. However, gut hormones have been shown to reduce following weight loss (152,154,160), as discussed in the introduction, while insulin secretion following weight loss is thought to be due to
improvements in insulin sensitivity (221, 224, 225, 263, 288, 289) making it unlikely that the modest weight reduction in this study was mediating the effects.

Unfortunately during the study, there were numerous problems with the Medtronic CGM system and only 7 complete sets of data were collected. It was intended that 5 days’ of data would be collected for each arm of the study, but again due to problems with the data collection, 16 of the arms had only 3 days’ data each. The CGM sensor is placed within the interstitial fluid and records the glucose concentration every 5 minutes. Unfortunately on many occasions the sensor had evidently been displaced during the recording of data, and data was missing for several hours. Since this displacement predominantly occurred during the night (when it can be reasonably suggested that it would be less likely to miss rapid changes in glucose excursions) for a number of subjects it also become displaced during the day. Therefore there is a possibility that the monitors did not pick up all post-prandial excursions and in that regard the post-prandial rise in glucose seen in MTT is a real effect.

The menstrual cycle is also known to affect glucose homeostasis (290, 291) with a decrease in insulin sensitivity in the luteal phase. Unfortunately in this investigation the menstrual cycle was not accounted for. In order for the subjects to build up tolerance to the maximum dose of inulin, it was necessary to have a 4 week run-in. Therefore in order to attempt to control for the menstrual cycle it would have been necessary to extend each crossover arm to 8 weeks. In order to reduce the burden on participants it was decided to have a 6 week long arm. There were 4 premenopausal women in this investigation. If this data is removed however there is no difference in the results (data not shown).

4.11 CONCLUSION

In conclusion, inulin significantly increased early phase insulin secretion in response to a MTT, and but has no effect on insulin sensitivity.
4.12 COMBINING RESULTS FROM INVESTIGATIONS 1 AND 2

All subjects in investigation 1 and 2 took the inulin and cellulose supplements for 6 weeks each, and all subjects attended the clinical research unit to be weighed and to have two fasting blood samples taken. Given the contrasting results in insulin sensitivity in investigations 1 and 2, and the known physiological differences between i-IFG and i-IGT (51,55,56,292), it was decided to compare the effect of inulin on glucose and insulin between these two prediabetic subtypes.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>i-IFG (N = 8)</th>
<th>i-IGT (N = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M:F)</td>
<td>7:1</td>
<td>5:7</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>63.8 ± 2.49</td>
<td>62.25 ± 3.59</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.51 ± 5.38</td>
<td>76.92 ± 4.51</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.11 ± 0.99</td>
<td>27.99 ± 0.80</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.96 ± 0.09</td>
<td>5.25 ± 0.06</td>
</tr>
<tr>
<td>2hPG (mmol/L)</td>
<td>5.04 ± 0.52</td>
<td>8.78 ± 0.22</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>41.25 ± 1.41</td>
<td>38.75 ± 0.94</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>22.0 ± 6.06</td>
<td>26.90 ± 5.27</td>
</tr>
</tbody>
</table>

Table 4.3: Subject characteristics for the prediabetic subtypes: i-IFG and i-IGT. All subjects combined from investigations 1 and 2.

4.12.1 FASTING INSULIN BY PREDIABETIC SUBTYPE

There was a significant reduction in fasting insulin in the subjects with i-IFG (Pre: 22.00 ± 6.06 μU/ml, Post: 16.67 ± 4.01μU/ml, N = 7, P = 0.0497. However, the delta change in fasting insulin in subjects with i-IFG was not significant between the groups (-5.33 ± 2.17μU/ml, N = 7 vs -0.64 ± 3.49μU/ml, N=6, P = 0.2642). Inulin had no effect on fasting insulin in subjects with i-IGT (Pre: 26.90 ± 5.27μU/ml, Post: 28.18 ± 5.74μU/ml N=11, P = 0.5859), and no effect between groups (1.28 ± 2.27 μU/ml vs -3.98 ± 4.39 μU/ml, N=8, P = 0.2652).
There was a significant reduction in fasting insulin in subjects with i-IFG (Pre: 22.00 ± 6.06, Post: 16.67 ± 4.01, N = 7, P = 0.0497). However the delta change between in the inulin and cellulose groups was not different. There were no differences in fasting insulin for any of the other prediabetic subtypes. Values are means and vertical bars are SEM.

4.12.2 FASTING GLUCOSE BY PREDIABETIC SUBTYPE

There was no effect of inulin supplementation on fasting plasma glucose either in subjects with i-IFG (Pre: 6.33 ± 0.20 mmol/L, Post: 6.17 ± 0.15 mmol/L, N=6, P = 0.4107) or subjects with i-IGT (Pre: 5.52 ± 0.19 mmol/L, Post: 5.56 ± 0.14 mmol/L, N=12, P = 0.936). There were no differences between the groups for i-IFG (-0.15 ± 0.17 mmol/L vs 0.04 ± 0.20 mmol/L, N=7, P = 0.4767) or i-IGT (0.01 ± 0.12 mmol/L vs 0.22± 0.13 mmol/L, N=9, P = 0.2671).

There were no differences in fasting inulin for any of the other prediabetic subtypes. Values are means and vertical bars are SEM.

Figure 4.13: Fasting insulin pre and post inulin and cellulose supplementation for subjects with a) i-IFG, and b) i-IGT.

Figure 4.14: Fasting glucose pre and post inulin and cellulose supplementation for subjects with a) i-IFG and b) i-IGT. There were no differences in fasting inulin for any of the other prediabetic subtypes. Values are means and vertical bars are SEM.

Figure 4.15: Delta change in fasting glucose pre and post inulin and cellulose supplementation for subjects with a) i-IFG and b) i-IGT. There were no significant differences in fasting plasma glucose either within or between prediabetic subtypes.
4.12.3 HOMA-IR BY PREDIABETIC SUBTYPE

There was a non-significant reduction in HOMA-IR in subjects with i-IFG, Pre: 6.33 ± 1.95, Post: 4.73 ± 1.16, N=7, P = 0.1205) but the within-group difference was not different (-1.60 ± 0.89 vs -0.15 ± 1.12, N = 6, P = 0.3260). There was no change in HOMA-IR following the inulin supplementation in subjects with i-IGT (Pre: 3.88 ± 0.53, Post: 3.80 ± 0.62, N=11, P = 0.8304) or between groups (-0.08 ± 0.39 vs -0.35 ± 0.54, P = 0.6875).

Figure 4.16: Delta change in HOMA-IR pre and post inulin and cellulose supplementation for subjects with a) i-IFG, b) i- and IGT. There were no significant differences in fasting plasma glucose either within or between prediabetic subtypes. Values are means and vertical bars are SEM.

4.12.4 WEIGHT BY PREDIABETIC SUBTYPE

There was no difference in weights between groups i-IFG vs i-IGT (-0.25 ± 0.43kg, N = 6 vs -0.28 ± 0.13kg, N=9, P = 0.9427) or i-IGT vs IFG/IGT (Figure 4.17).

Figure 4.17: Delta change in body weight (kg) pre and post inulin and cellulose supplementation for subjects with i-IFG (N=6) and i-IGT (N=9). There were no significant difference in body weight between prediabetic subtypes. Values are means and vertical bars are SEM.
4.13 DISCUSSION OF RESULTS FROM SUB-ANALYSIS ON PREDIABETIC SUBTYPES.

Insulin sensitivity only improved in subjects with i-IFG but not with i-IGT, despite no differences in weight loss between the i-IFG and i-IGT groups. Since subjects with i-IGT have peripheral insulin resistance and subjects with i-IFG have hepatic insulin resistance (55-57), these results indicate that inulin may be effective at specifically improving hepatic, ie fasting, but not peripheral insulin resistance. The reduction in HOMA-IR but not Matsuda Index indicates that the effect is on fasting inulin sensitivity only. Given the small sample size of each of these subtypes, further studies with larger sample sizes should be done to clarify the results, with an a priori hypothesis to determine whether there are differences between groups.

A number of mechanistic pathways may be behind this possible metabolic improvement. Further studies should be carried out to confirm this effect and to delineate the mechanism(s) involved.

While the pancreatic effects of GLP-1 are well understood, there are less well known extrapancreatic effects of GLP-1 in regulating hepatic insulin sensitivity. In an interventional study in mice, Cani et al demonstrated that the FCHO-mediated suppression of endogenous glucose production was dependent upon a functional hepatic GLP-1 receptor (293), indicating that extra-pancreatic effects of GLP-1 may be an important mediator of FCHO’s suppression of FPG. In rat hepatocytes, GLP-1 promotes glycogen accumulation via increases in glycogen synthase, and decreases in glycogen phosphorylase (294). The same authors also found a significant increase in labelled glucose into glycogen. Interestingly this process was glucose dependent, and increase with increasing concentrations of glucose (294). Potentially, these are further mechanistic possibilities explaining the effect of inulin on fasting glucose homeostasis. In contrast, GLP-1 has not been shown to influence peripheral insulin sensitivity independent of effects on weight loss which may explain why inulin appears to be effective at improving hepatic glucose homeostasis but not peripheral.
Fasting glucose is principally determined by the relative concentrations of insulin and glucagon in the fasting state (295). Work over the past 3 decades has established a critical role of insulin pulsatility in maintaining basal and post-load insulin sensitivity (296-299), and it has been shown that insulin and/or glucagon pulsatility is impaired in subjects with prediabetes and type 2 diabetes (300-302). Pulsatility is a physiologically important phenomenon, not least because pulsatile secretion of hormones prevents down-regulation of their receptors (296). In particular, a number of studies have demonstrated that the cyclic oscillations of insulin and glucagon prevent down-regulation of their hepatic receptors (298). Recently GLP-1 has been shown to restore pulsatility in subjects with type 2 diabetes and with IGT (303), an effect potentially due to restored synchrony of Ca^2+ oscillations in beta-cells (304). Therefore the apparent distinct effects of inulin on tissue-specific insulin sensitivity in this body of work may be due to the role of GLP-1.

