Measuring appetite and food intake; the effects of acute fibre exposure on biological, psychological and behavioural measures.

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by

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Declaration

No portion of this work has been submitted in support of any other application for degree or qualification at this or any other institute of learning.

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Abstract

Background: Obesity action plans have highlighted the need to develop satiating products to reduce food intake and encourage individuals to make healthier choices, particularly in relation to food and diet. Fibre supplements have been shown to reduce appetite however current data is equivocal on a range of fibres tested due to inconsistencies in the methodology. While some fibres can reduce energy intake their precise effects on appetite and specific modes of action have seldom been tested. Drinks designed to maximise satiety were explored to identify the optimal combination of satiating ingredients and investigate their consumer acceptability. ***Methods:*** a combination of methods including a systematic review, laboratory studies and a questionnaire were used to address the aims. **(Chapter 3):** A systematic review explored the acute effects of fibre on appetite and food intake. **(Chapters 4/5):** Explored the preload study design; specifically, the preload formulation and *ad-libitum* test meal in a laboratory study. **(Chapters 6/7):** Explored the different modes of action of fibres in isolation/combination on biological markers, appetite and food intake in the laboratory. **(Chapter 8):** an online questionnaire explored consumer perceptions of fibre related health claims. ***Key Findings:*** *Aim 1: Identify the specific fibre types/doses that reliably increase satiation and satiety* **(Chapter 3):** a systematic review identified fibres with viscous properties were more effective at reducing appetite and fibres with fermentable properties were more effective at reducing energy intake. The most effective fibres and dosage were identified as inulin and b-glucan. *Aim 2: Assess the optimal appetite study design*. **(Chapter 4):** A BMI Scaled preload was most appropriate to detect an effect of fibre on appetite and food intake in participants who are obese. **(Chapter 5):** A limited variety buffet meal detected the effects of a fibre preload on appetite and food intake in obese participants. Increasing variety in a buffet meal increased food intake in all participants, with both normal weight and obese participants compensating for the increased intake. *Aim 3 Assess the influence of different fibres physical properties on satiation and post meal satiety to explore their modes of action* **(Chapter 6):** Viscous fibre did not increase satiety in the immediate post-prandial period, but an effect on hunger was demonstrated 7 h after the preload for b-glucan and inulin in isolation, with markers of fermentation showing similar effects. *Aim 4 Explore any potential synergistic effects for fibres with different physiochemical properties on appetite and food intake* **(Chapter 6/7)**: There was an additive effect for appetite and biomarkers, with biomarkers increasing at an earlier timepoint when fibres were combined. Food intake was reduced in a combined fibre condition but this effect did not significantly increase beyond the combined effects of each fibre in isolation, suggesting no enhanced effects on food intake. *Explore consumer perceptions of drinks carrying fibre related health claims.* (**Chapter 8):** Health claims did not significantly influence drink choice; but significantly affected perceptions of the drinks, personal factors such as motivation to eat for health and weight management predicted those perceptions. ***Implications*:** This thesis increases the understanding of the acute effects of fibre on appetite and food intake, identifying new fibre combinations to reduce appetite and food intake. It improves methodology which will help to improve research to identify more products to help reduce appetite and food intake. This research also explores fibre related health claims and evaluates how such health claims are perceived, to help develop strategies to improve consumer acceptance.

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Glossary of Acronyms

BMI	Body mass index
CKK	cholecystokinin
EFSA	European Food Safety Authority
EI	Energy Intake
FDF	Food and Drink Federation
FOS	Fructooligosaccharides
ITF	Inulin Type Fructans
NW	Normal weight
OB	Obese
OW	Overweight
PYY	Peptide YY
SCFA	Short Chain Fatty Acids
SSS	Sensory Specific Satiety
WHO	World Health Organisation

Chapter One

1. Appetite and Fibre; a Review of the Literature

This literature review begins by examining obesity and its adverse health consequences, establishing that there is a need to address the global epidemic. The science underpinning appetite regulation is explored looking at the complex behavioural, psychological, physiological and metabolic interactions involved in appetite regulation. The classification of fibre and its different physiochemical properties are discussed to explore how their different modes of action may work together to reduce appetite. Fibres which warrant further investigation are identified and reviewed. Nutrition and health claims are discussed and the importance of the preload study design in substantiating claims related to appetite and food intake control are highlighted. The gaps in knowledge that this thesis seeks to address are identified and the chapter concludes with an explicit statement of the aims and objectives of this thesis.

1.1 Defining obesity and weight status

Obesity can be defined as “as abnormal or excessive fat accumulation that presents a risk to health” (WHO, 2017). Adult weight status is defined using BMI, a person’s weight (in kilograms) divided by the square of their height (in metres) to provide a numerical index that can be used to ascertain which category of body mass an individual falls in to. Established cut off points for BMI categories exist (WHO, 2017) and are summarised in Table 1.1. These cut-off points are based on associations between chronic disease and mortality (WHO, 2017).

Table 1-1 BMI categories and cut-off points

Classification	BMI range
Underweight	< 18.5kg/m^2
Healthy weight	18.5 kg/m^2 – 24.9 kg/m^2
Overweight	25.0 kg/m^2 – 29.9 kg/m^2
Obese	30.0 kg/m^2 – 40.0 kg/m^2
Morbidly obese	> 40 kg/m^2

1.1.1 Obesity: The Scale of the Problem

Obesity has become a worldwide epidemic within developed countries. According to WHO (2016), more than 1.9 billion adults 18 years and older, were overweight of these over 650 million were obese. In 2017, UK figures indicated 62% of women and 67% of men were overweight or obese, with obesity prevalence reaching 27% for women. It has been predicted that 60% of the U.K population will be obese by 2050. Obesity has many health consequences and has a large-scale impact on society as a whole. Obesity is a risk factor for serious conditions, such as type 2 diabetes, coronary heart disease, stroke and types of cancer (WHO 1998). In 2015/16 there were 525,000 admissions in NHS hospitals where obesity was recorded as a factor (NHS England, 2017). Moreover, 44% of the burden of ill health associated with obesity is made up from diabetes, 23% from ischaemic heart disease, and between 7% up to 41% from cancer (WHO, 2008 – 2013). Each year it is estimated that around 3 million adults die due to complications of obesity and being over-weight.

The growing prevalence of obesity worldwide has led to an urgent need to determine the factors that cause obesity to try to tackle the problem head on. The rise of marketed fast foods and sedentary lifestyles the so called “obesogenic environment” is often linked to the increase in obesity prevalence. Obesity is a complex condition with many interacting factors that may play a part in its prevalence including psychological, genetic, environmental and economic factors. Although the aetiology of obesity has been established as highly complex, this can be summarised as the

result of energy imbalance over time. If energy intake via food and drink is greater than the amount of energy expended a positive energy balance occurs which, if persistent, leads to weight gain and ultimately overweight and obesity.

1.1.3 Obesity addressing the problem

Over the years, a variety of approaches have been developed to try to tackle obesity from diets and pharmacological weight loss agents to more extreme forms of surgery. The long-term efficacy and safety of these diets, drugs and radical surgery has been questioned and many uncertainties remain. Due to the ineffectiveness and associated adverse side effects there has been a move towards investigating dietary components as a means of identifying potential products that could impact on appetite. This has led to an increased interest in functional foods. Functional foods are foods that have a positive effect on health beyond basic nutrition (Nicoletti, 2012).

1.1.3 Reformulating foods – Identifying Ingredients

In 2018 Public Health England announced a government strategy aimed to challenge the food industry to reduce calories in products consumed by 20% by 2024. Under pressure from the government food companies attempt to improve how foods are formulated and invest in research to investigate dietary components as a means of identifying potential products that could not only reduce calorie content but also improve health. Fibre is one such ingredient due to its physiochemical properties can be used in a variety of ways not only to increase fibre content but can be used to replace of some sugars and to improve the textural and sensual qualities when fat content is reduced (Yang et al., 2017). There is a well-established body of research into the many health benefits of dietary fibre such as improving digestive health (Carlson, Erickson, Lloyd, & Slavin, 2018), reducing cholesterol, glycaemia (Cassidy, McSorley, & Allsopp, 2018) and improving diabetes control (Pick et al., 1996). Dietary

fibre has also been shown to help reduce appetite and regulate body weight (Alviña & Araya, 2016).

Advice to increase the intake of dietary fibre from grains, vegetables, and fruits has become part of current nutritional policies and recommendations around the world. In 2015 the Government Scientific Advisory Committee increased the recommended daily amount (RDA) for dietary fibre in England (for men and women aged 19 and over) from 24g/day to 30g/day. Just 9% of the adult population in England currently meet the recommended 30g/day, on average men consume 71% and women 60% of the recommended amount of fibre, respectively. Interestingly individuals who are normal weight consume more fibre than their age and height matched overweight/obese counterparts (Davis et al., 2006). Reformulation of foods to improve the healthfulness can help consumers achieve RDAs and can remove the barriers to access healthy foods for all populations including low-income, rural, vulnerable and minority populations. Given the extensive scientific evidence that corroborate the multiple and varied health benefits of dietary fibre, and the risks associated with a diet that lacks fibre, the optimisation of fibre within our diets represents an important public health strategy which could help to not only reduce obesity but also improve overall health.

1.2 Appetite

1.2.1 Satiety and Satiation

To help develop targeted approaches to obesity such as food reformulation it is important to understand the processes that underpin the control of human appetite. Human eating is organised into discrete eating episodes i.e., meals and snacks. Satiation refers to a feeling of fullness which develops over the course of a meal and marks the end of an eating episode. Satiety is the absence of hunger between meals.

These eating episodes are underpinned by a number of factors broadly divided into the interaction between the psychological cues (sight, smell and recognition) and peripheral physiological signals (adipose tissue, pancreas, and the gastrointestinal tract) (Berthoud, 2006; Yeomans et al., 2004). Increasing satiety and satiation with functional foods such as fibre has the potential to reduce appetite and food intake.

1.2.2. Appetite Expression

Appetite is the instinctive physical drive for the search, choice and ingestion of food. Appetite encompasses various aspects of eating patterns, choices of high fat or low-fat foods, energy density of foods consumed, variety of foods, palatability and variability in food intake. Traditional models of appetite control suggested food intake was regulated by two complementary drives; the homeostatic pathway, this evolved to defend against an energy deficit by increasing motivation to eat. In contrast, hedonic or reward-based regulation was thought to override the homeostatic pathway during periods of relative energy abundance by increasing the desire to consume foods that are highly palatable (Lowe & Butryn, 2007; Halford et al., 2004). The internal homeostatic regulation of energy was able to cope with a daily fluctuation in food intake, however homeostatic regulation was less sensitive to overconsumption therefore it allowed for weight gain much more readily than weight loss (Berthoud, 2006).

1.2.3 Hedonic Hunger

Studies investigating the hedonic control of appetite have focused on environmental appetite-stimulating factors such as advertising (Halford et al., 2004) and increasing readily available high calorific, palatable food (Gillis & Bar-Or, 2003) which were thought to be responsible for the increase in obesity. These external cues encourage us to increase food intake, consume more calories leading to weight gain. External

cues often override the homeostatic mechanisms that tell us when and how much to eat (Yeomans et al., 2004). Hedonically driven motivation to eat has been extensively associated with obesity, with the odds of being obese approximately doubling for each unit increase on the power of food scale (G. Ribeiro et al., 2018).

1.2.4 3-System Integrated Model of Appetite

Traditional models of appetite control such as the dual-factor model of obesity proposed by Lowe and Levine (2005) have emphasised food intake is driven by these homeostatic and hedonic factors which act both independently and in combination. More recently the distinction between these homeostatic and hedonic systems has been abandoned in favour of a more comprehensive model for appetite control that integrates cognitive, homeostatic and reward mechanisms which are modulated by metabolic signals (Higgs et al., 2017) (see Figure 1-1). The neural control of eating involves activity in brain circuits that process signals of nutritional state and food reward value. When food is consumed the incentive value of food reduces, which is reflected in decreased activity in reward-related brain areas (Higgs & Spetter, 2018; Thomas et al., 2015). Evidence suggests everyday appetite control is influenced by higher level neurocognitive processes involving memories, attention, expectations, and evaluations about food and the consequences of eating (Higgs, 2016). It has been suggested that alterations in these higher cognitive functions via metabolic signals may also have indirect effects on food reward processing (Thomas et al., 2014).

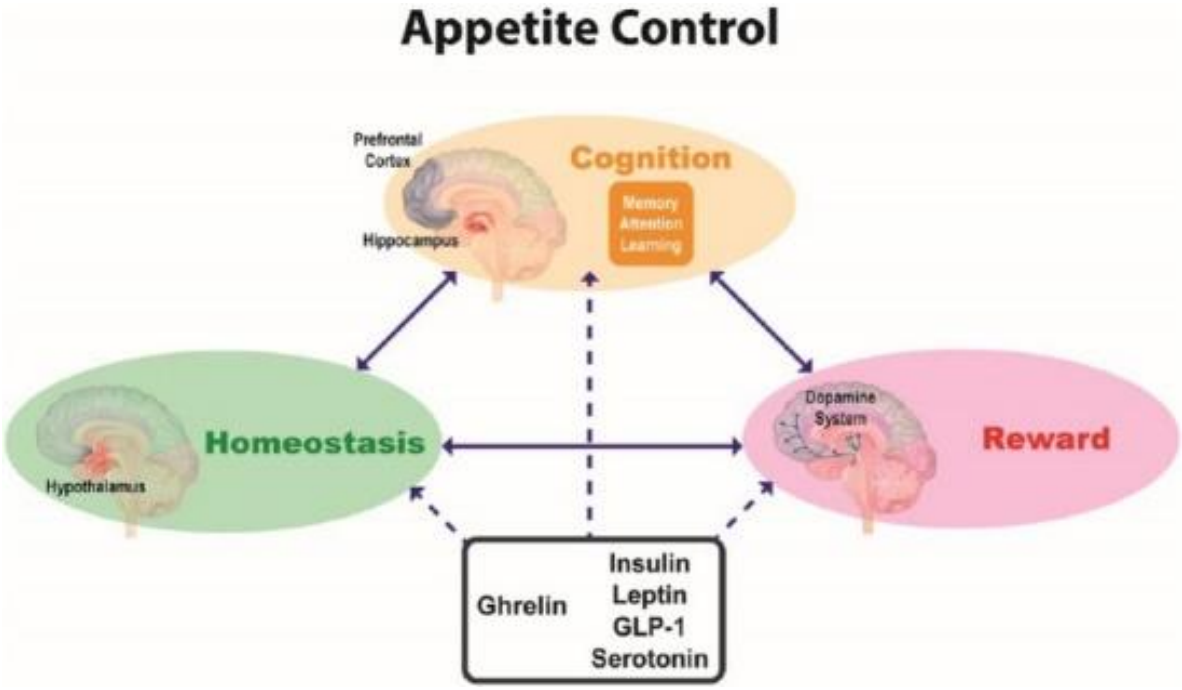

Figure 1-1 Diagram outlining a model of appetite control involving interactions between homeostatic, reward and cognitive processes (indicated by solid arrows) and the modulation of these processes by metabolic signals (indicated by dashed arrows) (Higgs et al., 2017).

1.2.5 The Satiety Cascade

The 'satiety cascade' describes a series of behavioural and physiological events that occur following food intake and that inhibit further eating until the return of hunger signals (Blundell & Halford 1994) (see Figure 1-2). They describe the processes that interact between the drive to eat and the satiating capacity of food on 3 levels; 1) psychological (hunger, perception and hedonic sensations) and behavioural events (meals, snacks and energy intakes); 2) peripheral physiology and metabolic events; 3) neurotransmitter and metabolic interactions in the brain.

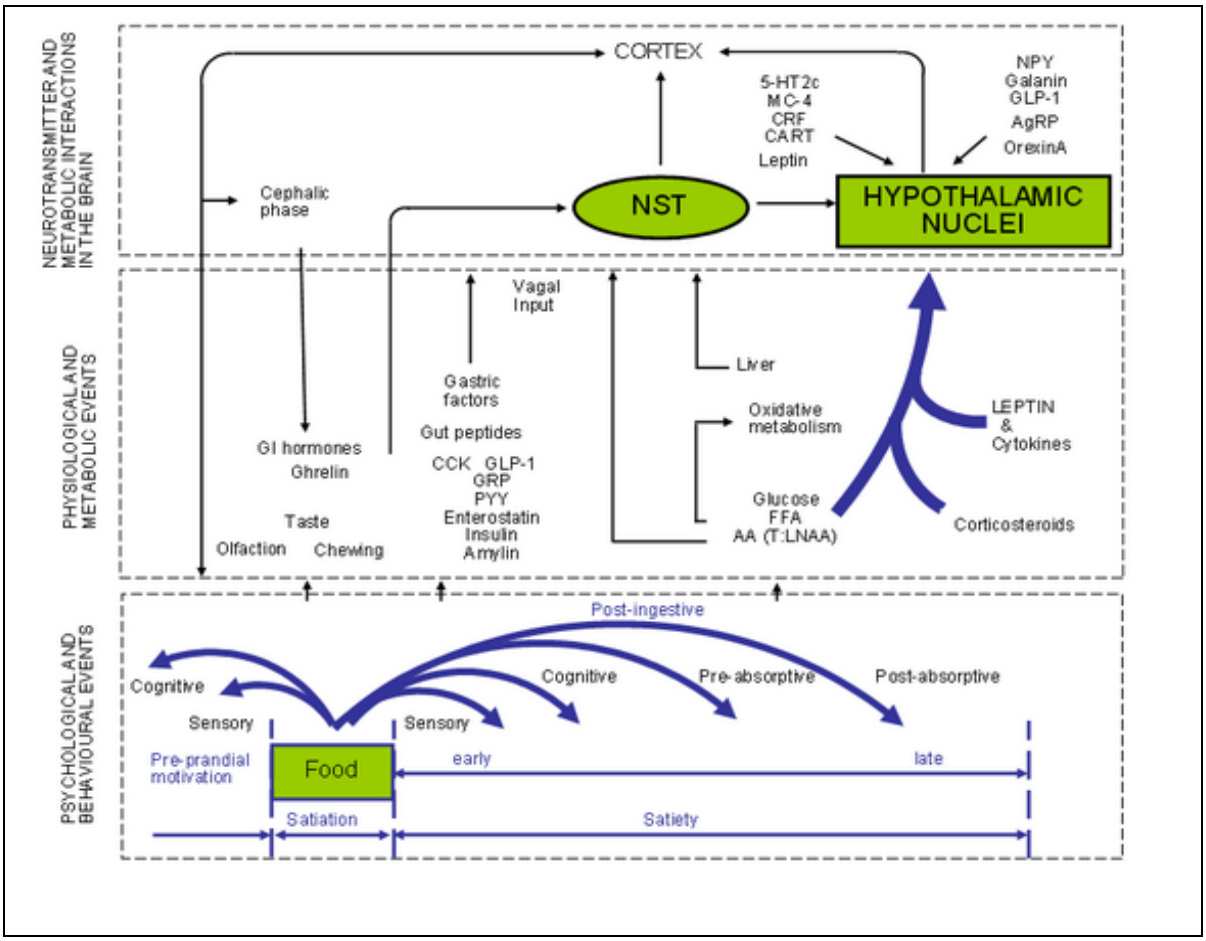

Figure 1-2 (Blundell & Halford, 1994) The satiety cascade - the complex interaction between physiological and psychological factors in eating behaviour.

The lower part of the model (see Figure 1-2) describes complex interaction between physiological and psychological factors in eating behaviour; the cascade is divided into 3 processes pre-prandial, post-ingestive and post-absorptive. Eating episodes are driven by psychological experiences which stimulate (pre-prandial motivation) and inhibit eating. Post-ingestive satiation brings the episodes of eating behaviour to an end. It is this state of satiation which eventually inhibits further eating (Blundell, 1991).

1.2.6 Sensory Cognitive effects

There is a temptation to ignore sensory and cognitive measures of satiety in favour of more advanced physiological measures, however subjective ratings of satiety can provide a valuable tool when evaluating the effectiveness of food ingredients (Sorensen et al., 2003). Initial sensory cognitive effects may impact on satiety even

before food has touched the mouth; this is more commonly known as the cephalic-phase of appetite. The cephalic-phase anticipates the ingestion of food, responses are then generated in many parts of the gastrointestinal tract (Smeets, Erkner, & de Graaf, 2010). Such anticipatory signals are mainly determined by sight and smell, recognition and previous memories for liking the taste. Food intake is positively related to sensory pleasantness (C. De Graaf, De Jong, & Lambers, 1999) and dietary variety (Raynor & Epstein, 2001). Studies show that when food variety increases food intake also increases, this is what is known as the "dessert effect". This phenomenon is caused by sensory-specific satiety, which was defined by Rolls et al, (Meiselman, Degraaf, & Lesher, 2000) as a "greater decrease in the pleasantness of an eaten food than in the pleasantness of an uneaten food". Sensory-specific satiety is an important driver for meal termination and the variety in food choices that humans make from meal to meal and from day to day (Meiselman et al., 2000; H. A. Raynor & Epstein, 2001)

1.2.6 Episodic and Tonic Signals for Appetite Control

Previously satiety signals were distinguished by short and long-term events in the regulation of appetite. However, Blundell (2000) proposed that distinguishing satiety signals in terms of episodic and tonic signals was far more appropriate for studies investigating appetite regulation. Feeding behaviour is controlled by a series of episodic hormonal and neural signals (see Figure 1-3) that derive from the gastrointestinal tract, for example cholecystokinin (CCK) is thought to inhibit eating, whereas, ghrelin initiates eating. Other hormones, such as insulin and leptin, indicate tonic signals which affect energy stores. These signals operate through neural sites and converge mainly on the hypothalamus which forms what is known as the energy homeostasis network (Halford & Blundell, 2000).

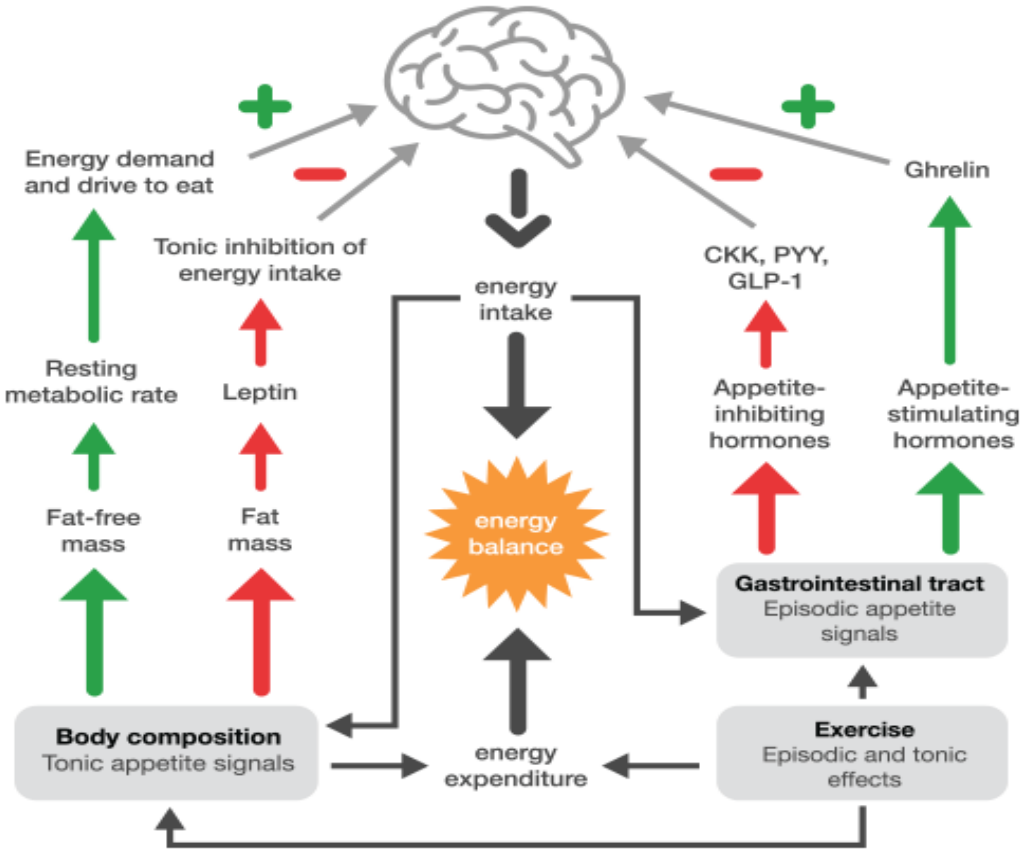

Figure 1-3 (Blundell et al., 2015) Episodic and Tonic signals for appetite control. Green arrows denote processes that stimulate feeding, while the red arrows denote processes that inhibit feeding. Episodic signals arise as a consequence of food consumption while tonic signals arise from body tissues and metabolism. The overall strength of the drive for food is the balance between the tonic excitatory and inhibitory processes. As adipose tissue accumulates in the body, the tonic inhibitory effect of fat on energy intake becomes weaker (due in part to leptin and insulin resistance). Therefore, as weight increases it becomes more difficult to control appetite.

1.2.6. Episodic Signals for Appetite Control

1.2.6.1 Glucagon-like-peptide (GLP)-1

GLP-1 is produced in the small intestine in response to fat and carbohydrates. GLP-1 works in part by increasing the period of post-prandial satiety and slowing gastric emptying (Flint, Raben, Astrup, & Holst, 1998; Naslund, Gutniak, Skogar, Rossner, & Hellstrom, 1998), this slows down the absorption of food in the gut, promoting feelings

of fullness and satiety, and therefore has the potential to reduce food intake (Flint et al., 1998). Blundell and Naslund et al., (1999) found it is unlikely that GLP-1 would affect the termination of the meal (satiation) since most meals are terminated within 20 minutes and GLP-1 reaches peak levels after approximately 60 minutes. This reflects the time it takes for nutrients to reach the ileum, but GLP-1 may contribute to inter-meal satiety and therefore influence eating at a later meal. Intravenous administration of GLP-1 is associated with a dose-dependent reduction in food intake and appetite in both normal weight and individuals who are obese (Tong & Sandoval, 2011; Gillis & Bar-Or, 2003), although (Neff & Kushner, 2010) found that individuals who are obese may be less responsive.

1.2.6.2 Cholecystokinin (CCK).

CCK mediates a number of physiological processes, involved in digestion and satiety. Fat or protein rich chyme entering the duodenum stimulates secretion of CCK by the duodenal and intestinal mucosa. CCK stimulates the gallbladder to contract and secrete pancreatic and gastric acid, this slows down the speed of digestion, so the small intestine can effectively digest the fats which subsequently suppresses energy intake (Little, Horowitz, & Feinle-Bisset, 2005). Along with GLP-1, CCK plays a key role in appetite control; scientific studies show that the two hormones have a synergistic effect working together and are more effective than either hormone alone (Gillis & Bar-Or, 2003).

1.2.6.3 Peptide $_{3–36}$ (PYY)

PYY is released primarily from the colon, and acts as a stimulator on the Y2 receptor in the hypothalamus. This receptor inhibits the release of neuropeptide Y, the most potent stimulant of appetite (Batterham et al., 2002). PYY is thought to be one of the causal agents in the appetite cascade, as Batterham et al., (2002) found PYY exerts

a suppressive effect on food intake. Batterham et al., (2003) found similar promising results as intravenous infusion of PYY in both individuals who were lean and individuals who were obese was shown to suppress 24-hour food intake. Plasma PYY levels were similar to the physiological levels after a meal. This suggests that, unlike leptin, the sensitivity of individuals to PYY is preserved in individuals who are obese. Others have found a blunted postprandial rise in PYY in individuals who are obese suggesting a possible association with impaired postprandial satiety in individuals who are obese (Chaudhri et al., 2008). However, data on the relationship between PYY and appetite are still very limited more research is required before PYY could be utilised as a reliable biomarker of satiety.

1.2.6.4. Amylin

Amylin decreases food intake through both central and peripheral mechanisms and indirectly by slowing gastric emptying (Morley et al., 1994). Amylin works together with insulin to suppress postprandial glucagon secretion (Smeets et al., 2010). Rushing et al., (2000) found chronic infusion of low dose amylin reduced long term food intake and body weight in rats. Consistent with this, in humans the mean basal amylin concentration is higher in individuals who are obese than in individuals who are lean, this suggests reduced amylin secretion may contribute to inadequate appetite regulation and obesity (Halford & Blundell, 2000).

1.2.7 Tonic Signals for appetite control

1.2.7.1 Leptin

Leptin has been regarded as a link between fat mass, food intake, and energy expenditure. The absence of leptin leads to uncontrolled food intake and subsequently obesity. Leptin is associated with long term appetite inhibition, in contrast to the rapid inhibition of eating attributed to CCK (Keim et al., 1998). For

example, leptin concentrations do not change acutely within 3–4 hours in response to meals, and most studies find that there is no relation between leptin concentrations and subjective measures of appetite before and after meals (Keim et al., 1998). Hunger ratings change dramatically after a meal, therefore there cannot be a strong direct relation between hunger ratings and leptin concentrations. Leptin also seems to have a role in the regulation of food intake when energy stores change. Energy deficits of more than a day have been found to lead to decreases in plasma leptin concentrations (Weigle et al., 2003), whereas an energy surplus over the course of a day increased leptin concentrations (van Dielen et al., 2001). Leptin is therefore a suitable long-term biomarker of satiety but cannot serve as a simple short-term biomarker.

1.2.7.2 Ghrelin

The highest concentrations of ghrelin are found in the stomach, and the small intestine. There appears to be a close correspondence between ghrelin concentrations and appetite, however unlike other peptides, ghrelin stimulates rather than inhibits feeding behaviour (Wren et al., 2001). Ghrelin appears to act both in the short term with meal initiation and in the longer term after weight loss, it demonstrates characteristics of both an episodic and tonic signals in appetite control (Cummings, 2006). Studies on ghrelin suggest that it may serve as an excellent biomarker for satiety (Weigle et al., 2003; Wren et al., 2001). Intravenous infusions of ghrelin in nine healthy participants were shown to enhance subjectively rated appetite and to increase energy intake during lunch by 28% (Wren et al., 2003). Interestingly it has been proposed that ghrelin also acts as a compensatory hormone; in individuals who are obese, ghrelin levels would be reduced in an apparent attempt to restore a normal body weight status (Cummings et al., 2002).

1.2.8 Reducing food intake

Controlling appetite is a complex process. Diets low in fats and energy suggested for individuals who are obese are often low satiating, therefore modifications to food structure that specifically increase the satiating effect and limit meal size may be more effective. Individuals who are obese ingest more energy than individuals who are normal weight (Hill et al., 2013) however there is no strong relationship between eating frequency and body weight, therefore a key factor in overconsumption in individuals who are obese could be meal size (Mook, 1992). Declining eating rates and emergence of fullness over a meal defines normal satiation. These changes are attenuated in individuals who are obese, hence slowing eating rate and amplifying fullness may be effective means of weight control (Watanabe, 2020; Hansen et al., 2019). However, others have found a decrease in hunger is not systematically associated with a decrease in intake (Touyarou et al., 2011) and increased satiety and reduction in short term food intake does not necessarily equate to long term weight reduction (Blundell, 1996).

There is also evidence that individuals after weight loss may not respond to modifications to food structure to limit meal size the same as individuals who have not undergone weight loss. The European Commission SATIN (Satiety Innovation) project investigated the acute and sustained effects of foods previously shown to reduce appetite and food intake (inulin and galactooligosaccharides (GOS)) (Andersen, et al., 2020). They found that inulin and GOS did not reduce acute or sustained appetite or improve weight loss maintenance as compared to control products in weight-reduced individuals. This raises the question whether appetite reducing foods are potent enough to impact on physiological mechanisms to such an extent that they counteract the strong biological pressures. This could potentially limit the application of such products for individuals following weight loss. This highlights

the need to understand the biological mechanisms that underpin appetite control, particularly in individuals who are obese or who have undergone weight loss to develop products which are effective at reducing appetite and food intake. Strategies which utilise a combination of cognitive, homeostatic, and reward mechanisms may represent the most promising approach to reduce food intake.

1.2.9 Summary Understanding Appetite Control

Before targeted approaches to reduce intake can be developed it is important to understand the complex mechanisms involved in appetite control. Satiety and satiation were defined along with how eating episodes are organised into meals and snacks. The satiety cascade was outlined to describe a series of behavioural and physiological events that occur following food intake that inhibit further eating until the return of hunger signals. Traditional models of appetite control were described before the more recent 3 factor model of eating was outlined, this is a more comprehensive model for appetite control that integrates cognitive, homeostatic and reward mechanisms. Episodic and tonic signals of appetite were discussed with a focus placed on potential biological markers of satiety, with lean/obese differences highlighted. Reducing appetite and food intake was discussed highlighting targeted approaches through modifications to food structure that specifically increase the satiating effect and limit meal size. Differences in responses for weight reduced individuals and people who are obese were discussed, highlighting the need to understand the biological mechanisms that underpin appetite control further and tailor products accordingly for different populations.

1.3 Measuring Appetite and Food Intake

To establish which ingredients, increase satiety and reduce food intake it is important that researchers and clinicians have access to procedures for the reliable and valid

measurement of the drive to eat, inhibitory processes and mechanisms associated with food consumption (Gibbons et al., 2019). Measuring appetite and satiety is a complex and multifaceted process where satiety and satiation are assessed by a combination of objective (energy intake) and subjective (ratings of appetite-related sensations) measures. Satiety is measured by the magnitude or duration of changes in subjective ratings of appetite-related sensations with or without measurement of energy intake at a test meal using Visual Analogue Scales (VAS) (Hill & Blundell, 1982). Satiation is measured experimentally through the study participant's *ad-libitum* consumption of the food under investigation during an eating occasion. The important distinction when measuring satiety and satiation is that satiety is an estimate, based on the sensory experience of eating, whereas satiation can be measured by meal termination (Benelam, 2009). Because of the large variation in test-meal energy intake between individuals, it is common practice to use cross-over (within-subject) designs in appetite studies, in which the different preloads are fed on separate days and preload order is counterbalanced across participants. Studies which combine these methods to measure short term appetite over part of or during a full day usually follow a preload design (Blundell et al., 2010).

Short-term, single day preload studies have become a cornerstone for appetite research, where short term postprandial effects of an intervention are assessed. The preload study is used to establish scientific evidence to substantiate health claims on food products. Claims relating to appetite and food intake are often difficult to substantiate due to the complex and individual nature of appetite expression. Food intake methodology is therefore particularly important to functional food research.

The preload study design is fraught with methodological issues that need to be considered when designing and interpreting satiety studies (Blundell et al., 2010), (Livingstone et al., 2000). A review by Blundell provided guidance on best practice

for evaluating the impact of a food or ingredient on appetite control, hunger and satiety. (Blundell et al., 2010). Blundell highlights the methodological issues with the preload study design including; *1)* the use of free-living or laboratory studies, *2)* the time interval between preload and subsequent test meal(s), *3)* the use of subjective ratings of satiety, *4)* the formulation of the preload, *5)* the formulation of the test meal(s). All of these arbitrary choices about the type of experiment carried out contribute to the strength of the claim being made.

Addressing the methodological issues is difficult as the experimental design applied will have limitations (Macdiarmid & Blundell, 1998). For example, the use of free-living studies though they increase external validity would reduce internal validity, a trade-off between naturalness and precision must be made (Petty et al., 2013). The precise measure of the laboratory is often chosen for this reason. The time between test meals is often fixed, this in part allows for procedures to be standardised for all participants and allows experimenters more control over the study day. The amount consumed at the *ad-libitum* test meal is commonly used as the outcome measure rather than onset of next meal. Subjective measures of appetite are used in combination with other measures, due to the individual nature of appetite. The results obtained from such studies are therefore the result of the experimental design applied and the derived conclusions must be carefully interpreted.