Finally, there are a number of other mechanisms which could potentially help to explain the effect of inulin on hepatic glucose homeostasis. A number of studies have shown that FCHO feeding reduces circulating inflammatory factors such as IL-6, TNF-α and IL-6 (305,306), potentially via leakage of lipopolysaccharides from gram-negative bacteria through gaps in the intestinal villae. Inflammatory factors and cytokines have been shown to disrupt cell signalling pathways in the liver (307,308). It has also been shown that GLP-2 modulates hepatic insulin sensitivity via increases activity of POMC neurons in the hypothalamus in mice (309). Consumption of FCHO increases circulating GLP-2 in healthy volunteers (310) and also significantly increases expression of POMC neurons in mice.

Since subjects lost a significant amount of weight when taking inulin, it cannot be said that the effects seen in this study were independent of weight. However, since there were no differences in weight loss between the prediabetic subtypes, and insulin sensitivity only improved in the i-IFG group it is likely that the effects are not due to weight loss.

Finally, since subjects with i-IFG have impaired first phase but intact second phase secretion, and marked hepatic but normal peripheral insulin sensitivity, FCHO consumption – which increases early
insulin secretion, GLP-1 secretion and may reduce fasting insulin resistance - may represent a targeted intervention.

CHAPTER FIVE:

EFFECT OF FERMENTABLE CARBOHYDRATE ON WEIGHT MANAGEMENT IN SUBJECTS WITH PREDIABETES
5.1 INVESTIGATION 3

This investigation was a randomised control trial comprising a 9 week weight loss phase followed by 9 week weight maintenance phase. All subjects attended four dietary counselling sessions between baseline and week 9 to meet their weight loss target of 5%. During the weight maintenance phase (week 9-18) subjects were asked to maintain the weight that they had lost. Study days took place at baseline, week 9 and week 18.

5.1 RECRUITMENT DATA

17 subjects were recruited to each arm of the study. One person dropped out of the inulin arm due to not liking the inulin supplement. Three people dropped out of the cellulose arm, two due to not liking the cellulose supplement, and one person due to time constraints. The data for the 15 participants who completed the study is shown in table 5.1. Subjects in the cellulose arm weighed less than subjects taking inulin but the difference was not significant (P = 0.1735).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inulin (N = 15)</th>
<th>Cellulose (N = 15)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M:F)</td>
<td>8:7</td>
<td>7:8</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>57.1 ± 3.2</td>
<td>59.3 ± 2.4</td>
<td>0.1735</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>90.1 ± 5.2</td>
<td>83.1 ± 1.9</td>
<td>0.1735</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 1.2</td>
<td>30.5 ± 0.6</td>
<td>0.5258</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.8 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>0.9419</td>
</tr>
<tr>
<td>2hPG (mmol/L)</td>
<td>7.4 ± 0.5</td>
<td>7.2 ± 0.5</td>
<td>0.5049</td>
</tr>
</tbody>
</table>

Table 5.1: Baseline demographic and biochemical data for the groups randomised to receive inulin or cellulose.

5.2 WEIGHT LOSS

Both groups lost approximately 5% of their body weight by week 9, and maintained this weight loss until week 18 (Figure 5.1.1). The inulin group had a mean weight change of -5.4 ± 0.32% (-4.7 ±
0.8kg) while the cellulose group lost 4.5 ± 0.16% (-3.8 ± 0.4kg) (P = 0.4) at week 9. However, following the weight maintenance phase from weeks 9-18, the inulin group had a further mean weight loss of 2.6 ± 0.05% (-2.2 ± 0.6kg) while the cellulose group had a mean weight loss of 0.61% (-0.5 ± 0.3kg) (P = 0.027). The overall weight loss (baseline to week 18) was not different between the groups (P = 0.088) (Figure 5.1).

5.3 BODY COMPOSITION

5.3.1 MRI DATA

Due to contraindications to the MRI scanner, including metal implants and claustrophobia, only 18 subjects were scanned in total, with 9 in each group.

5.3.1.1 BODY FAT

There was a slight, but not significant difference in percentage weight loss between groups. (Inulin: -0.05 ± 0.01% vs Cellulose: -0.04 ± 0.001%, P = 0.2996) (Figure 5.2).
Figure 5.2 Delta change in percentage weight loss at 9 weeks for inulin (N = 9) and cellulose (N = 9) arms for the sub-set of subjects undergoing an MRI Scan. There were no significant differences between groups. Values are means and vertical bars are SEM.

Figure 5.3: Changes in body fat content expressed as a) delta change in percentage of total adipose tissue (tAT) following inulin and cellulose supplementation at 9 weeks. b) the percentage of weight lost as fat during the 9 weeks. Values are means and vertical bars are SEM. The inulin group had a lower fat percentage following the inulin supplementation (-1.93 ± 0.71% vs -0.278 ± 0.54%, P= 0.0826), as they had lost more weight as fat during the trial (66.63 ± 9.77% vs 42.36 ± 17.95%, P = 0.2523).

Subjects in the inulin group tended to lose more fat than the control group. The percentage fat lost was greater (-1.93 ± 0.71% vs -0.278 ± 0.54% N=9) but did not reach significance (P=0.0826). The weight lost as fat demonstrated that in the inulin group 66.63 ± 9.77% of the weight lost was fat mass vs 42.36 ± 17.95% N=9 in the cellulose group, P = 0.2523).

Given the slightly different degrees of weight loss at 9 weeks (Inulin: -0.05 ± 0.01% vs Cellulose: -0.04 ± 0.01%, P = 0.2996) all further analysis on changes in fat depots was done on the raw data, and then controlled for body weight.

5.3.1.2 IHCL

The inulin group lost more intrahepatocellular lipid (IHCL) than the cellulose group, but the difference was not significant when all the subjects were analysed together (-9.16 ± 3.09 vs -2.16 ±
2.91, P = 0.1183). However, if only subjects with elevated IHCL (>3%) were included in the analysis, the inulin group lost significantly more IHCL than subjects taking cellulose (-15.44 ± 3.49 vs -2.16 ± 2.91, P = 0.0153)

![Figure 5.4: Delta change in IHCL following inulin and cellulose supplementation for a) all subjects and b) only subjects with IHCL >3%. Values are means and vertical bars are SEM. The inulin group (N = 9) lost more IHCL than the cellulose group (N = 9) when all subjects were analysed together (-9.16 ± 3.09 vs -2.16 ± 2.91 P = 0.1183) and this result reached significance if only subjects with elevated IHCL (>3%) were included (-15.44 ± 3.485 vs -2.162 ± 2.908, Inulin: N = 5; Cellulose: 9, P = 0.0153)](image)

Following adjustment for weight loss, the ratio of IHCL was not different between the groups (2.67 ± 1.09 vs 1.37 ± 0.78, N=9, P = 0.3477). However, when the data was analysed with subjects with normal levels of IHCL removed, the result was borderline significant (4.61 ± 1.47, N=5 vs 1.37 ± 0.78, N=9, P = 0.0519).

![Figure 5.5: a) Delta change in IHCL adjusted for weight loss following cellulose and inulin supplementation at week 9 for all subjects (2.67 ± 1.09, N=9 vs 1.37 ± 0.78,N = 9, P = 0.3477), and b) those subjects with non-normal levels of IHCL (>3%), (4.61 ± 1.47, N=5 vs 1.37 ± 0.78, N=9, P = 0.0519). Values are means and vertical bars are SEM.](image)
5.3.1.3 PANCREAS

Due to problems with the MRI scanner it was not possible to obtain MRS data for pancreatic fat for 3
of the follow-up subjects in the cellulose group. There were therefore only 9 paired sets of data for
the inulin group and 6 paired sets of data for the cellulose group. There was no difference in
pancreatic fat between groups either adjusted (-0.28 ± 0.19 N=9 vs -0.261 ± 0.25, N=6, P=0.9461) or
unadjusted for weight loss (-2.13 ± 1.13 N=9 vs 0.58 ± 0.50, N = 6, P = 0.3046).

Figure: 5.6: Delta change in pancreatic fat a) unadjusted and b) unadjusted for weight loss. There was no effect of inulin
on pancreatic fat unadjusted (-2.13 ± 1.13 N=9 vs 0.58 ± 0.50, N = 6, P = 0.3046) or adjusted for weight loss (-0.28 ± 0.19
N=9 vs -0.26 ± 0.25, N=6, P=0.9461). Values are means and vertical bars are SEM.

5.3.1.4 VISCERAL ADIPOSE TISSUE

There were no significant differences in the delta change for visceral adipose tissue (VAT) between
the inulin (N = 9) and cellulose groups (N = 9) either unadjusted (-0.79 ± 0.26 N=9 vs -0.27 ± 0.23, P =
0.147), or adjusted for weight lost (-0.14 ± 0.04 vs -0.06 ± 0.08, P = 0.3719).
5.3.1.5 NON-VISCERAL FAT

There was no difference in non-VAT unadjusted for differences in weight loss (-0.43 ± 0.22 vs 0.06 ± 0.13, P = 0.0751) or adjusted for weight loss (0.07 ± 0.04 vs -0.01 ± 0.05, P = 0.3638).

5.3.1.6 IMCL

Intramyocellular lipids (IMCL) were measured in two muscles – the soleus muscle and tibialis muscle. These muscles were selected as the former is an oxidative muscle while the latter is a glycolytic muscle. Inulin supplementation reduced fat content in the soleus unadjusted (-1.51 ± 1.18 vs 2.56 ± 1.65, P = 0.061, N = 18) for weight loss. When the data was adjusted for weight loss the difference was significant (-0.31 ± 0.30 vs -0.92 ± 0.49, N = 20, P = 0.047).
On the other hand, there was no effect of inulin supplementation on the tibialis muscle. The delta change for IMCL following inulin and cellulose supplementation unadjusted and adjusted for weight loss was -1.19 ± 1.14 vs -1.01 ± 1.35 (P = 0.9236) and 0.18 ± 0.38 vs 0.68 ± 0.84 (P = 0.5981) respectively.

There were significant reductions in percent total fat, percent subcutaneous fat, percent subcutaneous abdominal fat, percent peripheral abdominal fat and percent visceral fat within group following inulin supplementation. However, there were no significant differences in regional fat depots between the groups. (Table 5.2).
Table 5.2: Fat content pre- and post-inulin and cellulose supplementation in regional fat depots. A) Paired t-test of within group difference. B) Independent t-test for between group difference. Sc-AT: Subcutaneous adipose tissue; Int-AT: Internal adipose tissue; Sc_AAT: subcutaneous abdominal adipose tissue; P-AAT: Peripheral Abdominal adipose tissue; VAT – Visceral adipose tissue.

5.4 BIOIMPEDANCE DATA

Bioimpedance data were completed for all 30 subjects and are shown below. Subjects in the inulin group lost significantly more weight as fat (-2.78 ± 0.57% vs -1.22 ± 0.42%, P = 0.039) and significantly less weight as free-fat mass (FFM) compared to control (0.05 ± 0.01% vs 0.02 ± 0.01%, P = 0.0036).

Figure 5.11: Change in percent body fat (2.78 ± 0.57 vs -1.22 ± 0.42, P = 0.039) and FFM (0.05 ± 0.01 vs 0.02 ± 0.01, P = 0.0036, N = 30) following inulin and cellulose supplementation. Values are means and vertical bars are SEM.
5.5 APPETITE
5.5.2. SUBJECTIVE APPETITE ASSESSMENT

The time-course data for each of the appetite questions for inulin and cellulose are shown on the next page. The VAS score is shown on the y axis and is shown in centimetres.
Figure 5.12: Time course data for each of the appetite questions. Values are means and vertical bars are SEM. The VAS (Visual Analogue Scale) score is on the Y axis and measured in cm.
Despite both groups losing approximately 5% of their body weight between weeks 1-9, there was no change in tAUC for hunger (Inulin within group difference: \( P = 0.1769 \); cellulose within group difference: \( P = 0.2159 \)) and pleasant to eat (Inulin within group difference: \( P = 0.1960 \); cellulose within group difference: \( P = 0.2715 \)) as measured on the week 9 study day (figure 5.12). Curiously there was a significant decrease in prospective food consumption following cellulose but not inulin supplementation (Inulin within group difference: \( P = 0.1260 \); cellulose within group difference: \( P = 0.0249 \)). However, the differences between the inulin and cellulose groups was not different for any of the scores measured (Hunger: \( P = 0.7536 \); Pleasant: \( P = 0.9913 \); Eat: \( P = 0.7077 \); Full: \( P = 0.1786 \)).

At 18 weeks, the inulin group has lost a further 2.6% of weight while the cellulose group had lost a further 0.6%. There was a significant rebound in tAUC hunger scores between weeks 9 and 18 for the cellulose group but no rebound in the inulin group (Inulin: \( P = 0.6455 \); Cellulose: \( P = 0.015 \)). There were no significant within-group differences for any of the other measures of appetite.
between weeks 9-18: tAUC pleasant (Inulin: P = 0.2275; Cellulose: P = 0.9547), tAUC eat: (Inulin: P = 0.9894; Cellulose: P = 0.1194) and tAUC fullness (Inulin: P = 0.6669; Cellulose: P = 0.2569). There were no between-group differences between groups for tAUC hunger at 18 weeks (P = 0.1788); tAUC pleasant (P = 0.5328), eat (P = 0.2468) or fullness (P = 0.1433).

5.5.2 AD LIBITUM MEAL TEST

Subjects taking inulin consumed less at the ad libitum meal at week 9 when compared to baseline (Baseline: 536.3 ± 83.04g, Week 9: 412.7 ± 46.79g, P = 0.1174), while subjects taking the control consumed more (Baseline: 490.6 ± 27.03g, Week: 533.6 ± 66.05g, P = 0.5955) (Figure 5.13). The difference between the groups was not significant (-133.9 ± 78.14g vs 8.58 ± 15.51g, P = 0.0762). At 18 weeks, the inulin group ate slightly less at the meal when compared to week 9 (Week 9: 412.7 ± 46.79g; Week 18: 407.5 ± 32.87g, P = 0.9274) while the cellulose group ate slightly more (Week 9: 490.6 ± 27.03g; Week 18: 497.3 ± 46.23g, P = 0.5955). There were no differences between the groups, P = 0.9862).

Figure 5.14a: Food intake (grams) in the ad libitum meal at baseline, week 9 and week 18 for the inulin and cellulose groups. Figure 5.14b: The delta change in food intake following the weight loss phase (week 9) and weight maintenance phase (week 18) for inulin and cellulose groups. The difference in food intake at the week 9 visit between the inulin and cellulose groups was not significant (-133.9 ± 78.14g vs 8.58 ± 15.51g, N =30, P = 0.0762).
5.6 BREATH HYDROGEN

There was no difference in breath hydrogen in the inulin group at week 9 compared to baseline (Baseline: 6.36 ± 2.09ppm; Week 9: 24.73 ± 11.01ppm, N = 15, P = 0.12) (Figure 5.15). The delta change between inulin and cellulose groups was not significantly different (P = 0.1138). At week 18 there was a non-significant decrease in breath hydrogen compared to week 9 in the inulin (Week 9: 24.73 ± 11.01ppm; Week 18: 15.55 ± 5.758ppm, P = 0.4683); and the cellulose group (Week 9: 6.50 ± 2.29ppm; Week 18: 3.30 ± 0.58ppm, P = 0.2283). The delta change in breath hydrogen from weeks 9-18 between the inulin and cellulose group was not significant (P = 0.7662).

Figure 5.15a: Breath hydrogen values at baseline, week 9 and week 18 for the inulin and cellulose groups. :Figure 5.14b: The delta change in breath hydrogen values at week 9 and week 18. Values are means and vertical bars are SEM. There were no significant differences between the inulin and cellulose groups. Inulin (N = 15); cellulose (N = 15).

5.7 COMPLIANCE

In this study, participants were asked to take 336 sachets of the supplement through the 18 week period. During weeks 1-9, it was initially decided to give the subjects the exact number of sachets needed to take them through to their next dietary counselling visit. However, in practice, many subjects had to change the date of their next dietary counselling visit due to job or family commitments, meaning that they would be going without supplement for a number of days.
Therefore to ensure days were not missed, subjects were given 5 days’ extra each time they came in. From weeks 9-18, subjects were given 189 sachets, and asked to return them at week 18. 14 subjects out of 30 returned the sachets, the average number of sachets returned was 9.

5.8 DISCUSSION

- The inulin group lost more weight between weeks 9-18 than the cellulose group.
- There was a significant difference in rebound hunger scores between 9 and 18 weeks.
- The inulin group lost significantly more intramyocellular lipid in the soleus muscle than the cellulose group.

5.8.1 WEIGHT LOSS

In this investigation, the inulin group lost more weight between weeks 9 and 18 than the cellulose group. There was also a positive effect on body composition, with the inulin group tending to lose a greater amount of body weight as fat than the cellulose group.

The results appear to support previous published data of the effect of FCHO on appetite and food intake. Both the inulin and the cellulose groups had lost approximately 5% of their body weight, and as such, had both undergone a sustained negative energy balance. It was expected based on previous studies (152,311) that this would result in an increase in food intake at the *ad libitum* meal at the follow-up visit. This was the case for the control group. However, the inulin group ate significantly less at week 9 and 18, despite further weight loss.

Similarly, weight loss of 5% has previously been shown to reliably increase measures of subjective appetite(151), and accordingly, there was a significant rebound in tAUC for hunger between weeks 9 and 18 for the cellulose group. However, there was no rebound in the inulin group, suggesting that inulin is able to suppress appetite through sustained and significant negative energy balance. The
scores for prospective food consumption also changed in the same direction, with a rebound at 18 weeks for cellulose but not for inulin. However, the scores for pleasantness to eat and fullness were contradictory. This may indicate specific effects on appetite, or there may be an element of difficulty in describing internal sensations as previously described in investigation 1. The error bars for these questions demonstrate the variability in responses.