Preload studies have generated variable outcomes, Blundell suggested a reduction in variance could be achieved with better standardisation of the test preload. Preloads can vary on a variety of dimensions including their physical state (liquid vs solid) (Almiron-Roig et al., 2013), energy content (de Graaf & Hulshof, 1996), macronutrient content (Bellissimo & Akhavan, 2015), energy density (Rouhani et al., 2017), weight or volume (Bell et al., 2003), sensory (Rogers & Shahrokni, 2018) and cognitive

characteristics (McCrickerd et al., 2016; Rolls, 2011; Brunstrom et al., 2010). Where preloads are not matched across conditions, the outcome is likely to be the result of the experimental design applied and not the variable under investigation. For example, Andersen et al., (2020) found there was no effect of inulin on appetite or weight loss in a parallel 12-week study yet the intervention products were not fully matched on energy content, which may have resulted in an over or underestimation of the effect size. This highlights the need to focus on the preload. Blundell (2010) further suggested that a reduction in variance may be achieved by adjusting the preload size according to individual energy requirements using measures such as BMI. Given the differences in appetite control between individuals who are normal weight or overweight creating preloads scaled to BMI and carefully matched to control preloads may improve methodology.

Blundell also identified the nature of the outcome test meal as an important factor in study design. The outcome test meal can have an impact on appetite and food intake as sensory and hedonic factors play an important role in meal termination (Zaremba et al., 2017). A limited choice meal is often favoured in studies measuring food intake (Gibbons et al.,2019). However, a limited item meal is at risk of the "portion size effect" as participants who are used to plate clearing finish the entire meal (Hetherington & Blundell-Birtill, 2018). Conversely, the multi item meal may encourage overconsumption in test and control conditions through variety as participants eat beyond satiation (Long et al., 2000), this has been linked to hedonic hunger (Espel-Huynh et al., 2018) and sensory specific satiety (Brondel et al., 2009).

One of the main issues with the test meal is that it is difficult to establish valid food selection methodology, consequently this area has not been developed extensively. It is difficult to determine the optimal test meal composition (McCrickerd & Forde,

2016) when this isn't something which has been extensively investigated. This lack of consensus is problematic since variation in this *ad libitum* meal may influence the primary outcome of the studies. Ensuring that the *ad-libitum* outcome meal is sensitive to manipulations is essential in all postprandial appetite studies.

The current thesis will attempt to address the issues with the preload formulation and outcome test meal, to establish if scaling the preload for BMI can improve outcome measures and whether a limited or multi-item outcome meal are more effective at detecting the effect of a preload. In appetite research, the optimal experimental protocol is likely to remain elusive because of the complex and multi-faceted nature of eating behaviour, never the less research should continue strive to improve the experimental protocol.

1.3.1 Summary Measuring Appetite

Measuring appetite is a complex process where a combination of objective (food intake) and subjective measures (appetite via VAS scales) are combined. The single day preload study has become the cornerstone of human appetite research and is used to provide scientific evidence to substantiate health claims. Conflicting data for effects of ingredients on appetite and food intake highlights potential issues with the preload study design which must be addressed. Blundell (2010) identified preload formulation and the test meal as two critical study design elements lacking empirical evidence. The optimal study design will vary with the research questions being addressed but further research is required to attempt to improve the methodology, the current thesis will attempt to address the issues with the preload formulation and outcome test meal to achieve this.

1.4 Classifying Fibre

1.4.1 Dietary Fibre

There have been incongruities between theory and practice that have resulted in confusion over the components that make up dietary fibre (DeVries et al., 2001). This has resulted in changes to the classification of fibre. The UK definition of dietary fibre has been used as a general term for "a complex mixture of substances with different chemical and physical properties which exert different types of physiological effects" (Department of Health 1991). The use of certain analytical methods to quantify dietary fibre by its nature of resisting digestion in the small intestine results in many other indigestible components being isolated along with the carbohydrate components of dietary fibre. Some carbohydrates that can now be included under this definition include oligosaccharides, resistant dextrins and resistant starches. Such components are likely to have physiological effects; therefore, it is more valid to classify fibre as a group of compounds with different physiological characteristics.

Sources of dietary fibres can be whole or isolated from foods into useful fibres with added benefits, these fibres can be added to processed foods and are referred to as functional fibres. Both whole and isolated fibres can be classified in terms of their physio-chemical structure based on solubility, viscosity and fermentability (see Figure 1-4). As the classification and definition of fibre has evolved, methods used to study fibre have also changed.

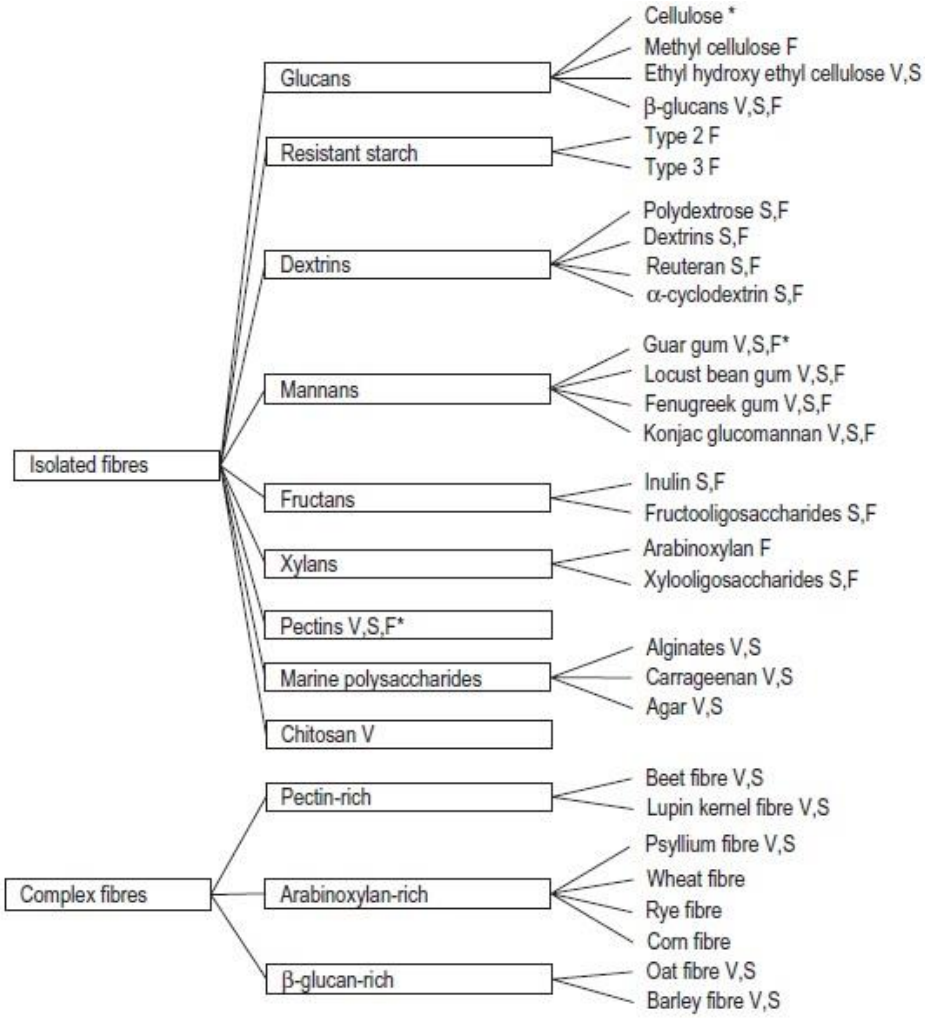

Figure 1-4 Fibres and their assumed physicochemical properties. F, more fermentable fibres; S, more soluble fibres; V, more viscous fibres; *, these fibres are often modified to adjust their physicochemical properties (Wanders, 2011).

1.4.1.1 Soluble/Insoluble Fibre

Many early studies have focused on solubility as the primary difference between fibres. Soluble dietary fibres readily dissolve in water and soluble fibre undergoes active metabolic processing via fermentation that yields end products with broad, significant health effects. Sources include *β-glucan*s, mucilages, psyllium, pectins and gums (see table 1-2). The rate and extent of fermentation in the colon is slower in insoluble fibres than soluble fibres. Sources of insoluble fibre include whole wheat, corn bran, flax seed, lignans, vegetables and potato skins. Study results for soluble and insoluble fibres were often unclear; this suggests solubility does not determine

satiety response. Research has seen a move towards defining fibre in terms of their physio-chemical properties such as fermentability and viscosity.

Table 1-2 Soluble and insoluble fibres

Soluble fibres	Insoluble fibres
Wheat dextrin	Cellulose
β-glucans	Lignin
Gums (e.g., guar gum, partially hydrolysed guar gum)	Some pectins
	Some hemicelluloses
Mucilages (e.g., psyllium)	Sources: wheat bran, some vegetables
Pectins	
Fructo-oligosaccharides	
Some hemicelluloses	
Sources: oat products, legumes (dry beans, peas, lentils)	

1.4.1.2 Viscous fibres

Viscosity refers to the thickness of a solution and its resistance to flow. Viscous fibres such as pectin, psyllium, β-glucan, glucomannan and guar gum have physio-chemical benefits and mix with food and human digesta in the gut, to form a firm soluble food matrix which increases satiety through their gel forming effect. This food matrix increases satiety by reducing post-prandial glycaemia through delaying gastric emptying; this slows transit time through the small intestine (Tomlin, 1995). Viscous fibres also alter blood glucose and cholesterol concentrations (Jenkins et al 1978; Schneeman 1987). Fibre viscosity was once thought to be the main physio-chemical property for enhancing satiety, however other dietary fibres, which do not exhibit gel-forming properties, indicate they are effective in the control of food intake. For example, non-viscous fructans were effective in increasing satiety and reducing food intake (Delzenne et al., 2003). This suggests other physiological mechanisms such as fermentation may explain such findings. Viscous fibres also once lacked the

versatility of non-viscous fibres; guar gum for example could not be added to liquids without affecting consistency. Methods to hydrolyse fibres are now available increasing the versatility, to allow them to be added to liquids and other products without affecting the consistency.

1.4.1.3 Fermentable and non-fermentable fibres

Pectins, *β-glucan*s, gums, inulin, oligofructose, wheat dextrin and resistant starch are dietary fibres that cannot be hydrolysed in the small intestine. They are, however, readily fermented by the microflora in the colon leading to the formation of SCFAs, (propionate, acetate and butyrate) and gases (carbon dioxide, methane, hydrogen). SCFAs are absorbed across the epithelium of the large intestine providing energy for the host (Topping & Clifton, 2001). Activation of receptors by SCFAs appears to evoke release of hormones that control variety of physiological processes such as appetite, gut motility and gastric emptying (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). These are supported by observations that exogenous administrations of several gut peptides such as GLP-1, PYY and CCK induce satiety and reduce food intake (Gutzwiller et al., 2004; Gutzwiller et al., 1999). Furthermore, several reports have associated satiety effects with intake of fermentable fibres in human dietary studies (Cani et al., 2006). Non fermentable fibres such as cellulose, lignin and wheat bran; though they add bulk, reduce calorie density and aid gut motility, do not benefit from the physiological and metabolic processes that increase satiety hormone secretion and modulate gut microbiota in fermentable fibres (J. Slavin & Green, 2007).

1.4.1.4 non-viscous functional fibres

The new UK definition of fibre has increased the number of products which can be classified as fibres. Non-viscous functional fibres (see Table 1-3) are of particular interest to the food industry because of their versatility; they can be added to foods

without affecting the texture or taste, examples include polydextrose, inulin, resistant dextrins, fructans, fructo-oligosaccharides and oligo- or polysaccharides (Chutkan et al., 2012). Resistant starches are often added to foods to reduce calorie content. Resistant starch has ~2 kcal/g compared to normal flour 4 kcal/g (Lockyer & Nugent, 2017; Sajilata et al., 2006). Inulins are hydrolysed into fructo-oligosaccharides which are more soluble and are often used as additive to sweeten low fat yogurts and other dairy products (Roberfroid, 2007).

There are physiological benefits as seen with fermentable fibres between the gut microbiota and functional food components, (Laparra & Sanz, 2010). This has led to an increased interest in this area of research with a wealth of studies indicating such functional fibres increase satiety (Cani et al., 2006; Lyly, et al., 2009; Perrigue et al., 2009) and reduces food intake both in the short term (Bodinham et al., 2010; Hess et al., 2011; Mathern et al., 2009) and long term (Parnell & Reimer, 2009; Pasmann et al., 1997; Pedersen et al.,1997).

Table 1-3 Non-Viscous Functional Fibres

Functional fibres	Description
Resistant dextrins (e.g., wheat dextrin)	Indigestible polysaccharides formed when starch is heated and treated with enzymes; includes resistant maltodextrins
Psyllium	Isolated from husks of psyllium seeds; also known as ispaghula husk
Chitin and chitosan	nondigestible carbohydrate from exoskeletons of crustaceans, e.g. crabs, lobsters; deacetylation of chitin gives chitosan, a nondigestible glucosamine polymer
Fructo-oligosaccharides (FOS)	Short synthetic fructose
Polydextrose and polyols	Synthetic polysaccharides used as bulking agents and sugar substitutes in foods

1.4.1.5 Prebiotic fibre

Prebiotics are a category of nutritional compounds grouped together based on ability to promote growth of specific beneficial (probiotic) gut bacteria. Prebiotics such as inulin and oligosaccharide occur naturally in foods such as leeks, asparagus, chicory, Jerusalem artichokes, garlic, onions, wheat, oats, and soybeans (van Loo, Coussement, de Leenheer, Hoebregs, & Smits, 1995) (see Table. 1.4). Prebiotics are like other carbohydrates that reach the cecum, such as non-starch polysaccharides, sugar alcohols, and resistant starch, as substrates for fermentation, therefore all prebiotic fibres can be classified as fermentable fibres but not all fermentable fibres are prebiotic fibres. Prebiotics are distinctive in their selective effect on the microflora.

Table 1-4 Whole foods containing prebiotics

Food	Prebiotic Fibre Content by Weight
Acacia Gum	85.6%
Raw Chicory Root	64.6%
Raw Jerusalem Artichoke	31.5%
Raw Dandelion Greens	24.3%
Raw Garlic	17.5%
Raw Leek	11.7%
Raw Onion	8.6%
Cooked Onion	5%
Raw Asparagus	5%
Raw Wheat bran	5%
Whole Wheat flour, Cooked	4.8%
Raw Banana	1%

According to Roberfroid's definition, a prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastro-intestinal microflora that confers benefits upon host well-being and health" (Roberfroid, 2000).

Gibson and Roberfroid (1995) specified that to be classified as a prebiotic requires scientific demonstration that the ingredient:

- Resists gastric acidity, hydrolysis by mammalian enzymes, and absorption in the upper gastrointestinal tract;
- Is fermented by the intestinal microflora;
- Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being.

There is an element of disagreement to which fibres fully meet the definition of a prebiotic. In his 2007 revisit of prebiotics, Roberfroid stated that only three fibres fully met this definition: galactooligosaccharides (GOS), oligofructose and inulin (Roberfroid, 2007) (see Table 1-5). Although not regarded by Roberfroid as true prebiotics, other authorities consider *β*-glucan, fructooligosaccharide (FOS) and resistant starch (RS) to meet all aspects of the definition. A number of studies have shown *β*-glucan daily doses of 5-10g have a similar probiotic activity to inulin**,** however they require further research before being formally classified as prebiotics (Kellow et al., 2014). Other isolated carbohydrates and carbohydrate-containing foods, including arabinoxylan (AX), transgalactooligosaccharides (TOS), polydextrose, wheat dextrin, acacia gum, psyllium, whole grain wheat, and whole grain corn have also demonstrated prebiotic effects both physically and in fermentation to SCFAs in the caecum and colon (Slavin, 2013).

Table 1-5 Prebiotic Status (Roberfroid, 2007)

Carbohydrate	Prebiotic status
Inulin and oligofructose	Yes
Galactooligosaccharides	Yes
β-glucan	Considered
fructooligosaccharide (FOS)	Considered
Resistant Starch (RS)	Considered
Isomaltooligosaccharides	No
Lactosucrose	No
Xylooligosaccharides	No
Soybean oligosaccharides	No
Glucooligosaccharides	No
transgalactooligosaccharides (TOS)	No
whole grain wheat	No
whole grain corn	No
Polydextrose	No
wheat dextrin	No
acacia gum	No
Arabinoxylan	No
Lactulose	No

1.4.1.6 Health Benefits of Prebiotics

Prebiotics have many health benefits; although there is still a limited amount of data compared to dietary fibre it is clear that prebiotics deliver many health benefits.

Prebiotics have been found to: -

- Reduce the inflammation and symptoms associated with inflammatory bowel disease (Steed et al.,2008).
- Exert a protective effect to prevent colon cancer. After the ingestion of prebiotics gut flora induces the chemo-preventive enzyme glutathione transferase in the colon, this leads to a reduced load of genotoxic agents in the gut and to an increased production of agents that deactivate toxic components. (Wollowski et al., 2001).
- Enhance the bioavailability and uptake of minerals, including calcium, and magnesium (Coxam, 2007).

- Lower some risk factors for cardiovascular disease via their serum or hepatic lipid lowering properties and exhibit cholesterol or triglyceride lowering effects (Delzenne & Williams, 2002).
- Enhance the immune response by modulating immunological processes at the level of the gut-associated lymphoid tissue (Seifert & Watzl, 2007)
- Promote satiety and weight loss and prevents obesity by modulating gut genes. This increases secretions of satiety gut hormones, releases energy from indigestible components of the diet, releasing SCFAs, increasing gut motility and stimulating the growth of beneficial bacteria (da Silva, dos Santos, & Bressan, 2013).

Although prebiotics confer such health benefits to the host there are some well documented side effects when they are consumed in large quantities. Some prebiotics, such as inulin, are associated with impaired gastrointestinal tolerance (Bonnema et al., 2010), other prebiotic fibres such as wheat dextrin, polydextrose exhibit high gastrointestinal tolerability (30–45g/day) (Pasman et al., 2006). Gastrointestinal intolerance can often create a barrier to the wider acceptance of prebiotics as a functional food (Cummings et al., 2001).

1.4.2 Summary Section Classifying Fibre

Defining and classifying fibre was outlined, discussing some of their physiological benefits and applications. The definition of fibre has changed, fibre is now defined as a group of compounds with different physiological characteristics. Fibres were once classified in terms of their solubility however, other physiochemical properties such as viscosity or fermentability are now focused on. Viscous fibres mix with food and human digesta in the gut, to increase satiety through their gel forming effect. Fermentable fibres are fermented by the microflora leading to the formation of SCFAs,

SCFAs evoke release of hormones to control appetite, gut motility and gastric emptying (Wong et al., 2006). Non-viscous functional fibres are of particular interest to the food industry because of their versatility to be added to foods reducing sugar content; with the added physiological benefits of fermentable fibres. Prebiotic fibre promotes growth of specific beneficial (probiotic) gut bacteria, they have many health benefits including promoting satiety and weight loss. Fibres with different modes of action warrant further investigation, to see if their benefits can be enhanced when fibres are combined.

1.5 Fibre and appetite

Fibre plays a key role in appetite regulation as previous reviews have demonstrated (Howarth et al., 2001; Slavin, 2005; Wanders et al., 2011). Several studies have shown increased fibre intake is associated with increased satiety, reduced food intake and lower obesity rates (Bodinham et al., 2010; Cani et al., 2011; Lyly et al., 2009; Mathern et al., 2009; Perrigue et al., 2009). Other beneficial effects such as reducing the risks of cancers, coronary heart disease, and diabetes are not disputed. However some fibres are more effective than others due to different physio-chemical properties and modes of action (Tomlin, 1995).

Fibre is thought to act as a physiological obstacle to energy intake in several ways (Heaton, 1973) :-

"1) Fibre displaces available calories and nutrients from the diet

2) Chewing promoting the secretion of saliva and gastric juice, which distend the stomach and promote satiety.

3) Fibre reduces the absorptive efficiency of the small intestine."

1.5.1 Fibre and central appetite regulation

Fibre affects central regulation of physiological mechanisms involved in appetite behaviour; these are divided into three main effects intrinsic, hormonal and colonic effects (Howarth et al., 2001) (see Figure 1-5). These mechanisms act to decrease food intake through increasing satiation (reduce energy intake at a meal) or satiety (longer duration between meals) or by influencing metabolic activities (increased fat oxidation and decreased fat storage) (Schweizer & Würsch, 1991). Due to their diverse physio-chemical properties and various bio-behavioural effects more research is required to untangle their specific mode of action to utilise their beneficial effects to help develop satiating lower energy dense products.

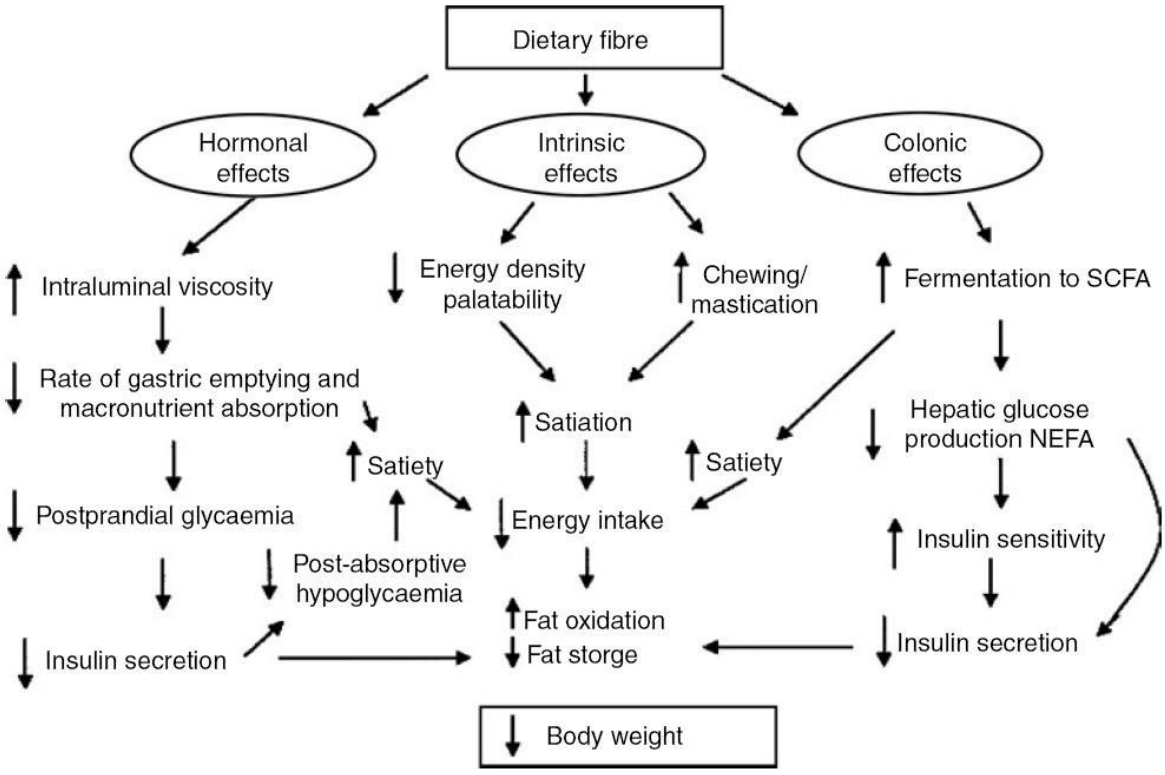

Figure 1-5 (Howarth et al., 2001) Effects of fibre on physiological mechanisms in appetite behaviour.

1.5.1.1 Intrinsic Effects

Texture influences satiety, foods with a chewier texture (e.g. whole grains) have a higher satiating capacity than less dense foods (de Graaf et al., 2004). Flood-Obbagy

and Rolls (2009) compared the satiating capacity of apple juice, apple sauce and whole apples; consuming whole fruit reduced ratings of satiety and food intake during an *ad-libitum* meal more than fruit juice this suggests texture and mouth feel impacts on early satiety signals. Fibre also adds bulk to food, consuming high fibre low calorie dense cereal has been shown to increase satiety and reduce energy intake when compared to low fibre equivalents (Rolls et al., 2005). Highly palatable foods, including those rich in fat and sugar, up-regulate the expression of hunger signals and satiety signals, blunting the response to satiety signals and activating the reward system, which often leads to over consumption (Erlanson-Albertsson, 2005).

1.5.1.2 Hormonal Effects

Dietary fibre promotes satiety and fat oxidation though several hormonal pathways (see Figure 1-6). Fibre intake increases secretions of PYY, CCK and GLP-1 and other factors that are assumed to have beneficial metabolic effects (Reimer & McBurney 1996; Tarini, 2010). Reimer (2012) found that inulin and oligofructose dose-dependently increase satiety hormones in rats; PYY levels were up-regulated, resulting in increased GLP-1.

Studies in this area often utilise animal models, however, equivalent dose cannot be tolerated in humans and leads to gastrointestinal upset (Robertson et al 2005; Robertson et al 2003; Verhoef et al 2011; Weickert et al 2005). Reimer & McBurney (1996) found a reduced dose exposure to highly fermentable resistant starch did not result in any detectable changes in circulating GLP-1 concentrations, despite significant improvement of insulin sensitivity. Often long-term supplementation of fibre is needed to show measurable changes of circulating satiety hormones (Weickert et al 2005). Freeland, (2010) found 9 months continuous supplementation was needed for a change in GLP-1 concentrations to be detected.

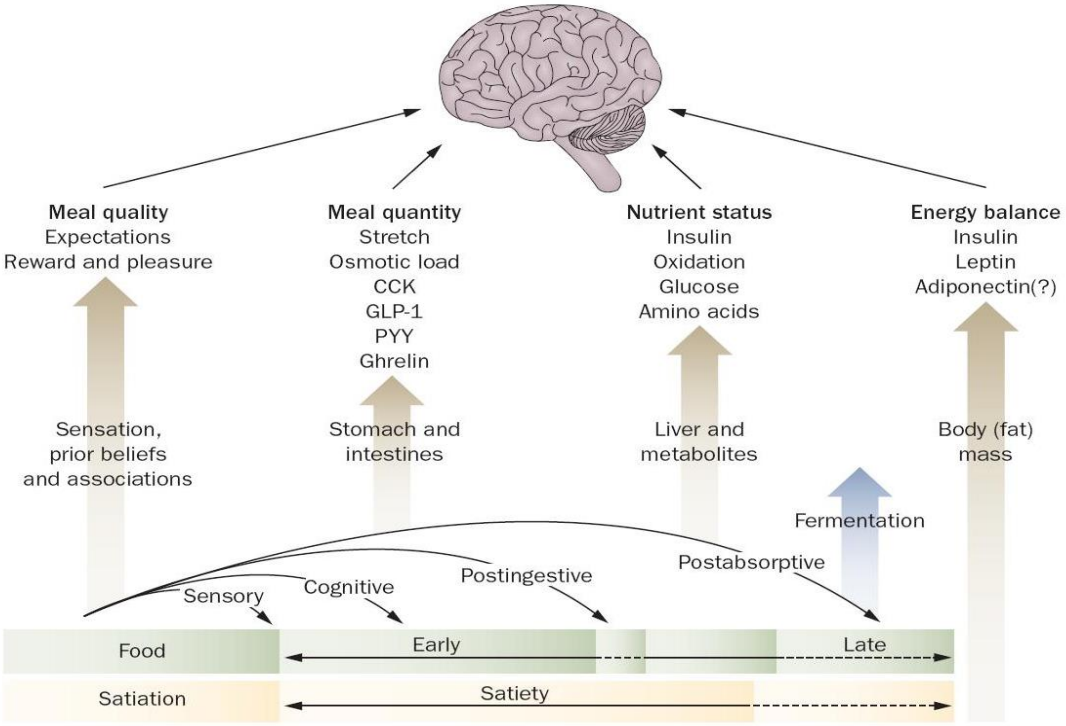

Figure 1-6 The satiety cascade and specific hormone effects

1.5.1.3 Colonic effects

Biologic effects of fibre in the large intestine have implications for hormonal and metabolic processes involved with appetite regulation. Non digestible carbohydrates exhibit different functional attributes; (Topping & Clifton, 2001) including modulation of the gut microbiota, induction of anti-inflammatory effects, reduction of food intake, modulation of bowel habits and regulation of alterations in lipid and glucose metabolism (Weigle et al., 2003). Gut microorganisms and their metabolic products regulate appetite through a series of processes; increasing secretions of satiety gut hormones, releasing energy from indigestible components of the diet, releasing SCFA, increasing gut motility and stimulating the growth of beneficial bacteria (Cani & Delzenne, 2009). The link between gut microbiota and fermentation of non-digestible carbohydrates and gut peptide secretion was proposed (Goodlad et al., 1987). They demonstrated that bulk fibre cannot stimulate fermentation in the colon, but fermentable fibre polysaccharides are susceptible to fermentation. Further to this

they discovered prebiotics can change gut microbiota, increase plasma levels of two gut peptides GLP-1 and PYY and decrease ghrelin.

1.5.2 Gut Microbiota

Gut microbiota have the potential to regulate appetite, influence weight gain and fat deposition through a variety of mechanisms (Weigle et al., 2003). Gut microbiota has an influence on both episodic and tonic signals of appetite regulation. Gut microorganisms and their metabolic products regulate appetite through a series of processes; increasing secretions of satiety gut hormones, releasing energy from indigestible components of the diet, releasing SCFA, increasing gut motility and stimulating the growth of beneficial bacteria (Cani & Delzenne, 2009).

1.5.2.1 Gut Motility

Dietary fibre can hold large quantities of water which can increase stomach distension this may also delay gastric emptying which prolongs the absorption of nutrients. The prolonged presence of nutrients in the small intestine can in turn trigger the release of gut peptides such as CCK, PYY and GLP-1, resulting in increased satiety and decreased food intake (van Dielen et al., 2001).

1.5.2.2 Satiety Hormones

Satiety hormones are released through long term fermentation and oxidation of nutrients by bacteria in the colon this process is known as thermogenesis (Westerterp-Plantenga, 2003). The bacteria in the colon can also be modified over time through diet to increase the secretion of satiety hormones. Parnell and Reimer (2012) found that prebiotic fibres dose-dependently increase satiety hormones and alter gut flora in rats.

1.5.2.3 Short Chain Fatty Acids (SCFAs)

When indigestible food components are fermented in the colon SCFAs are produced. Highly fermentable fibres such as oligofructose and resistant starch produce SCFAs. SCFAs acetate, butyrate and propionate enter the portal circulation and effect glucose homeostasis in a variety of ways. SCFAs regulate appetite as they increase the satiating properties of food and regulate intestinal fat absorption. Butyrate is utilised by enterocytes in the liver and generally regarded as a healthy metabolite as it impairs lipid transport (Westerterp-Plantenga, 2003) acetate on the other hand contributes to lipid and cholesterol synthesis in the liver. These actions alter insulin sensitivity, insulin secretion, metabolic fuels and regulation of satiety (Darzi et al., 2011; Frost et al., 2014).

SCFAs can be delivered directly within food or indirectly via fermentation of non-digestible carbohydrates in the colon. Many studies have reported that the direct administration of SCFAs can prevent or attenuate long-term body weight gain by increasing energy expenditure through increased lipid oxidation (Alia, Aaron, Gary, & Edward, 2019). Ostman et al., (2005) found that oral ingestion of SCFAs was associated with enhanced satiety. Frost et al. (2014) found that in a mouse model acetate, derived from the colonic fermentation of inulin, acts to directly supress appetite through central hypothalamic mechanisms involving changes in transcellular neurotransmitter cycles. However, Darzi et al. (2011) found this may be explained by increased food palatability rather than the physiological effect of SCFAs. Production of short chained fatty acids are also subject to individual variation, dependant on type of fibre, hosts microbiota and gut transit (Frost et al., 2014). SCFAs, delivered indirectly via fermentation of non-digestible carbohydrates in the colon, can take longer to have an effect on metabolic actions or satiety. Hess et al (2011) on the other hand found that increased fermentation and production of SCFA observed over the

short term did not induce satiety. Hydrogen excretion (a by-product of fermentation collected via breath hydrogen to measure fermentation) indicated that fermentation was taking place however this does not indicate that the fibre had undergone complete fermentation. Fermentation may continue for several hours therefore the ability to affect satiety and food intake may not be immediate.

1.5.2.4 Energy Harvest

Microorganisms in the large intestine release energy by fermenting otherwise indigestible components of the diet; commonly referred to as energy harvest. Evidence for the role of gut microbiota in energy harvesting from the diet, came from studies performed in germ-free mice. Cummings (2006) transplanted caecal microbiota from lean and obese mice to germ free recipients and after only two weeks, mice harbouring the microbiota from obese mice had increased adipose tissue and extracted more calories from their food compared to the lean mice having received the gut microbiota from lean mouse donors. This suggests any changes in gut microbiota composition that influence energy expenditure, satiety, and food intake have the potential to alter weight gain and weight loss (Tremaroli, Kovatcheva-Datchary, & Bäckhed, 2010).

1.2.8.5 Beneficial Bacteria

Indigestible components can also stimulate the growth of beneficial bacteria such as bifidobacteria in the colon. Bifidobacteria is thought to be beneficial because it produces lactic acid which increases the acidity of the large intestine and deters further growth of other harmful bacteria. This regulates the microbial balance in the small and large intestines and aids digestion and absorption (Mitsuoka, 1990). The species composition of the gut microbiota changes with diet composition, as has been shown in studies with individuals who are obese after reduced carbohydrate weight

loss diets, or diets containing different non-digestible carbohydrates. Gut microbiota composition also differs between individuals who are obese and individuals who are normal weight, individuals who are obese have fewer bacteroidetes and correspondingly more firmicutes than that of their lean counterparts, suggesting that there could be differences in caloric extraction (DiBaise et al., 2008). However, Weickert et al., (2011) found that even if fibre supplementation changes the dominant groups of gut microbiota, they do not necessarily increase differences between groups in various markers of colonic carbohydrate fermentation including faecal SCFAs and breath hydrogen concentration. Most of these effects are derived from their structural resistance to mammalian digestive enzymes and their ability to stimulate the growth of beneficial bacteria in the colon and to increase SCFA production with diverse biological roles (Rodriguez-Cabezas et al (Parnell & Reimer, 2012).

1.5.3 Candidate Fibres

To identify candidate ingredients to explore in the current thesis it was important to consider how the different physio-chemical properties of the fibre types discussed in this literature review could be exploited to reduce appetite and food intake. A systematic review by Wanders et al. (2011) found that fibres with the greatest number of comparisons showing a reduction in acute energy intake were β-glucan-rich fibres, arabinoxylan-rich fibres, mannans, and resistant starch. When fibres were grouped according to physiochemical properties, more fermentable fibres such as fructans reduced appetite and intake significantly more than non-fermentable fibres. With this in mind the potential to exploit the viscous and fermentable proprieties of different fibres was focused upon and following a systematic review in the current thesis the fibres inulin and β-glucan were identified as target ingredients.

1.5.3.1 Inulin

Inulin, an isolated fibre, is one of the most researched prebiotic dietary fibres. It is extracted exclusively from chicory root and is one of only three prebiotic fibres that fulfils Roberfroid's prebiotic criteria. It is a naturally occurring oligosaccharide belonging to a group of carbohydrates known as fructans. Unlike most carbohydrates, inulin is non-digestible. This allows it to pass through the small intestine and ferment in the large intestine.

Inulin is not simply one molecule; it is a polydisperse β (2—1) fructan. The fructose units in this mixture of linear fructose polymers and oligomers are each linked by β (2—1) bonds. Inulin has a unique structure due to its β (2—1) bonds as a result inulin is non-digestible unlike most carbohydrates (Franck, 2002). Through the fermentation process inulin promotes growth of healthy intestinal micro flora (bifidobacterium), a dietary supplementation of inulin at a dose of 8g per day or greater increases bifidobacteria (Gibson et al., 1995; Kolida et al., 2007; Ramirez-Farias et al., 2009; Rao, 1999; Salazar et al., 2004). Not only does inulin actively promote the growth of existing strains of probiotics in the colon, it has also shown to encourage survival and growth of newly added probiotics (Bezkorovainy, 2001).

Inulin can be added to food or drink as a soluble powder with little impact on the texture whilst adding fibre, inulin displays a slightly sweet flavour, without raising blood glucose levels (Barclay et al.,2010). This makes inulin a very attractive food ingredient for food companies, as it can be used in low calorie products. The sugar content is replaced by inulin which retains the sweet flavour but with additional health benefits. Previous studies have demonstrated inulin can increase satiety (Cani et al., 2006), reduce food intake (Whelan, 2006) and encourage a healthy digestive system (Kolida, 2002). However, some of the research findings for other studies using inulin-

type prebiotics yield mixed results (Howarth, et al., 2001). Review articles summarising the research (Salleh et al., 2019; Clark & Slavin, 2013) have suggested the variable methods, dosage and lack of standardisation in such studies could explain the conflicting data.

The importance of dosage to reduce appetite and food intake has been investigated but results for the dose dependant relationship between inulin, satiety and food intake are mixed. Buckley, Thorp, Murphy, and Howe (2006) added 2, 5 or 10 g of alpha-cyclodextrin to white bread, higher doses resulted in greater satiety. However, Hess et al. (2011) found no difference in satiety with 0g, 5g or 8g of FOS added to hot chocolate twice a day. Further, Genta et al. (2009) found that fibre dose adjusted for BMI (0.14g/kg) decreased food intake and increased weight loss over four months. Conflicting data for inulin dosage warrants further investigation, to see if there could be an effect when an optimal dose is utilised.

1.5.3.2 β-glucan

Inulin has gained considerable interest as a prebiotic in the past 10 years, but new fibres with prebiotic characteristics are being discovered that exhibit the same beneficial effects. β-glucan is a complex fibre and a potential prebiotic compound, they represent 50% of dietary fibres and are present in oats (Hughes et al., 2007) (Vardakou et al., 2008) (Grootaert et al., 2007). In the GI tract, β-glucan acts much like a soluble fibre being fermented by the microflora of the colon. Whereas some studies demonstrated a beneficial effect on satiety (Barone Lumaga, Azzali, Fogliano, Scalfi, & Vitaglione, 2012), others showed no effect (Doyon et al., 2015). Thus, it appears that differences in the properties of β-glucan influence the physiological response. A limitation of existing prebiotics such as FOS and inulin is their rapid fermentation in the proximal colon. β-glucan may be fermented more gradually along

the colon, particularly in the distal colon which could enhance between meal satiety over longer periods. β-glucan can also be modified into shorter fractions so is a versatile potential prebiotic.

β-glucan has the potential to increase satiety immediately after ingestion via viscosity as well as after several hours via fermentation. Lu et al., (2000) found that postprandial glucose and insulin responses were improved by ingestion of β-glucan fibre. Fourteen healthy participants consumed three breakfasts in random order on 3 mornings, 3 days apart, after an overnight fast, containing 0 g, 6 g, and 12 g oat β-glucan. Venous blood was collected at regular intervals over 2 hours and was analysed for glucose and insulin. Compared with the control meal postprandial glucose levels were significantly lower with only 6 g of β-glucan rich fibre supplementation while 12 g produced the greatest benefit.

Garcia et al. (2007) found that oat β-glucan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin responses. Seven female and four male adults with impaired glucose tolerance (BMI 30.1kg/m^2) received either placebo or 15g oat β-glucan supplement for 6 weeks with a 6-week washout period in-between. The oat β-glucan consumption improved postprandial metabolic responses and reduced total ghrelin response indicating there could be an effect on long term appetite control. Lu, Walker, Muir, and O'Dea (2004) also found that a supplement of 15g/day of oat β-glucan can significantly improve glycaemic control in people with Type II diabetes. Fifteen participants with Type II diabetes supplemented their usual diet with control bread and muffins or with β-glucan bread and muffins and completed a 7-day food diary. At 0 and 5 weeks, venous blood was collected for determination of fasting and 2-hour glucose, insulin and blood lipids. Consumption of the β-glucan diet significantly lowered fasting and

2-hour plasma glucose and 2-hour insulin and serum. The mode of action behind β-glucan on improving glucose tolerance is unknown. However, it is thought to be due to the high viscosity inside the GI tract, thereby slowing the rate of glucose absorption.

Summary Section Fibre and Appetite

Fibre affects the central regulation of appetite behaviour through three main effects intrinsic, hormonal and colonic. Intrinsic effects such as texture influences satiety as foods with a chewier texture have a higher satiating capacity than less dense foods. Fibre also adds bulk to food, consuming high fibre low calorie foods increases satiety and reduces energy intake. Hormonal effects are observed as dietary fibre promotes satiety and fat oxidation though increasing secretions of PYY, CCK and GLP-1. Colonic effects regulate appetite through a series of processes; increasing secretions of satiety gut hormones, releasing energy from indigestible components of the diet, releasing SCFA, increasing gut motility and stimulating the growth of beneficial bacteria. Candidate ingredients inulin and β-glucan, with different physio-chemical properties to reduce appetite and food intake were identified. Inulin and β-glucan have properties which could be exploited, to enhance the effects of the fibres in isolation. The effects of these fibres on appetite, food intake and biological markers will be explored in isolation and combined in a preload study.

1.5 Food Reformulation and Health Claims

Health claims are a means for the food industry to communicate health or functional benefits of foods to consumers. Information from nutrition claims, health claims and front-of-pack labels can help consumers to make healthier food choices, to achieve a healthy, balanced diet (Benson et al., 2018). Consumers' interest in healthy eating could be increased by adopting appropriate communication strategies on food packaging (Hung et al., 2019). Health claims are attractive to industry for marketing

foods and may be an incentive to industry to innovate and develop foods with health or functional benefits. The global functional food and drink market was worth an estimated 210 billion pounds in 2020 with the market exhibiting an impressive 11% growth in 2020. Although there are marketing opportunities for functional products the food industry faces balancing what consumers want with what is important from a public health perspective, recognising that there is often a tension between consumers' desire for choice and choice editing by manufacturers. Some of the successful reformulation efforts have been achieved without consumer awareness. Manufacturers often want to retain the characteristics of the product that are attractive to consumers, sometimes making reformulation a costly and time-consuming business.

1.6 Nutrition and Health Claims

A nutrition claim is any message conveyed in text or images about a food product that states, suggests or implies that a food has beneficial nutritional properties, typically the presence or level of a nutrient such as "low fat" or "high in fibre". A nutrition claim simply states the presence of a nutrient it does not link this to any specific health benefit or risk reduction. A health claim similar to a nutrition claim but further states, suggests or implies that health benefits can result from consuming a given food, for instance that a food can "reduce post prandial glycaemic response."

EFSA who regulate EU food claims divide health claims into 2 distinct categories. These include 1) the 'Functional Health Claims' relating to the growth, development and functions of the body**,** psychological and behavioural functions**,** slimming or weight-control. 2) The 'Risk Reduction Claims' Reducing a risk factor in the development of a disease. For example: "Plant stanol esters have been shown to

reduce blood cholesterol. Blood cholesterol is a risk factor in the development of coronary heart disease".

1.6.1 Substantiating Claims

An increasing number of foods sold in the EU bear nutrition and health claims. European health claims are closely regulated as food companies battle for dominance in the consumer market, exaggerating claims to improve marketing and sales is not unheard of. Consumers need to be confident in the products they are consuming that the nutritional or functional health benefit they imply to have are true (Pravst et al., 2018). EFSA is responsible for evaluating the scientific evidence supporting health claims. Union rules on nutrition and health claims have been established by Regulation (EC) No 1924/2006 this came into effect on 1 July 2007. The regulation is a legal framework which not only protects consumers, but also promotes innovation and ensures fair competition. The Commission authorises health claims provided they are based on scientific evidence and phrased without using overly complex scientific wordings, in order to be meaningful for consumers. (Hung, Hieke, Grunert, & Verbeke, 2019). In the UK, claims relating to fat such as 'low in fat' are the most common nutrition claims, while claims relating to the digestive system and the cardiovascular system are the most prevalent health claims (A. Kaur et al., 2016).

1.6.2 Claims specific to fibre

The health benefits of fibre are well documented with UK government policy in place for RDAs, of these benefits there are many health-related claims already discussed here in this literature review which are substantiated by EFSA for specific types of fibre, with new fibres being added as research continues. EFSA has approved claims for dietary fibre for the following claimed effects; normal blood glucose concentrations, normal blood cholesterol concentrations, normal bowel function and regularity,

reduction of postprandial glycaemic response, decreasing potentially pathogenic gastro-intestinal microorganisms, increasing the number of gastrointestinal microorganisms, fat absorption, weight management, and satiety. The health benefits of fibre are well documented however claims specific to fibre can have negative connotations with consumers, studies have demonstrated high fibre products are associated with bland taste and gastrointestinal upset (Dhingra et al., 2012), this may be a disincentive for manufactures considering embarking on expensive reformulation work to add fibre.

Fibre, satiety and weight loss claims

Fibre related claims for satiety and weight loss have been a topic of discussion for many years. Fibre has been found to be a useful tool for weight management and appetite control (Wanders et al., 2011). It is arguably beneficial for consumers to be informed on the satiating effects of foods where this is been adequately substantiated and described, as such information may provide a means to support appetite control and weight loss. However, the claims relating to fibre satiety and weight loss are often difficult to substantiate and sometimes misunderstood by consumers.

Concerns have been raised over whether satiety and weight loss claims are over interpreted by consumers (de Ridder et al., 2017) found that the general population overestimated the effects that a product can have on appetite. However, (van Kleef et al., 2005) tested consumer understanding of satiety related claims in four European countries, they found that participants did not over interpret satiety related claims but the type of product carrying the claim had a profound impact on consumer perception. Consumers also considered other factors such as pleasure, taste, cognition, and anticipated reduction of hunger. Booth and Nouwen (2010) suggested claims to boost satiety that are used to sell materials to dieters could potentially worsen consumers

problems with body weight and could even increase the prevalence of obesity. Many consumers are unaware health claims are regulated, with some consumers sceptical about the claims being made. It remains unclear how the presence of health claims regarding fibre and appetite may influences not only consumer choice but also consumer perception of such products. Understanding consumer acceptability for such products will help to identify a potential target market and move towards a wholesome approach to develop products carrying fibre related health claims.

1.6.4 Summary Section Health Claims

Understanding the acceptability of products carrying fibre related claims is key for both the food industry and consumers. Health claims communicate health or functional benefits of foods to consumers and can help consumers to make healthier food choices. They can be used by the food to industry to market foods and may be an incentive to develop foods with health or functional benefits. A nutrition claim states the presence of a nutrient whilst a health claim further states the health benefits from consuming a given food. Claims have to be substantiated and are regulated by EFSA. Claims specific to fibre were discussed and current EFSA authorised claimed were outlined. Fibre related claims for satiety and weight loss were discussed and their benefits informing consumers on the satiating effects of foods outlined. Concerns with satiety related claims have been raised such as over interpreting the effects of the claims. Consumer acceptability and communicating the added benefits through health claims is essential when reformulating products. The effect of fibre related claims on consumers choice and perception has not been tested and warrants further investigation.

1.7 The Current Thesis

The current thesis aims to address the gaps in the research to identify potential ingredients that could help to reduce appetite and food intake. Obesity action plans have highlighted the need to develop satiating products to reduce food intake and encourage individuals to make healthier choices, particularly in relation to food and diet. Given the plethora of scientific evidence that corroborate the multiple and varied health benefits of dietary fibre in this literature review, the optimisation of fibre within our diets represents an important public health strategy which could help to not only reduce obesity but also improve overall health. In this literature review it was identified that fibre supplements have been shown to reduce appetite through effects on postprandial satiety but may have beneficial intra-meal effects by satisfying appetite to mark the end of eating through satiation however, current data is equivocal on a range of fibres tested. To address this a systematic review will identify fibres which warrant further investigation.

While some fibres can reduce energy intake their precise effects on appetite have seldom been tested; nor has there been systematic analysis of the mechanisms whereby any changes are induced. Fibres with different physiochemical properties will be explored to identify the optimal combination of satiating ingredients. To develop products to target appetite the complex nature of appetite control and how it is measured must be understood. As discussed, there is currently a lack of scientific consensus regarding the preload study design, key methodological issues with little or no empirical evidence were identified which could explain the equivocal results. To address this the current thesis will explore the preload formulation and outcome test meal to improve the methodology both within this thesis and wider appetite research. As this literature review highlighted understanding consumer acceptance of functional ingredients is essential to develop targeted products that will appeal to the general

population. Fibre related health claims have not been extensively tested. The effect of fibre related health claims on choice and perception will be tested to help understand how health claims influence choice, how consumers perceive fibre related health claims and also identify specific population groups that would likely benefit from such products.

1.8 Aims and Hypothesis

This thesis examines the impact of satiety-inducing dietary components on appetite, food intake and biological markers. Human intervention studies were employed to determine the specific modes of action of these dietary ingredients and a questionnaire explored consumer perceptions.

Specifically, this project: -

1) **Identified the specific fibre types/doses that reliably increase satiation and satiety.** To address this, a systematic review was conducted which 1) identified the most effective dietary fibre type to reduce acute appetite and energy intake 2) identified the most effective fibres in terms of their physiochemical properties 3) identified the optimal dose for such an effect and 4) identified study design issues; measuring the effects of fibre on acute appetite and food intake.

2) **Assessed the optimal study design, scaling a preload according to BMI.** To address this aim it was hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) and food intake measures after a preload adjusted for body mass compared to a standard fixed inulin preload for participants who are obese.

3) **Identified the optimal number of ad libitum test meal items to detect an effect on appetite and food intake.** It was hypothesised that i) There will be an increase in food intake and a reduction in appetite in a high variety buffet meal compared to a low variety buffet meal for participants who are normal weight and participants who are obese. ii) participants who are normal weight but not participants who are obese will compensate for the increased intake at the high variety meal. iii) A high variety meal will decrease the chances of observing an effect of a fibre preload on appetite and food intake in participants who are obese.

4) **Assessed the influence of meal enrichment with fibres of different physical properties on satiation and post meal satiety and explored the relative contribution**

of proximal psychological and distal gut/neuroendocrine factors to prandial/post prandial behaviour. Identified probable mechanisms of ingredient effects on appetite. It was hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) and glycaemic response after a preload with inulin or β-glucan in isolation compared to a control, this decrease will be further enhanced after a preload with inulin and β-glucan in combination. ii) There will be an increase in colonic fermentation after a preload with inulin or β-glucan in isolation compared to a control, this increase will be further enhanced after a preload with inulin and β -glucan in combination. iii) There will be a decrease in glycaemic response after a preload with inulin or β-glucan in isolation compared to a control, this increase will be further enhanced after a preload with inulin and β -glucan in combination.

5) **Explored any potential synergistic effects for fibres with different physiochemical properties on appetite and food intake.** It hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) after a preload with inulin or β-glucan in isolation compared to a control, this decrease will be further enhanced after a preload with inulin and β-glucan in combination. ii) There will be a decrease in food intake after a preload with inulin or β-glucan in isolation compared to a control, this decrease will be further enhanced after a preload with inulin and β -glucan in combination.

6) **Explored consumer perceptions of products carrying fibre related health claims.** Specifically, it was hypothesised that i) Participants will choose a fibre drink with a health claim present significantly more than a drink without a health claim present. ii) Personal factors (demographics, nutritional/health claim knowledge, motivation to eat) will positively predict drink choice. iii) The presence of a health claim will positively affect participants perceptions (willingness to buy, taste, heath, weight management, fullness) of a drink. iv) Personal factors (demographics, nutritional/health claim knowledge, motivation to eat) will predict how participants perceive a drink with a health claims present.

1.9 Chapter Overview

Chapter 2 provides a detailed description of the materials, methods and measures used in this research. The chapter also considers the preload test meal paradigm, evaluating the reliability and validity. A clear rationale for use of these methods is provided.

Chapter 3 A systematic review was conducted to summarise the available literature on the relationship between specific dietary fibre types, subjective appetite and energy intake. Dietary fibres relevantly reduced appetite in 61.9% of comparisons and reduced appetite on average by 13%. Food intake was reduced in 57.6% of comparisons by an average of 83.1kcal (8.5%). More viscous fibres appeared to increase satiety more, whereas fermentable fibres were more effective at reducing food intake. The equivocal data for an effect of fibre on appetite and food intake suggested the methodology may play a part in the variable data.

Chapter 4 assesses the optimal study design through scaling a preload according to BMI**.** Participants were 24 females who were normal weight or obese who took part in a laboratory study over 6 weeks, attending the laboratory for 6 study days 1 week apart. A scaled preload was tested to see if it was more effective at reducing appetite and food intake in an acute study. After scaling the preload for BMI, the participants who are obese reduced appetite, feelings of hunger and reduced total food intake in the adjusted load condition above the compensation required for the increase in preload calories.

Chapter 5 identifies the optimal number of ad libitum test meal items to detect an effect on appetite and food intake. Participants were 24 females who were normal weight or obese who took part in a laboratory study over 6 weeks, attending the laboratory for 6 study days, 1 week apart. A limited variety meal detected the effect of the fibre preload on appetite and food intake in participants who are obese. The preload significantly reduced hunger and total food intake in the limited item condition

Furthermore food intake at the test meal significantly increased with increasing number of items for all participants at lunch, but there were no significant differences in total food intake for the participants who are obese or the participants who are normal weight participants, participants compensated for the increased calories

Chapter 6 explores the influence of meal enrichment with fibres of different physical properties on satiation and post meal satiety, to identify probable mechanisms of ingredient effects on appetite**.** 15 normal weight and overweight female participants took part in a laboratory study over 4 weeks, attending the laboratory for 4 test days 1 weeks apart. Changes in appetite in response to fibre preloads were explored both in isolation and combination, in an acute study over 6 h**.** Reliable biomarkers were utilised to determine the efficiency of different fibres and were combined with subjective measures of appetite. It was demonstrated β-glucan a viscous fibre did not affect hunger in the immediate post-ingestive period, as the effects on satiation and satiety may be mediated by oro-sensory exposure. There was a reduction in hunger after 7h for both β-glucan and inulin in isolation, consistent with these findings there was an increase in H_2 breath production, suggesting colonic fermentation. Combining fibres with different physio-properties had a significant effect on glycaemic response, colonic fermentation and appetite when the fibres (β-glucan and inulin) were combined which was significantly greater than when they were offered in isolation, suggesting there was a additive effect.

Chapter 7 explores the effects of combining fibres with different physiochemical properties on appetite and food intake. 18 normal weight and overweight female participants took part in a laboratory study over 4 weeks, attending the laboratory for 4 test days 1 week apart. Fibres with different physiochemical properties were combined to see if they improve the outcome relative to fibres in isolation over 8 h. Inulin and β-glucan in isolation reduced total food intake compared to the control condition, suggesting they strengthen within meal satiation. In combination calorie

intake was not significantly suppressed beyond the combined reduction of each fibre in isolation. Changes in subjective appetite ratings did become distinct in the combined fibre condition however, there were no significant differences when the fibres were tested in isolation compared to the combined fibres. This suggested there was no synergy or additive effect on appetite or food intake in the combined fibre condition.

Chapter 8 A questionnaire study assessed consumer perceptions of drinks carrying fibre related health claims to see if health claims predict choice and acceptability. 207 male and female participants completed an online questionnaire to gain insight into the effects of nutrition and health claims on purchasing intent and perceptions of a fibre drink. Health claims did not significantly influence drink choice; however, participants chose the “maintains blood sugar” drink slightly more than the “fuller for longer” drink compared to the control, participants chose the “high in fibre” drink less than the control drink. Personal factors did not predict drink choice. However, health claims did significantly affect perceptions of the drinks and personal factors predicted those perceptions.

Chapter 9 Collates the original research findings from Chapters 3-8 and integrates them with the literature reviewed. The contribution of this research to current knowledge of this field is discussed and implications for future research are considered.

Chapter Two

Methodology

This chapter describes the experimental methods used and the recruitment of participants that were performed in the studies described in this thesis. This research sought to add to the literature regarding fibre, appetite and food intake using a number of methodologies. The use of the established preload paradigm was enhanced in Chapter 4 by scaling the fibre preload. The buffet test meal was explored to assess its sensitivity to detect changes in appetite and food intake after a fibre preload (Chapter 5). In Chapter 6, biological measures were combined with subjective appetite measures and applied systematically to assess the effects fibres on appetite and biological markers over 6 hours, previous studies have tested over 4 hours. These results were combined to assess the effects on food intake for 8 hours in the laboratory and 24 hours including food diaries (Chapter 7). An online questionnaire study using the Qualtrics program tested the effect of different fibre related health claims on choice and perception (chapter 8).

2.1 Study design

The studies described in Chapters 4 and 5, employed a single-blind mixed-measures design. The within subject design allowed for a robust assessment of the effects of the preload on appetite and food intake measures, each participant acted as their own control. Between-subject comparisons were carried out for weight status. Participants were randomly allocated to receive each of the conditions in a counterbalanced sequence with each visit to the laboratory separated by a week. Randomisation to the studies was conducted by means of Latin squares. A within-subjects repeated measures design was employed for the studies described in Chapter 6 and 7. This was most appropriate to address the aims of the studies. Each participant took part

in every condition and acted as their own control. Appetite and food intake have such individual variability a repeated measures design is thought to be the optimal design (Yeomans, 2018). A randomised crossover design ensured the conditions were administered in a counterbalanced sequence and a Latin square ensured randomisation. Chapter 8 was a questionnaire study using the Qualtrics program, with a between-subjects design.

Power calculations using G*power 3.1 determined the sample size for each study based on 80% power, for a within-subjects design to find significant interactions and differences between conditions on measures of appetite and food intake, of medium effect sizes (Horner et al 2014; Heap et al., 2016; Vitaglione et al., 2009; Beck et al., 2009; Cani et al., 2006). Analyses by Flint et al. (2000) indicated that 12 participants would be required to identify a 10 mm (10%) difference in mean appetite ratings over 4 hours, with 0.8 level of power for a within-subjects design. Blundell suggests under good experimental conditions, 15 participants is generally sufficient to capture a 10% difference in mean or AUC appetite ratings between foods. A 10% difference is typically also seen as a reasonable and realistic difference (Fint et al., 2000). Using a within-subject design Gregersen et al., (2008) suggested a minimum of 16 participants to detect a 120kcal difference in *ad-libitum* food intake. There were 24 participants completed the studies in chapter 4 and 5, 15 participated in the study described in chapter 6 (food intake was not measured) and 18 participated in the study described in chapter 7.

2.2 Participants

2.2.1 Recruitment

For the experimental studies (chapters 4-7) participants were females aged 18 – 55 years. The experimental chapters in this thesis focused on female only participants

because previous studies suggest that there are differences between men and women in appetite response to nutrient manipulations. Women are more sensitive to overfeeding (Cornier et al 2004, Bédard, et al., 2015) and macronutrient changes (Westerterp-Plantenga et al 2009), leading to greater changes in appetite sensation ratings and/or subsequent energy intake compared to men. As the systematic review in chapter 3 demonstrated there were very few studies which found a significant reduction in appetite and food intake for men. It isn't clear if this is due to physiological differences or whether men are more prone to overconsume in all conditions (ceiling effects), removing the satiating effects of the preload manipulations in all conditions in the laboratory. Further to this in England, the obesity rate is slightly higher for women (29%) than for men (26%) (NHS Digital, 2020). In the UK, it has been estimated that increased energy intake accounted for the entirety of the increase in body weight in women between 1986 and 2000, but not in men. For men, the increase in body weight over this period is likely to be due to a combination of increased total energy intake and reduced physical activity levels. Women are also more likely to be invested in diet than men (Wardle et al., 2004) which would suggest designing products targeted towards a female market would have greater public health impact, as males with excess weight are more likely to exercise to reduce their weight than women.

Recruitment for the studies in chapter 4-7 followed the same procedure and timeline (see Appendix 1 for recruitment procedure form). Participants were recruited via advertisement from the University of Liverpool and surrounding areas in the North West of England. Volunteers responded to study advertisements; posters were displayed in local areas (e.g., on university notice boards), the university portal system and external websites. The advertisements (see Appendix 2) were study specific and consisted of information on the purpose of study, the experimental procedures to be

used and the potential (minimal) risks involved. Participants who responded to the initial advertisement and who fulfilled the criteria for inclusion stated, were given full information on the study and the process of informed consent. After completing the email assessment to initially determine their eligibility participants were then invited to a full screening to ensure suitability for the study and protection from harm.

2.2.2 Screening

After the initial telephone/email assessment, potential participants received detailed information on the protocol and after adequate time were invited to the study centre, (The Kissileff Ingestive Behaviour Laboratory in the School of Psychology, The University of Liverpool), for a screening no more than 21 days before commencing the study. All volunteers signed an informed consent form (see Appendix 3) before any study-specific procedures were undertaken. The consent form stated that participants have read and understood the information sheet. The protocol and consent were approved by the School of Psychology Research Ethics Committee before recruitment commenced. It was conveyed to participants that this research was undertaken on a voluntary basis and that they could withdraw from the research at any time without being obliged to explain their withdrawal. Volunteers received financial compensation for their time.

At the full screening, height, using a stadiometer to the nearest cm, and weight, using standard calibrated digital scales (Tanita) to the nearest 0.1 kg, were verified. Participants were barefoot and wore light clothing during body mass measurements. The body mass index (BMI) was calculated by dividing participants' weight (in kilograms) by the square of their height (in metres). Participant's BMI was categorised using the World Health Organisation definitions; *underweight*, *normal weight*, *overweight*, or *obese* based on the value. A medical history (See Appendix

4), measures of eating behaviour and dieting history were taken (see Appendix 5). Participants also completed the restraint scale of The Dutch Eating Behaviour Questionnaire (DEBQ-R) (van Strien, Frijters, Bergers, & Defares, 1986) (see Appendix 6). The DEBQ-R assesses both intentions to restrict food intake (3 items) and actual behavioural restraint (7 items). Studies in general population's samples have shown that the DEBQ-R is a reliable instrument (Jane Wardle, 1987). An intolerance/liking for study foods questionnaire was also administered (see Appendix 7).

2.2.3 Exclusion criteria

Following screening, participants were excluded from the studies if they reported any of the following: a history of anaphylaxis to food of any sort, known food allergies or food intolerances to any food; significant health issues likely to affect their well-being and/or appetite; taking medication known to affect appetite; systemic or local treatment likely to interfere with evaluation of the study parameters; current adherence to a specific food avoidance diet; having abnormal eating behaviour (restricted eaters measured by the DEBQ-R and with a cut-off of point of more than 4 on the scale); pregnancy; breastfeeding; dislike of more than 25% of the *ad-libitum* study foods.. Participants not able to attend the study centre at the requisite times or unable to follow the strict protocols were also excluded. Individuals employed in nutrition, dietetics, food research or the food manufacturing industry were not eligible to take part. Participants were also required to have a BMI +of 20kg/m^2-24.9kg/m^2 or 30kg/m^2-40kg/m^2 for the studies in chapter 4 and 5 and 18.5kg/m^2 – 30kg/m^2 for the studies in chapter 6 and 7.

2.2.4 Data Confidentiality

Participants were allocated a number upon recruitment and were not identified by name or initials on any study materials. Screening details and all study data in manual form were stored in a locked filing cabinet. Electronic study data were stored on a PC with password protection and anonymised.

2.2.5 Ethical considerations

The University of Liverpool 'Code of Practice for Experiments with Human Volunteers' applies wherever the possibility exists that an experiment may introduce special hazards or intensify everyday hazards. This code was followed at all times. The studies contained in this thesis were consistent with the associated generic approval, therefore the detailed risk assessment within the generic approval was considered sufficient to address the risks associated with the studies. The methods incorporated followed the methodology ethically approved in the generic approval RETH000565. Approval for each individual study was sought from Institute of Health and Society Research Ethics Committee before recruitment commenced under this existing generic approval.

The research took place within the Kissileff Ingestive Behaviour Laboratory, under the guidance of the Laboratory Supervisor. The Laboratory Supervisor is trained in food hygiene (Level 2 Award in Food Safety in Catering from a validated awarding body regulated on the Qualifications and Credit Framework e.g., Chartered Institute of Environmental Health or Highfield Awarding Body for Compliance). Training took place by the Laboratory Supervisor in food hygiene and laboratory skills prior to commencement of studies.

2.2.6 Risks identified to Researcher and Participants

The studies were minimal risk. All foods and drinks offered in the studies are commercially available to the public (a detailed study food list for each study is included). The fibre products being examined are common ingredients of commercially available products, they are consumed as components of the standard European diet and are available in Europe and the UK. The risk of side effects occurring was minimal as the amount of fibre did not exceed the recommended amounts or amounts consumed habitually in processed foods.

If information on eating behaviour, obesity, dieting or related matters was requested by the participant no specific advice was offered by the researchers, but participants were advised where this can be found.

2.3 Materials and Tools/Methods of Measurement

The research took place within the Kissileff Ingestive Behaviour Laboratory which is a purpose-built laboratory for the study of ingestive behaviour. The appetite studies in this thesis were conducted in tightly controlled laboratory test settings as they offer the highest degree of sensitivity and control over potentially confounding variables and provide the optimum conditions for disentangling the determinants of eating behaviour (Stubbs, Johnstone, O'Reilly, & Poppitt, 1998). Controlled laboratory testing provides a highly controlled environment to measure appetite, but the effects detected are not necessarily detectable in free-living conditions where intervening variables exert a strong influence on appetite-related sensations and eating behaviour. Knowing that food intake is being monitored prompts many participants to under eat (Robinson et al. 2014; 2015). Compromises were therefore made about the requirements for internal and external validity, between precision and naturalness when choosing to conduct laboratory-based studies.

Participants were naïve to the precise aims of the study, however, to provide fully informed consent they were informed about the general purpose of the study. Appetite studies requiring a single study visit often use a cover story or cover task to conceal the true aims of the study. A cover story is false information about the entire study, a cover task is false information about the specific task to be performed by the participant. It was difficult to employ such a technique in the current thesis as the studies involved measuring appetite or food intake during several study visits which following specific protocols (e.g., only consuming the foods provided in the laboratory). The research was conducted in the laboratory as measuring habitual food intake is prone to bias, usually under reporting of energy intake (Kye et al., 2014), therefore the precision of laboratory measurement was preferred for this research.

2.3.1 Methods for Measuring Satiation, Satiety and Energy Intake

Due to the complex nature of appetite expression measuring appetite and satiety is a complex and multifaceted process (Gibbons et al., 2019). Satiety and satiation were assessed by a combination of objective (energy intake) and subjective (ratings of appetite-related sensations) measures. Satiety was measured by the magnitude or duration of changes in subjective ratings of appetite-related sensations with measurement of energy intake at a test meal. Satiation was measured experimentally through the study participant's *ad libitum* consumption of the food under investigation during lunch and dinner in chapters 4, 5 and 7. The important distinction between satiety and satiation is that *satiety is an estimate, based on the sensory experience of eating.* Studies which combine these methods to measure short term appetite over part of or during a full day follow a preload design (Blundell et al., 2010). The use of single independent measures has major limitations, multiple measures are preferred and provide the most insightful data. The combinations of measures in this thesis

provided insights that could not have been found with simple intake or appetite measures alone (Gibbons et al.,2014).

2.3.2 Food Intake

The amount of food and water was determined by weighing foods on a balance (Sartorius Model BP8100, Sartorius Ltd., Epsom, UK; 0.1g accuracy) before and after the opportunity for consumption. This was necessary in order to analyse the consumed food, which was the difference between the remaining weight and the initial weight of the food contents. Energy intake in kilocalories and individual macronutrients was calculated from the manufacturers' nutritional values of each food to assess the energy and macronutrient content.

Where total food intake is calculated this includes intake at all fixed and *ad-libitum* meals/snacks as well as the fixed load preload, total food intake can be used to establish if there is any compensatory intake during the course of the entire study day in the laboratory and once participants have left the laboratory (via the evening snack box). Total *ad-libitum* food intake (kcal/g) was calculated in each study, this included all *ad-libitum* intake; lunch, dinner and the evening snack box.

Compensatory Intake

Energy compensation is defined as "the adjustment of energy intake provoked by the previous ingestion of a given stimulus (preload), whether a meal, a snack, or a beverage" (Blundell et al., 2010). Insufficient energy compensation both in the short and the long term has been associated with increased energy intakes and positive energy balance, leading to obesity (Almiron-Roig., et al, Jebb et al., 2006). Compensatory intake is calculated to see if there is a reduction in food intake (kcal) after the calories in the preload are accounted for. This can be calculated at each *ad-*

libitum meal or over the course of the day through total food intake. Energy compensation can be calculated using the following formula: -

$$\%EC = [\ (EI\ control\ condition - EI\ preload\ condition)/EP\ ] * 100$$

In this equation, EI represents energy intake in the control condition or preload condition, (excluding the energy in the preload itself); EP represents the energy in the preload (or the difference in preload energy if the control has a matched preload). Values of 100% indicate perfect compensation. Values <100% indicate partial compensation, values <0% indicate eating additional energy beyond the preload energy content. For example, 50% energy compensation after consumption of a 300kcal preload versus water indicates participants consumed 150kcal less at the next meal after the preload than after consuming water, while -50% EC indicates participants consumed 150kcal more after the preload than the water (overeating). Values above 100% indicate that the preload supressed subsequent intake to an extent greater than the energy content of the preload (overcompensation). Energy compensation value of 150% indicates participants consumed 450 kcal less at the next meal after a 300kcal preload than after water.

2.3.4 Subjective Appetite

Subjective appetite was measured using Visual Analogue Scales (VAS). VAS are a standard tool used to rate degrees of subjective appetite such as hunger, satiety, fullness, prospective food consumption, desire to eat, palatability, thirst and nausea (Flint 2000). They provide a greater insight into feeding behaviour than can be determined from measures of food intake alone. For example, hunger is rated along a 100 mm line that is preceded by the question "how hungry do you feel at this moment?" and anchored on the left by "not at all hungry" and on the right by

"extremely hungry". VAS consists of questions that assess subjective appetite-related sensations (see Figure 2-1) in response to an eating occasion right before and after consuming a preload or a test meal, and then at regular time intervals. The subjective sensation is quantified by measuring in millimetres from the left-hand end of the line to the point that the participant marks. When used in this context, VAS has an acceptable degree of validity and reliability (Stubbs 2000, Livingstone 2000, Arvaniti 2000; Flint et al., 2000). These questionnaires were completed immediately before and after each meal and at various specified time intervals throughout the test day during each study to monitor subjective appetite during the study day.

INSTRUCTIONS FOR PARTICIPANTS:

Please read each question and then put a mark through the line that best represents how you are feeling in relation to that particular sensation at this moment.

EXAMPLE:

How **HUNGRY** do you feel **at this moment?**

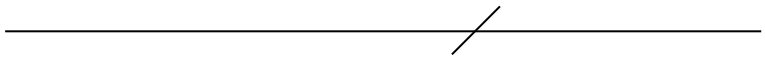

Figure 2-1 VAS Instructions for participants

2.3.5 GI Questionnaire

A GI questionnaire was also completed at the end of each test day to monitor any possible GI side effects (see Appendix 8). Participants completed 5 VAS scales “How bloated have you felt today?”, “How comfortable have you felt today?” “How flatulent have you felt today?”, “How tight has your stomach felt today?” and “How much abdominal discomfort (e.g., stomach cramps) have you felt today?”. The risk of side

effects occurring was minimal as the fibre doses did not exceed the recommended amounts or amounts consumed habitually in everyday foods. Products under investigation could potentially reduce food intake by generating side effects rather than by targeting the specific controls of appetite and food intake. Including both measures of subjective appetite and food intake with a battery of ratings of potential adverse reactions, helps to determine the mechanism leading to reduced intake. This also protects participants from harm.

2.3.6 Food and Activity Diary

On each day preceding the study day participants were asked to keep their food intake, fluid intake and activity levels similar and to record these in a diary from 5 pm until they retired for the night. The food and activity diary (see Appendix 9) was used for participants to record their meals, snacks, and any physical activities undertaken from 5 pm the evening before a study session up until the start of the study session. It served both as a check that participants kept to the study restrictions regarding food / alcohol intake and strenuous exercise on the day before the experiment, and that participants consumed and exercised roughly similar amounts before each session. There is always the possibility that participants would not be truthful when completing their diary, but it did provide a method for identifying participants who did not adhere to the study restrictions.

2.3.7 Biological and physiological measures

2.3.7.1 Blood glucose sampling

To evaluate the effects of glycaemia on experimental results (Chapter 6), the Accu-Chek Aviva Blood Glucose Monitor (Roche Diagnostics ltd., UK) was used to measure blood glucose levels before, immediately after and over the course of three and a half hours post-preload across four test days to assess glycaemic response. This system

is available over the counter for self-monitoring and was selected due to its ease of use and experimentally demonstrated accuracy (Freckmann et al., 2012). The meters used require a small blood sample and are designed to ensure minimal pain and tissue damage. A single-use needle, housed in a single-use lancet stick, was used to prick the tip of participant's finger and a drop of blood was placed on a test strip, and the strip inserted into the glucose monitor to obtain a reading. To prevent infection risk from potentially contaminated blood appropriate PPE were worn. Additionally, test strips containing participants' blood and other clinical waste were discarded in clinical waste bags. Furthermore, to prevent needle stick injury, all sharps were disposed of in sharps bins.