Interestingly there was also no increase in hunger scores in the control group at week 9, despite this group also having lost approximately 5% body weight. Between baseline and week 9 all participants had attended 4 dietary counselling sessions during which behavioural techniques were used to guide the participants towards sensible dietary changes, with the last dietary counselling session taking place 2-3 weeks before the 9 week visit. Behavioural change interventions have previously been shown to reduce appetite ratings (312). Therefore, the appetite ratings at the week 9 visit may have been affected by the recent counselling session, while at week 18, the effect of the counselling had subsided.

Alternatively the lack of change in appetite scores in the control group may be due to changes in dietary intake in the first 9 weeks. During this period, all subjects in the inulin and control group were offered the same dietary advice, including increase fruits, vegetables and wholegrains. These are foods which are likely to include some fermentable fibre (313). Therefore the cellulose group may have had increases in fermentable carbohydrate intakes between weeks 0-9, causing a decrease in sensations of hunger and increases in fullness. This interpretation is partly supported by moderate increases in breath hydrogen in the cellulose group between weeks 0-9. However, since a food diary was not completed at week 9 or 18, this cannot be confirmed.

Investigation 3 is the first controlled study to test the effectiveness of FCHO alongside a calorie-reduced weight loss program. Since the sustained negative energy balance required to lose weight results in compensatory increases in appetite - potentially via adaptations in appetite hormones (152, 156, 159, 160) - it was hypothesised that a dietary agent which can reduce appetite – potentially
via those same mechanisms – would result in more successful maintenance of weight lost. In this investigation, the hypothesis proved correct, and the subjects taking inulin maintained significantly more weight than the control.

Both inulin and cellulose groups met their weight loss target of 5% body weight at 9 weeks, demonstrating that dietary restraint short-term is effective. It was then expected that each group would gradually begin to regain the weight lost during the weight maintenance phase (314). However this did not happen in either group. The cellulose group lost a mean 1kg more, while the inulin group lose a mean 4kg more. There may be several reasons for this. Firstly, the length of the follow-up period may not have been long enough. Second, while the participants did not attend dietary counselling visits or receive any further advice, they were still contacted by the researcher every couple of weeks to ensure compliance with the supplementation. Third the behaviour change techniques during the dietary counselling sessions may be been effective at achieving longer-lasting lifestyle changes. Fourth, the cellulose supplementation in the control group may have helped weight loss, albeit not to the same degree as the inulin group. Fifth, these prediabetic study subjects may not be representative of the population as a whole.

When selecting the length of the weight loss and weight maintenance phase, a balance was sought between degree of participant burden and feasibility. A number of studies have successfully shown 5% weight loss via portion control and dietary modification (as opposed to meal replacement) is feasible within 9 weeks (315-317). Due to not unduly burden the participants, it was thought that an additional 9 weeks would be long enough to see weight regain. However, it appears that this was not long enough as the participants were still losing weight. While the results still suggest that rate of weight loss slows down more quickly in the control group compared to inulin, longer studies of at least 6 months’ duration should be carried out to determine the effect of true maintenance.

Second, it is also important to acknowledge the important impact the Hawthorne effect has on participant behaviour. Trial subjects are known to change their behaviour when they are being
observed (318). However, to ensure compliance they were contacted frequently during the weight maintenance period. Outside of a clinical trial, it is therefore possible that the subjects may have begun to regain weight more quickly.

Third, it has been demonstrated that techniques such as motivational interviewing can change behaviours longer-term and help prevent weight regain relative to standard dietary advice (319, 320). Part of the appeal of using an FCHO-based intervention is to reduce appetite cravings naturally, therefore limiting the need for structured intervention. Since both inulin and behavioural change interventions have been shown to reduce appetite (174, 312), an intervention comparing inulin to control with only standard dietary advice given may have greater sensitivity.

Cellulose was selected as the control in this study as it is not considered fermentable (321). However, during the dietary intervention subjects were advised to increase their intake of fruits and vegetables to at least 5 servings per day. Additional dietary changes were also made such as selecting whole-grain products which would also increase the daily fibre intake. It is therefore possible that the control group were also benefiting from the products of fermentation which may have limited the differences between groups.

However, overall, these data support a role for inulin in promoting long-term weight management.

5.8.2 BODY COMPOSITION

In addition to promoting weight loss, inulin also appears to promote favourable changes in body composition, with subjects taking inulin losing more weight as fat, although the difference did not meet significance between groups. The bioimpedance data supports this view, with a significant reduction in percentage of body weight as fat and a significant increase in percentage of body weight as free fat mass (FFM). While caution is required when interpreting bioimpedance data - which will be discussed below - this data is supported by the study by Parnell and Reimer.
demonstrating that 12 weeks’ supplementation with OFS led to greater weight loss as fat, as compared to FFM, measured by x-ray absorptiometry(173).

Comparisons of the percentage of weight lost as fat in this study with those in the literature is challenging because factors such as age, baseline weight, duration, speed and degree of weight loss all affect changes in body composition (322,323). Subjects losing a mean 10% of body weight over 6 months lost a mean 22% of fat mass measured by x-ray absorptiometry(324). This is more than the 20% in the inulin group and 5% in the cellulose group in this study. However, the degree of weight loss was much greater, as were the baseline body weights and fat mass (FM)(324). A 3-month long study evaluating a 500kcal calorie deficit dietary intervention found that two-thirds of weight lost was FM. However, the intervention was longer, and subjects had higher baseline % FM (325).

Nonetheless, in this study, the two groups underwent the same dietary intervention for the same duration. There were no differences in baseline body weights or absolute or relative fat mass, gender balance, or age between the groups and therefore it appears likely that any effect on weight lost as body fat in the experimental group would be due to the effects of inulin.

The mechanism by which FCHO is able to reduce body fat is unclear, and appears to occur independent of changes in lifestyle (173), during HFD-induced weight gain (ie, FCHO ameliorates fat deposition relative to control)(326), and following significant weight loss induced by a lifestyle intervention. Work in cells and animals has demonstrated a role for SCFA in regulating adipogenesis(183,187-190). Propionate significantly increases GPCR43 expression and levels of PPAR-γ2 (an important transcription factor involved in adipogenesis) during adipocyte differentiation(187). In theory this would result in the development of multiple smaller adipocytes, which has been shown in mice fed a high-RS diet(327). Smaller adipocytes are associated with improved insulin resistance(328). However, if FCHO’s role is in promoting adipogenesis, this seems contrary to results demonstrating a reduction in fat mass. Therefore it appears likely the reduction in fat mass is more likely due to an increase in fat oxidation.
Carnitine palmitoyltransferase 1B (CPT-1B) is the rate-limiting enzyme in transport of fatty acids into the mitochondria, and therefore the rate-limiting step of fat oxidation. Rats fed a diet high in viscous (fermentable) fibres have increased muscle expression of CPT-1B, PPARγ 1α, PPARδ and uncoupling protein 3 (UCP3), and citrate synthase (CS) activity, all enzymes and transcription factors involved in fat oxidation(329). In particular, these enzymes were measured in the soleus muscle, indicating that decreased fat in this muscle seen in this body of work could be due to an increase in fat oxidation. In humans fed a low-glycaemic index diet, fat oxidation is increased during exercise as measured by substrate oxidation rates calculated from VO₂ max and CO₂ production during exercise(330). The subjects ate the low glycaemic index breakfast 3 hours before the exercise, indicating that fermentation of undigested fibres may have partly influenced oxidation rates.

The changes in body composition following weight loss have been suggested to be one of the causes of rebound weight gain, as a loss of FFM lowers the basal metabolic rate (BMR). Without a sustained reduction in energy intake to meet the new lower energy expenditure, weight regain will occur. Therefore, if inulin can promote maintenance of FFM during weight loss it may increase the likelihood of maintenance of that weight loss. However, there are limitations with bioimpedance which must be taken into account (331). FFM comprises the non-fat components of the body, including water (73%) and protein (23%) as its major components, and the equations used in determining FFM are not able to discriminate effectively between water and protein. During weight loss, glycogen stores decrease with a concomitant decrease in water. A study comparing the use of bioimpedance with gold-standard densitometry demonstrates this limitation. Following a 10kg very-low-calorie-diet-induced weight loss, bioimpedance estimated that only 0.6kg was lost as FFM (6%), while densitometry estimated this as 2.3kg (23%). While Parnell and Reimer’s study used x-ray absorptiometry, the subjects only lost a mean 1.0kg (1%) of weight. It is unknown whether the result would be similar following further weight loss. Therefore, further studies are required using more accurate methods to determine whether FCHO may promote maintenance of muscle tissue during significant weight loss.
In addition, while this study was not designed to examine the effect of inulin on body composition during weight regain, the effects seen on visceral fat and sparing of lean body mass potentially have consequences on long-term maintenance of body composition. There is a variety of data from re-feeding studies in anorectic patients, from cross-sectional studies on normal and over women and from the Minnesota semi-starvation study which suggest that weight cycling leads to preferential deposition of visceral fat. While these are extreme examples, a recent study which followed 97 women for one year after they had lost a mean 12kg via an 800kcal/day diet, found that weight regain was associated with a 25% increase in visceral fat in non-exercisers (332). Interestingly, exercise, whether aerobic or resistance attenuated both the regain in weight and tendency to regain visceral fat, with the differences between both exercise groups and non-exercisers being significantly different. Therefore the finding that a dietary compound may have similar effects in tissue remodelling is promising. In a study comparing the effects of exercise vs resistant starch (RS) during weight regain in rodents, RS was as effective as exercise at preventing regain as visceral adipose tissue. Furthermore, RS was better able to promote regain of lean body mass at the expense of fat mass.