2.3.7.2 Hydrogen Breath Test

In Chapter 6 participants completed hydrogen breath test measures before, after and over the course of 6 hours post-preload across four test days to assess carbohydrate fermentation. The GastroCH_4ECK® Gastrolyser was utilised which measures both hydrogen (H_2) and methane (CH_4) levels in expired breath samples in response to appropriate substrates. Measuring hydrogen and methane in the breath may provide useful and practical biomarkers of colonic fermentation. Hydrogen and methane are end products of fermentation that are absorbed into the portal bloodstream and excreted via expired air. The basis for breath testing in these circumstances is that bacteria in the intestine can break down carbohydrates to produce the gases (Lebet, Arrigoni, & Amadò, 1998). The sole source of the gases is bacterial fermentation of carbohydrate in the gut, so estimation of hydrogen in breath samples can be used to study the passage of carbohydrates through the gut (Simrén & Stotzer, 2006). The hydrogen generated in the intestines passes through the intestinal wall, ends up in the bloodstream, this is transported to the lungs and is excreted on exhalation (Uday et al., 2011). Evidence suggests that the exhaled hydrogen indicates the quantity and

the metabolic activity of anaerobic bacteria in the intestines (Rumessen, 1992). Levels of hydrogen in expired breath have been shown to correlate very well (r = 0.9) with concentrations produced in the large intestine (Le Marchand et al., 1992). These breath gases have been used mainly as measures of colonic fermentation (Ghoshal, 2011). Participants exhaled directly into the GastroCH_4ECK® monitor via a disposable mouthpiece and results were recorded immediately.

2.4 Methods

2.4.1 Study procedure/standardised Instructions

The protocol used in the studies was based around the European consensus document which outlines recommendations for food intake studies Blundell (2010). For the studies contained in this thesis the preload–test meal paradigm was selected to study the short-term regulation of food intake, carried out during a single day. The effects of a fixed preload on postprandial appetite-related ratings using VAS, biological markers and *ad libitum* food intake from subsequent test meals was measured. Because of the large variation in test meal energy intake between individuals, a cross-over (within-subject) design was adopted, in which the different preloads were fed on separate days and preload order is counterbalanced across participants.

2.4.2 Test Meal Paradigm

All foods and drinks offered in the studies are readily and commercially available to the public. They were prepared in accordance with the manufacturer's instructions, the Guidelines for Human Nutrition Research and the individual standard operating procedures prepared for the equipment or specific food items used. The food was prepared in the Kissileff Laboratory kitchen and served in individual booths in the separate eating area.

2.4.2.1 Fixed Breakfast

Participants consumed around 25% of their recommended daily calorie allowance during breakfast. This standardised meal included items such as toast and cornflakes and required participants to consume the entire meal to ensure that every participant reached a moderate to high level of satiation, as assessed by VAS responses. Had we provided an *ad-libitum* meal; some participants would most likely have consumed a small breakfast which would not have allowed them to feel satiated. In Chapter 6 and 7 the breakfast food intake was also scaled for BMI to ensure overweight participants reached the same level of satiation as normal weight participants.

2.4.2.2 Test Meal Formulation

The *ad-libitum* meals (lunch and supper) were designed to offer a selection of high and low-fat savoury and sweet food items. The test meals were based on the sensory nutrient relationships of four sensory nutrient food groups; high fat savoury items, low fat savoury items, high fat sweet items and low-fat sweet items. Salad items were not included within these four sensory nutrient food groups but provided in addition to the distinct four groups. This model has been validated as a method to measure food preference and intake and has been used in a variety of studies to test sensory food preference.

Participants were presented with the buffet items on separate plates on serving trays and instructed to select the items and amount they would like to eat. Each food was presented in excess. Once the buffet was accessible, participants were told that they had 30 minutes to consume food until they were satisfied. Participants were kept in isolation throughout the meal in order to limit social factors from influencing food intake. Selected foods were weighed, and leftovers were used to calculate intake. Liquid and semi-solid foods were limited at the buffet to prevent participants from

consuming amounts similar to those consumed habitually, water was however provided at each meal. An evening snack box was also provided (table 3-1) for participants to consume in the evening. This allowed for any compensatory intake to be measured after participants had left the laboratory. Participants were instructed return the snack box with any empty wrappers/waste/uneaten food on their next study day.

Table 3-1 Nutrient and energy profiles of foods provided in the evening snack box.

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
Rivita minis	1 packet	30	2.6	2.2	22.8	113
Tesco marshmallows	1 packet	200	9	0.2	156	670
1 apple or banana	1 piece	80/100	0.2/1.2	Trace/0.3	9.8/23.2	44/105
bar of chocolate	1 bar	66	2	11	29.8	228
Mini Cheddars	1 packet	131	2.7	7.5	12.9	131

2.4.2.3 Ad Libitum Meals

Providing an *ad libitum* meal during the test session more closely modelled real life eating behaviour (Zaremba et al., 2017). Satiation is provoked by accumulating anorectic signalling as food is consumed (Bellisle et al. 2012). It is generally defined as being achieved when someone stops eating of their own accord, not when they stop merely because they have eaten all of the available food.

There are theoretical advantages and disadvantages to both single-item restricted meal and multi-item buffet test meals (Zaremba et al., 2017). Nutrient intake in food choice studies is clearly better addressed by presenting a multi-item buffet meal (Blundell et al., 2010) which allows the participant to make wide choices from foods which differ in energy, energy density and macronutrient content. Multi-item buffet meals increase the variety of items offered which in turn can promote greater intake

from the meal (Hetherington et al., 2006, Rolls et al.,1981). However, the single item *ad libitum* test meal allows little or no choice and typically has a lower palatability rating which may further decline as multiple treatments are completed, this can lead to rapid onset of sensory specific satiety (SSS). Weissing (2012) attempted to determine whether restricted single-item or multi-item test meals are better able to detect prior changes in hunger and fullness when assessing *ad libitum* eating behaviour. They found that increasing the variety of an *ad libitum* test meal did not decrease the sensitivity to detect changes in hunger and fullness as participants adjusted their intake accordingly in the multi-item condition.

The Multi-item buffet test meal is used as the outcome measure, however there is very little research into the validation of this meal (Zaremba et al., 2017). One of the main issues is that it is difficult to establish valid food selection methodology, consequently this area has not been developed extensively. It is therefore difficult to determine the optimal test meal composition (McCrickerd & Forde, 2016). This lack of consensus has been considered problematic since variation in this *ad libitum* meal may potentially influence the primary outcome of the study. High hedonic value of many of the foods offered in excess may induce over consumption in all conditions (ceiling effects), removing the satiating effects of the preload manipulations. Conversely, where little variety is offered in the test meal monotony is likely to ensue, an attribute likely to limit consumption within a study as a whole (floor effect), irrespective of the enhanced satiating potential of one of the preloads.

The use of familiar foods in the buffet test meal may overcome the constraints of using manipulated diets to recreate the real-life feeding situation, yet, the choice of foods provided in the laboratory is inevitably limited. In the real-world individuals seldom face such a variety of foods from which they can freely choose in one sitting, however,

to accurately measure food intake and particularly differences in macronutrient intake there needs to be a variety of foods in the buffet meal. Ensuring that the *ad-libitum* outcome meal is sensitive to manipulations made within the fixed preload test meal is essential in all postprandial appetite studies. A strong methodology will exert strong control over the nutritional and sensory aspects of each item. Chapter 5 looks more closely at the formulation of the *ad-libitum* test meal.

2.4.3 Timing

Preload studies assess the extent to which physiological mechanisms can compensate for the ingestion of a preload at the subsequent meal. Multiple psychological and physiological mechanisms are active at varying times during the phases of the satiety cascade. Therefore, the duration of the interval between the preload and the subsequent test meal will be decisive in determining the extent of subsequent energy and/or macronutrient compensation (Chungchunlam et al., 2012). This study focused on both the effect of pre-absorptive gastrointestinal factors on satiety and any post-absorptive inhibitory effects such as fermentation of fermentable fibres. This was reflected in the timings of data collection; 30 minutes or less is required for pre-absorptive factors (Slavin & Green, 2007), while several hours is required for post absorptive effects, however not so long that the effects of the preload are no longer detectable (Brighenti et al., 2006).

The preload in chapter 4 and 5 was administered 30 minutes before the lunch time meals was served, allowing both pre-absorptive gastrointestinal factors and post-absorptive inhibitory effects at dinner to be investigated. In chapters 5 and 6 the preload was administered before breakfast to allow the maximum amount of time to measure the effects of a fibre under laboratory conditions. Lunch was fixed 4 hours later in chapters 5 and 6, and in chapter 6 the dinner meal was 4 hours after lunch.

Differences in the interval between preload and subsequent meal could account for much of the variability in the results of preload studies (Warwick & Weingarten, 1994). Length of time interval can range from no time delay (Gray et al., 2003; Yeomans et al., 2001) to several hours (Zhu et al., 2013). Unfortunately, many study protocols designed to assess the relative satiating properties of various preloads fail to account for the time course of the post-absorptive satiating effects of each of the preloads is highly variable. In the current thesis the time interval was adapted in relation to the research question being addressed for each individual study to use the optimal timing for each study.

2.4.4 Preload formulation

In the current thesis a liquid preload was chosen for the studies described in chapters 4-7. The number of adults consuming drinks with functional claims continues to rise. The global functional beverage market is predicted to grow at a compound annual growth rate of 9% in 2019-2024. Demand for new innovative functional beverages is high and supports the need to develop such beverages that could benefit consumer health.

From an experimental perspective a liquid preload is preferred due to the ease of manipulating the contents, matching the sensory attributes across conditions and reducing prior association. Prior association in memory between sensory cues and particular post-ingestional consequences of specific foods have been found to affect appetite and intake (Boon et al., 1998; Worsley, 2002). When looking at the effects of fibre in whole foods it is difficult to untangle the previous experience/memory from the true satiating capacity of a food or ingredient (Gadah et al., 2016; Yeomans, 2012). Using a liquid preload may help to eliminate some of the issues associated

with a solid preload such as previous experience, however selecting a liquid preload isn't without issue. Solid and liquid preloads affect appetite in distinct ways. Liquid preloads have been found to be more satiating and reduce food intake more than the equivalent dose in solid form (Pan & Hu, 2011; Cassady et al., 2012; Drewnowski, 1998; Rolls et al., 2000). . Yet others such as E. Almiron-Roig et al. (2003) point out liquids fail to trigger physiological satiety mechanisms, so that compensation for energy consumed as beverages may be imprecise and incomplete. However, when the manipulation was a fibre, Peters et al., (2009) found no effect on appetite or food intake with a 16g dose of inulin in a solid bar in an acute study, whereas Hess et al. (2011) found that a 16g dose of inulin in a preload drink reduced appetite and food intake over 24 hours.

The preloads in the current thesis were carefully formulated and taste tested to ensure there were minimal sensory differences between conditions. Preload formulation is important as the characteristics of the preload could potentially influence the study outcome (Rolls et al., 1991; Sorensen et al., 2003). In chapters 4 and 5 the preload was scaled for BMI by increasing the overall volume of the preload drink. The preload drink provided a fixed or adjusted load according to each participant's BMI. Guided by the systematic review chapter 3 the optimal inulin dose of 0.66g/kg/m^2 was selected, the minimum fibre dose for individuals who are normal weight was 15.2g and the maximum dose for individuals who are obese was 23.1g. In the systematic review inulin reduced appetite and food intake with a dose of 15g in participants who were normal weight and in participants who are obese this dose was 21g. Previous research suggested the dose selected was considered to be well tolerated and safe for participants as doses of 30g-40g/day have been shown to cause adverse gastrointestinal symptoms. In the current thesis the fixed load contained 15g inulin, 15g black current squash and 200g water. The adjusted load provided 0.66g/kg/m^2

inulin, 0.66g/kg/m^2 of squash and 8.88g/kg/m^2 water, the volume of the drink varied from 221.7ml for participants who were normal weight to 333.1.ml for participants who were obese. More details can be found in chapter 4.

In chapters 6 and 7 the drinks contained inulin, oat β-glucan or a combination there of (plus a no fibre control). The inulin dose remained the same as chapter 4 and 5. The scaled β-glucan dose was guided by the literature, with the optimal β-glucan dose ranging from 2.2g to 6g/day. Due to the viscosity of the β-glucan fibre a smoothie was selected to mask the difference in consistency. The test drink was developed and taste tested in the Kisseliff laboratory to ensure the drinks were matched on taste and sensory dimensions across all 4 conditions. The β-glucan has a distinctive smell therefore the smoothie was served in a cup with a lid and a straw in all 4 conditions to reduce the sensory variation. Each ingredient was scaled for BMI so that the drink volume increased for the participants who are overweight compared to participants who are normal weight, but all other properties of the drink remained unchanged. Due to the difference in calorie content and mass of the fibres under investigation there were slight differences in the absolute energy content, macronutrient composition and volume across the 4 conditions which was considered when calculating energy compensation (Warwick & Weingarten, 1994), (Drewnowski, 1998; Rolls et al., 2000). De Graaf & Hulshof (1996) found the energy loads of the manipulations appear to be particularly critical, as relatively large energy differences of preloads within the literature may have been responsible for yielding negative results with respect to energy compensation in some studies (Almiron-Roig et al., 2013).

2.5 Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows (SPSS Inc., Chicago, I1, USA), software version 22-25. Data

conformed to the requirements for parametric analysis therefore Analysis of Variance (ANOVA) was used. If the assumptions of sphericity were violated, Greenhouse Geisser correction was employed. Where appropriate, post-hoc planned t-tests were carried out to identify the location of significant differences (with Bonferroni adjustments for multiple comparisons). Two-tailed comparisons were used, and statistical significance was taken at the 0.05 level unless otherwise stated. All tests were two tailed, and a P value <0.05 was considered significant.

Intake at the test meals was analysed for amount consumed (in grams and kcal) using a within-subjects ANOVA. In chapters 4 and 5 a 2 (preload fixed/preload adjusted) x 3 (variety levels: 5, 10, 20) repeated measure general linear model ANOVA was used. The food items offered at the *ad-libitum* lunch were further analysed according to variation in fat content and taste. BMI was also examined as a between-subjects factor in the analysis. Post hoc analyses correcting for Bonferroni adjustments were carried out to identify where differences lay. In chapter 6 food intake was analysed for amount consumed using paired t-tests (in grams and kcal).

Subjective parameters (e.g., hunger, fullness) rated on the VAS were analysed using a within-subjects repeated measures ANOVA with condition (preload fixed/preload adjusted) and time (prebreakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch, post-lunch, 2 pm, 3 pm, 4 pm, pre-dinner and post dinner; T1–T12) as within-subject factors. If a time-by-condition interaction effect was found significant, paired t-tests were conducted at each rating time between conditions. An appetite score was also calculated using the formula ([hunger + prospective food consumption + desire to eat + (100−fullness) + (100−satisfaction)]/5) (Stubbs et al. 2000) for each condition in order to reduce variance in the appetite data.

1.5.1 Satiety Quotients (SQ)

Satiety Quotients were calculated to integrate both the energy content of food ingested during a meal and the associated change in appetite sensations, Green and collaborators developed a SQ as an indicator of the satiating efficiency of food (Green et al., 1997). The SQ is calculated by dividing the change in subjective appetite sensations in response to a meal by the energy content of the meal.

1.5.2 Biological Markers

Biological markers were analysed (Chapter 6) using a within-subjects repeated measures ANOVA with condition (control, combined, β-glucan or inulin) and time (prebreakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch, post lunch, 2 pm, 3 pm, T1–T12) as within-subject factors for the H_2 breath test. If a time-by-condition interaction effect was found significant, paired t-tests were conducted at each rating time between conditions. Glycaemic response was analysed using the same method only the time pointed varied (prebreakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch, T1- T11). Where necessary, the 'Trapezoidal Rule' was applied to calculate area under the curve (AUC) VAS variable and differences in AUC VAS ratings were assessed using repeated measures ANCOVA with baseline values serving as covariant.

Chapter 3

3. The Effects of Fibre Supplementation in a liquid or semisolid preload on Energy Intake and appetite in Healthy Adults: Evidence from Systematic Review

The benefits of dietary fibre are well established. Several studies have shown increased fibre intake is associated with increased satiety, reduced food intake and lower obesity rates (Bodinham et al., 2010; Cani et al., 2006; Hess et al., 2011; Lyly et al., 2009; Mathern et al., 2009a; Perrigue et al., 2009; Howarth, 2001). However, not all fibres are equal, some fibres are more effective than others due to different physio-chemical properties and modes of action (Tomlin, 1995). Multiple mechanisms by which dietary fibres affect appetite and energy intake have been suggested (Slavin & Green, 2007), however, most of the physiological benefits are attributed to two characteristics, viscosity in the small intestine and fermentability in the large intestine (Poutanen et al., 2017).

Viscosity refers to the thickness of a solution and its resistance to flow. Viscous fibres such as pectin, psyllium, and guar gum have physio-chemical benefits, and mix with food and digesta in the gut, to form a firm soluble food matrix which increases satiety through their gel forming effect. This food matrix increases satiety by reducing post-prandial glycaemia through delaying gastric emptying; this slows transit time through the small intestine (Tomlin, 1995). Viscous fibres also alter blood glucose and cholesterol concentrations (Jenkins et al 1978; Schneeman 1987). Fibre viscosity was once thought to be the main physio-chemical property for enhancing satiety however other dietary fibres, which do not exhibit gel-forming properties, indicate they are effective in the control of food intake. For example non-viscous fructans are effective in increasing satiety and reducing food intake (Delzenne et al., 2003). This

suggests other physiological mechanisms such as fermentation may explain such findings.

Viscous fibres were once thought to lack the versatility of non-viscous fibres; they could not be added to liquids without affecting the consistency of the product. Methods to hydrolyse fibres are now available to add viscous fibres to liquids and other products without affecting the consistency. Pectins, *β-glucan*s, gums, inulin, oligofructose and resistant starch are dietary fibres that cannot be hydrolysed in the small intestine. They are, however, readily fermented by the microflora in the colon leading to the formation of SCFAs, (propionate, acetate and butyrate) and gases (carbon dioxide, methane, hydrogen). SCFAs are absorbed across the epithelium of the large intestine providing energy for the host (Topping & Clifton, 2001). Activation of receptors by SCFAs appears to evoke release of hormones that control variety of physiological processes such as appetite, gut motility and gastric emptying (Wong et al., 2006). These are supported by observations that exogenous administrations of several gut peptides such as GLP-1, PYY and CCK induce satiety and reduce food intake (Gutzwiller et al., 2004; Gutzwiller et al., 1999). Furthermore, several reports have associated satiety effects with intake of fermentable fibres in human dietary studies (Cani et al., 2006). Non fermentable fibres though they add bulk, reduce calorie density and aid gut motility, do not benefit from the physiological and metabolic processes that increase satiety hormone secretion in fermentable fibres (Slavin & Green, 2007).

Dietary fibre can influence appetite without a corresponding direct effect on food intake. As Touyarou et al., (2011) found a decrease in hunger is not systematically associated with a decrease in intake. Disassociation between appetite and food intake is common in studies measuring both appetite and food intake. However, a reduction

in eating motivations in the absence of food intake reduction may still be beneficial for reducing dysphoria and improving compliance with a controlled-energy regimen. This suggests strategies which utilise a combination of homeostatic and hedonistic control mechanisms may represent the most promising approach to reduce food intake.

The food industry plays a key role in trying to reduce obesity by improving food formulation to develop healthy products with functional ingredients. At present, beverages are by far the most active functional food category, possibly because of convenience, ease of distribution and storage (Nazir et al., 2019). Interestingly, in a systematic review conducted in 2010, Wanders (2013) concluded that fibres were more satiating when added to liquids. Fibre added to beverages can be used to enhance flavours and help to improve the textural and sensual qualities associated with low fat products (Yang, Ma, Wang, & Zheng, 2017).

Non-viscous functional fibres are of particular interest to the food industry because of their versatility; they can be added to foods without affecting the texture or taste, examples include polydextrose, inulin, resistant dextrins, fructans, cellulose, fructo-oligosaccharides and oligo- or polysaccharides (Fossiez et al., 1996). Resistant starch is often added to foods to reduce calorie content (Sajilata et al., 2006). Inulins on the other hand are hydrolysed into fructo-oligosaccharides which are more soluble and are often used as additive to sweeten low fat yogurts and other dairy products (M. B. Roberfroid, 2007). There are physiological benefits as seen with fermentable fibres between the gut microbiota and functional food components (Laparra & Sanz, 2010). This has led to an increased interest in this area of research with a wealth of studies indicating such functional fibres increase satiety (Cani et al., 2006; Lyly et al., 2009; Perrigue et al., 2009) and reduce acute food intake (Bodinham et al., 2010; Hess et al., 2011; Mathern et al., 2009).

There are inconsistencies in the findings regarding the benefits of soluble dietary fibre on appetite and food intake. Studies have shown the effects of soluble fibre to depend on factors such as dose, physiochemical properties, food matrix and methodology. The potential for the methodology to affect the outcome measures is particularly important in acute studies where their effect is measured over a short duration. To date no systematic review has looked at the acute effects of fibres in a liquid or semi solid matrix in healthy populations.

3.1.1 Aims

A systematic review to summarise the available literature on the relationship between specific dietary fibre types, subjective appetite and energy intake. In this review we will specifically 1) identify the most effective dietary fibre type to reduce acute appetite and energy intake 2) identify the most effective fibres in terms of their physiochemical properties 3) identify the optimal dose for such an effect and 4) identify study design issues in current research practices to measure the acute effects of fibre on appetite and food intake.

3.2 Methods

3.2.1 Eligibility Criteria

Laboratory studies examining the acute (<24 hours) effects of isolated fibres on appetite and food intake in healthy male or female adults aged 18 years or above were selected. Studies were included if they were limited to humans, utilised a repeated measures crossover design, measuring food intake and/or appetite with VAS measures in the laboratory. Studies also had a low fibre or no fibre control comparison. Publications were limited to English Language and published between 1996 to 2016.

Studies were excluded if they were observational in nature or failed to accurately measure food intake in the laboratory. Longitudinal studies were excluded at the screening stage (day 1 measures were included). Clinical populations as well as children and animals, were excluded as their heterogeneity may interfere with the results. If the comparator groups were absent or not specified or the study investigated combined fibres, they were also excluded. Only full-text English articles were included in this review. Studies written in languages other than English were excluded due to potential bias of information resulting from poor translation. Due to the improvements in methodology and measurement, over the past 20 years, we excluded studies published prior to 1996.

3.2.2 Search Strategy

Three different electronic databases PubMed, Scopus and Web of Science were used to systematically search the literature as well as previous review papers from January 1996 up to January 2017. PubMed was selected as it contains over 20 million biomedical studies from MEDLINE and life science journals. Scopus was chosen as it is the largest database for scientific journals covering the fields of science and medicine. Web of Science was chosen as it encompasses over 15,000 journals, the coverage includes the sciences and goes across all disciplines. For Scopus, only articles in the fields of 'agriculture and biological science', 'biochemistry' and 'genetics and molecular biology' were included. Boolean operators were included in the keyword searches of all three electronic databases. Full details of the Boolean search strategy can be found in appendix 10.

3.2.3 Data Management and Analysis

Data from included papers were extracted on the types of fibres, sample size, dosage, participant characteristics (sex, BMI, age), preload formulation (liquid or semi-solid),

study design, study duration, control condition, test meal, and the main outcomes; subjective appetite and energy intake. The titles and abstracts were reviewed based on the pre-defined criteria described above. Full-text articles were reviewed to determine whether the articles met the inclusion criteria. Primary data extraction was done to identify studies investigating the effects of soluble fibre in a liquid or semi-solid matrix on energy intake and appetite as outcome measures. Secondary data extraction was done to exclude studies that did not meet the defined criteria.

3.2.4 Evaluation of Studies and Data Synthesis

Studies included were laboratory-based with a preload study design where energy intake was measured accurately. Changes in energy intake after fibre admission were calculated as absolute changes (kcal) and relative changes (%) compared to the control treatment. Effect rates were calculated as the proportion (%) of all available comparisons that reduced energy intake. Effect sizes were calculated as an average (% and calories), weighted by the number of participants who completed the study. A negative value meant energy intake has been suppressed after fibre administration relative to the control. Effect rates (%) and effect sizes (calories and %) weighted for number of participants were also calculated for the fibres grouped according to their physiochemical properties, viscosity and fermentability.

Many different methodologies are used to measure appetite. In the current study, ratings of subjective appetite expressed on visual analogue scales measured up to 24 h after the fibre preload were included. Ratings of subjective appetite are often measured by one or more of the following six items: hunger, appetite, satiety, fullness, desire to eat, and prospective consumption. There isn't one common measure for appetite across all studies. Where possible hunger ratings were extracted if hunger ratings were not available, appetite or reverse fullness or satiety was used. An effect

of fibre on appetite was present if there was a significantly smaller area under the curve for fibre compared to control or there was a significant reduction in mean appetite ratings. Percentage differences in subjective appetite ratings between fibre and control were reported or calculated from AUC data or changes from baseline data. A negative value means appetite has been suppressed after fibre administration relative to the control. Effect rates (%) and effect sizes (%) weighted for number of participants for appetite were also calculated for the fibres grouped according to viscosity and fermentability.

3.3 Results

3.3.1Study Selection

Figure 3-1 illustrates the study selection. An initial sample of 7219 articles were identified. Of these, 3352 articles were from Scopus®, 2641 articles were from PubMed, and the remaining 1216 articles were from Web of Science. Duplicates were excluded and all articles were screened based on their titles and abstracts. A total of 143 full-text articles were retrieved to assess their eligibility, and 26 articles met the defined criteria and were subsequently included in the analysis.

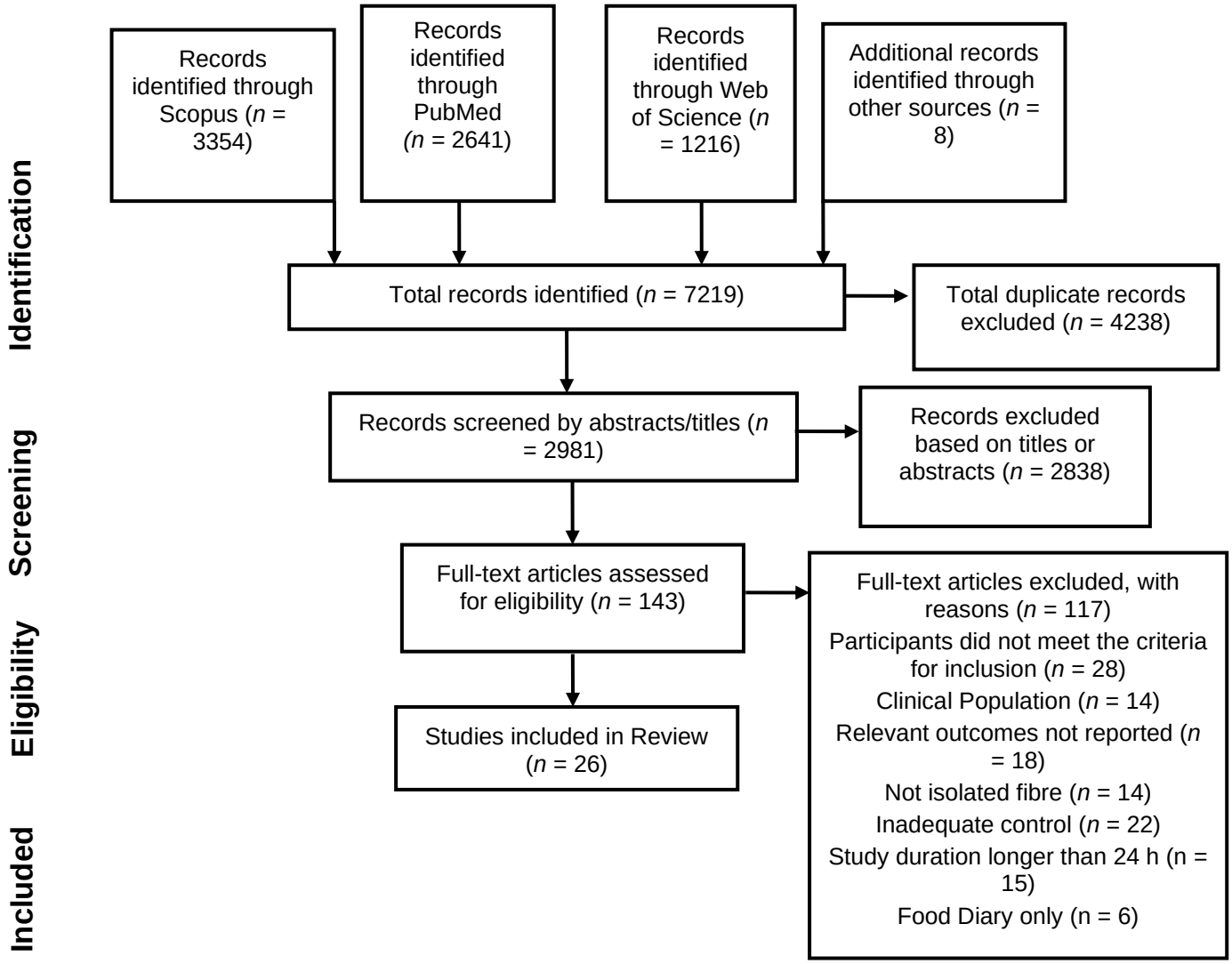

Figure 3-1 Study selection.

3.3.2 Study Characteristics

Table 3-1 presents study characteristics and effects of fibres on subjective appetite ratings by fibre group (*n* = 26), there were a total of 42 fibre control comparisons included in this review. A total of twelve soluble dietary fibres were identified: alginate (*n* = 3), fenugreek (*n* = 2), β-glucans (*n* = 8), guar gum (*n* = 5), inulin (*n* = 5), pectin (*n* = 5), polydextrose (*n* = 7) psyllium (*n* = 1), yellow pea fibre (*n* = 2), dextrin (*n* = 1), flaxseed (*n* = 1) and fenugreek (*n* =2). Table 3-1 presents Study characteristics and effects of fibres on food intake by fibre group (*n* = 26), there were a total of 33 comparisons.

Of the 26 studies, 3 were double-blinded and 23 were single-blinded. A total sample size of 1080 participants were included in the appetite comparisons, whilst food intake comparisons included 836 participants. The sample size ranged from 4 to 74 participants for the appetite comparisons whilst they ranged from 14 to 58 for food intake. Weighted BMI in all studies was 23.9kg/m^2. Participants from 5 comparisons were classified as overweight and 3 comparisons were obese. The studies were made up of a total of 422 males and 488 females, 28 studies included both males and females, 4 included only males whilst 5 included only females and 5 studies failed to report participant's gender.

The doses of fibre used in the studies were in the range of 0.5g to 25g and supplemented in either liquid (*n* = 18) or semi-solid (*n* = 24) form. The lowest dose of soluble fibre included in this systematic review was 0.5g of β-glucan (Doyon et al., 2015). The highest dose was found in 2 fibres, 3 different comparisons; resistant starch 25g (Klosterbuer et al., 2012) and polydextrose 25g (Astbury et al., 2005). The average weighted dose for the appetite measures was 11.1g while food intake the weighted average was 9.7g.

The duration of outcome measures for energy intake and appetite measures in the laboratory ranged from 90 to 615 min, with the weighted mean for appetite 257.2 minutes and the weighted mean of 260.6 minutes for food intake. The shortest duration of food intake measures was 90 minutes (Astbury et al., 2013), while the longest was 570 minutes (Hull et al., 2012). Fermentable fibres also tended to be measured over a longer time period compared to more viscous fibres, with a weighted mean of 225.9 minutes compared to 295.9 minutes for studies measuring appetite and 225.6 minutes compared to 287.7 minutes for studies measuring food intake. Five studies measured food intake over 24 hours via food diaries in eight comparisons

including β-glucan, inulin, polydextrose, pectin and psyllium after the standardised test meal in the laboratory.

The preload formulation differed between the studies, of the 42 appetite comparisons 24/42 (57.1%) were liquid and 18/42 (42.9%) were semi solid. Of these 18 semi solid comparisons 7 were porridge, 8 were yoghurt and 3 were soup. For the food intake comparisons 7/33 were semi solid and 24/33 were liquid. All studies provided a matched control; however, it was not explicitly clear in many studies whether the preload had been matched on calorie content, macronutrient content or sensory aspects such as taste and texture.

All studies provided a fixed breakfast. The breakfast consisted of the test product itself in 17 out of the total 42 comparisons. One study invited participants to self-select their portion size on the first test day, this was weighed, and the breakfast provided on subsequent test days were matched (Hull et al., 2012). There was a large degree in variability in the test meals. Some studies provided a fixed single item isocaloric meal (*n* = 11), whereas others opted for a buffet style meal. The buffet meals ranged from a limited number of items, less than 10 items *(n* = 13), to over 10 items (*n* = 9), this was considered high variety.

3.3.3 Outcome Measures

3.3.3.1 Appetite

Twenty-six comparisons (61.9%) relevantly reduced appetite (Table 3.2). Irrespective of the fibre group, fibre reduced appetite on average by 10.6% over the time interval with a mean fibre dose of 11.1g. Weighted average study duration was 257.2 minutes. Figure 3.1 shows the mean change in subjective appetite ratings by fibre

dose, weighted by number of participants per comparison, for all comparisons that reported dose and effect size (*n* = 26).

Fibre groups with the largest proportion of appetite-reducing effects were psyllium (one out of one comparison 100%), flaxseed (one out of one comparison 100%) and β-glucan (seven out of eight comparisons, 87.5%). The fibres with the biggest effect size on appetite were pectin (-18.9%), β-glucan (-17.9%) and alginate (-16.8) these are also viscous fibres. For appetite there was no significant effect for resistant starch, dextrin or yellow pea fibre.