Subjects in the inulin group lost more triglyceride in the liver compared with control, although the result was only borderline significant once controlled for differences in weight lost. If this effect is real, it is of particular importance given the strong relationship between fat content of the liver and metabolic disease, particularly diabetes (333). FCHO has previously been shown to reduce or ameliorate the deposition of fat in the liver in rodent studies (220,220,334). SCFA can also significantly increase the activity of AMP-activated Protein Kinase (AMPK) in obese mice (335). AMPK is a recognised “master” switch - when activated, AMPK increases fat oxidation and decreases fatty acid synthesis via multiple pathways (335,336). Pyra et al’s recent finding that prebiotics themselves increase the expression of AMPK mRNA in obese rats is therefore interesting (281), and makes it
likely that decreased de novo lipogenesis and increased fat oxidation both contribute to the reduction in liver fat seen with FCHO feeding.

Of particular interest is the finding that inulin significantly decreases the lipid content of the soleus muscle, relative to cellulose, whereas inulin has no effect on lipid content of the tibialis muscle. Previous studies have found an increase in lipid content of the soleus muscle but not the tibialis in the off-spring of subjects with type II diabetes, which was linked to insulin resistance (337). Indian males with diabetes have significantly higher IMCL-content in the soleus compared to healthy controls (338). It has also previously been shown that viscous fibre feeding increases the expression of AMPK, citrate synthase, CPT-1B, PGC-1α, PPARδ and UCP3 in the soleus muscle of rats fed a high viscous diet, all genes involved in fat oxidation, (329).

With the exception of the liver and soleus muscle, the loss of adipose tissue did not appear to be fat depot-specific, with no differences in abdominal, trunk, internal, viscous and non-viscous fat. It appears interesting that subjects taking inulin lost a significant amount of visceral fat (VAT) compared to baseline, but the cellulose group did not. However, the between group difference was not different. VAT has been considered a risk factor for metabolic disease, and interventions which reduce VAT have been shown to improve insulin resistance and glucose homeostasis. However, studies which measure both VAT and hepatic lipid have shown a 0.79 concordance between the two depots(339,340), indicating that it may be changes in hepatic fat which is most critical for improvement of metabolic health.

Finally, there was no difference in loss of pancreatic fat between the two groups. While loss of liver fat occurs early on during weight loss, pancreatic fat may be one of the last depots to lose fat(276).

Therefore the combined effects of reduced weight, reduced fat mass, and reduced fat deposition in the liver and soleus muscle would all be expected to have positive effects on glucose homeostasis. Since FCHO has been shown to improve measures of glucose homeostasis independent of weight,
and that FCHO promotes weight loss, a useful next step would be to measure the insulin and glucose concentrations of the participants at week 9 and 18.

**5.8.3 LIMITATIONS**

Weight change occurs when there are alterations in the balance of energy in versus energy out. While data was not collected on food intake or physical activity in investigation 3, the results on food intake from the first study, and the reductions in appetite and intake from the test meal strongly suggest the differences between the inulin and control group were predominantly due to food intake.

The recruitment process itself may also have introduced bias into the analysis. Firstly, people who were identified as being at risk of diabetes were invited into the clinical research unit for an OGTT. For many of these individuals, this was the first time they had been made aware of their glycaemic status and for many there was also a time-lag of up to 3 months between their screening date and their first study visit. It is therefore possible that some subjects made alterations in their lifestyle which could have affected the results of the study. In particular, there was a concern that changes in physical activity in particular could influence the results as exercise has been shown to both promote weight loss and also improve insulin sensitivity independent of changes in weight. Since neither food diaries nor physical activity monitors were used during this study it is possible that the inulin group were more active by chance.

The recruitment process may also have introduced sampling bias. In the process of recruitment, over 8000 letters in total were sent to potentially eligible individuals. Approximately 12% of these people responded. Since these individuals were already more engaged and motivated than other members of the population, the weight loss seen in investigation 3 may not necessarily be extrapolated to all individuals with prediabetes.
In this investigation, subjects were asked to take 336 supplements through the 18 week study, with returned sachets taken as a measure of compliance. Unfortunately only 14 subjects returned sachets, 8 in the inulin group. Many of the subjects complained that, once opened, the sachets became sticky and it was not practical for them to keep them for a long period of time. In addition, other studies have been able to monitor compliance with subjects taking one or two sachets per day, and therefore could consume one at breakfast and one before bed. However, in this investigation, subjects also took a sachet in the middle of the day, and for many subjects out at work or doing the shopping, it was not practical for them to keep it in a bag. The majority of participants (N = 16) said that they always threw away the lunchtime sachet. Therefore compliance in this study cannot be estimated with complete confidence.

The fact that the breath hydrogen measures declined from week 9 to 18 also calls into question whether the participants were compliant in this study, particularly during the weight loss phase. No previous study has examined the effect of FCHO on breath hydrogen levels for as long as this study. In addition, no study has measured breath hydrogen levels in the middle and end of an interventional study using FCHO. Therefore it is unclear whether the decline in breath hydrogen concentrations reflects adaptation by the gut microbiota to increased levels of FCHO in the diet, or a decline in compliance.
CHAPTER SIX: GENERAL

DISCUSSION
6.0 DISCUSSION

This body of work was designed to evaluate the potential of a fermentable carbohydrate inulin on weight management and glucose homeostasis in people at prediabetes. These investigations found that subjects taking inulin consumed less calories and lost more weight than subjects taking cellulose. However, inulin had unexpected effects on glucose homeostasis. There was a reduction in insulin and improvement in HOMA-IR only in those subjects with Impaired Fasting Glucose (IFG), a significant increase in early insulin secretion and a small, but statistically significant increase in post-prandial glucose.

Food intake was assessed by 3-day food diary and ad libitum meal in investigation 1, and by ad libitum meal intake in investigation 3. In investigation 1, subjects consumed approximately 273 kcal less while taking inulin. This calorie deficit matched the weight loss at the end of the 6 week supplementation period. Interestingly, food intake was reduced at the ad libitum meal at the post-inulin study day despite these subjects arriving with a significant energy deficit. Even short-term and moderate energy restriction has been shown to increase food intake at a subsequent meal. Therefore the reduction in food intake by inulin can be interpreted as preventing rebound hunger, and potentially could help to promote weight maintenance long-term. This interpretation is supported by data from investigation 3. Both the inulin and the cellulose groups had lost approximately 5% of their body weight, and as such, had both undergone a sustained negative energy balance. It was expected based on previous studies (152,311) that this would result in an increase in food intake at the ad libitum meal at the follow-up visit. This was the case for the control group. However, the inulin group ate significantly less at week 9 and 18, despite further weight loss.

The greater effect on inulin on the ad libitum meal in investigation 3 compared to investigation 1 likely has multiple causes. Firstly, previous studies have shown that consumption of FCHO has an appetite suppressing effect at the second meal (232,252). In this investigation 1, the supplement was taken the night before, but not the morning of the study day. Hence the second meal would have
been the breakfast. However, since the protocol for investigation 1 required that all participants eat all of the breakfast that had been provided, it cannot be determined whether there was any effect of inulin on the second meal. In the third investigation, the meal test was done at the second meal, where the effects of inulin would be expected to be greatest. Secondly, it has been suggested that the effect of FCHO on GLP-1 is cumulative and takes longer than 6 weeks to occur (173, 212), and therefore 9 and 18 weeks supplementation versus 6 would be expected to have a different magnitude of effect on hunger. Finally, the shorter study day seemed less arduous on volunteers. It was also made clear to volunteers that there were remaining study tasks to be completed after the meal, and therefore there was no direct link between completing the meal and leaving the unit. Therefore, taken together, these data support the findings of previous studies that FCHO reduces food intake.

In investigation 1, there was no change in appetite scores. As mentioned in the discussion in chapter 3, this may be partly due to methodological problems such as subject error, questionnaire fatigue; or the inherent difficulty in describing complex appetite sensations. However, it may also reflect an amelioration of the expected weight-loss mediated increase in hunger. This interpretation is supported by the findings in investigation 3, where subjects taking cellulose had a significant rebound in hunger scores between weeks 9 and 18. No rebound occurred in the inulin group, despite having lost more weight. In addition, there was also a non-significant decline in scores for hunger. Therefore these data suggest that inulin can ameliorate the rebound in hunger sensations following a period of weight loss. Further studies using weight-matched controls would help to confirm this.

Despite the positive effects seen on both food intake and appetite scores, there was no effect of inulin on PYY and GLP-1 in investigation 1, yet there was a significant early post-prandial increase in GLP-1 in investigation 2. While the inulin supplement was given on the morning of the follow-up in investigation 2, but not investigation 1, this is unlikely to be the cause of the discrepancy in results.
between the two investigations. Data from a human ileostomy model suggests the oro-caecal transit time of 30g inulin-type fructan is 3.5 hours (278).