Table 3-2 Study characteristics and effects of the different groups of fibre on acute Appetite

Fibre	Fibre Properties V/F	Comparisons (*n*)*	Studies (*n*)	Participants (*n*)	Blinding S/D	Sex m/f/x	BMI (kg/m2)	Food/Sup	Liquid/Semi Liquid	Test Meal m/lb/vb	Fibre Dose (g)			Mean Study Duration (min)	Overall Effect Rate (%)	Effect Rate			References
											Mean	Min	Max			AUC	VAS	Effect Size	
Alginate	V	3	2	70	1/2	20/50	23	0/3	3/0	3/0/0	7.8	5	9.9	227.1	66.7	2/3	0/1	-16.8	(Arshad et al., 2016; T. P. Rao et al., 2015)
B-glucan	V/F	8	7	207	7/1	78/108/21	24.9	2/6	3/5	0/0/5	3.6	0.5	11	235.3	87.5	7/7	0/0	-17.9	(C. Rebello et al., 2015) (C. J. Rebello et al., 2013) (Doyon et al., 2015) (Pentikainen et al., 2014) (Barone Lumaga et al., 2012) (Beck, Tosh, Batterham, Tapsell, & Huang, 2009b) (M. Lyly, Liukkonen, Salmenkallio-Marttila, Karhunen, Poutanen, & Lahteenmaki, 2009)
Inulin	F	5	5	157	5/0	59/98	22.8	0/5	3/2	2/1/2	11.3	2.4	16	349	60	3/4	0/1	-11.1	(Doyon et al., 2015) (Harrold, Hughes, O'Shiel, et al., 2013) (Hess et al., 2011) (Lee et al., 2016) (Perrigue et al., 2009)
Guar Gum	V/F	5	4	82	4/1	57/6/19	26.3	0/5	3/2	1/0/0	6.4	2	8	161.7	80	2/3	2/5	-8.8	(Heini et al., 1998) (T. P. Rao et al., 2015) (Arshad et al., 2016) (M. Lyly, Liukkonen, Salmenkallio-Marttila, Karhunen, Poutanen, & Lahteenmaki, 2009)
Resistant Starch	F	2	2	56	2/0	24/32	22.7	0/2	2/0	0/0/1	16.5	11.8	25	222.9	0	0/2	0/0	-9.4	(Klosterbuer et al., 2012) (Monsivais, Carter, Christiansen, Perrigue, & Drewnowski, 2011)
Dextrin	F	1	1	36	1/0	14/22	22.6	0/1	1/0	0/0/1	12	12	12	230	0	0/1	0/0	-11.9	(Monsivais et al., 2011)
Polydextrose	F	7	3	183	7/0	78/105	22.9	0/7	5/2	3/1/3	13	6.25	25	325.7	57.1	0/1	4/6	-10.4	(King et al., 2005) (Astbury et al., 2013) (Hull et al., 2012)
Pectin	V/F	5	3	175	5/0	55/30/90	23.1	0/5	4/1	0/0/4	11.2	2.5	14	240.7	60	3/3	0/0	-18.9	(Wanders et al., 2014) (Tiwary et al., 1997) (Lumaga et al., 2012)
psyllium	V	1	1	14	1/0	7/7	20.9	0/1	1/0	1/0/0	7.4	7.4	7.4	360	100	1/1	0/0	-13	(Rigaud et al., 1998)
Yellow pea fibre	V	2	1	40	2/0	40/0	21.8	0/2	0/2	0/2/0	15	10	20	195	0	2/2	0/0	x	(Smith et al., 2012)
Flaxseed	F	1	1	24	1/0	10/14	22.4	0/1	1/0	1/0/0	2.5	2.5	2.5	120	100	1/1	0/0	-8.5	(Ibrugger et al., 2012)
Fenugreek	B	2	1	36	2/0	20/16	36	0/2	2/0	0/2/0	6	4	8	210	50	0/2	0/0	-9.8	(Mathern et al., 2009)
Total Fibre	24/18/20	42	24	1080	38/4	422/488/165	23.9	2/40	31/11	16/12/8/6	11.1	5.8	12.9	257.2	61.9	23/34	4/9	13	

*Number of fibre control comparisons; one study can result in multiple comparisons. †Abbreviations used for study design characteristics: nb, not blind; b, blind; db, double blind; ?, missing; x, crossover; p, parallel; f, food or drink; sup, supplement; l, liquid; s, solid. AUC, Area Under the Curve; VAS, Visual Analogue Scale; M single item test meal; LB, limited buffet meal (≤10 food items); VB, Varied buffet meal (>11 food items); ‡Mean fibre dose, weighted by the number of participants s per comparison. §Mean study duration in hours, weighted by the number of participants per comparison. If the fibre treatment reduced energy intake compared to control, this was rated as an effect. The effect rate is given as an effect total in %. **Change in energy intake in kcal and %, weighted by number of participants per comparison. A negative effect size means a reduction in appetite after fibre treatment

3.3.3.2 Effects on acute energy intake

Study characteristics and effects of the different groups of fibre on acute energy intake are shown in Table 3.2, 19 out of 33 comparisons (57.6%) showed an absolute reduction in energy intake. Weighted average reduction in food intake across the studies was 83.1kcal (8.5%). The average study duration was 260.6 minutes with an average fibre dose of 9.7g. Participants consumed more than the control in three fibre control comparison this was fenugreek, a fermentable fibre which increased intake by 67kcal (5.2%) and yellow pea fibre which increased intake by 47.7kcal (3.5%) and 62.5kcal (4.6%).

Fibre types with the highest effect rate for energy intake reduction were flaxseed (100%), psyllium (100%), resistant starch (100%) and guar gum (100%) however, there was only 1 comparison available for flaxseed, resistant starch, guar gum and psyllium. Fibres with the biggest effect sizes were β-glucan which reduced food intake by 132.3kcal (12.8%) compared to the control, inulin 121.6 (10.4%) and polydextrose 112.2kcal (9.7%). There were no effects found for fenugreek and yellow pea fibre.

Table 3-1 Study characteristics and effects of the different groups of fibre on acute energy intake

Fibre	Fibre Properties V/F	Comparisons (*n*)	Studies (*n*)	Participants (*n*)	Blinding S/D	sex m/f/x	BMI (kg/m2)	Food/ Sup	Liquid /Semi Liquid	Test Meal M/LB/VB	Fibre Dose (g)			Mean Study Duration (min)	Effect Rate (%)	Effect Size (Kcal)			Effect Size (%)			References
											Mean	Min	Max			Mean	Min	Max	Mean	Min	Max	
Alginate	V	3	2	70	½	20/50	23	0/3	3/0	3/0/0	7.8	5	9.9	227.1	66.7	-72.4	-66	-100	-10.3	-5	-15.4	(Arshad et al., 2016; T. P. Rao et al., 2015) (C. Rebello et al., 2015) (C. J. Rebello et al., 2013) (Doyon et al., 2015) (Pentikainen et al., 2014) (Lumaga et al., 2012) (Beck et al., 2009) (Lyly et al., 2009)
B-glucan	V/F	5	5	110	4/1	61/49	25.6	3/2	1/4	0//3/2	2.6	0.5	5.7	260	80	-132.3	-85	-191	-12.8	-9.1	-19	
Inulin	F	4	4	136	3/1	48/88	22.5	0/4	3/1	1/0/3	10.8	2.4	16	328.8	75	-121.6	-80	-183	-10.4	-7.6	-11.7	(Doyon et al., 2015) (Harrold, Hughes, O'Shiel, et al., 2013) (Hess et al., 2011) (Lee et al., 2016) (Perrigue et al., 2009)
Guar Gum	V/F	1	1	30	1/0	0/30	22.3	0/1	1/0	1/0/0	5	5	5	170	100	-50	-50	-50	-7.7	-7.7	-7.7	(Heini et al., 1998) (T. P. Rao et al., 2015) (Arshad et al., 2016) (Lyly et al., 2009)
Resistant Starch	F	2	2	56	2/0	24/42	22.7	0/2	2/0	0/2/0	16.5	12	25	222.9	100	-8.4	-2	-11.9	-1.1	-0.2	-1.4	(Klosterbuer et al., 2012) (Monsivais et al., 2011)
Dextrin	F	1	1	36	1/0	14/22	22.6	0/1	1/0	0/0/1	12	12	12	230	100	-90.8	-90.8	-90.8	-11	-11	-11	(Monsivais et al., 2011)
Polydextrose	F	7	4	183	7/0	92/91	22.9	0/7	4/3	3/3/1	13	6.3	25	325.7	57.1	-112.2	-32.2	-332	-9.7	-4.2	-24.2	(King et al., 2005) (Astbury et al., 2013) (Hull et al., 2012) (Wanders et al., 2014)
Pectin	V/F	4	2	101	4/0	55/30/16	21.9	0/4	3/1	0/3/1	9	2.5	14.2	197.2	25	-87.1	-49	-168	-8.1	-4.1	-18	(Tiwary et al., 1997) (Lumaga et al., 2012)
psyllium	V	1	1	14	1/0	7/7	20.9	0/1	1/0	1/0/0	7.6	7.6	7.6	360	100	-70	-70	-70	-7.9	-7.9	-7.9	(Rigaud etc al., 1998) (Smith et al., 2012)
yellow pea fibre	V	2	1	40	2/0	40/0	21.8	0/2	0/2	0/2/0	15	10	20	195	0	55.1	47.7	62.5	4.1	3.5	4.6	
flaxseed	F	1	1	24	1/0	10/14	22.4	0/1	1/0	1/0/0	2.5	2.5	2.5	120	100	-66.2	-66.2	-66.2	-9	-9	-9	(Ibrugger et al., 2012)
fenugreek	V/F	2	1	36	2/0	16/20	36	0/2	2/0	0/0/2	6	4	8	210	0	-30.5	67	-128	-2.4	5.2	-10	(Mathern et al., 2009)
Total Fibre	16/17/12	33	26	836	29/4	387/443/6	23.5	3/30	22/11	10/11/10	9.7	5.8	12.6	260.6	57.6	-83.1	-56	-100	-8.5	-6.2	-11.1	

*Number of fibre control comparisons; one study can result in multiple comparisons. †Abbreviations used for study design characteristics: nb, not blind; b, blind; db, double blind; ?, missing; x, crossover; p, parallel; f, food or drink; sup, supplement; l, liquid; s, solid. AUC, Area Under the Curve; VAS, Visual Analogue Scale; M single item test meal; LB, limited buffet meal (≤10 food items); VB, Varied buffet meal (>11 food items); ‡Mean fibre dose, weighted by the number of participants s per comparison. §Mean study duration in hours, weighted by the number of participants per comparison. If the fibre treatment reduced energy intake compared to control, this was rated as an effect. The effect rate is given as an effect total in %. **Change in energy intake in kcal and %, weighted by number of participants per comparison. A negative effect size means a reduction in energy intake after fibre treatment.

Dose appears to vary according to fibre type (see table 3.3). The lowest dose of soluble fibre in this systematic review was 0.5g of β-glucan (Doyon et al., 2015) there were no significant effects on appetite or food intake. The highest dose was found for resistant starch 25g (Klosterbuer et al., 2012) and two studies with polydextrose 25g (Astbury et al., 2013; King et al., 2005). Only one study for polydextrose significantly reduced food intake at the test meal (Astbury et al., 2013), however the reduction was considerable with a reduction of 333.2kcal (24.2%) in the fibre condition compared to the control. The very low dose in the β-glucan study 0.5g may have explained the negative effect found for both appetite and food intake. The dosage tolerance for each type of fibre varies considerably, for example 4g/day of β-glucan is well tolerated, whereas for inulin is 15g/day, resistant starch is well tolerated up to 20g/day.

Table 3-3 Minimal fibre dose to detect a significant difference in appetite and food intake.

Fibre	Min Fibre Dose (g) Appetite	Min Fibre Dose (g) Food Intake
Alginate	5	5
B-glucan	2.2	3
Inulin	15	15
Guar Gum	5	5
Resistant Starch	25	25
Dextrin	-	12
Polydextrose	6.3	6.3
Pectin	11	14.2
Psyllium	7.4	7.6
yellow pea fibre	-	-
Flaxseed	2.5	2.5
Fenugreek	8	-

3.3.3.3 Viscous and Fermentable Fibres

When fibres were grouped according to their physiochemical properties, 17 out of 24 comparisons (70.8%) with more viscous fibres reduced appetite with an effect size of

16.6% (see table 3-4). Whereas nine out of 18 comparisons (50%) with fermentable fibres reduced appetite with an effect size of 10.5%. For food intake (see table 3-5) more viscous fibres significantly reduced food intake in 8 out of 16 comparisons (50%) with a mean reduction of 90.7kcal (9.5%). Fermentable fibres reduced food intake in 11 out of 17 comparisons (64.7%) by 92.3kcal (8.4%). More viscous fibres had a lower mean fibre dose of 7.1g compared to more fermentable fibres of 11.6g. The duration of the studies also differed with the more viscous fibre studies measuring over a period of 225.9 minutes compared to the fermentable fibre studies which measured over 295.9 minutes.

Four fibres had both fermentable and viscous properties; β-glucan, pectin, guar gum, and fenugreek. For appetite measures these fibres grouped had an effect rate of 75% (15/20 comparisons) with a weighted effect size of 16.3%. For food intake 9 out of 12 studies (75%) demonstrated a significant reduction in food intake with a mean reduction of 93.7kcal (14.9%)

Table 3-4 Study Characteristics and effects of the different groups of fibre on acute appetite grouped according to physiochemical properties

Fibre	Comparisons (*n*)	Studies (*n*)	Participants (*n*)	Blinding S/D	Sex m/f/x	BMI (kg/m2)	Food/Sup	Liquid/Semi Liquid	Test Meal m/lb/vb	Fibre Dose (g)			Mean Study Duration (min)	Overall Effect Rate (%)	Effect Rate			References
										Mean	Min	Max			AUC	VAS	Effect Size	
Viscous	24	18	588	20/4	257/201/130	24.0	2/22	14/10	7/6/11	7.6	0.5	20.0	225.9	70.8	17/19	2/6	16.6	(Arshad et al., 2016; T. P. Rao et al., 2015)(C. Rebello et al., 2015) (C. J. Rebello et al., 2013) (Doyon et al., 2015) (Pentikainen et al., 2014) (Lumaga et al., 2012) (Beck et al., 2009) (Lyly et al., 2009) (Heini et al., 1998) (T. P. Rao et al., 2015) (Arshad et al., 2016) (Lyly et al., 2009)(Wanders et al., 2014) (Tiwary et al., 1997) (Lumaga et al., 2012)(Rigaud etc al., 1998) (Smith et al., 2012) (Ibrugger et al., 2012) (Mathern et al., 2009) (Doyon et al., 2015) (Harrold, Hughes, O'Shiel, et al., 2013) (Hess et al., 2011) (Lee et al., 2016) (Perrigue et al., 2009) (Klosterbuer et al., 2012) (Monsivais et al., 2011)(Monsivais et al., 2011) (King et al., 2005) (Astbury et al., 2013) (Hull et al., 2012) (Wanders et al., 2014) (Tiwary et al., 1997) (Lumaga et al., 2012) (C. Rebello et al., 2015) (C. J. Rebello et al., 2013) (Doyon et al., 2015) (Pentikainen et al., 2014) (Lumaga et al., 2012) (Beck et al., 2009) (Lyly et al., 2009)(Heini et al., 1998) (T. P. Rao et al., 2015) (Arshad et al., 2016) (Lyly et al., 2009)
Fermentable	18	13	492	18.0	205/287	23.8	0/18	14/4	6/4/7	11.8	2.4	25.0	295.9	50.0	4/11	4/7	10.5	
Viscous/ Fermentable	20	15	500	18/2	210/160/130	25.3	2/18	12/8	39845	6.9	0.5	25	223.3	75	12/15	2/5	16.3	

*Number of fibre control comparisons; one study can result in multiple comparisons. †Abbreviations used for study design characteristics: nb, not blind; b, blind; db, double blind; ?, missing; x, crossover; p, parallel; f, food or drink; sup, supplement; l, liquid; s, solid. AUC, Area Under the Curve; VAS, Visual Analogue Scale; M single item test meal; LB, limited buffet meal (≤10 food items); VB, Varied buffet meal (>11 food items); ‡Mean fibre dose, weighted by the number of participants s per comparison. §Mean study duration in hours, weighted by the number of participants per comparison. If the fibre treatment reduced energy intake compared to control, this was rated as an effect. The effect rate is given as an effect total in %. **Change in energy intake in kcal and %, weighted by number of participants per comparison. A negative effect size means a reduction in appetite after fibre treatment.

Table 3-5 Study Characteristics and effects of the different groups of fibre on acute food intake grouped according to physiochemical properties

Fibre	Comp (*n*)	Studies (*n*)	(*n*)	Blinding S/D	sex m/f/x	BMI (kg/m2)	Food /Sup	Liquid/Semi Liquid	Test Meal M/LB/VB	Fibre Dose (g)			Mean Study Durat (min)	Overall Effect Rate (%)	Effect Size (Kcal)			Effect Size (%)			References
										Mean	Min	Max			Mean	Min	Max	Mean	Mn	Mx	
Viscous	16	12	365	13/03	183/166	23.2	3/13	9/7	5/6/5	7.1	0.5	20.0	225.6	50.0	90.7	63.9	136.9	9.5	6.2	15.1	Arshad et al., 2016; T. P. Rao et al., 2015)(C. Rebello et al., 2015) (C. J. Rebello et al., 2013) (Doyon et al., 2015) (Pentikainen et al., 2014) (Lumaga et al., 2012) (Beck et al., 2009) (Lyly et al., 2009) (Heini et al., 1998) (T. P. Rao et al., 2015) (Arshad et al., 2016) (Lyly et al.,2009)(Wanders et al., 2014) (Tiwary et al., 1997) (Lumaga et al., 2012)(Rigaud etc al., 1998) (Smith et al.,2012) (Ibrugger et al., 2012) (Mathern et al., 2009) (Doyon et al., 2015) (Harrold, Hughes, O'Shiel, et al., 2013) (Hess et al., 2011) (Lee et al., 2016) (Perrigue et al., 2009) (Klosterbuer et al., 2012) (Monsivais et al., 2011)(Monsivais et al., 2011) (King et al., 2005) (Astbury et al., 2013) (Hull et al., 2012) (Wanders et al., 2014) (Tiwary et al., 1997) (Lumaga et al., 2012) (C. Rebello et al., 2015) (C. J. Rebello et al., 2013) (Doyon et al., 2015) (Pentikainen et al., 2014) (Lumaga et al., 2012) (Beck et al., 2009) (Lyly et al., 2009)(Heini et al., 1998) (T. P. Rao et al., 2015) (Arshad et al., 2016) (Lyly et al., 2009)
Ferm	17	13	471	16/1	204/267	23.7	0/17	13/4	5/5/7	11.6	2.4	25.0	287.7	64.7	92.3	51.3	203.5	8.4	5.5	15.0	
Viscous / Ferm	12	9	277	11/1		25.2	3/9	7/5	1/4/7	5.6			220.9	41.6	93.7	73.7	151.3	8.6	5.9	14.9	

*Number of fibre control comparisons; one study can result in multiple comparisons. †Abbreviations used for study design characteristics: nb, not blind; b, blind; db, double blind; ?, missing; x, crossover; p, parallel; f, food or drink; sup, supplement; l, liquid; s, solid. AUC, Area Under the Curve; VAS, Visual Analogue Scale; M single item test meal; LB, limited buffet meal (≤10 food items); VB, Varied buffet meal (>11 food items); ‡Mean fibre dose, weighted by the number of participants s per comparison. §Mean study duration in hours, weighted by the number of participants per comparison. If the fibre treatment reduced energy intake compared to control, this was rated as an effect. The effect rate is given as an effect total in %. **Change in energy intake in kcal and %, weighted by number of participants per comparison. A negative effect size means a reduction in food intake after fibre treatment.

3.3.3.4 Methodological Considerations

3.3.3.4.1 Participants

Thirty-four studies included participants who were normal weight, eight studies included participants who were overweight and obese, average overall weighted BMI was 23.9kg/m^2. Participants from 5 comparisons were classified as overweight of these, appetite was significantly reduced in 4 comparisons. Of the 3 comparisons with participants who are obese there were no significant results for appetite or food intake. Of the 42 comparisons 28 included both males (n = 422) and females (n = 488), 4 included only males whilst 5 included only females and 5 studies failed to report this. Interestingly there were no significant differences in appetite or food intake for the 4 comparisons including all men (Doyon et al., 2015; Smith et al., 2012). Whereas there was a significant reduction in appetite and food intake in 4 out of the 5 comparisons which included all females (Arshad et al., 2016; Harrold et al., 2013; Pentikainen et al., 2014).

3.3.3.4.2 Test Meal

Of the 11 comparisons that included a single item meal appetite and food intake was significantly reduced in 11 comparisons. Thirteen comparisons included a limited buffet meal and 6 significantly reduced appetite or food intake. The multi-item buffet was included in 9 comparisons appetite or food intake was reduced in 6 comparisons. The studies with the highest variety 16 item (Monsivais et al., 2011) and 33 item buffets (Doyon et al., 2015) found no effect in 4 comparisons for both food intake and appetite.

3.3.3.4.2 Preload formulation

Of the 42 appetite comparisons 18 included a semisolid preload of these, 9 preloads (50%) significantly reduced appetite. Of the 24 liquid preloads 17 (70.8%) significantly reduced appetite. The 33 comparisons measuring food intake 6 used a semisolid preload of these, 5 significantly reduced food intake (83.3%). There were 27 comparisons with a liquid preload measuring food intake, of these 14 (42.4%) significantly reduced food intake.

Table 3–6 Summarised effect rates of dietary fibre on subjective appetite and acute energy intake.

Fibre	Subjective Appetite		Acute Energy Intake	
	Comparisons	Effect Rate	Comparisons	Effect Rate
Alginate	3	66.7	3	66.7
B-glucan	8	87.5	5	80
Inulin	5	60	4	75
Guar Gum	5	80	1	100
Resistant Starch	2	0	2	100
Dextrin	1	0	1	100
Polydextrose	7	28.6	7	57.1
Pectin	5	60	4	25
psyllium	1	100	1	100
yellow pea fibre	2	0	2	0
flaxseed	1	100	1	100
fenugreek	2	50	2	0
Total Fibre	42	61.9	33	57.6

3.4 Discussion

This review has demonstrated that different dietary fibres in liquid or semi-solid matrices affect acute appetite and food intake differently. The most effective dietary fibre type and dosages to reduce acute appetite and energy intake were identified. Fibres were also grouped in terms of their physicochemical properties to explore how

they may contribute reductions in appetite and food intake. Key methodological issues were identified to identify how the current methodology could be improved. A total of twelve soluble fibres were identified alginate, guar gum, β-glucan, pectin, polydextrose, inulin, dextrin, flaxseed, fenugreek, yellow pea fibre, resistant starch and psyllium. In this review, two outcome measures; appetite and energy intake were focused on. Some studies focused on measuring appetite in isolation (*n* = 9) whilst others measured both appetite and energy intake (*n* =33). Out of 42 fibre–control comparisons, 26 comparisons (61.9%) relevantly reduced appetite (Table 3.1). Irrespective of the fibre group, fibre reduced appetite on average by 13% over the time interval with a mean fibre dose of 11.1g. This is higher than previous reviews have found (Clark & Slavin, 2013; Wanders et al., 2011a), however the exclusion of solid matrices may explain the results.

There was an absolute reduction in energy intake in 19 out of 33 comparisons (57.6%). Weighted average reduction in food intake across the studies was 83.1kcal or 8.5% with a mean fibre dose of 9.7g. Effect sizes were relatively small in many studies and differed largely between studies. Previous reviews have discredited reductions of less than 10%. Nonetheless food intake was reduced though not always significantly in 32/33 studies, even small differences may be relevant. Participants consumed more than the control in two fibre control comparisons (Mathern et al., 2009; Smith et al., 2012) this was fenugreek a fermentable fibre with an increase in intake of 67kcal (5.2%) and yellow pea fibre a viscous fibre with an increase in intake of 47.7kcal (3.5%). The low fibre dose may have explained the negative effects found.

The current review builds on the previous reviews by Wanders et al., (2011) and Clark & Slavin (2013). Previous reviews have found slightly different results where reviews have included fibres in a solid matrix as well as liquid and semi-solid. Clark & Slavin,

2013 found that fibre reduced appetite in 39% of comparisons and food intake in 22%, whereas Wanders found fibre reduced appetite in 43% of comparisons and food intake in 54% of comparisons. The literature searches for these reviews were carried out in February 2010 (Wanders et al., 2011) and April 2010 (Clark & Slavin, 2013), respectively. It was important to carry out a current review to capture the most recent studies in this growing area of research. The previous reviews predate many of the studies included in the current review. This review also focused on liquid/semisolid preloads as previous studies demonstrate it is difficult to untangle the previous experience/memory of the whole food from the true satiating capacity of the fibre under investigation (Gadah et al., 2012), not only this but demand for new innovative functional beverages is high and warrants further investigation, therefore studies testing fibre in whole foods/solids were excluded.

The current review also included stricter search criterion excluding clinical populations to reduce clinical heterogeneity in the data as for example acute hyperglycaemia (high blood glucose level) experienced by diabetic patients suppresses hunger and food intake after a nutrient preload (Russell, 2001). The current review included single ingredients under investigation only, existing commercially developed products were excluded and studies lacking in a no fibre matched control condition were excluded, the previous reviews included studies with low fibre control conditions. The current review also excluded studies conducted prior to 1996, prior to this there was very little corroboration in the methodology used to test the ingredients. In the current review the results were summarised into tables with additional information about the participants and study characteristics, test meal information, percentage benefit, fibre dose, and duration were calculated using weighted means rather than arbitrary cut-off points as other studies such as Wanders (2011) and Clarke & Slavin, (2013) did. Including such measures provides greater insights.

The most effective dietary fibre type to reduce acute appetite and energy intake were identified. A 100% effect rates for both appetite and food intake measures was lacking, however β-glucan appeared to be the most consistent fibre across the measures and also had the highest number of comparisons. Fibre groups with the largest proportion of appetite-reducing effects were psyllium (100%), flaxseed (100%), guar gum (100%) and β-glucan (87.5%). The fibres with the biggest effect size on appetite were pectin (-18.9%), β-glucan (-17.9%) and alginate (-16.8) these are also viscous fibres. This is not surprising given the immediate post-ingestive effects of viscous fibres on appetite.

For appetite there was no significant effects for resistant starch, dextrin or yellow pea fibre. Research shows resistant starch is usually more effective at reducing appetite in solid matrices, this could explain the lack of effect. Fibre types with the highest effect rate for energy intake reduction were, resistant starch (100%), dextrins (100%), guar gum (100%) and pectin (100%), psyllium (100%) and flaxseed (100%). Four out of the five fibres are fermentable fibres (psyllium is a viscous fibre), whilst pectin and guar gum exhibit both fermentable and viscous properties. Fibres with the highest effect size on food intake were β-glucan (-132.3kcal, -12.8%), inulin (-121.6kcal, -10.4%) and polydextrose (-112.2kcal, -9.7%), all three fibres are fermentable. The timing of the test meals in the acute studies (>240 minutes) could potentially favour fermentable fibres. For food intake there were no effects found for fenugeek and yellow pea fibre.

A disassociation between appetite and food intake was observed, a greater percentage of treatments reduced appetite (61.9%) than reduced food intake (57.6%), however some studies found no effect on appetite but a significant reduction in food

intake. The complex nature of appetite and food intake regulation may go some way to explain this disassociation but equally there are limitations intrinsic to the measurement of appetite and food intake. VAS scales are subjective tools that are open to interpretation (Livingstone et al., 2000). *Ad libitum* meals may not represent typical eating behaviours (Zaremba et al., 2017). Furthermore, appetite and food intake may be sensitive to uncontrolled food and individual participant factors, such as sensory-specific satiety (Wilkinson & Brunstrom, 2016), environmental and social cues (Johnson, 2013; McCrickerd, 2016), palatability of the food (Johnson & Wardle, 2014), stress levels (Yau & Potenza, 2013) and sleeping habits (Knutson, 2007). These factors may be more controlled in a single study than when comparing results between studies.

To help identify the optimal fibre dose for each fibre type the minimal dosage for a significant reduction in appetite and food intake was identified. Increasing fibre dose increased effect rate in the majority of comparisons. Where an insufficient dose was provided there were no significant effects, for example there was no significant effect of 0.5g of β-glucan on appetite and food intake. Different fibres require a different dose to have a similar effect on appetite and food intake 3.5g/day of β-glucan is thought to have an optimal effect (Khoury et al., 2012), whereas resistant starch is 20g/day (Lockyer & Nugent, 2017). Within the same fibre group higher dose appeared to be more effective than a low dose. However, care must be taken not to exceed the recommended dose for each fibre type. Many studies in humans err on the side of caution as a large dose of fibre can lead to unpleasant GI symptoms. In animal models bigger effect sizes with higher doses are found, however these doses would not be well tolerated in humans (Hervik & Svihus, 2019). Beyond fibre dose fibre composition may also have an impact on outcome measures in this review, even among fibre from the same source for example (Wanders et al., 2014) compared 3

types of pectin with the same dose (10g). They demonstrated that viscous and bulking pectin had similar effects on energy reduction however gelled pectin did not significantly reduce food intake. Product viscosity appeared to reduce *ad libitum* intake. For fermentable fibres chain length is important a shorter chain length ferment quicker and therefore have an effect over a shorter timeframe (Stewart et al., 2008), this would be particularly important testing in an acute study. It can be concluded that it is important to consider both the fibre dose and composition when considering the results of this review.

Fibres were also grouped in terms of their physicochemical properties to explore how their physiochemical propertied may contribute reductions in appetite and food intake. When fibres were grouped according to physiochemical properties more viscous fibres appear to be more effective at reducing appetite (73.7%, 16.2%), whereas fermentable fibres appear to be more effective at reducing food intake (52.8%, 13.5%). It is worth noting the effect size on food intake was very similar for viscous and fermentable fibres. The effects found could be due to the different physiochemical properties. Acute studies which are conducted in 240 minutes or less may favour viscous fibres as the effects of viscous fibres are more likely to be detected in the immediate post-ingestive period (Rebello et al., 2016). Therefore, the effects on appetite are more likely to be apparent in the 240-minute time frame many acute studies adopt. Fermentable fibres on the other hand take longer to ferment in the colon therefore the effects are often only detected 240 minutes onwards (Salmean, 2017). Had the test meal been served at a later timepoint or a second meal provided the effect sizes may have been bigger.

In the current review fermentable fibre studies were tested over a slightly longer period, however this difference was quite small. Four fibres have both fermentable

and viscous properties β-glucan, pectin, guar gum, and fenugreek when grouped they had a 75% effect rate with weighted mean effect size of 16.3% on appetite. This effect rate was higher than viscous or fermentable fibres grouped and could suggest that fibres which exhibit both fermentable and viscous properties could potentially be more effective at reducing appetite and food intake.

Several different mechanisms of action for effects of viscous and fermentable fibres have been proposed. Viscous fibres may increase sensory delivered appetite by increased exposure time in the oral cavity (Chambers, 2016). Because viscous dietary fibres can hold large quantities of water, they can increase stomach distension which may trigger afferent vagal signals of fullness (Grabauskas & Owyang, 2017). They may also delay gastric emptying and thereby prolong the absorption of nutrients (Yu, et al., 2014; Qi, 2018). Furthermore, the increased viscosity of digesta in the small intestine can also result in prolonged presence of nutrients in the small intestine which in turn affects the release of appetite-regulating peptides throughout the intestine, such as cholecystokinin (CCK) in the duodenum and peptide tyrosine (PYY) and glucagon-like peptide 1 (GLP-1) in the distal ileum and proximal colon (Grundy et al., 2016; Prinz, 2017). As a result, subjective appetite may be reduced. Highly fermentable fibres on the other hand produce SCFAs, acetate, butyrate and propionate which enter the portal circulation and effect glucose homeostasis in a variety of ways (den Besten et al., 2013). Butyrate is utilised by enterocytes in the liver and generally regarded as a healthy metabolite as it impairs lipid transport (Liu et al., 2018). Acetate contributes to lipid and cholesterol synthesis in the liver, propionate inhibits the effects of acetate (De Vadder et al., 2016). These actions alter insulin sensitivity, insulin secretion, metabolic fuels and regulate appetite (Darzi et al., 2011; Frost et al. 2014).

We identified the two most effective and consistent fibres. β-glucan and inulin reduced food intake by 132.3kcal (12.8%) and 121.6kcal (10.4%). They also had the biggest effect rate for food intake with 80% for β-glucan and 75% for inulin. β-glucan also had the highest effect rate for appetite (87.5%) with a reduction in appetite of 17.9%. Inulin had a slightly lower effect rate of 60% and an effect size of 11.1%. As β-glucan exhibits both viscous and fermentable properties it can increase stomach distension and delay gastric emptying and thereby prolong the absorption of nutrients. In the GI tract, β-glucan acts much like a soluble fibre being rapidly fermented by the microflora of the colon. Previous research suggests inulin is rapidly fermented in the proximal colon; however, the fermentation rate is largely dependent on the chain length of individual fibres. B-glucan may be fermented more gradually along the colon, particularly in the distal colon. B-glucan can also be modified into shorter fractions so is a versatile fibre. B-glucan has the potential to increase satiety immediately after ingestion via viscosity as well as after several hours via fermentation.

Study design

This review extends the work of Wanders (2011) and Clark & Slavin (2013) to provide details about current research practices to measure the acute effects of a fibre preload on appetite and food intake. This systematic review aimed to identify where there could be design weaknesses and establish how these issues could be addressed to improve the preload study design. This review included preload studies with standardised VAS appetite ratings, with or without a subsequent *ad libitum* test meal. Despite this there was some degree of variation in the study designs used, with homogeneous methods and reporting which creates difficulties when comparing the effects across studies. Although studies use VAS to measure appetite there are large variation in how often appetite measures are collected and how they are reported

which makes comparisons difficult. The follow-up time in these single-preload acute studies varied from 90min to 24 h, the latter affording opportunity to observe any second meal effects later in the day. In many cases, the background diet was not reported, this poses a challenge, because they always contain other sources of fibre.

Participants

Participant selection may have an impact on the outcome measures. Research has suggested appetite control differs in individuals who are overweight and obese (MacLean et al., 2017), they are less sensitive to manipulations as they exhibit weaker satiety signals (Lean et al., 2018) and they are more susceptible to hedonic hunger (Gabriela Ribeiro et al., 2018) and eating in the absence of hunger (Perez-Moraleset al., 2014). This could potentially explain why there were no significant differences in appetite or food intake in the 3 comparisons with a mean BMI in the obese range. Gender may also have an impact on outcome. Studies included males and females in the majority of comparisons. Only 4 comparisons included all males, interestingly there were no significant differences in appetite or food intake for these comparisons (Doyon et al., 2015; Smith et al., 2012). However, for the studies including all females, there was a significant reduction in appetite and food intake in 4 out of the 5 comparisons (Arshad et al., 2016; Harrold, Hughes, O'Shiel, et al., 2013; Pentikainen et al., 2014). This suggests there could potentially be some gender differences in appetite and food intake, males consume more than females habitually, but they may also be more susceptible to overconsuming in all conditions in a test setting (Ello-Martin et al., 2005).

Test Meal

Previous research has shown that test meal can have an impact on appetite and food intake as sensory and hedonic factors play an important role in meal termination

(Zaremba et al., 2017). A limited choice meal is often favoured as the meal of choice in studies measuring food intake (Gibbons et al., 2019). However, a limited item meal is at risk of the "portion size effect" as participants who are used to plate clearing finish the entire meal (Hetherington & Blundell-Birtill, 2018). Of the 11 comparisons that included a single item meal appetite or food intake was significantly reduced in 11 comparisons. Conversely, the multi item meal may encourage overconsumption in test and control conditions through variety as participants eat beyond satiation (Long, et al., 2000), this has been linked to hedonic hunger (Espel-Huynh et al., 2018) and sensory specific satiety (Brondel et al., 2009). In the current review thirteen comparisons included a limited buffet meal appetite or food intake was significantly reduced in 6 (46.2%) comparisons. The multi-item buffet was included in 9 comparisons appetite or food intake was reduced in 6 (66.7%) comparisons. The studies with the highest variety test meals, 16 buffet items (Monsivais et al., 2011) and 33 item buffets (Doyon et al., 2015) found no effect in 4 comparisons for both food intake and appetite. However, (Wiessing et al., 2012) found that there was no difference in the sensitivity to detect an effect on appetite or food intake between a buffet meal and single item meal.

There are theoretical advantages and disadvantages to both single-item restricted meal and multi-item buffet test meals (Zaremba et al., 2017). Nutrient intake in food choice studies is clearly better addressed by presenting a multi-item buffet meal (Blundell et al., 2010) which allows the participant to make wide choices from foods which differ in energy, energy density and macronutrient content. There is however very little research into the validation of this multi-item buffet meal (Zaremba et al., 2017). One of the main issues is that it is difficult to establish valid food selection methodology, consequently this area has not been developed extensively, indeed none of the studies presented in this review provide any empirical evidence for why

particular outcome test meals are selected. It is difficult to determine the optimal test meal composition (McCrickerd & Forde, 2016) when this isn't something which has been extensively investigated. As this review highlights this lack of consensus is problematic since variation in this *ad libitum* meal may have potentially influenced the primary outcome of the studies.

The use of familiar foods in the buffet test meal may overcome the constraints of using manipulated diets to recreate the real-life feeding situation, yet, the choice of foods provided in the laboratory is inevitably limited. In the real-world individuals seldom face such a variety of foods from which they can freely choose in one sitting, however, to accurately measure food intake and particularly differences in macronutrient intake there needs to be a variety of foods in the buffet meal. Ensuring that the *ad-libitum* outcome meal is sensitive to manipulations made within the fixed preload test meal is essential in all postprandial appetite studies. A strong methodology will exert strong control over the nutritional and sensory aspects of each item. Chapter 5 looks more closely at the formulation of the ad libitum test meal.