A contributory factor could be the fact that subjects taking inulin lost weight. Since it is clear from the literature that weight loss can reduce secretion of gut hormones, and even modest weight loss of 1.5kg (similar in magnitude to the weight loss in this investigation 1) significantly decreases 24-hr PYY concentrations (255). However, subjects in investigation 2 also lost a similar amount of weight, suggesting this interpretation may not be correct.

Instead, the discrepancy between the two investigations is more likely to be partly due to the composition of the meal provided in investigation 2. Both GLP-1 and PYY are known to be released in proportion to calories consumed (131, 256). In investigation 1, the maximum energy content of the breakfast was 408kcal, while the mean was 293kcal. In contrast, all subjects in investigation 2 took 450kcal. And while the lunch in investigation 1 did have a substantial energy content, FCHO is known to act via a second meal effect. Since the supplement was not provided on the morning of the study day, the lunch was the “third” meal post supplementation, and therefore fermentation – and its products - may have declined from their peak.

There may also have been differences in compliance between the groups. Unfortunately, a breath hydrogen measure was only taken at 420 minutes during the investigation 2 study day, and not prior to the inulin being added to the MTT. Therefore it is unclear whether there were differences in compliance between the groups. The results from investigation 1 (both the reduction in food intake and the side-effect scores) suggest that the subjects were taking the inulin at least in the 7 days prior to the study day in investigation 1, so differences in compliance seem an unlikely explanation for the differences in gut hormone secretion seen between the two investigations.

The data within this body of work therefore support previous findings that inulin supplementation reduces weight primarily by reducing food intake. In addition, the data on body composition and
BMR may suggest that long-term, inulin may help by promoting fat loss while sparing other lean body mass.

In this study, inulin significantly increased early phase insulin secretion in response to a MTT, and improved insulin sensitivity in subjects with isolated-IFG. These effects were independent of any changes in lifestyle. Interestingly, there did not appear to be any reduction in glucose levels in investigations 1 and 2, except for a reduction (not-significant) in fasting glucose in the i-IFG group. However, as stated in the introduction, the hypothesis was that inulin works via a two-pronged effect 1) via improvements in insulin sensitivity independent of changes in lifestyle and 2) by promoting weight loss which itself reduces glucose levels. Therefore an important next step would be to measure glucose and insulin concentrations in investigation 3 to determine whether the greater weight loss at week 18 translates into greater reductions in glucose.

6.1 LIMITATIONS

While this study demonstrated significant effects of inulin on appetite, food intake and weight loss, there are still questions remaining about the exact mechanism involved. An increase in gut hormones has been accepted as the principal mediator, yet FCHO supplementation - even where it affects weight loss and food intake - does not always increase the gut hormones. It is also interesting that resistant starch has not been shown to affect GLP-1 or PYY concentrations, but has been shown to reduce food intake (252); and while it has been shown to reduce food intake when taken acutely, this has not translated into an effect on weight loss when taken continuously, even up to 12 weeks (289).

Unfortunately, due to limited resources we were not able to measure other peptides involved in appetite. Appetite is a complex process and the number of peptides involved in its regulation have continued to increase since secretin and gastrin were discovered in the 60s (131).
over 20 peptides which play some role in the regulation of appetite including the well known, such as leptin, CCK, PYY and GLP-1 (131), to the less well understood such as GLP-2 or peptide histidine isoleucine (PHI) (341). Since FCHO has been shown to decrease ghrelin concentrations (173,342) it is possible that some of the effects on weight loss seen in this body of work and other studies was mediated by reduction in this “hunger” hormone. Finally, if indeed the effects of weight loss are mediated by gut hormones, then we would expect to see no change or an increase in these hormones following inulin supplementation in investigation 3. This would be a useful next step (see future work).

Inhibition of eating can occur via satiety and satiation. Satiety is the state in which further eating is inhibited and follows the end of an eating episode. Satiation develops during the course of eating and eventually brings the period of eating to an end (343). It is generally accepted that GLP-1 and PYY are satiation hormones which regulate the size and/or timing of subsequent meals (131,344). However, whether the reduction in food intake in this study occurred by eating smaller meals (satiety) or fewer episodes of eating between meals (satiation) was not examined.

The recruitment process itself may also have introduced bias into the analysis. Firstly, people who were identified as being at risk of diabetes were invited into the clinical research unit for an OGTT. For many of these individuals, this was the first time they had been made aware of their glycaemic status. There was a concern in designing the trials that this may result in individuals changing their lifestyle habits in the weeks preceding their first study visit, or just as they commenced one of the trials. For this reason a crossover trial design was utilised to reduce the influence of such confounders in investigations 1 and 2. However, in investigation 3, this was not possible. For many subjects there was a time-lag of up to 3 months between their screening date and their first study visit. It is therefore possible that some subjects made alterations in their lifestyle which could have affected the results of the study. In particular, there was a concern that changes in physical activity could influence the results as exercise has been shown to both promote weight loss and also
improve insulin sensitivity independent of changes in weight. Since neither food diaries nor physical activity monitors were used during this study it is possible that the inulin group were more active by chance.

Compliance in dietary studies is always extremely challenging. Subjects throughout these trials were asked to take 10g of a fibre 3 times a day for a total of 16 weeks in investigations 1 and 2, and for 18 weeks in investigation 3. For investigations 1 and 2, subjects were asked to return their empty packets on their follow-up study day. However, less than half of subjects did this. Therefore the return of sachets could not provide a reliable estimate of compliance in this study. Nevertheless, the fact that breath hydrogen significantly increased in investigation 1 and 2 provides some confidence that the supplement was being taken. However, a significant step forward in nutritional interventions studies would be reliable biochemical measures of compliance.

We have related to metabolic effects seen in this body of work with the products of fermentation, namely, the SCFAs acetate, butyrate and propionate. Breath hydrogen was used as a proxy of fermentation in this study as it has consistently been shown to increase following FCHO supplementation. However, it is important to note that fermentation is a complex and dynamic process. While typical fermentation includes the production of the gases carbon dioxide, methane and hydrogen, many microorganisms are adaptive, and are able to use other gases as substrates. For example, both sulphate-reducing bacteria and methanogenic bacteria can consume hydrogen produced by secondary fermenters (345). Some studies have suggested that transfer of hydrogen between fermentative bacteria (hydrogen-producing) and methanogenic bacteria produces the large amounts of acetate seen in methane-producers (346), while substrates such as oligofructose have been shown to promote the bacterial conversion of acetate and lactate to butyrate (347). Therefore, while the breath hydrogen levels in this study give some confidence in compliance with the supplementation, no conclusions can be drawn between the concentration of hydrogen and likely concentration of acetate, butyrate or propionate.
If the biochemical changes as described in these investigations were truly the result of changes in fermentation, whether direct (ie, GLP-1) or indirect (FFA), it would be expected that a correlation would be identified between breath hydrogen levels and the delta change in a given metabolite. Indeed, these was shown by Cani and Delzenne who demonstrated that breath hydrogen levels correlated with changes in plasma glucose following 2 weeks’ of OFS. However, in reality, the variation in breath hydrogen levels at baseline and following the intervention was so great in these investigations that this would not be realistic. Plasma concentrations of short chain fatty acids (SCFA) are notoriously difficult to measure. However, even a single fasting measure of acetate – which is the most highly concentration SCFA circulating in the blood would aid the interpretation of these investigations.

6.2 CONCLUSIONS

Inulin supplementation reduces food intake and body weight, and promotes long-term weight loss maintenance. Inulin also appears to promote changes in body composition which may be metabolically beneficial long-term. Independent of changes in lifestyle, inulin also significantly increases early insulin secretion and appears to improve fasting insulin sensitivity in subjects with i-IFG. Inulin may be a useful adjunct to lifestyle interventions for subjects with IGT, and may also be effective at preventing diabetes in subjects with i-IFG independent of lifestyle changes.

6.3 FUTURE WORK

This body of work found that subjects taking inulin had better weight maintenance at 18 weeks compared to cellulose. Given that weight regain is associated with decreases in PYY and GLP-1, a critical next step will be to measure both PYY and GLP-1 at weeks 9 and 18 of investigation 3.
Similarly, while the findings from this study indicate that inulin does not improve insulin sensitivity in subjects with IGT independent of lifestyle change, it may do so indirectly via its effects on weight loss. Therefore, measurement of glucose and insulin from investigation 3 should be measured to determine whether this is the case.

The finding that inulin appears to be effective at improving fasting insulin sensitivity in subjects with i-IFG, and also improves first-phase insulin secretion indicates the inulin may be an effective targeted intervention at preventing the development of diabetes in subjects with i-IFG. This is all the more critical as there is emerging evidence from diet, exercise and pharmaceutical studies that existing interventions are not effective in this prediabetic sub-group (348-351). Therefore an interventional trial, powered to determine differences in fasting glucose should be carried out to determine the effect on inulin on risk of diabetes in subjects with i-IFG.