Preload Formulation

The preload formulation can potentially influence appetite and food intake beyond increasing the fibre dose. The food matrix by which the fibre is delivered has a pivotal role, in this review fibres in liquid form appeared to reduce appetite in more comparisons than semi solid fibres. In the current review there was a significant effect for inulin with 6g in yoghurt (Perrigue et al., 2009), however Karalus et al. (2012) found no effect on appetite or food intake for 10g inulin in chocolate crisp bars. Similarly, most studies have failed to show any significant effect of β-glucan on satiety when prepared in solid or semi-solid compared with liquid preloads. This could be because soluble fibres, when prepared in liquid form, absorb more water and increase stomach

distension, increasing fullness. Though we selected studies with a low fibre, or no fibre control it was difficult to tell whether the preloads were matched in terms of palatability and texture. Adding some fibres to liquids can alter the texture, particularly some viscous fibres which may have an impact on outcome measures. An increase in calories between the control and test preload often isn't reported and when testing takes place over part of the day the preload calories are often not included in the food intake calculations.

Fibres in liquid form reduced appetite in more comparisons than semi-solid fibre, but this was not the case for food intake. This reconfirms the disassociation between food intake and appetite in the literature (Yeomans, 2018). Potentially prior association in memory between sensory cues and particular post-ingestional consequences for the semi-solid preload reduced food intake (Boon et al.,1998; A. Worsley, 2002) as it is difficult to untangle the previous experience/memory from the true satiating capacity of a food or ingredient. (Gadah et al., 2012). Previous experiences/memory guide meal anticipation and learned associations with anticipated reward and pleasure. The semi-solid preload may also provide post-ingestive information via the stomach and intestines more effectively than the liquid preload where a non-viscous fibre is present through the physical signals of distension, providing feedback related to meal quantity releasing gut peptide hormones including GLP-1, CCK and PYY as digesta pass through the gastrointestinal tract inhibiting food intake.

Intrameal Interval

Different fibre types may favour different intrameal intervals. More viscous fibres may favour a shorter test period whereas fermentable fibres may favour a longer test window. When fibres were grouped according to physiochemical properties viscous fibres the mean duration was 225.9 minutes whereas fermentable fibres were tested

over 295.9 minutes. This may explain the results in (Doyon et al., 2015) study that found no effect for β-glucan on food intake with a intrameal interval of 270 minutes, whereas (Barone Lumaga et al., 2012) found that β-glucan reduced appetite and food intake after 180 minutes. For viscous fibres, it may be important to administer the *ad libitum* meal shortly after the fibre preload, potentially as soon as 30–120 minutes after consumption when gastric emptying is still ongoing (Müller et al., 2018).

An intrameal interval of 240 minutes or more may be more suitable for fermentable fibres. There was no significant effect on food intake for polydextrose with intrameal time interval of 200 minutes (Monsivais et al., 2011). However, another study found polydextrose with a longer time interval of 615 minutes, testing over 2 meals significantly reduced appetite and food intake (Hull et al., 2012). This supports previous papers suggesting there could be second meal effects, where fermentable fibre may modulate not only the first subsequent meal after consumption but also later meals on the same day. Only 8 comparisons included full day food intake analysis using food diaries in the evening, food intake and appetite was significantly reduced in all 8 comparisons, with much larger average reduction in food intake observed (347.6kcal, 15.2%). This highlights the need to test beyond 4 hours and suggests that a slightly longer test frame may work better across fibre types as bigger effects were found later in the day after a second meal.

Combining fibres

Combining fibres with different physiochemical properties could have a synergy effect on food intake and appetite (Salleh et al., 2019). Viscous fibres have immediate post-ingestive effect and fermentable fibres have an effect after 240 minutes, combing fibre with such properties warrants further investigation. It would not be feasible to combine two highly viscous fibres due to the potential to create a drink too viscous to consume.

B-glucan could be combined with fibres that display a sweet taste such as inulin to improve palatability. Inulin can be added to any food or drink as a soluble powder, with little impact on the texture, making inulin an ideal fibre to combine with a more viscous fibre such as β-glucan (Barclay et al., 2010).

A systematic review to summarise the available literature on the relationship between specific dietary fibre types, subjective appetite and energy intake. In this review we will specifically 1) identify the most effective dietary fibre type to reduce acute appetite and energy intake 2) identify the most effective fibres in terms of their physiochemical properties 3) identify the optimal dose for such an effect and 4) Report current research practices to measure the acute effects of a fibre preload on appetite and food intake and identify study design issues.

Conclusion

This review was conducted to determine the effects of soluble dietary fibre in a liquid or semi-solid matrix on appetite and food intake. The most effective dietary fibre types to reduce acute appetite and energy intake were identified. Overall, 61.9% of comparisons reduced appetite. For food intake, 57.6% of comparisons significantly reduced intake. An optimal dose for a significant reduction in appetite and food intake was identified for each fibre. Not all soluble fibres have similar effects, fibres with different physiochemical properties affect appetite and food intake differently. Viscous fibres were identified as being more effective at reducing appetite, whereas fermentable fibres were more effective at reducing food intake. More viscous fibres favoured a short intrameal interval, whereas fermentable fibres favoured a longer interval. Current research practices were also explored to identify study design issues as the effects of fibre may be confounded by other factors such as study design, particularly preload formulation, meal size and study duration. Future research should

aim to improve the methodology of the preload study design to standardise procedures and determine whether combining soluble fibres with different physiochemical properties could enhance the reduction in appetite and food intake.

Chapter Four

4. Optimising the preload design; Scaling a liquid fibre preload for BMI reduces appetite and food intake above the compensation required for the preload.

Overview

Chapter four and five utilise the same study data but address distinct research questions within a 2 x 3 design. Chapter 4 aims to directly assess whether scaling the preload for BMI in an acute prebiotic study could improve the overall effectiveness to detect an effect on appetite and food intake. We hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) and food intake measures after a preload adjusted for body mass compared to a standard fixed inulin preload for participants who are obese. Chapter 5 identifies the optimal number of *ad-libitum* test meal items to detect an effect on appetite and food intake. We hypothesised that i) There will be an increase in food intake and a reduction in appetite in a high variety buffet meal compared to a low variety buffet meal for normal weight and participants who are obese. ii) Normal weight but not participants who are obese will compensate for the increased intake at the high variety meal. iii) A high variety meal will decrease the chances of observing an effect of a fibre preload on appetite and food intake in participants who are obese. The studies are presented separately however the methodology for both studies can be found on page 120.

4.1 introduction

Preload studies measure the effects of a preload on postprandial appetite related ratings such as hunger and fullness using VAS and *ad-libitum* food intake from one or more subsequent test meals. The design of the preload study is complex, with many methodological considerations influencing appetite and food intake outcome measures (Blundell et al., 2010). The simplicity of the preload design and its

subsequent wide use has led to large and widely varying literature for the effects of fibre on appetite and food intake, with limited consensus (K. S. Poutanen et al., 2017) (Blundell et al., 2010). Preload studies are often used to validate claims made by food manufacturers (Pravst et al., 2018) therefore food intake methodology is particularly important to functional food research. Previous studies have explored various characteristics of the preload study design to try to standardise methodology, such as test meal timing (Chungchunlam et al., 2012), study duration (Hobden et al., 2017) and the characteristics of the test meal itself (Brondel et al., 2009) (Williams, Roe, & Rolls, 2014). However, to date there has been very little research into the preload characteristics (Eva Almiron-Roig et al., 2013). Studies often include both normal weight and overweight participants with little or no attention paid to the differences in appetite control. Whether scaling the preload for BMI in an acute prebiotic study could improve the overall effectiveness to detect an effect on appetite and food intake remains to be explored.

Characteristics of the preload can vary on many dimensions; absolute energy content (Rouhani et al., 2017), macro nutrient content (Bellissimo & Akhavan, 2015), cognitive aspects (Brunstrom et al., 2010), sensory aspects (Rogers & Shahrokni, 2018) and state (Almiron-Roig et al., 2013). These differences can often be observed within the same study, between different conditions, potentially influencing the study outcomes (Rebello et al., 2016). How these characteristics influence appetite and intake maybe heightened in participants who are obese (Ribeiro et al., 2018). Very little attention is paid to the design of the preload when considering how appetite control differs between overweight and normal weight participants. Potentially a preload that finds an effect in normal weight participants may fail to detect an effect in overweight participants. Whether the preload could be improved to detect an affect in both overweight and normal weight participants needs to be explored further.

Macronutrient Content

Macronutrient content is thought to play an important role in preload formulation. Fat, carbohydrates, and proteins do not provide the same amount of energy. A gram of proteins or carbohydrates provide about 4 calories and a gram of fat about 9 calories. The number of calories per gram of food and the extent to which a product provides satiety are not linear. Proteins are most satiating, however the satiating effects of protein is mainly observed using solid preloads in the form of familiar foods (Luhovyy et al., 2007), and not with liquid stimuli (Tieken et al., 2007). Fats are also more satiating in solid form (Hulshof et al., 1993). Some research suggests carbohydrates do not appear to differ in their satiating capacity in liquid or solid form (Martens et al., 2012). Whilst others suggest they are more satiating in liquid form (Wanders et al., 2011).

Fibre is considered a type of carbohydrate, unlike other carbohydrates, fibres differ in how well they are digested and how much energy is available to the body. Fibre is one of the main food constituents which can contribute to a lower energy density but can also increase satiety (Burton-Freeman, 2000). Soluble fibres, either absorb water and become gels, or dissolve in water and reach the intestine where they are digested by bacteria. As they are digested by bacteria soluble fibres produce short-chain fatty acids (SCFAs) that provide the body with energy. It is estimated that fibres fermented by bacteria provide about 2 kcal/g of fibre compared to other carbohydrates which provide about 4 kcal/g. Research has shown individuals who are normal weight and individuals who are obese have different bacteria present in the gut which may produce different SCFA, affecting both the rate that the fibre is fermented and also how much energy is harvested from the fibre. SCFAs are thought to be linked to satiety (Byrne et al., 2015), which could explain the differences in satiety between

normal weight and overweight after the same fibre preload. It isn't clear whether a larger fibre dose could increase satiety in participants who are obese.

Absolute energy content

Some discrepancies in results are thought to be due to differences in the absolute energy content. Energy compensation is the adjustment of energy intake provoked by the ingestion of a preload, whether a meal, a snack, or a beverage (J. Blundell et al., 2010). Insufficient energy compensation both in the short and the long term has been associated with increased energy intakes and positive energy balance, leading to obesity (Almiron-Roig et al., 2003). A high calorie preload may reduce any compensatory effects (Warwick & Weingarten, 1994), whilst a higher macronutrient composition may artificially inflate the satiety effects. The energy loads of the manipulations appear to be particularly critical as de Graaf and Hulshof (1996) found. Hence the relatively small energy differences of preloads may have been responsible for yielding negative results with respect to energy compensation in some studies (Warwick & Weingarten, 1994). It could potentially be easier to detect an effect of a fibre on food intake in normal weight participants as normal weight participants are able to compensate better after calories in a preload than participants who are obese (Almiron-Roig et al., 2013). Almiron-Roig et al., (2013) suggested that participants who are obese either have weaker satiety signals or are less sensitive to them. Improving the preload formulation may improve the chances of detecting a significant effect in an obese population.

Palatability

Variation in palatability and sensory characteristics may lead to an effect on satiety as a result of the palatability rather than the effects of the product itself (Rogers & Shahrokni, 2018). Increasing palatability is thought to be linked to increases in satiety

(Sorensen et al., 2003) (B. J. Rolls et al., 1991; Sorensen et al., 2003). Not only that, orosensory cues, such as food texture, have been shown to play a role in satiety, as a thicker textured beverage increased satiety (Martin R. Yeomans & Lucy Chambers, 2011). It is therefore important manipulations are covert. Matching a test preload on taste, texture, and palatability is important as small variations between the test preload and control preload can lead to significant differences in satiety due to the sensory differences rather than the manipulation in question. It is particularly difficult to untangle such effects when testing whole foods. It is possible to match whole food preloads for calorie content and in some cases macronutrient content but other sensory stimuli such as visual appeal, texture, and taste are difficult to replicate. Highly palatable preloads even when matched on taste, texture and other sensory measures across different conditions may lead to increased satiety in both control and experimental conditions, reducing the effects of the test product. It is unclear whether taste quality can moderate satiety through postprandial metabolic responses to the energy consumed. Tey, Salleh, Henry, and Forde (2018) on the other hand found that energy density rather than taste is more important when predicting energy compensation after a preload. Therefore, both palatability and energy density must be controlled during study design.

Cognitive

Cognitive factors modulate sensory perceptions such as palatability (McCrickerd & Forde, 2016; Rolls et al., 2011). Prior association in memory between sensory cues and particular post-ingestional consequences of eating have been found to affect appetite and intake (Boon et al.,1998; Worsley, 2002). When looking at the effects of fibre in whole foods it is difficult to untangle the previous experience/memory from the true satiating capacity of a food or ingredient. Even when the preload is matched for palatability, taste, and texture there is the potential with repeated measures design

for the learned effects of a more satiating experimental condition to be carried over to subsequent test days (Gadah et al., 2016; Yeomans, 2012). Familiarity could also be an issue as participants often consume more in the test-meal on the second test occasion than on the first due to familiarity with the preload and eating environment. Using a liquid preload may help to eliminate some of the issues associated with a solid preload such as previous experience, however selecting a liquid preload isn't without issue.

State: Solid vs. liquid

Solid and liquid preloads affect appetite in distinct ways as people's ability to compensate after a preload varies according to its form (Almiron-Roig et al., 2013). Liquid preloads have been found to be more satiating and reduce food intake more than the equivalent dose in solid form (Pan & Hu, 2011; Cassady et al.,2012; Drewnowski, 1998; Rolls et al., 2000). On average, beverages are around ten-fold lower in energy density than foods because of their high-water content which may explain such results. Yet others such as Almiron-Roig et al. (2003) point out liquids fail to trigger physiological satiety mechanisms, so that compensation for energy consumed as beverages may be imprecise and incomplete. However, when the manipulation was a fibre, Peters et al., (2009) found no effect on appetite or food intake with a 16g dose of inulin in a solid bar in an acute study, whereas Hess et al. (2011) found that a 16g dose of inulin in a preload drink reduced appetite and food intake by 370kcal over 24 hours. It could be that it is easier to covertly manipulate a drink removing all other sensory aspects, particularly with soluble fibres which can easily be added to drinks.

Preload Volume

Volume plays an important role in satiety, the higher weight or volume in the liquid preload is thought to increase stomach distension and lead to a feeling of fullness. (Bell et al., 2003) found that doubling the volume of the liquid food that was consumed, without changing the energy content, significantly increased sensory-specific satiety. Doubling the energy content of the food without changing its volume, however, had no additional effect on sensory-specific satiety. These results suggest that the volume of food that is consumed has a greater influence on satiety does its energy content. Similarly, Almiron-Roig et al., (2013) found that when participants consumed a liquid preload their adjusted intake at the next meal compensated for only 71% of the preload energy compared to 109% in solid form (*e.g.,* bread). Providing evidence that liquid calories elicit weaker effects on satiety even when food volume is increased. Such effects could potentially be heightened in participants who are obese.

Obese Physiology

Participants who are obese and participants who are normal weight often receive the same preload, despite the observed differences in appetite control between normal weight and overweight participants. Some studies have found an effect for fibre on appetite but only in normal weight participants (Howarth et al., 2001). The systematic review in chapter 3 demonstrated there were no significant differences in appetite or food intake in the 3 comparisons that focused exclusively on participants who were obese. Those with a large body mass may require a larger preload dose to elicit the same effect on appetite as normal weight participants (Smiljanec et al., 2017). individuals who are obese/overweight have weaker satiety signals (Batterham et al., 2003). A review by Hellstrom (2013) produced evidence that individuals who are obese are less responsive to internal physiological cues indicative of hunger or satiety. Individuals who are obese have a decreased ability to respond to post-

ingestive satiety signals, they experience less activation of higher brain centres in association with a meal and therefore compensate with increased meal size or frequent food intake. Women who are obese have also been shown to be less sensitive than lean women to covert variations in the energy content of orally ingested preloads (Rolls & Roe, 2002). In another study, lean participants adjusted their intake to changes in energy density, while participants who are obese failed to regulate their energy intake in response to an ad libitum meal (Rolls et al., 1999). Meyer-Gerspach et al. (2014) also found that participants who are obese could require more calories before their maximal satiation is reached and they stop eating this is because for individuals who are obese gastric emptying can be impaired with delayed interaction of nutrients with the intestine resulting in decreased GLP-1 and PYY secretion.

Previous research has shown equivocal results for the same fibre for normal weight and participants who are obese. There was no effect on appetite or food intake for participants who are obese with a premeal 10g dose of inulin in 500ml of water (Smiljanec et al., 2017). Parnell and Reimer (2009) also found no effects for FOS on subjective hunger or appetite in participants who are obese who consumed a significantly higher dose of 21g FOS/d. Giuntini et al., (2015) found that 16g inulin significantly decreased hunger and food intake by 140kcal in normal weight participants yet, Doyon et al., (2015) found no effect for 2.4g of inulin on appetite or food intake in normal weight participants. This may suggest a minimum dose of 16g/d could be required for an affect in normal weight participants, but also a much larger fibre dose may be required to achieve the same outcome in participants who are obese. Fibre dose cannot simply be increased without caution. Excessive fibre consumption may cause intestinal discomfort and flatulence because of their high fermentation rate and production of gases. A FOS dose over 40 g/day is reported to cause diarrhoea (Salmeron et al., 1997). It is difficult to evaluate what is an

acceptable dose as individual differences exist in terms of intestinal discomfort (Roberfroid, 2000). Studies should err on the side of caution with dose to minimise such discomfort.

Whether a scaled preload dose proportional to BMI could increase satiety signals is unclear. In one study normal and overweight women consumed two separate doses of FOS 0g, 5g, or 8g at breakfast and *Ad libitum* food intake was measured at a lunch meal at 240 min. The high dose of FOS significantly reduced food intake and hunger more in the 16g condition than the 10g condition, with a reduction of 32kcal. This suggests FOS reduces hunger and has an additive effect on food intake with increasing dose in women (Hess et al., 2011). Adjusting the preload size according to body mass in acute prebiotic studies is yet to be tested but given the results of Genta et al. (2009) who found that fibre dose adjusted for BMI (0.66g/kg/m^2) decreased food intake and increased weight loss over four months, such results suggest there could potentially be an effect in acute preload studies.

At present, beverages are by far the most active functional food category possibly because of convenience and ease of distribution and storage (Nazir et al., 2019). The need to reliably test and substantiate any claims made by such products, supports the need to improve research methodology in this area. Despite the studies looking at various aspects of the preload formulation, there is yet to be a study investigating scaling the preload for BMI in an acute study. The literature does not provide any clear indication that scaling a preload according to BMI, could increase the likelihood of observing significant changes in hunger and fullness and energy intake. Scaling a preload according to BMI increases the energy compensation required to detect a significant effect of the preload. If the effects of the preload are only small increasing the calories in the preload could remove any potential effects on food intake.

Increasing the fibre dose may not induce effects strong enough to compensate for this increase.

A short-chained inulin fibre preload was selected in the current study to test the effects of scaling a preload. Inulin is a prebiotic, dietary fibre extracted exclusively from chicory root. It is a naturally occurring oligosaccharide belonging to a group of carbohydrates known as fructans. Unlike most carbohydrates, inulin is non-digestible. This allows it to pass through the small intestine and ferment in the large intestine. Inulin as a soluble powder can be added to any food or drink with little impact on the texture whilst adding fibre and displays a slightly sweet, pleasant flavour, without raising blood sugar levels. This makes inulin a very attractive food ingredient for the food industry as it can be used as a replacement for sugar due to its sweet flavour and lower calorie content. Previous unpublished and published studies have demonstrated inulin can increase satiety (Cani et al., 2006) and reduce food intake (Whelan, 2006 ; Harrold et al., 2013).

The current study aims to directly assess whether scaling the preload for BMI in an acute prebiotic study could improve the overall effectiveness to detect an effect on appetite and food intake. Results from the current study will help to quantify the optimal preload design to improve methodology used in preload studies in general and specific studies during this PhD. This research is important to develop a standardised methodology in laboratory procedures for an agreed standard of working for theory and commercial development.

4.1.2 Hypothesis

We hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) and food intake measures after a preload adjusted for body mass compared to a standard fixed inulin preload for participants who are obese.

Methods 4.2
4.2.1 Materials and methods

4.2.2 Participants

Twenty-four healthy women, aged 18–55, with a body mass index (BMI) between 18.5 – 24.9 kg/m² (n =12) and 30- 34.9 kg/m² (n = 12) completed the study between September 2013 and August 2014. Given the requirements for volunteers to attend for six full day visits, participants were compensated for their time.

4.2.3 Study design

This was a single-blind, within subject study, using a randomised crossover design to test the effects of the number of *ad libitum* meal items on overall energy intake after a fixed or variable (adjusted for body mass) preload. Visual analogue scales (VAS) and *ad libitum* energy intake was assessed in 6 conditions 5, 10 and 20 food items with a standard preload and 5, 10 and 20 items with an adjusted preload. Participants were allocated to receive each of the six treatment arms in randomised order (i) 5 items; fixed preload (ii) 10 items; fixed preload (iii) 20 items; fixed preload iv) 5 items; adjusted preload (v) 10 items; adjusted preload (vi) 20 items; adjusted preload. The conditions were administered in a counterbalanced sequence. Participants' visits to the lab were separated by a 1-week washout period. At the end of six visits each participant had received each of the six conditions. The sample size (24 female participants; 12 normal weight and 12 obese) was calculated based on the previous research and a Power calculation Using G*power 3.1. For a paired design based on 0.8 power with medium effect sizes, a minimum of 12 participants would be needed to detect a 10 mm change in 8 h postprandial mean ratings (Flint et al., 2000) and a sample size of 16 to detect a 120kcal difference in ad libitum food intake (Gregersen et al., 2008). Previous studies testing the effect of a fibre preload on appetite and food intake have demonstrated similar findings with similar sample sizes (Vitaglione et al.,

2009; Beck et al., 2009; Cani et al., 2006). Randomisation to the study was conducted by means of Latin squares. Allocation of participants to conditions was performed by the experimenter.

4.2.4 Study procedure/standardised Instructions

On each day preceding the study day participants were asked to keep their food intake and activity levels similar and to record these in a diary from 5pm until they retired for the night. They were asked not to consume any alcohol and not to eat or drink anything except water from 12midnight until they attended the study centre the following morning. On each test day participants attended the research centre for breakfast (between 0830 and 9:00 hours), lunch (between 12:30 and 13:00 hours) and dinner (between 16:30 and 17:00 hours).

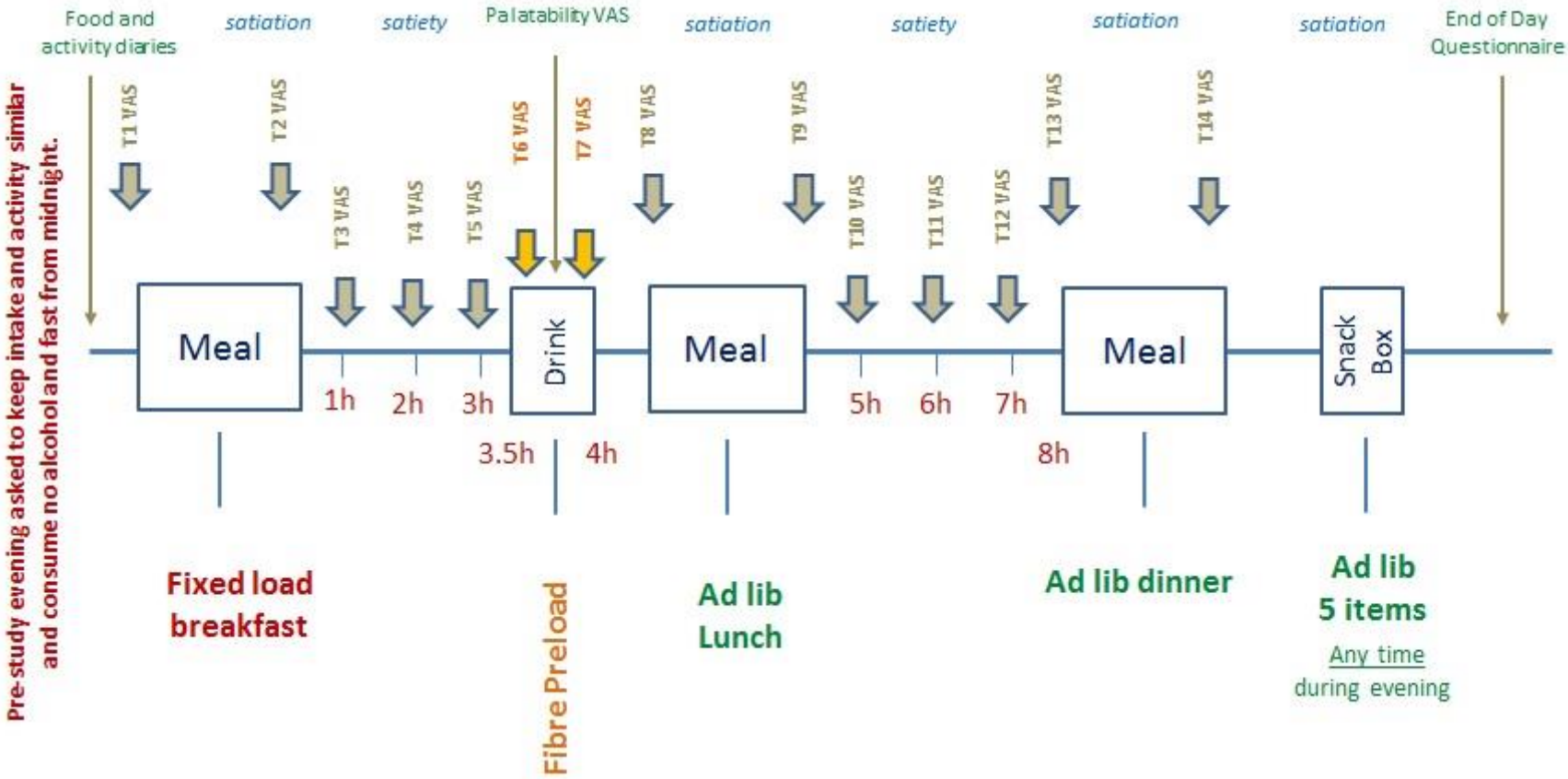

Figure 4–1 Protocol diagram outlining the study day.

Test Day Protocol

1. At ~8.30 am participants attended the study centre. On arrival participants were seated in an experimental booth (meals were consumed in isolation so that social influence did not affect food selection and intake). Evening food intake and activity diary were collected.
2. Participants then completed several VAS to rate their appetite sensations (hunger, fullness, desire to eat and prospective consumption) and thirst.
3. Participants were then asked to consume the fixed breakfast.
4. Upon finishing their meal, participants were asked to rate their appetite sensations and pleasantness of the food.
5. Participants were then free to leave the study centre once the researcher had provided some further questionnaires to complete hourly. (Participants were instructed to abstain from eating or drinking between breakfast and lunch except water that was provided by the study).
6. Thirty minutes before lunch (three and a half hours after breakfast). Participants returned to the lab and rate their appetite sensations (hunger, fullness, desire to eat, prospective consumption) and thirst. Between meal VAS were collected.
7. A preload cordial drink containing 15g inulin or 0.66g/kg/m^2 participant's body mass was served. Manufacturers of Metamucil inulin recommend a dose of 15g/day for adults over 12 years of age. Participants were asked to consume the entire drink within 10 minutes. Participants then completed a VAS to rate the pleasantness of the preload drink and appetite sensations.
8. Four hours after breakfast an *ad-libitum* lunch was served. All items were served in moderate excess with the intent that participants would not consume the entirety of any single item. Participants were instructed to eat as much as they liked from the choice

of foods and water offered, taking as long as they wished (up to 30 minutes), signalling when they have finished.

9. Upon finishing their meal, participants again rated their appetite sensations and pleasantness of the food. The researcher provided some further questionnaires to complete hourly until the next meal.
10. Participants returned for supper four hours later and were served a hot *ad-libitum* evening meal. Between meal VAS were collected. Participants were asked to consume as much as they would like, taking as long as they wish (up to 30 minutes) and signalling when they have finished.
11. The study day was then complete, and participants were provided with an evening snack, they were instructed to eat only from the snack box for the remainder of the day. Participants were asked to complete a diary to record food intake for the remainder of the 24h period, an end of day questionnaire to assess overall appetite experiences of the study day and a GI symptoms questionnaire. Arrangements were made to return the snack box containing any uneaten snacks/empty wrappers at the next visit.

The same procedure was followed on all test days with the six conditions randomised. The foods provided were familiar everyday food items available from Tesco stores (see p168 for individual food items provided).

Test Meals

Breakfast

A standard breakfast (496 kcal) was dispensed to participants (Table 4-1). In addition to the fixed-load breakfast, at the first visit, participants were offered a hot drink of tea or coffee with additional milk (35 g) and sugar if desired. If requested, this drink was to be consumed on each subsequent visit.

Table 4-1 Nutrient and energy profiles of foods served at breakfast.

Amount and energy composition of fixed load breakfast	
Food	**Amount (g)**
Kellogg's Cornflakes	30
Semi-skimmed UHT Milk	125
Orange Juice	200
Sliced White Bread (toasted)	60
Flora Margarine	10
Strawberry Jam	20
hot drink, 35 g milk and sugar (if required)	
TOTAL WEIGHT	**445**
TOTAL KCALS	496
10% Energy from Protein, 17% Energy from Fat, 73% Energy from Carbohydrate	

Ad-libitum meals

The *ad-libitum* meals (lunch and supper) were designed to offer a selection of high and low-fat savoury and sweet food items. The test meals were based on the sensory nutrient relationships of four sensory nutrient food groups; high fat savoury items, low fat savoury items, high fat sweet items and low-fat sweet items. Salad items are not included within these four sensory nutrient food groups but provided in addition to the distinct four groups. This model has been validated as a method to measure food preference and intake and has been used in a variety of studies to test sensory food preference (J. Blundell et al., 2010). See chapter 5 (p 168) for further details on test meal formulation.

Lunch was fixed at precisely 4 hours after breakfast and supper 4 hours after lunch. All meals were served in individual booths in the Kisseliff Laboratory. Cold food items appropriate for the meal occasion were served for the buffet lunch meal (see page 168). Participants were presented with the buffet items on separate plates on serving trays and instructed to select the

items and amount they would like to eat. At dinner a single course meal was served with a selection of desserts (see page 178). For lunch and dinner each food was presented in excess. Selected foods were weighed, and leftovers were used in intake calculations. Liquid and semi-solid foods were limited at the test meals; milk or juices were not provided to prevent the participants from consuming amounts similar to those consumed habitually, water was however provided at each meal.

4.2.3.3 Preload Formulation

An inulin preload was provided in a blackcurrant cordial diluted with chilled water 30minutes before the *ad-libitum* lunch. A cordial drink was selected due to the low-calorie content and the ability to mask the slightly sweet taste of the fibre. The preload was scaled for BMI by increasing the overall volume of the drink. The preload drink provided a fixed or adjusted load (15g or 0.66g/kg/m^2) according to each participant's BMI. Guided by the systematic review chapter 3 the optimal inulin dose of 0.66g/kg/m^2 was selected, In the systematic review inulin reduced appetite and food intake with a dose of 15g in participants who were normal weight and in participants who are obese this dose was 21g. Previous research suggested the dose selected was considered to be well tolerated and safe for participants as doses of 30g-40g/day have been shown to cause adverse gastrointestinal symptoms. Participants who were normal weight received an average dose of 15.2g. and participants who were obese received a dose of 21.7g (see Table 4-3 for preload composition). The fixed load contained 15g inulin, 15g black current squash and 200g water. The adjusted load provided 0.66g/kg/m^2 inulin, 0.66g/kg/m^2 of squash and 8.88g/kg/m^2 water, the volume of the drink varied from 240.6ml for participants who were normal weight to 333.1.ml. for participants who were obese.

Table 4 -2 Composition of the fixed or adjusted load preload drinks. Values are mean for 12 participants who are obese and 12 normal weight participants.

	Normal weight Fixed Load			Normal Weight Adjusted Load			Overweigh Fixed Load			Obese Adjusted Load		
		SE	±Std		±SE	±Std		±SE	±Std		±SE	±Std
Fibre												
Gram Intake (g)	15	0	0	15.2	0.2	0.9	15	0	0	21.7	0.4	1.4
Energy Intake (Kcal)	64.65	0	0	67	1	3.8	64.65	0	0	93.6	1.7	6.1
Squash												
Gram Intake (g)	15	0	0	15.2	0.2	0.9	15	0	0	21.7	0.4	1.4
Energy Intake (Kcal)	2.6	0	0	2.8	0	0.1	2.6	0	0	3.7	0.1	0.2
Water												
Gram Intake (g)	200	0	0	210.2	3.3	11.3	200	0	0	289.6	5.4	19
Energy Intake (Kcal)	0	0	0	0	0	0	0	0	0	0	0	0
Total Preload												
Gram Intake (g)	230	0	0	240.6	3.6	13	230	0	0	333.1	6.2	21.8
Energy Intake (Kcal)	67.2	0	0	68.8	1	3.9	67.2	0	0	97.3	1.8	6.3

Metamucil® inulin has been used in previous ethically approved unpublished studies in the Kisseliff laboratory which has provided evidence for an effect on appetite and intake. Metamucil® is produced by Proctor and Gamble. It is a commercially available inulin fibre which can be purchased as an individual ingredient or as a component ingredient of other commercially available products. It is consumed as part of the standard European diet and was available to purchase both in the UK and Europe. It was available to purchase in a variety of stores in the UK however, due to low demand it was removed from the UK retail market but was available to purchase online. Metamucil® was selected as it is 100% natural inulin fibre and can be easily incorporated into beverages.

4.3 Statistical analysis

All data collected was recorded and analysed in SPSS (Statistical Package for the Social Sciences) 22. Data conformed to the requirements for parametric analysis therefore Analysis of Variance (ANOVA) was used. If the assumptions of sphericity were violated, Greenhouse

Geisser correction was employed. All tests were two tailed, and a *P* value < 0.05 was considered significant. Data was analysed in three main steps to assess the effect of BMI adjusted load on food intake and appetite, number of items on appetite and food intake and the effects of the load and food items. Where an interaction was present further ANOVAs were carried out separately for each weight category.

Intake at the test meals was analysed for amount consumed (in grams and kcal) using a repeated measure analysis of variance (ANOVA) with the fixed load/adjusted load as the within-subjects factor. Weight (over or normal weight) was also examined as a between-subjects factor in the analysis to test for an interaction for weight status and load. *Post hoc* analyses correcting for Bonferroni adjustments were carried out to identify where differences lay. Where an interaction was present further ANOVAs were carried out separately for each weight category.

All Participants Interaction

Weight (obese or normal weight) was also examined as a between-subjects factor in the analysis to test for an interaction for weight status and load. *Post hoc* analyses correcting for bonferroni adjustments were carried out to identify where differences lay. Intake at the test meals was analysed for amount consumed (in grams and kcal) using a repeated-measures analysis of variance (ANOVA) with the fixed load/adjusted load as the within-subjects factor.

Changes in ratings of appetite such as hunger, fullness, prospective consumption, and desire to eat, assessed the nature of any reductions in food intake. These parameters rated on the VAS were analysed using within-subject ANOVA for repeated measures with condition (fixed load and adjusted load) and time (pre-breakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-drink, post drink, pre-lunch, post-lunch, 2 pm, 3 pm, 4 pm, pre-supper and post-supper) as within-subject factors. If a time-by-condition interaction effect was found significant, paired t-

tests would have been conducted at each rating time between conditions. An appetite score was also calculated using the formula ((hunger + desire to eat + prospective consumption) + (100 - fullness) /4)) for each condition in order to reduce variance in the appetite data. The trapezoid rule was used to calculate area under the curve (AUC) in accordance with the recommendations of Blundell et al. (2010), for each VAS variable and differences in AUC VAS ratings were assessed using repeated measures ANCOVA with baseline values serving as covariant. Separate ANOVAS were also performed for each weight category.

4.3 Results

Participants

In total, 41 participants were screened, 26 were recruited and 24 completed the study. Two participants withdrew due to reasons unrelated to the study. The screening measures, demographic (age) and anthropometric (weight, height, BMI) characteristics of the completing participants are shown in Table 4-3.

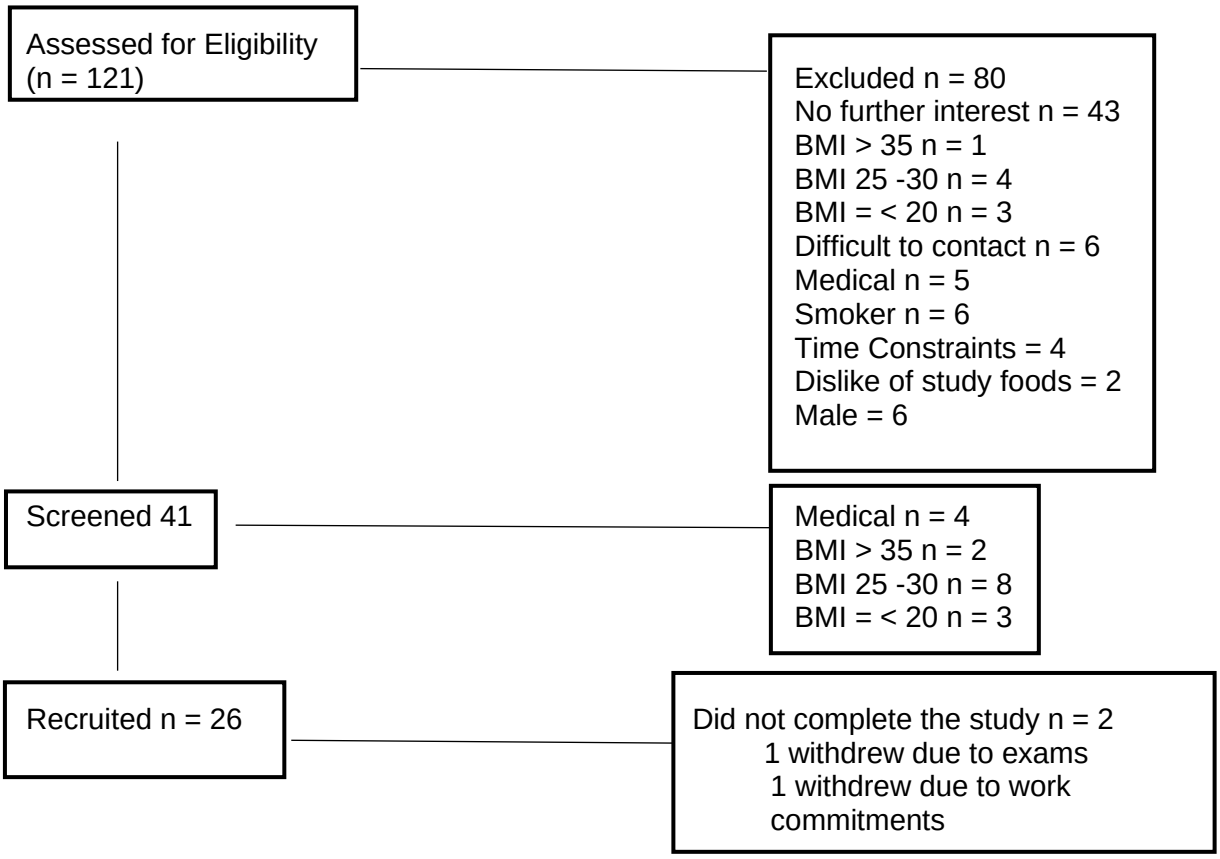

Figure 4-2 Total enquiries, participants screened and recruited into the study

Table 4- 3 Baseline characteristics of participants who completed the study

	Obese (*n* =12)			Normal weight (*n* = 12)			*p*
	Mean	±SE *a*	±Std *c*	Mean	±SE *a*	±Std *c*	
Age (years)	34	3.25	11.3	28	2.3	8	0.131
Height (m)	1.63	0.02	0.7	1.67	0.02	0.08	0.177
Weight (kg)	86.5	2.6	9.1	60.8	2.3	7.9	0.001*
BMI (kg m2)	32.6	0.6	2.1	21.7	0.4	1.2	0.001*
DEBQ *b*) restraint score)	2.33	0.19	0.68	2.29	0.14	0.49	0.864

a Standard error. *b* Dutch eating behaviour questionnaire. *c* Standard deviation. *P <0.05 normal weight vs obese.

Load Composition

There was a significant difference in total preload (g) for the adjusted preload compared to the fixed preload (g) for the participants who are obese t (11) = -16.741, $p < 0.001$ and normal weight participants t (11) = 2.322, p = 0.04. There was a significant difference in total preload calories for the participants who are obese t (11) = -16.741, $p < 0.001$ and normal weight participants t (11) = 2.322, p = 0.04.

Preload Drink Taste Perceptions

There were no significant differences in taste t (23) = -1.504, p = .146, palatability t (23) = -1.514, p = .120, pleasantness t (23) = -1.743, p = .095, sweetness t (23) = -0.661, p = .515 or saltiness t (23) = 0.034, p = .973 for the fibre drink in the adjusted or fixed load conditions for all participants. There was no significant difference in pleasantness t (11) = 1.153, p = 0.273, palatability t (11) = -0.190, p = 0.853, taste t (23) = -0.929, p = 0.373, sweetness t (11) = 0.233, p = 0.820 and saltiness t (11) = 1.321, p = 0.213 for the normal weight participants. There were no significant differences in pleasantness t (11) = -1.153, p = 0.273, paletability t (11) = -0.190, p = 0.853, taste t (11) = -0.929, p = 0.373, sweetness t (11) = 0.233, p = 0.820 and saltiness t (11) = 0.321, p = 0.213 for the participants who are obese.

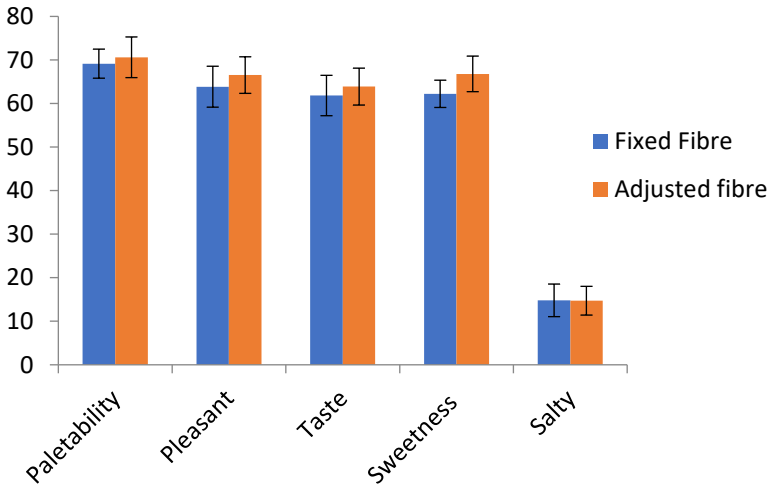

Figure 4-3 Effect of fixed or adjusted load on the different dimensions of taste for the fibre drink. Values are mean for 24 participants.

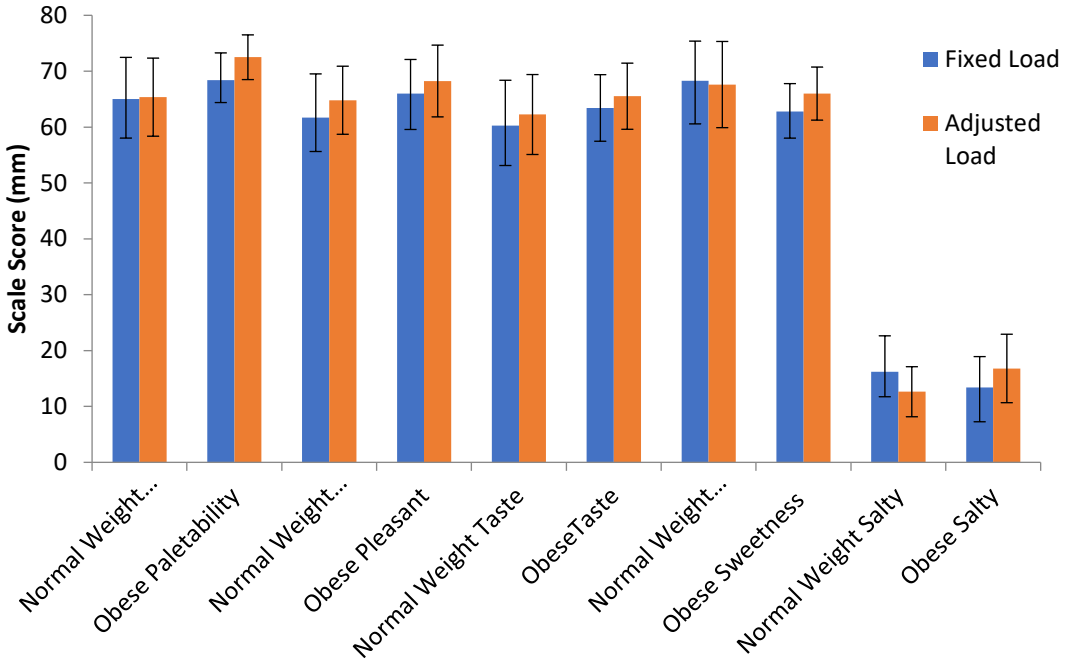

Figure 4 -4 Effect of fixed or adjusted load on the different dimensions of taste for the fibre drink. Values are mean for 12 normal weight and 12 participants who are obese.

GI Symptoms

There were no adverse reactions reported and no significant differences in GI symptoms reported in participants who are normal or overweight on any of the 5 measures assessing; how bloated, comfortable, flatulent, tight has your stomach felt and how much abdominal discomfort experienced, compared to the control condition.

Results Summary Food Intake

All Participants

Intake at the test meals was analysed for amount consumed (in grams and kcal) using a repeated-measures analysis of variance (ANOVA) with the fixed load/adjusted load as the within-subjects factor.

Intake at Test Meal for Fixed and Adjusted load Condition
All Participants

Intake at lunch, dinner, total ad libitum and total intake (in grams and kcal) was calculated for each condition. Food Intake was analysed using a repeated mixed measures ANOVA with the fixed and adjusted load conditions as the within subject effects and weight category as the between subject effect.

Lunch

There was no significant main effect for load on calories consumed at lunch f (1, 22) = 2.87, p = .598 or food consumed at lunch (g) f (1, 22) = .076, .785.

Dinner

There was a main effect of condition on food intake at dinner (kcal) f (1, 22) = 9.398, p = .006 with a 7.31% reduction in calories for the adjusted load condition compared to fixed load condition. There was also a reduction in food intake (g) at dinner f (1, 22) = 8.983, p = .007 (6.3%).

Total Ad Libitum Intake

There was a main effect of condition on total ad libitum calories consumed f (1, 22) = 4.977, p = .036 with a 5.6% reduction in the adjusted load condition. There was a trend for condition on ad libitum food intake (g) f (1, 22) = 3.160, p = .089 with a 4% reduction food intake (g) in the adjusted condition.

Total Intake

A trend for total calorie intake (kcal) was also found f (1, 22) = 3.694, p =.068 with a reduction in food intake (3.6%) for the adjusted load condition compared to the fixed

condition and a trend for total food intake (g) f (1, 22) = 3.567, p = .072 with a 3% reduction in intake.

Table 4 - 4 Intake at Test Meal for Fixed and Adjusted load Condition all participants

	Fixed load			Adjusted load			
		±SE	±Std		±SE	±Std	%
Lunch							
Gram Intake (g)	343.7	18.1	88.6	346.7	19.4	94.9	.9
Energy Intake (Kcal)	647.5	39	191.2	634.4	4.1	196.6	-2.1
Dinner							
Gram Intake (g)	617.4*	35.8	175.4	578.5*	39.7	194.3	-6.7
Energy Intake (Kcal)	914.9*	45	22.4	848.0*	45.7	223.7	-7.9
Total Ad Libitum							
Gram Intake (g)	1045	5.9	249.4	1002.8	58.1	284.5	-4.2
Energy Intake (Kcal)	184.9*	84.9	415.8	1741.4*	84.8	415.3	-5.7
Total Day							
Gram Intake (g)	1519.4	51.4	251.8	1474.3	58.6	287.2	-3.1
Energy Intake (Kcal)	241.5	85.2	417.3	2323.7	84.7	415	-3.7

*P <.05 fixed load vs. adjusted load.

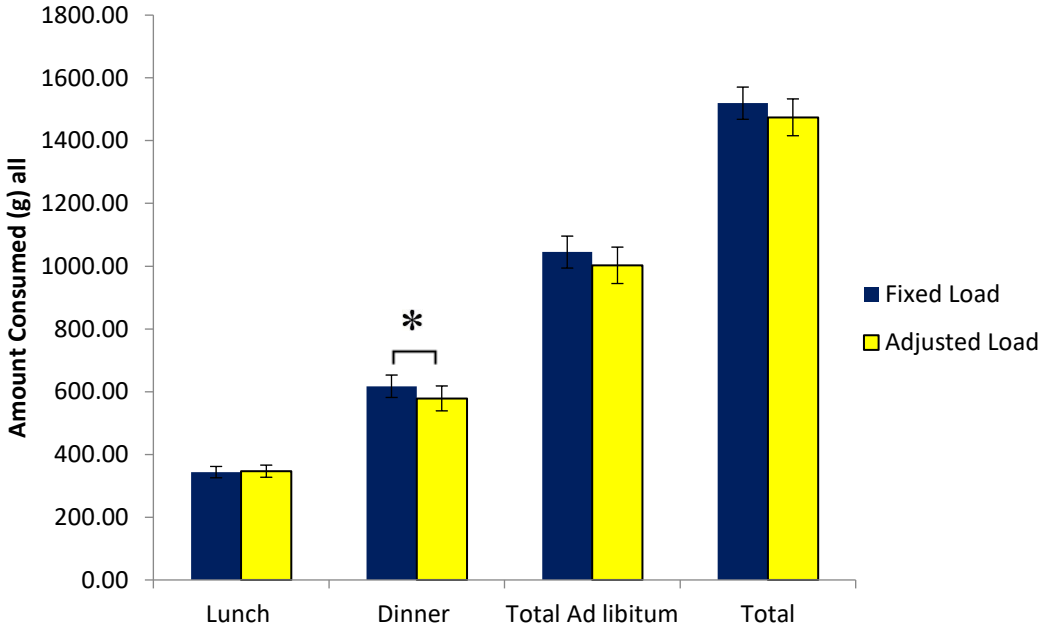

Figure 4-5 Effect of load condition on food intake at the ad libitum test meals. Values are mean for 24 participants. *P < .05 fixed load vs. adjusted load.

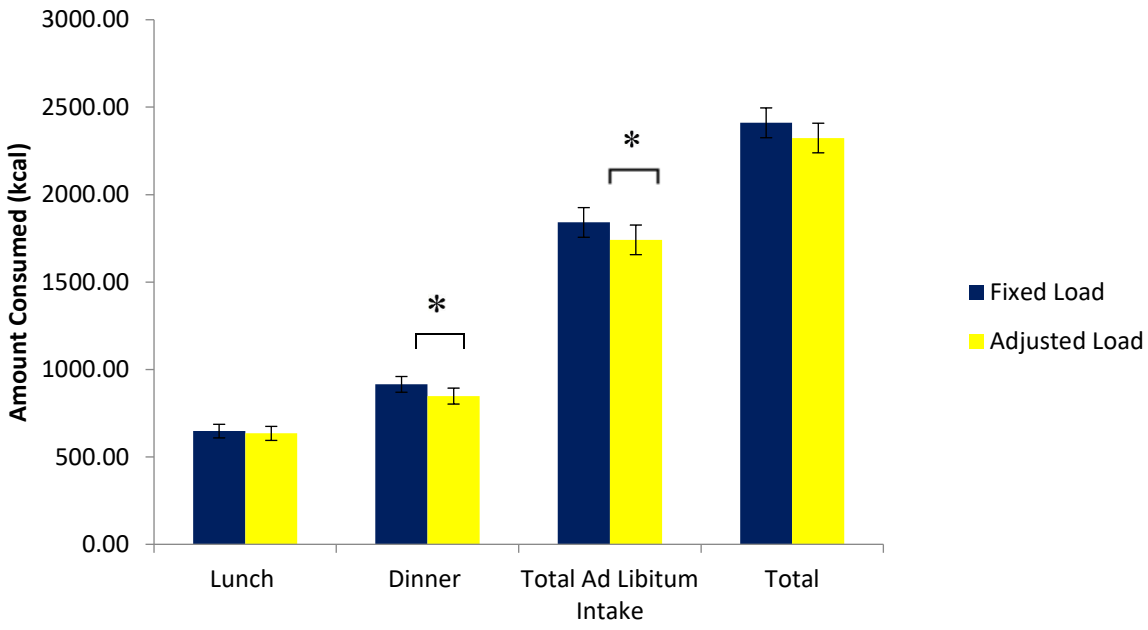

Figure 4 - 6 Effect of load condition on food intake at the ad libitum test meals. Values are mean for 24 participants. *P < .05 fixed load vs adjusted load

Weight Category analysis for Load Condition

Lunch

There was a significant interaction for load condition and weight category for lunch calories f (1, 22) = 3.620, p = .07 normal weight participants consumed 33.2kcal (5.6%) more in the adjusted condition while the participants who are obese reduced their intake by 59.2kcal (8.4%) in the adjusted condition. There was no significant interaction for food intake at lunch (grams) f (1,22) = 2.656, p = .117.

Dinner

A trend for interaction between load condition and group f (1, 22) = 3.706, p = .067 on energy intake at dinner was observed. There was a calorie reduction at dinner for both participants who were normal weight and participants who are obese with normal weight participants consuming 24.8kcal (2.8%) less calories and participants who are obese consuming 108.9kcal (11.7%) less calories in the adjusted load condition. There was a significant interaction between load condition and group for food intake

(g) f (1, 22) = 4.489, p = .046 the normal weight participants consumed 22.5g (1.8%) less food in the adjusted load condition and the participants who are obese consumed 66.5g (11.2%) less in the adjusted condition.

Total Ad Libitum

There was a significant interaction between the load condition and group for total ad libitum calories consumed f (1, 22) = 7.266, p = .013. The participants who are obese consumed 219.7kcal (11.6%) less in the adjusted condition compared to the fixed condition while the normal weight participants consumed 2.8kcal (1.2%) more. There was a significant interaction between the load condition and group for total ad libitum food intake (g) f (1, 22) = 6.271, p = .02. The participants who are obese consumed 101.6g (9.8%) less food while the normal weight participants consumed 17.3kcal (1.6%) more in the adjusted condition compared to the fixed condition.

Total Intake

A significant interaction between load condition and group f (1, 22) = 5.210, p = .032 on total energy intake (kcal) over the entire study day was observed. The obese group saw a calorie reduction in the adjusted compared to fixed conditions with a 19.3kcal (7.7%) reduction in total food intake while the normal weight group consumed a similar total amount with 16kcal (.7%) more.

There was a significant interaction between load condition and group f (1, 22) = 5.713, p = .026 on total weight of food consumed was observed. The obese group saw a reduction in the food (g) consumed throughout the day in the adjusted compared to fixed condition. The obese group consumed 102.3g (6.8%) less food while the normal weight group consumed 12g (.8%) more food.

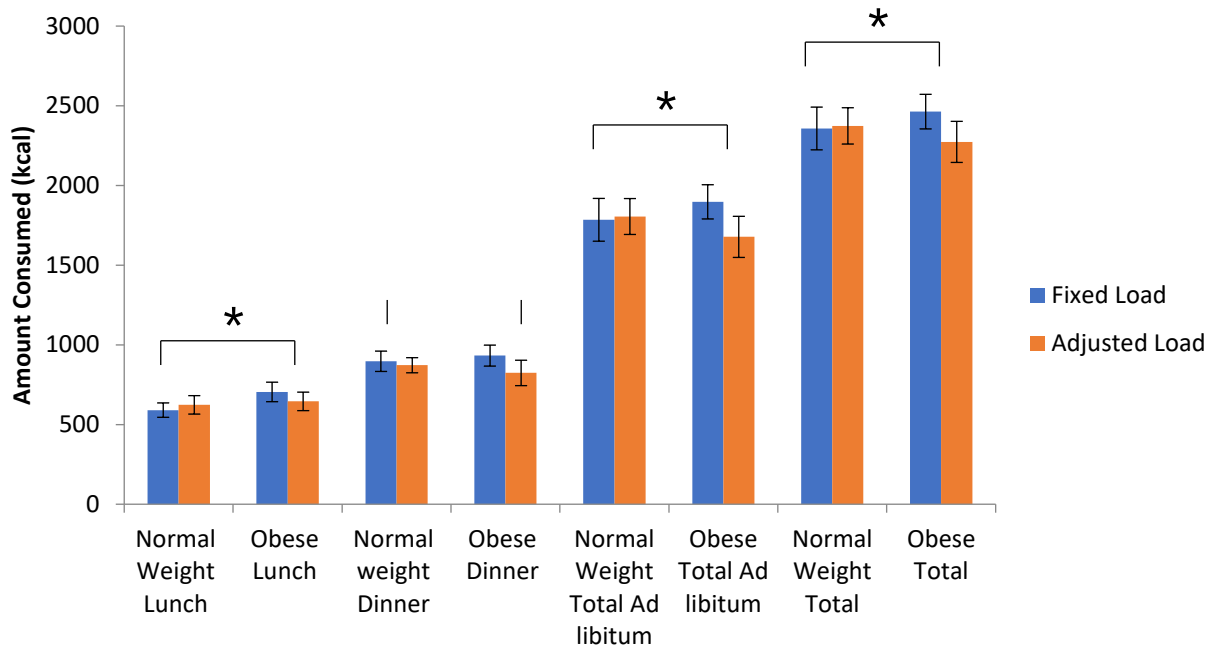

Figure 4 - 7 Effect of load condition on food intake at the ad libitum test meals. Values are mean for 12 normal weight and 12 participants who are obese. *P < .05 interaction load and weight status for total ad libitum intake. +P < .05 interaction load and weight status for total intake

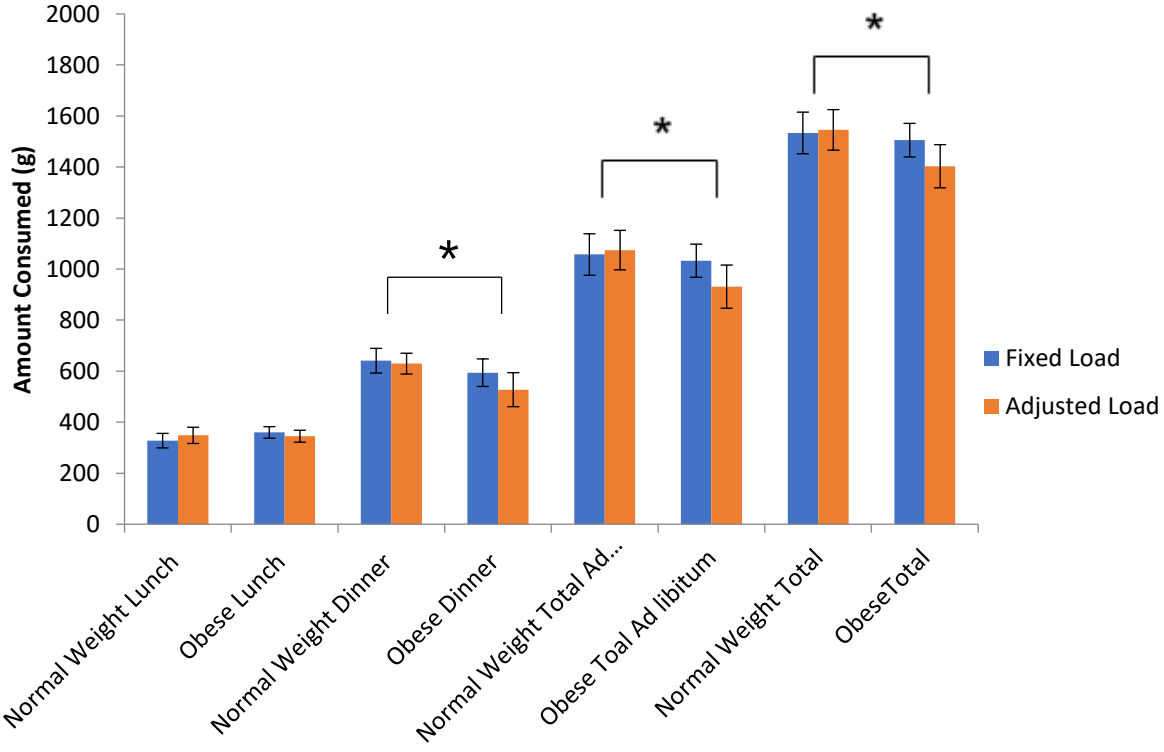

Figure 4-8 Effect of load condition on food intake at the ad libitum test meals. Values are mean for 12 normal weight and 12 participants who are obese. *P < .05 interaction load and weight status.

Table 4–5 Results summary for food intake for fixed load and adjusted load for 12 normal weight and 12 participants who are obese.

	Obese Fixed load			Obese Adjusted load			Normal Weight Fixed load			Normal Weight Adjusted load		
		±SE	±Std		±SE	±Std		±SE	±Std		±SE	±Std
Lunch												
Gram Intake (g)	359.8	22.6	141.3	345	23.4	150	327.6	38.4	133.1	348.4	35	121.2
Energy Intake (Kcal)	704.5	7.4	243.9	645.3	72.4	251	59.4	52.9	183.2	623.6	63.6	22.4
Dinner												
Gram Intake (g)	593.9*	60	207.8	527.4*	69.8	242	641.0*	58.7	203.5	629.5*	46.1	159.7
Energy Intake (Kcal)	932.8	72.8	252.2	823.9	85	295	896.9	73.2	253.6	872.1	58.5	202.8
Total Ad Libitum												
Gram Intake (g)	1032.8*	64.9	224.8	931.2*	84.7	293.3	1057.1*	81.3	281.5	1074.4*	77.5	268.4
Energy Intake (Kcal)	1897.2*	107.4	372.2	1677.5*	129	446.8	1784.5*	134.2	464.8	1805.3*	112.6	390
Total Day												
Gram Intake (g)	1505.3*	84	29.9	1403.0*	9.9	315	1534.0*	92.3	319.6	1546.0*	83.5	289.3
Energy Intake (Kcal)	2463.3*	122.3	423.6	2273.0*	138	480	2358.0*	149.2	516.8	2374.0*	126	435.9

*P <.05 fixed load vs. adjusted load.

Table 4-6 Percentage Change in Food Intake for the fixed and adjusted load conditions for 12 normal weight and 12 participants who are obese.

	Obese				Normal Weight			
	Fixed	Adjusted	Intake	%	Fixed	Adjusted	Intake	%
Lunch								
Gram Intake (g)	359.8	345	14.8	4.1	327.6	348.4	-2.8	-6.34921
Energy Intake (Kcal)	704.5	645.3	59.2	8.4	59.4	623.6	-33.2	-5.62331
Dinner								
Gram Intake (g)	593.9	527.4	66.5	11.2	641	629.5	11.5	1.794072
Energy Intake (Kcal)	932.8	823.9	108.9	11.7	896.9	872.1	24.8	2.76508
Total Ad Libitum								
Gram Intake (g)	1032.8	931.2	101.6	9.8	1057.1	1074.4	-17.3	-1.63655
Energy Intake (Kcal)	1897.2	1677.5	219.7	11.6	1784.5	1805.3	-2.8	-1.16559
Total Day								
Gram Intake (g)	1505.3	1403	102.3	6.8	1534	1546	-12	-.78227
Energy Intake (Kcal)	2463.3	2273	19.3	7.7	2358	2374	-16	-.67854

*P <.05 fixed load vs. adjusted load.

Food Intake Load Condition Participants Who Are Obese

Lunch

A trend for calories consumed at lunch f (1, 11) = 4.046, p = .069 suggested a reduction for the adjusted load condition compared to fix load condition. Participants who are obese consumed 59.2kcal (8.4%) less in the adjusted load condition, after compensatory intake for the preload they consumed 29.1kcal (3.9%). There was no significant main effect for load on food intake at lunch (grams) f (1, 11) = 1.037, p = .033.

Dinner

There was a significant main effect for load on calories consumed at dinner f (1, 11) = 15.330, p = .002 with a 108.9kcal (11.7%) reduction in intake for the adjusted load compared to fixed. After compensatory intake participants consumed 78.8kcal (8.6%) less in the adjusted condition. There was also a significant main effect for grams of food consumed at dinner (f (1, 11) = 1.903, p = .007 with a 66.5g (11.2%) reduction in food intake in the adjusted condition. After compensatory intake for the preload food intake was reduced by 36.6g (4.3%) in the adjusted condition.

Total Ad Libitum Intake

A significant main effect for total ad libitum calories f (1, 11) = 1.552, p = .008 was found with a 219.7kcal (11.6%) reduction in intake in the adjusted load condition compared to the fixed load condition. After compensatory intake for the preload food intake was reduced by 189.6kcal (1.7%) in the adjusted load condition. There was also a significant reduction in total ad libitum intake (g) f (1, 11) = 5.985, p = .032 with a 101.6g (9.8%) reduction in intake in the adjusted load condition compared to the fixed load condition. After compensatory intake for the preload participants who are obese consumed 1.5g (.1%) more in the adjusted condition.

Total Intake

There was a significant main effect for total calories consumed f (1, 21, = 7.501, p = .019 with a 19.3kcal (7.7%) reduction in total intake and a main effect of total food grams consumed f (1, 11) = 5.913, p = .033 with a 102.3g (6.8%) reduction in the adjusted condition.

and a main effect of total food intake (g) f (1, 11) = 5.913, p = .033 with a 6.79% reduction.

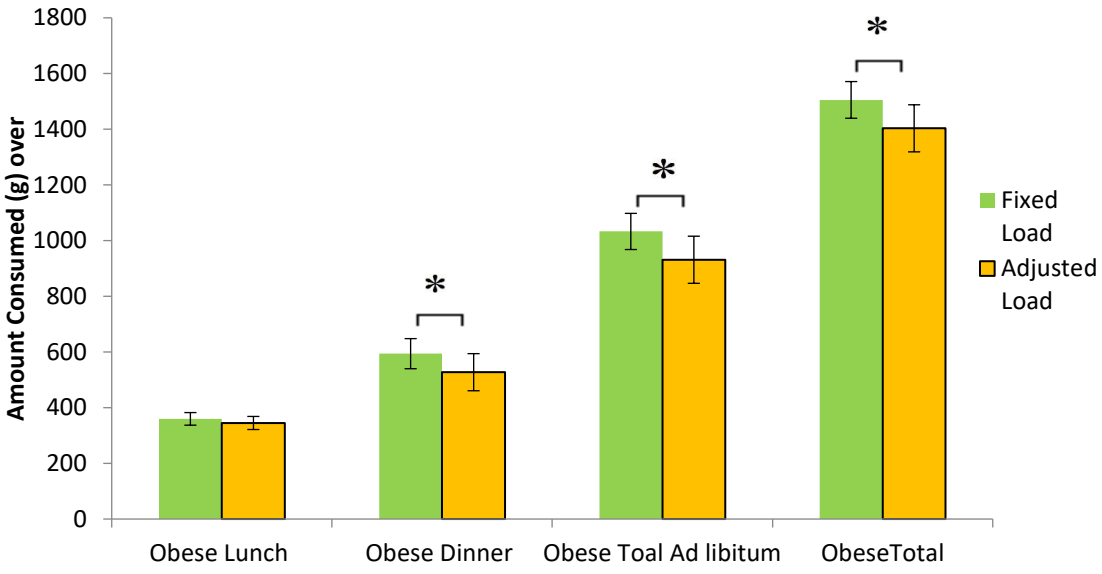

Figure 4-9 Effect of condition on food intake at the ad libitum test meals. Values are mean for 12 participants who are obese. *P <.05 fixed load vs. adjusted load.

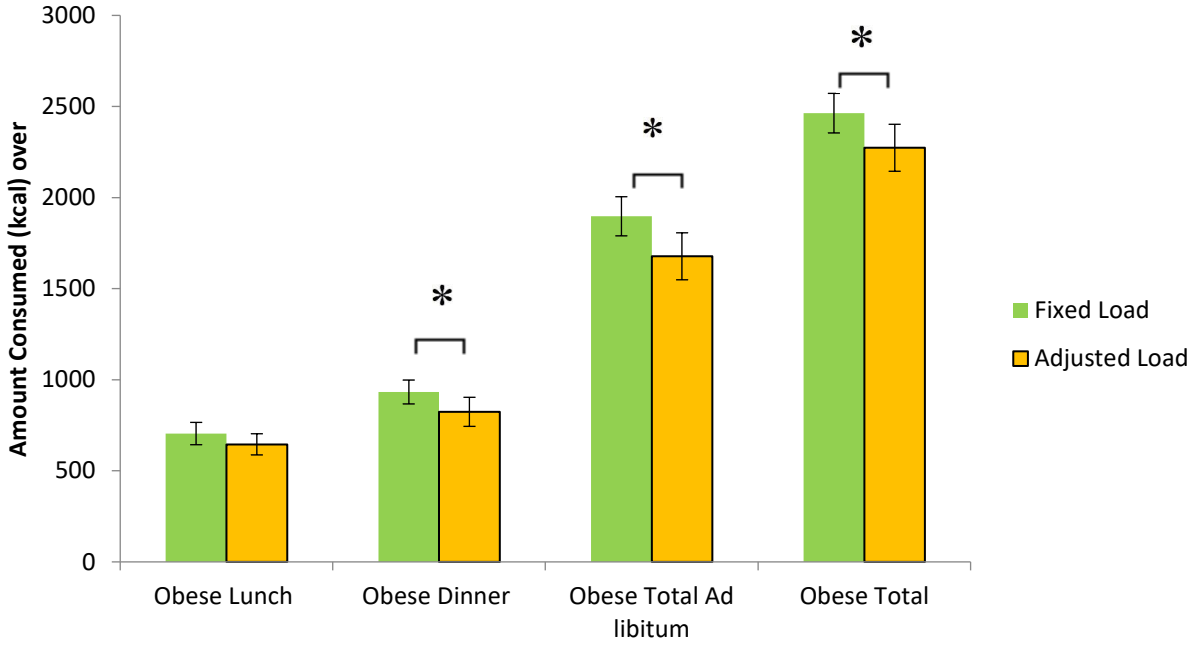

Figure 4–10 Effect of condition on food intake at the ad libitum test meals. Values are mean for 12 participants who are obese. *P <.05 fixed load vs. adjusted load.

Table 4-7 Results summary for food intake for fixed load and adjusted load for 12 participants who are obese

	Obese Fixed load			Obese Adjusted load		
		±SE	±Std		±SE	±Std
Lunch						
Gram Intake (g)	359.8	22.6	141.3	345.0	23.4	15.0
Energy Intake (Kcal)	704.5	7.4	243.9	645.3	72.4	251.0
Dinner						
Gram Intake (g)	593.9*	6.0	207.8	527.4*	69.8	242.0
Energy Intake (Kcal)	932.8*	72.8	252.2	823.9*	85.0	295.0
Total Ad Libitum						
Gram Intake (g)	1032.8*	64.9	224.8	931.2*	84.7	293.3
Energy Intake (Kcal)	1897.2*	107.4	372.2	1677.5*	129.0	446.8
Total Day						
Gram Intake (g)	1505.3*	84.0	29.9	1403.0*	9.9	315.0
Energy Intake (Kcal)	2463.3*	122.3	423.6	2275.4*	138.0	48.0

. *P <.05 fixed load vs. adjusted load.

Table 4- 8 Percentage Change in Energy Intake after compensatory intake for the fixed and adjusted load conditions for 12 normal weight and 12 participants who are obese.

	Obese				Normal Weight			
	Fixed	Adjusted	Intake	Over%	Fixed	Adjusted	Intake	Normal%
Lunch								
Gram Intake (g)	589.8	678.1	88.3	13.0	557.6	57.1	12.5	2.2
Energy Intake (Kcal)	771.7	742.6	-29.1	-3.9	657.6	688.4	3.8	4.5
Dinner								
Gram Intake (g)	823.9*	86.5*	36.6	4.3	871.0	851.2	-19.8	-2.3
Energy Intake (Kcal)	100.0*	921.2*	-78.8	-8.6	964.1	936.9	-27.2	-2.9
Total Ad Libitum								
Gram Intake (g)	1262.8*	1264.3*	1.5	.1	1287.1	1296.1	9.0	.7
Energy Intake (Kcal)	1964.4*	1774.8*	-189.6	-1.7	1851.7	187.1	18.4	1.0
Total Day								
Gram Intake (g)	1505.3*	1403.0*	-102.3	-7.3	1534.0	1546.0	12.0	.8
Energy Intake (Kcal)	2463.3*	2273.0*	-19.3	-8.4	2358.0	2374.0	16.0	.7

*P <0.05 fixed load vs. adjusted load.