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APPENDICES
Appendix A: Blood Sampling Timetables for each investigation:

Investigation 1

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>PYY</th>
<th>GLP-1</th>
<th>Glucose</th>
<th>Insulin</th>
<th>VAS</th>
<th>Breath H²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>-10</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>0</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>30</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>60</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>90</td>
<td>600 µl</td>
<td>400 µl</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>120</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>180</td>
<td>600 µl</td>
<td>400 µl</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>240</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>280</td>
<td>600 µl</td>
<td>400 µl</td>
<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>320</td>
<td>600 µl</td>
<td>400 µl</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
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<td>Lunch</td>
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<td></td>
</tr>
<tr>
<td>Lunch</td>
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<td>500 µl</td>
<td>500 µl</td>
<td>+</td>
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</tr>
<tr>
<td>Ad Libitum Meal</td>
<td>36</td>
<td></td>
<td>27</td>
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</table>

Investigation 2

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>Breath H²</th>
<th>GLP-1</th>
<th>FFA</th>
<th>Glucose</th>
<th>Insulin</th>
</tr>
</thead>
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<td>MTT</td>
<td>-30</td>
<td>500 µl</td>
<td>2ml</td>
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<td></td>
<td></td>
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<td>-15</td>
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<td>1ml</td>
<td>500 µl</td>
<td>2ml</td>
<td></td>
</tr>
<tr>
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<td>1.5ml</td>
<td>1ml</td>
<td>500 µl</td>
<td>2ml</td>
<td></td>
</tr>
<tr>
<td>MTT</td>
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<td>500 µl</td>
<td></td>
<td>2ml</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td>1ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT</td>
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<td>500 µl</td>
<td></td>
<td>1ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT</td>
<td>20</td>
<td>500 µl</td>
<td></td>
<td>1ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT</td>
<td>30</td>
<td>1.5ml</td>
<td>1ml</td>
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<td>1ml</td>
<td></td>
</tr>
<tr>
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<td>2ml</td>
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<td></td>
</tr>
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<td>1.5ml</td>
<td>1ml</td>
<td>500 µl</td>
<td>1ml</td>
<td></td>
</tr>
<tr>
<td>MTT</td>
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<td>1.5ml</td>
<td></td>
<td>1ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT</td>
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<td>1ml</td>
<td>500 µl</td>
<td>1ml</td>
<td></td>
</tr>
<tr>
<td>MTT</td>
<td>150</td>
<td>1.5ml</td>
<td></td>
<td>1ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTT</td>
<td>180</td>
<td>1.5ml</td>
<td>1ml</td>
<td>500 µl</td>
<td>1ml</td>
<td></td>
</tr>
<tr>
<td>MTT</td>
<td>240</td>
<td>1.5ml</td>
<td>1ml</td>
<td>500 µl</td>
<td>2ml</td>
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</tr>
</tbody>
</table>

MTT: Meal Tolerance Test
Investigation 3

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>VAS</th>
<th>Breath H²</th>
<th>PYY?</th>
<th>GLP-1?</th>
<th>FFA</th>
<th>Glucose</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>-15</td>
<td></td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>2ml</td>
<td>500μl</td>
<td>2ml</td>
</tr>
<tr>
<td>0</td>
<td>VAS</td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>500μl</td>
<td>2ml</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500μl</td>
<td>2ml</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500μl</td>
<td>1ml</td>
</tr>
<tr>
<td>15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>500μl</td>
<td>1ml</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500μl</td>
<td>1ml</td>
</tr>
<tr>
<td>30</td>
<td>VAS</td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>500μl</td>
<td>1ml</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>500μl</td>
<td>1ml</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>VAS</td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td></td>
<td></td>
<td>500μl</td>
<td>1ml</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>500μl</td>
<td>1ml</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>VAS</td>
<td>H</td>
<td>1.5ml</td>
<td>1.5ml</td>
<td></td>
<td></td>
<td>500μl</td>
<td>1ml</td>
</tr>
<tr>
<td>180</td>
<td>VAS</td>
<td></td>
<td>1.5ml</td>
<td>1.5ml</td>
<td>2ml</td>
<td>500μl</td>
<td>1ml</td>
<td></td>
</tr>
</tbody>
</table>

Further work: We intend to measure glucose and insulin. Based on the results from the change loss seen in this study, we will measure PYY and GLP-1 in the first instance.
Appendix B: Tables of macronutrient content of meals used in the study.

Table 1: Nutritional information for breakfast and lunch for investigation 1.

<table>
<thead>
<tr>
<th></th>
<th>Serving size</th>
<th>Energy (kcal)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Sugar (g)</th>
<th>Fibre (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornflakes</td>
<td>30-60g</td>
<td>113.4-226.8</td>
<td>25.2-71.4</td>
<td>0.3-0.5</td>
<td>2.1-4.2</td>
<td>2.4-4.8</td>
<td>0.9-1.8</td>
</tr>
<tr>
<td>Rice Krispies</td>
<td>30-60g</td>
<td>114.9-229.8</td>
<td>34.5-69.0</td>
<td>0.3-0.6</td>
<td>1.8-3.6</td>
<td>3.0-6.0</td>
<td>0.3-0.6</td>
</tr>
<tr>
<td>Milk (semi-skim)</td>
<td>90-170ml</td>
<td>49.0-83.3</td>
<td>5.0-8.5</td>
<td>1.7-2.9</td>
<td>3.4-5.8</td>
<td>5.0-8.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Orange juice</td>
<td>200ml</td>
<td>94.0</td>
<td>20.8</td>
<td>0.2</td>
<td>1.0</td>
<td>20.8</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Sub-total</strong></td>
<td></td>
<td><strong>256.4-407.1</strong></td>
<td><strong>51.0-100.7</strong></td>
<td><strong>2.2-3.7</strong></td>
<td><strong>6.2-11.0</strong></td>
<td><strong>28.2-35.3</strong></td>
<td><strong>0.3-1.8</strong></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White bread</td>
<td>2 slices</td>
<td>190.0</td>
<td>35.6</td>
<td>1.6</td>
<td>7.2</td>
<td>3.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Margarine</td>
<td>10</td>
<td>40</td>
<td>0.0</td>
<td>4.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Crisps</td>
<td>15-25g</td>
<td>66-132</td>
<td>6.45-12.9</td>
<td>4.8</td>
<td>0.75-1.5</td>
<td>0.05-0.1</td>
<td>0.05-1.1</td>
</tr>
<tr>
<td>Yogurt</td>
<td>125g</td>
<td>120</td>
<td>19.1</td>
<td>2.4</td>
<td>4.5</td>
<td>16.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Kit-kat</td>
<td>11.5-23g</td>
<td>53.5-107</td>
<td>6.65-13.3</td>
<td>2.7-5.3</td>
<td>0.65-1.3</td>
<td>5.2-10.4</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td><strong>Sub-total</strong></td>
<td></td>
<td><strong>469.5-589.0</strong></td>
<td><strong>67.8-80.9</strong></td>
<td><strong>15.2-21.8</strong></td>
<td><strong>13.1-14.5</strong></td>
<td><strong>24.4-29.6</strong></td>
<td><strong>2.8-4.0</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>725.9-996.1</strong></td>
<td><strong>118.8-181.6</strong></td>
<td><strong>17.4-25.5</strong></td>
<td><strong>19.3-25.5</strong></td>
<td><strong>52.6-64.9</strong></td>
<td><strong>3.1-5.8</strong></td>
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</table>
Table 2: Nutritional information (per 100g) for *ad libitum* meals (Investigations 1 & 3).

<table>
<thead>
<tr>
<th>Meal</th>
<th>Kcal</th>
<th>CHO</th>
<th>Fat</th>
<th>Protein</th>
<th>Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato &amp; Mozzarella Pasta Bake</td>
<td>127</td>
<td>18.2</td>
<td>3.5</td>
<td>4.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Spaghetti Bolognaise</td>
<td>125</td>
<td>11.5</td>
<td>5.7</td>
<td>6.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Vegetable Biryani</td>
<td>131</td>
<td>20.4</td>
<td>3.5</td>
<td>3.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 3: Nutritional information for Meal Tolerance Test (Investigation 2)

<table>
<thead>
<tr>
<th>Product</th>
<th>Energy</th>
<th>CHO</th>
<th>Fat</th>
<th>Protein</th>
<th>Sugar</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ensure Plus (220ml)</td>
<td>330</td>
<td>44.4</td>
<td>10.8</td>
<td>13.8</td>
<td></td>
<td></td>
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<tr>
<td>Kellogg’s Nutrigrain (37g)</td>
<td>120</td>
<td>24</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>450</td>
<td>68.4</td>
<td>13.8</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C: The Visual Analogue Scale questions used in the study

How hungry are you right now?

________________________________________________________________________
Not at all                                                        Very

How pleasant would it be to eat right now?

________________________________________________________________________
Not at all                                                        Very

How much do you think you could eat right now?

________________________________________________________________________
Not at all                                                        A lot

How full do you feel right now?

________________________________________________________________________
Not at all                                                        A lot

How anxious do you feel right now?

________________________________________________________________________
Not at all                                                        A lot
How sick do you feel right now?

__________________________________________
Not at all       A lot

How much stomach discomfort have you experienced in the last hour?

__________________________________________
Not at all       A lot

How much flatulence have you experienced in the past hour?

__________________________________________
Not at all       A lot

How sick have you felt in the past hour?

__________________________________________
Not at all       Very

How much diarrhoea have you experienced in the past hour?

__________________________________________
Not at all       A lot
NAME: __________________________________________

DATE: _____________________

This record is designed to obtain accurate information about the type and quantity of food that you eat.

Please answer the General Question section and then go on to the Food Record.

Please return to: Nicola Guess on your next visit
Tel: 07951 252395
GENERAL QUESTIONS

Which type of bread do you usually eat?

- White
- Brown/Hovis
- Granary
- Wholemeal
- None

Do you usually buy large or small loaves, sliced or unsliced?

- Large
- Small
- Sliced
- Unsliced

If you eat any type of biscuit regularly, please specify which brands?

__________________________________________________________________

__________________________________________________________________

Which type of milk do you usually use?

- Full cream milk (blue top)
- Semi-skimmed milk (green top)
- Skimmed milk (red top)
- None

How much milk do you usually use?

- 1-2 pints daily
- ½-1 pint
- ¼-½ pint
- None
How many tablespoons of milk do you take in tea and coffee?

_____ tablespoons milk in a cup of tea

_____ tablespoons milk in coffee

_____ None.

Which kind of fat do you usually use on bread, crispbreads etc?

- Butter
- Margarine
- Low fat spread

Which brand do you usually use? ___________________

What do you do with the visible fat on your meat?

- Eat most of the fat
- Eat as little as possible
- Eat some of the fat
- Don't eat meat

How often do you eat food that is fried?

- Daily
- 1-3 times/week
- 4-6 times/week
- Less than once/week

Do you drink alcoholic drinks?

YES ☐ NO ☐

If the answer is Yes, please indicate how many units you drink per week?

1 unit = ½ pint beer/lager
1 glass wine,
1 tot spirit.

_____ units per week.
FOOD RECORD

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

We would like you to record, as accurately as possible, what you eat and drink for 3 days. The first day should be a Sunday, so keep the record for Sunday, Monday and Tuesday.

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

You should include all meals and snacks, plus sweets, drinks etc. When recording food eaten at meals, please include any sauces, dressing or extras eg: gravy, salad dressing, pickles, as well as the main food.

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

**Guidelines for describing food & drink:**

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

2. Give as many details as possible about the type of food you eat:
   
   a) **State brand name where applicable**

      eg: 'Princes' sardines in tomato sauce OR 'Sainsburys' half-fat Edam cheese.

   b) **Name the type of biscuit, cake or cereal**

      eg: Rich Tea, Madeira, Branflakes.

   c) **Name the type of cheese, fish or meat**

      eg: Cheshire cheese, haddock fillet, pork chop.
3. Suggestions for recording quantity of food and drink:

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

   ![Diagram](Level)
   ![Diagram](Rounded)
   ![Diagram](Heaped)

b) All convenience foods have their weight on the packaging and this can be quoted
   
   eg: 150g carton Ski raspberry yoghurt OR
        ½ 15 oz can baked beans.

c) Bread, fruit loaves etc. Indicate the size of the loaf and the thickness of the slice
   
   eg: 1 thick slice granary bread, small loaf.

d) Cheese, fish, meat. When possible, please weigh your portions of these foods. Otherwise describe as well as you can.
   
   eg: 2 large thin slices ham OR
        2 small lamb chops (no fat eaten) OR
        Medium fillet of cod grilled with 1 tsp flora OR
        Cube of cheddar cheese the size of a matchbox.

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

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

**THANK YOU VERY MUCH FOR YOUR HELP**
DIETARY RECORD SHEET - EXAMPLE

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

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

<table>
<thead>
<tr>
<th>MEAL/SNACK</th>
<th>QUANTITY EATEN</th>
<th>DETAILS OF FOOD &amp; DRINK</th>
<th>Leave Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early Morning:</strong></td>
<td>1 cup 1 tbsp</td>
<td>Tea with Skimmed milk</td>
<td></td>
</tr>
<tr>
<td><strong>Breakfast:</strong></td>
<td>3 heaped tbsp 1/4 pint 1 medium slice 1 tsp 2 mugs</td>
<td>Branflakes (Kellogg's) Skimmed milk for cereal &amp; drinks Wholemeal bread (large loaf) Flora extra light margarine Coffee</td>
<td></td>
</tr>
<tr>
<td><strong>During Morning:</strong></td>
<td>1 mug 1 tbsp 1 medium</td>
<td>Coffee with skimmed milk Apple (eaten with skin)</td>
<td></td>
</tr>
<tr>
<td><strong>Midday:</strong></td>
<td>4 slices 4 level tsp 2 thin slices 1 large 1 large 1 can (330ml)</td>
<td>Sandwiches: wholemeal bread (Allinsons) large loaf, sliced Flora extra light margarine Ham (no fat) Tomato Banana Diet Tango</td>
<td></td>
</tr>
</tbody>
</table>

DAY: Example       DATE: 1st June 1994
<table>
<thead>
<tr>
<th>MEAL/ SNACK</th>
<th>QUANTITY EATEN</th>
<th>DETAILS OF FOOD &amp; DRINK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>During Afternoon:</strong></td>
<td>1 glass</td>
<td>Low Calorie squash made with concentrated squash</td>
</tr>
<tr>
<td></td>
<td>25g pkt</td>
<td>KP roasted salted peanuts</td>
</tr>
<tr>
<td><strong>Evening Meal:</strong></td>
<td>4 heaped tbsp</td>
<td>Chicken &amp; mushroom casserole (home-made with skimmed milk in the sauce)</td>
</tr>
<tr>
<td></td>
<td>1 apple sized</td>
<td>Jacket potato</td>
</tr>
<tr>
<td></td>
<td>3 tbsp</td>
<td>Broccoli, boiled</td>
</tr>
<tr>
<td></td>
<td>1 x 150g tub</td>
<td>Shape raspberry yoghurt</td>
</tr>
<tr>
<td></td>
<td>1 glass</td>
<td>Half mineral water/half natural orange juice</td>
</tr>
<tr>
<td></td>
<td>1 cup</td>
<td>Tea with skimmed milk</td>
</tr>
<tr>
<td><strong>During Evening:</strong></td>
<td>1 mug</td>
<td></td>
</tr>
<tr>
<td><strong>Bedtime Snack:</strong></td>
<td>1 tsp</td>
<td>Ovaltine made with:</td>
</tr>
<tr>
<td></td>
<td>½ mug</td>
<td>Ovaltine</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>ordinary silver top milk, the rest water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rich Tea biscuits (Sainsburys).</td>
</tr>
</tbody>
</table>
DIETARY RECORD SHEET

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

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

DAY 1: ______________________   DATE: ______________________

<table>
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</thead>
<tbody>
<tr>
<td>Early Morning:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During Morning:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Midday:</td>
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<tr>
<td>MEAL/ SNACK</td>
<td>QUANTITY EATEN</td>
<td>DETAILS OF FOOD &amp; DRINK</td>
<td>Leave Blank</td>
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<td>-------------</td>
</tr>
<tr>
<td>During Afternoon:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening Meal:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>During Evening:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bedtime Snack:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DIETARY RECORD SHEET

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

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

DAY 2: __________________________ DATE: _____________________________

<table>
<thead>
<tr>
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<th>DETAILS OF FOOD &amp; DRINK</th>
<th>Leave Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Morning:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>During Morning:</td>
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<td>Midday:</td>
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<tr>
<td>MEAL/SNACK</td>
<td>QUANTITY EATEN</td>
<td>DETAILS OF FOOD &amp; DRINK</td>
<td>Leave Blank</td>
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<tr>
<td>During Afternoon:</td>
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<tr>
<td>Evening Meal:</td>
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<td>During Evening:</td>
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<tr>
<td>Bedtime Snack:</td>
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</table>
**DIETARY RECORD SHEET**

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

Record method of cooking, type and quantity of food

*eg:* 6 tbsp boiled wholemeal spaghetti
2 egg sized roast potatoes.

DAY 3: ___________________________ DATE: _____________________________

<table>
<thead>
<tr>
<th>MEAL/ SNACK</th>
<th>QUANTITY EATEN</th>
<th>DETAILS OF FOOD &amp; DRINK</th>
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<tbody>
<tr>
<td><em>Early Morning:</em></td>
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<tr>
<td><em>Breakfast:</em></td>
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<td><em>During Morning:</em></td>
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<tr>
<td><em>Midday:</em></td>
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<tr>
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<td>-------------</td>
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<tr>
<td><strong>During Afternoon:</strong></td>
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<td><strong>Evening Meal:</strong></td>
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<td><strong>During Evening:</strong></td>
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<tr>
<td><strong>Bedtime Snack:</strong></td>
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</table>

*dietrec.doc/c:diets/JUN02*
Appendix E: Description of measures of glycaemic variability

**Mean Amplitude of Glycaemic Excursions (MAGE):** The MAGE is calculated using the formula as the mean height of excursions (greater than 1 SD).

**Continuous Overlapping Net Glycaemic Action (CONGA):** CONGA is calculated by determining the difference between values at different set intervals, and the difference is then applied to the CONGA formula.

**Mean of Daily Differences (MODD):** The mean of daily differences (MODD) formula is calculated as the average of the difference between values on different days but at the same time.

**Low Blood Glucose Index (LBGI) and High Blood Glucose Index (HBGI):** The LBGI and HBGI formulae are implemented by converting glucose values into risk scores. If the glucose risk score is below 0, then the risk is labeled as LBGI, and if it is above 0, then it is labeled as HBGI.

**Average Daily Risk Ratio (ADRR):** ADRR is calculated by transforming each glucose value using a formula and then attributing a risk value to the transformed point.