Food Intake Load Condition Normal Weight Participants

Lunch

There was no significant main effect of load on calories consumed at lunch f (1, 11) = .738, p = .408 and food intake at lunch (g) f (1, 11) = 1.627, p = .228.

Dinner

There was no main effect of load on calories consumed at dinner f (1, 11) = .547, p = .475 food intake at dinner (g) f (1, 11) = .482, p = .502.

Total Ad Libitum

There was no main effect of load on total ad libitum calorie intake f (1, 11) = .127, p = .728 and total ad libitum intake (g) f (1, 11) = .564.

Total Intake

Total calories f (1, 11) = .079, p = .784 and total grams consumed f (1, 11) = .278, p = .608.

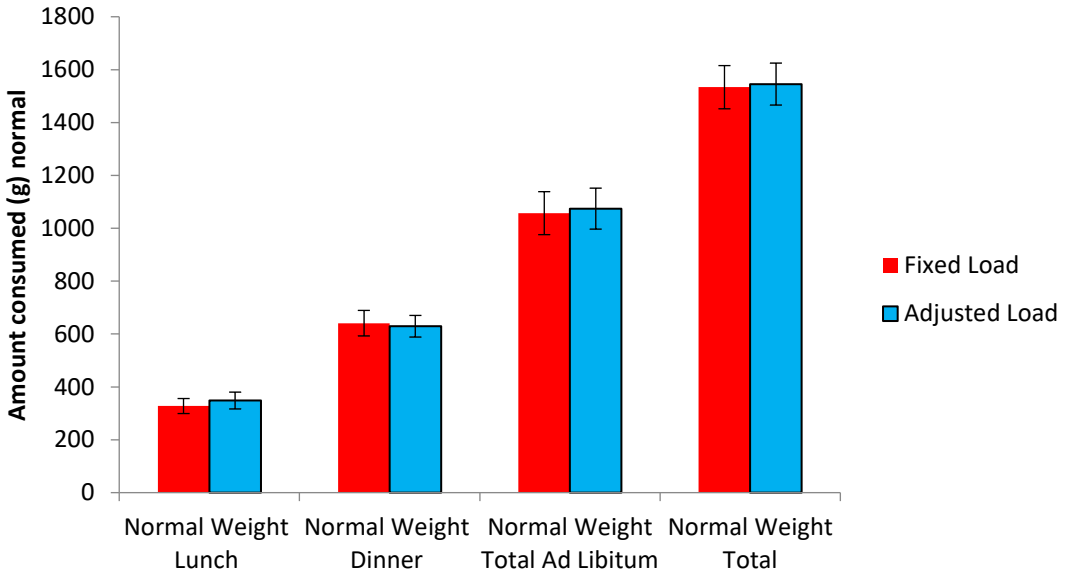

Figure 4–11 Effect of condition on food intake (grams) at the ad libitum test meals. Values are mean for 12 normal weight participants

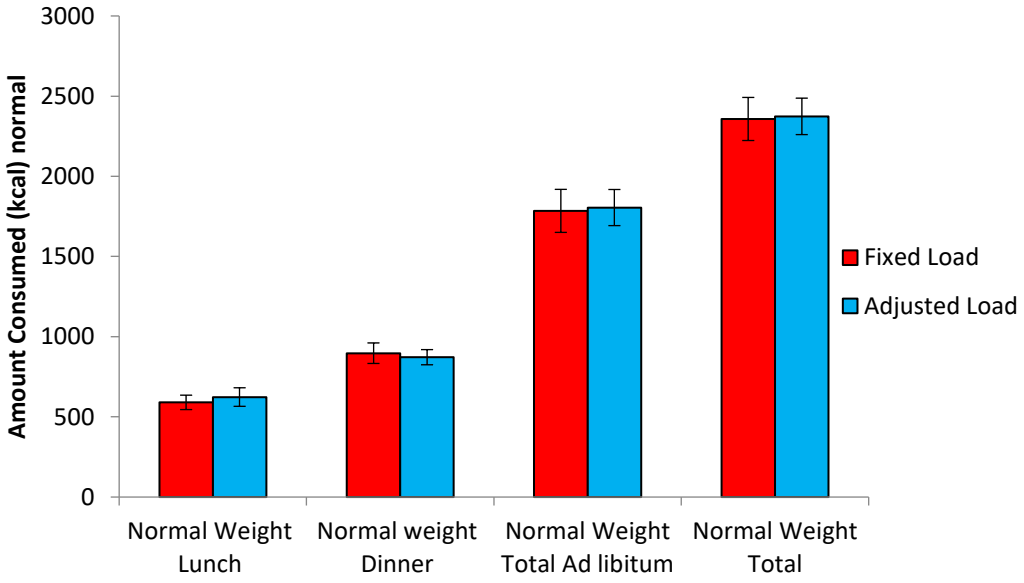

Figure 4-12 Effect of load condition on food intake (kcal) at the ad libitum test meals. Values are mean for 12 normal weight participants

Table 4–9 Food Intake fixed vs adjusted Load Condition Normal Weight Participants

	Normal Weight Fixed load			Normal Weight Adjusted load		
		±SE	±Std		±SE	±Std
Lunch						
Gram Intake (g)	327.6	38.4	133.1	348.4	35.0	121.2
Energy Intake (Kcal)	59.4	52.9	183.2	623.6	63.6	22.4
Dinner						
Gram Intake (g)	641.0	58.7	203.5	629.5	46.1	159.7
Energy Intake (Kcal)	896.9	73.2	253.6	872.1	58.5	202.8
Total Ad Libitum						
Gram Intake (g)	1057.1	81.3	281.5	1074.4	77.5	268.4
Energy Intake (Kcal)	1784.5	134.2	464.8	1805.3	112.6	39.0
Total Day						
Gram Intake (g)	1534.0	92.3	319.6	1546.0	83.5	289.3
Energy Intake (Kcal)	2358.0	149.2	516.8	2374.0	126.0	435.9

Total *Ad-Libitum* Kcal

For the participants who are obese there was a significant main effect for total ad libitum calories f (1, 11) = 1.552, p = .008 was found with a 219.7kcal (11.6%) reduction in calories in the adjusted load condition compared to the fixed load condition. After compensatory intake the reduction was 189.6kcal (1.7%). There were no significant differences for normal weight participants, they consumed a similar amount in each condition.

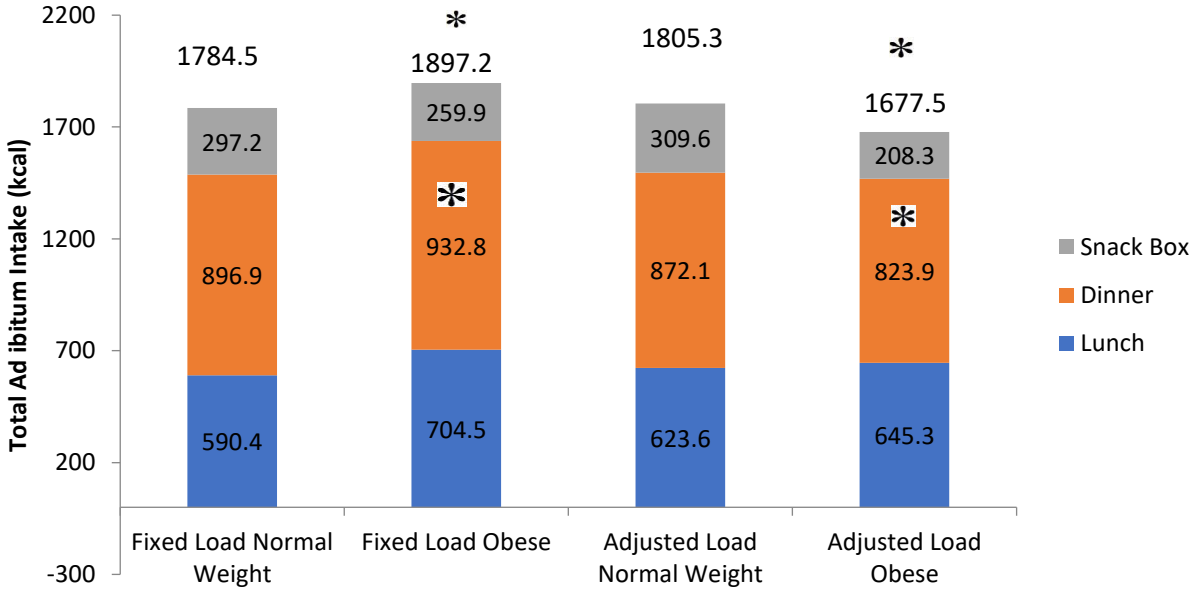

Figure 4-13 Effect of load condition on food intake at the ad libitum test meals. Values are mean for 12 normal weight and 12 participants who are obese. *P <.05 fixed load vs. adjusted load.

Total *Ad-Libitum* Food and Drink

There was a significant difference in total ad libitum food and drink intake p = .008. Total ad libitum food and drink consumption was reduced by 424.1g (16.9%), after compensatory intake the reduction was 323g (12.9%). There were no significant differences found for normal weight participants.

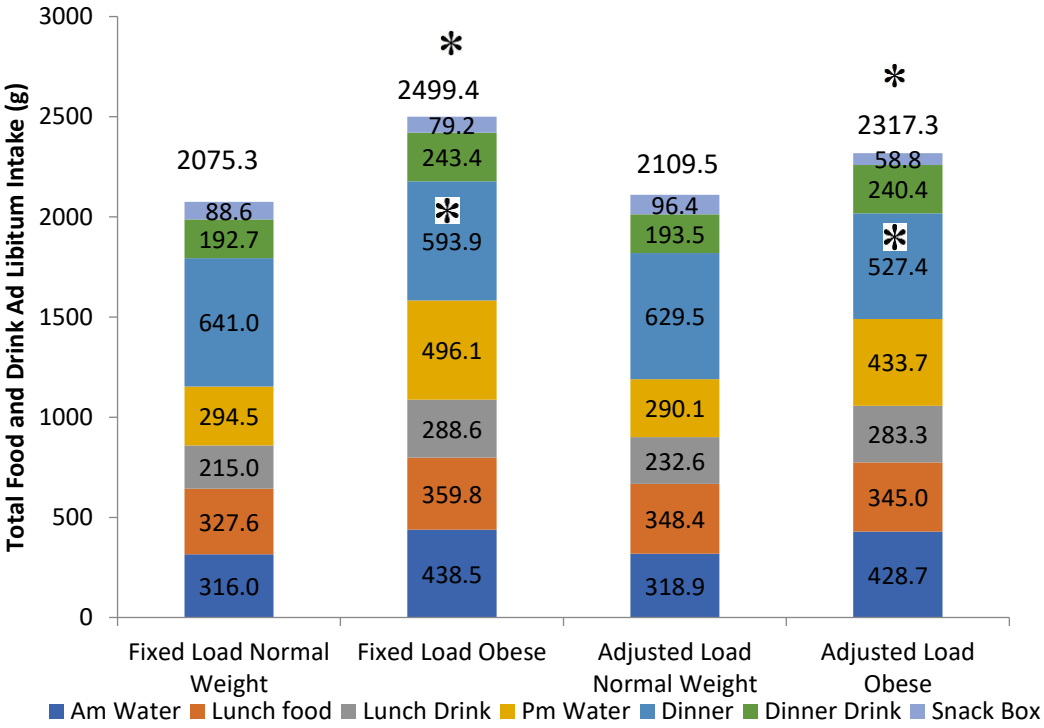

Figure 4-14 Effect of load condition on total food and drink intake throughout the day. Values are mean for 12 normal weight and 12 participants who are obese. *P <.05 fixed load vs. adjusted load.

Table 4 – 10 Total Ad libitum food and drink intake (grams)

	Fixed Load Obese			Adjusted Load Obese				Fixed Load Normal Weight			Adjusted Load Normal Weight			
		±SE	±Std		±SE	±Std	*p*		±SE	±Std		±SE	±Std	*p*
AM Water	438.5	5.0	173.2	428.7	42.4	147.0	.801	316.0	51.2	177.5	318.9	41.9	145.0	.911
Lunch Water	288.6	34.4	119.3	283.3	36.8	127.4	.607	215.0	33.7	116.9	232.6	36.0	124.8	.458
Lunch Food and Drink	648.4	41.4	143.4	628.3	51.5	178.4	.234	542.6	47.3	163.9	581.0	47.1	163.3	.271
PM Water	496.1	58.0	20.9	433.7	49.6	172.0	.290	294.5	31.9	11.4	29.1	39.0	135.0	.891
Dinner Water	243.4	3.2	104.7	24.4	32.7	113.2	.897	192.7	3.6	106.1	193.5	28.9	10.2	.950
Dinner Food and Drink	837.3	7.8	245.3	767.8	82.8	286.8	.071	833.6	62.1	215.2	823.1	52.4	181.6	.569
Total Ad Libitum Food and Drink	2499.4*	168.8	584.7	2317.3*	165.6	573.7	.008	214.9	153.4	531.5	2108.5	131.7	456.4	.563
Total Food and Drink	3448.4	156.3	541.5	3271.1	165.7	574.0	.345	303.7	145.7	504.7	3055.0	131.7	456.3	.642

*P <0.05 fixed load vs. adjusted load.

Total Intake Kcal

There was also a significant main effect for total calories consumed f (1, 21, = 7.501, p = .019 with a 19.3 kcal (7.7%) reduction in total calories consumed. There were no significant differences in Kcal consumed for normal weight participants.

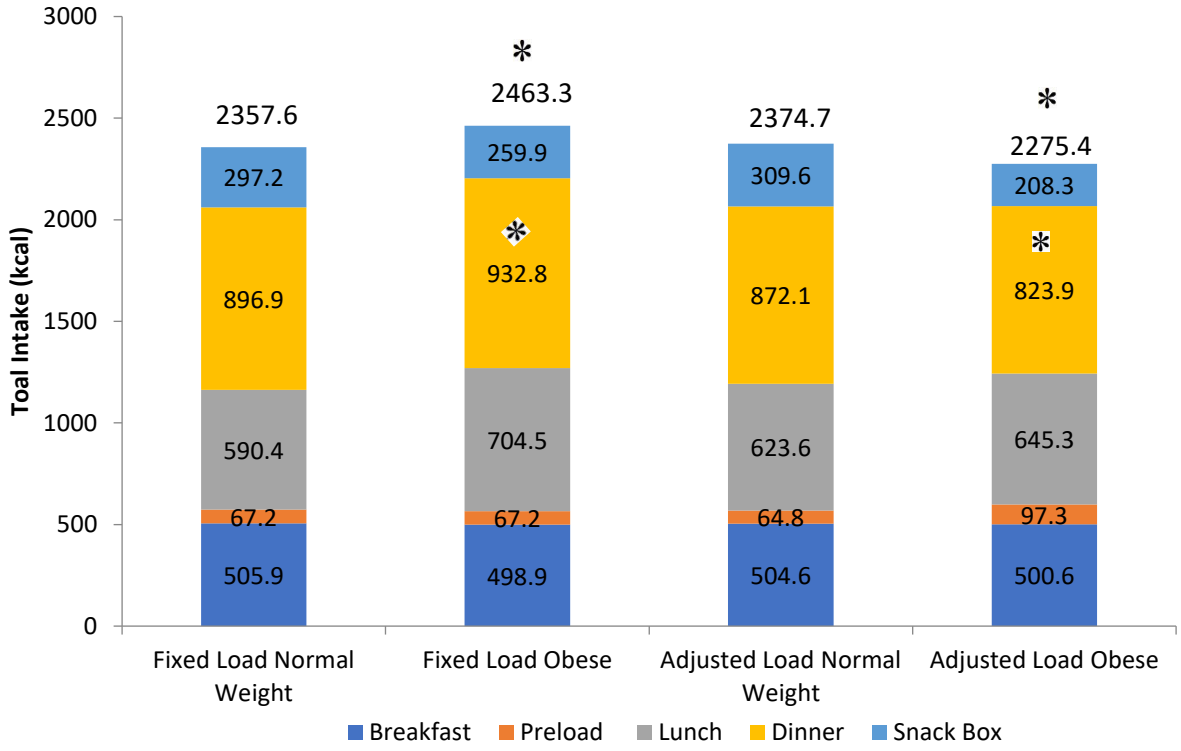

Figure 4-15 Effect of load condition on total food intake with preload. Values are mean for 12 normal weight and 12 participants who are obese. *P <.05 fixed load vs. adjusted load.

Total Food and Drink Intake (Grams)

There was a trend for total food and drink (gram) combined f (1,11) = 4.474, p =.058, participants consumed less in the adjusted condition with a reduction of 176.5 (5.1%). Normal weight participants consumed very similar amounts in the 2 conditions.

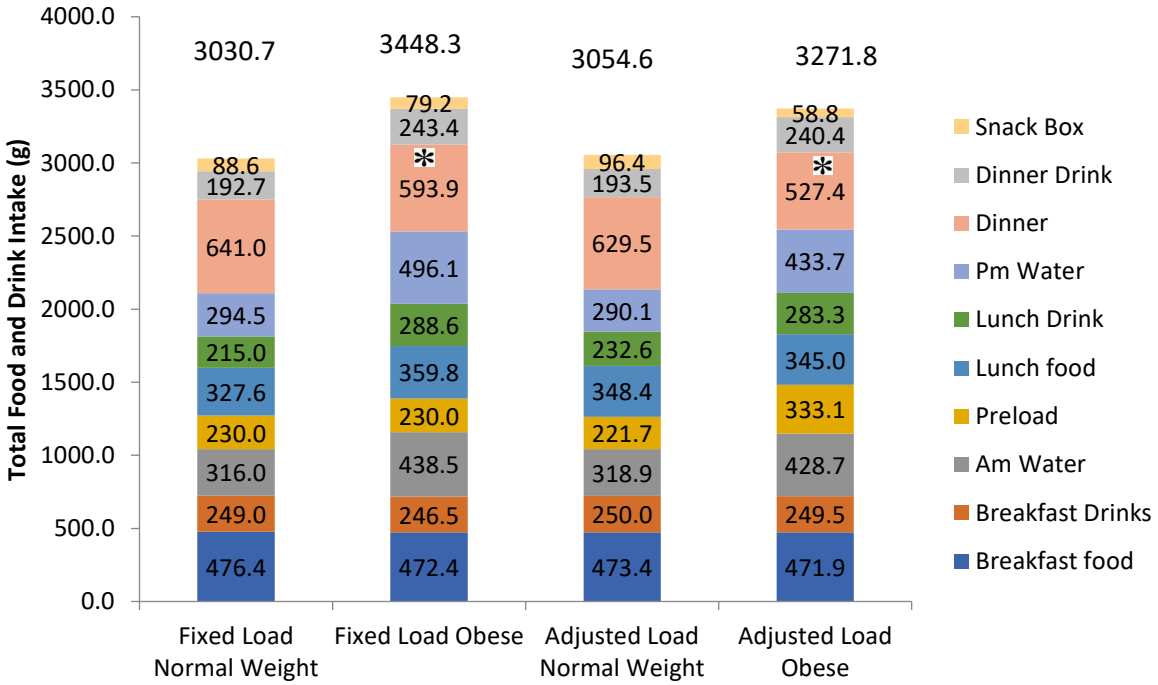

Figure 4-16 Effect of load condition on total food and drink intake throughout the day. Values are mean for 12 normal weight and 12 participants who are obese. *P <.05 fixed load vs. adjusted load.

Appetite Ratings load Conditions All Participants

Using standard time by condition ANOVA analysis of absolute VAS ratings a significant interaction for weight category, load condition and time for appetite score f (26, 576) = 93.203, p = .05.

Appetite Score

There was a significant interaction for load condition and time for appetite score for all participants f (4.410, 97.014) = 6.619, p = .035. Paired t-tests revealed a significant reduction in appetite score 3 hours after lunch t (23) = -2.209, p = .037 and immediately before dinner t (23) = -2.154, p = .042.

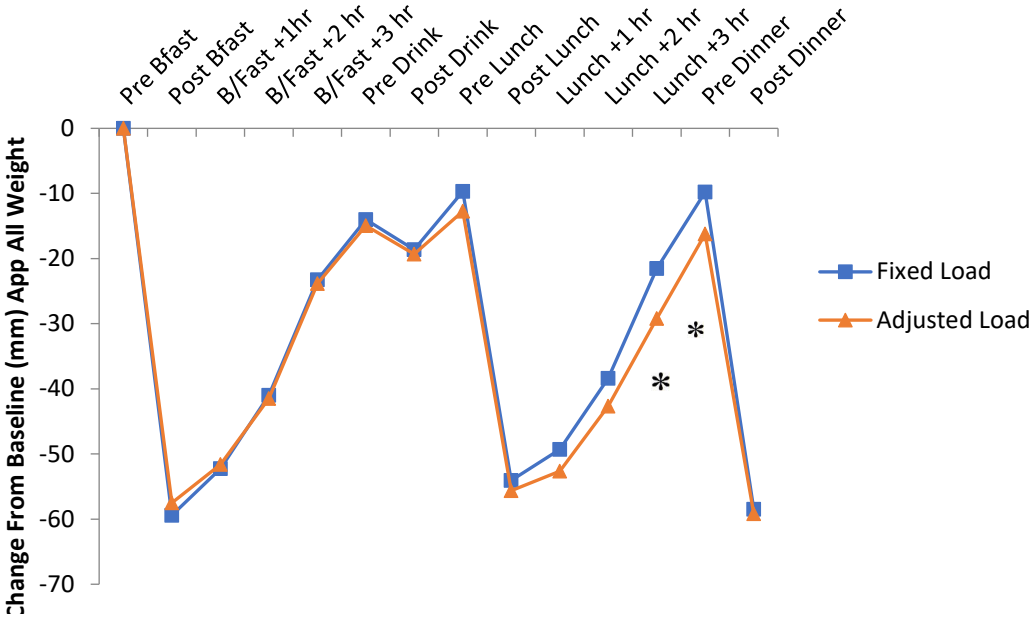

Figure 4-17 - Visual analogue scale (VAS) ratings for overall appetite score. Values are presented as changes from baseline score and are means for 24 participants. *P <.05 fixed load vs. adjusted load.

Area Under the Curve T1- T14 All Participants

There was no significant difference in AUC for appetite for the fixed and adjusted load conditions f (1, 22) = 2.570, p = .123. There was a significant difference in hunger AUC f (1, 23) = 7.246, p = .013 with hunger significantly lower in the adjusted load condition compared to the fixed load. There was no significant difference in AUC for the fixed and adjusted load conditions for desire to eat f (1, 22) = 2.818, p = .107, prospective consumption f (1, 23) = .504, p = .485, fullness f (1, 22) = .011, p = .917 and satisfaction f (1, 22) = .1, p = .923.

Appetite Measures Participants who are obese T1-T14

Appetite Score

Using standard time by condition ANOVA analysis of absolute VAS ratings there was a trend for an interaction for load condition and time for appetite score f (5.149, 49.610) = 2.123, p = .07 with a reduction in appetite immediately before dinner in the adjusted load condition t (1, 11) = -2.252, p = .046.

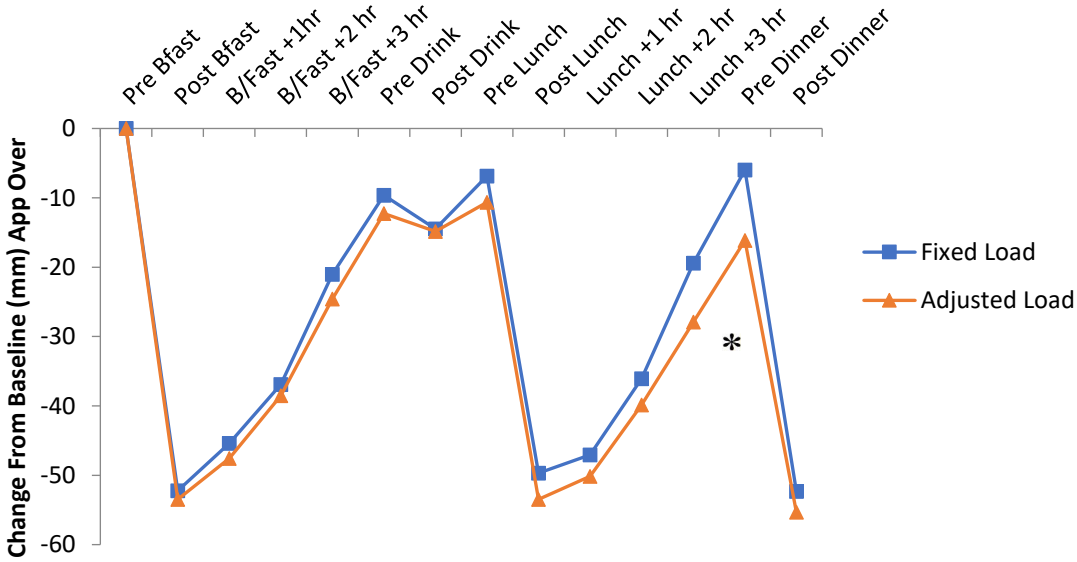

Figure 4–18 Visual analogue scale (VAS) ratings for appetite scores for the adjusted load and fixed load conditions T1-T14. Values are presented as changes from baseline score and are means for 12 participants who are obese.

AUC T1-T14 Fixed and Adjusted Load Participants who are obese

There was a trend for a difference in AUC for appetite in the adjusted load condition compared to the fixed condition f (1, 22) = 4.276, p = .063. There was a significant difference in AUC for hunger f (1, 22) = 8.645, p = .013 with hunger reduced in the adjusted load condition compared to the fixed load condition. There was no significant difference in AUC for desire to each f (1, 21) – 2.045, p =.180, prospective consumption f (1, 21) = 2.269, p = .160, fullness f (1, 22) = .199, p = .664 and satisfaction f (1, 22) = .029, p = .867.

Appetite Measures Normal Weight T1-T14

Appetite Score

There was no significant interaction for the load condition with time for appetite f (2.759, 3.347) = 1.442, p = .251.

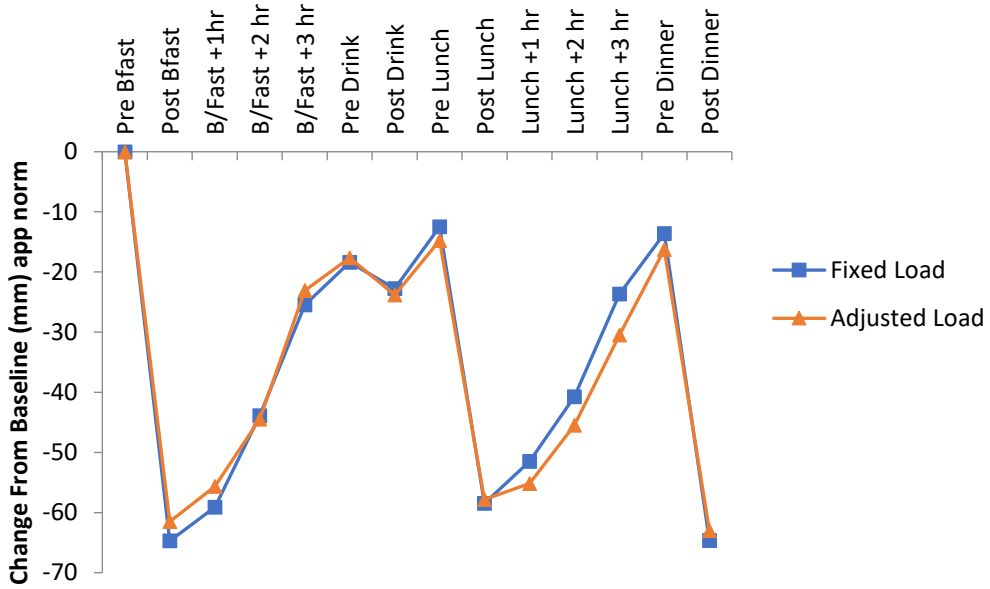

Figure 4 - 19 Visual analogue scale (VAS) ratings for appetite scores for the adjusted load and fixed load conditions T1-T14. Values are presented as changes from baseline score and are means for 12 normal weight participants.

AUC Normal Weight T1-T14

There was no significant difference in AUC for appetite f (1, 11) = 1.0239, p = .635, hunger f (1, 11) = 1.245, p = .288, desire to eat f (1, 11) .917, p =.358, prospective consumption f (1, 11) = .002, p = .967, fullness f (1, 11) = .255, p = .624 and satisfaction f (1, 11) = .001, p = .975 for the fixed and adjusted load conditions.

4.4 Discussion

The preload study design is often adopted as the method of choice to test the short-term effects of a preload and is widely used of to substantiate claims; however, it is fraught with methodological issues. It was unclear if scaling the preload for BMI in an acute prebiotic preload study could potentially improve the overall effectiveness to detect an effect on appetite and food intake in participants who are obese. We found that after scaling the preload for BMI, the participants who are obese reduced appetite, feelings of hunger and reduced total food intake in the adjusted load condition above the compensation required for the increase in preload calories. As predicted, there were no significant differences in appetite or food intake for normal weight participants for the fixed and adjusted loads. This indicated that the level of scaling was appropriate for both normal weight and participants who are obese and that there could be a case for individual scaling of a preload for BMI in acute preload studies.

Intake at lunch time was explored to see if there were any significant effects immediately after the preload. There was a trend for an interaction for load and weight category for calories consumed at lunch. After compensation for the preload participants who are obese reduced their intake by 29.0kcal (3.9%) to 742.6kcal although this was not statistically significant, meanwhile normal weight participants increased their intake by 3.8kcal (4.5%). Our results were consistent with Parretti et al. (2015) who found that a fibre preload had a significant effect on intake in the participants who are obese after just 30 minutes, however participants did not sufficiently compensate for the preload calories. Thirty minutes in the current study may not have been long enough to observe a significant effect and for compensatory intake to take place. Almiron-Roig et al., (2013) recommended an inter-meal interval of 30-120 minutes for optimal energy compensation after a preload. Gastric and possibly post gastric responses are more likely to explain the trend for reduced in

calorie intake at lunch for the participants who are obese. Food in the stomach causes gastric distension and arouses sensations of fullness, which can influence food intake.

In the current study the preload volume varied by an average of 103.1ml for participants who are obese, the reduction in food intake at lunch was surprising given volume is more influential when preloads are large. Van Walleghen, Orr, Gentile, and Davy (2007) found that 200ml premeal water reduces food intake 30 minutes before a meal in normal weight participants. In a similar study with participants who are obese the volume of water was much bigger (500ml) for a smaller significant reduction in calories (58kcal) (Parretti et al., 2015). However, (Rolls & Roe, 2002) infused overweight females with either 200 or 400 ml of isocaloric liquid they found food intake was reduced by 77 kcal or 13%, demonstrating that a relatively small difference in volume can affect intake in obese females. Habitual intake may explain the failure to compensate for the preload volume, obese individuals consumed a consistent fixed amount of food at lunch in both conditions (359.8g in the fixed condition and 345.0g in the adjusted condition). Previous research has also shown that regardless of the energy source, drinks elicit a weaker compensatory dietary response particularly in obese individuals. Obese individuals may therefore be at particular risk for positive energy balance due to beverage consumption.

Despite the trend for a reduction in food intake at lunch time appetite measures did not indicate any significant differences immediately after the scaled preload or before lunch. A significant difference was only observed 4 hours after lunch which may suggest a longer time frame could be required for the post-ingestive effects of the fibre preload on appetite to be observed. It is commonly assumed that appetitive sensations serve to link energy need with energy intake, but this is not reliably observed (Mattes, 2006). The present data reconfirms this, as despite the lack of response in appetite measures to the preload, there was a difference in the

compensatory dietary response they elicited. Our findings are not surprising given the multitude of factors that independently influence appetite.

There was a significant effect for intake (grams and calories) at dinner for participants who are obese, the adjusted condition significantly reduced calorie intake by 108.9kcal (11.7%) and 66.5g (11.2%). After compensatory intake for the preload intake grams increased by 36.6g (4.3%), however calorie intake was reduced by 78.8kcal (8.6%). This may suggest that the time elapsed was more appropriate to test the effects of scaling a fermentable fibre preload, dinner was served 4.5 hours after the preload. There was also a significant reduction in hunger and appetite immediately before dinner for participants who are obese. These results were consistent with previous studies which demonstrated inulin increases satiety and reduced food intake after 240 minutes (Hess et al., 2011). Had we used a more viscous fibre as a test vehicle we potentially would have observed different results as more viscous fibres tend to have immediate post-ingestive effects. This highlights the importance of study design.

Studies indicate inulin undergoes fermentation after 4 hours, however other studies suggest this may continue for several hours, increasing SCFA production and satiety hormones, potentially reducing appetite and food intake over a longer period. This could explain why we observed a further reduction in intake after participants left the laboratory. When total intake for the participants who are obese was observed there was significant reduction in total food intake with a reduction of 190kcal (7.7%), 102g (6.79%). These results were consistent with a significant difference in total appetite AUC for the participants who are obese. Normal weight participants on the other hand consumed a similar amount during the test day, 16kcal (.7%), 12g (.78%). This demonstrates that scaling the preload for BMI (.66g/kg) was appropriate for participants who are obese.

Often acute studies conducted over a few hours do not consider the compensatory intake required after a preload at the test meal, reporting significant effects on food intake at the test meal without considering the calories in the preload. Once the compensatory intake is considered effects on intake are often minimal. We calculated the compensatory intake for each meal as well as the total ad libitum intakes and total intake to ensure compensatory intake was carefully considered. The participants who are obese fully compensated for difference in the preload at dinner as well as total ad libitum calorie intake. The time required to compensate is important in acute studies, often such studies are conducted over 240 minutes. The ad libitum meals were served at 30minutes and 270 minutes post preload. The significant reduction in intake and significant overcompensation took place at dinner. Previous research has shown that individuals who are obese do not compensate for preload calories to the same extent as normal weight participants. In the present study, it was established that the participants who are obese did overcompensate for the calories in the preload, but this was after 270 minutes. It isn't clear whether normal weight participants would have overcompensated at an earlier time point as the differences in the preload for normal weight participants were not large enough to detect an effect.

Participants were tested over 2 test meals; this was to explore the immediate effects of the increasing volume of the preload and the potential effects of the fibre at the second test meal. The second meal effect is a phenomenon whereby the glycaemic response to a meal is influenced by the preceding meal. Acute studies usually present the preload either on arrival or after a short time interval of up to 60 minutes before presenting a test meal up to 240 minutes later. The current study followed the standard preload test meal design, participants arrived after an overnight fast and a fixed breakfast was provided 3.5 hours prior to the preload to ensure the preceding meal did not have an impact on appetite or food intake. We then presented the

preload and after a short interval of 30 minutes served the first *ad-libitum* test meal. This allowed us to test the immediate effects on both appetite and food intake. Four and a half hours after the preload we presented the second ad libitum meal. We also measured intake for the rest of the day. This method allowed us to gain further insight into the effects of the preload over the course of the test day as the systematic review in chapter 3 demonstrated this was more appropriate than simply testing over the four-hour protocol that many acute studies adopt.

The preload drink was carefully formulated to minimise any differences in macronutrient value, to achieve this we suspended the fibre in cordial diluted with water. Had the preload contained a large difference in macronutrient content there is a potential risk that the effects observed in the study could have been a result of the macronutrient variation rather than the scaling. There were no significant differences in taste or palatability between the two fibre drinks, the drink was proportionality scaled, only the amount increased. The preload was presented in a glass the same shape with a slight increase in diameter to covertly increase the volume in the preload. The maximum dose for the adjusted load was carefully considered to reduce the risk of adverse GI symptoms. A bigger dose may have inferred bigger effects in the adjusted load condition however as the inulin fibre is a fermentable fibre care is needed to avoid intestinal discomfort (Roberfroid, 2005). In the current study, the fibre dose was well tolerated with no reported symptoms of GI discomfort or nausea throughout the 24 h test period.

This study also highlights how participant characteristics such as BMI and variable preload formulation may explain some of the mixed results for inulin fibre. Harrold, Hughes, O'Shiel, et al. (2013) found an effect with 15g of inulin with a reduction of 80kcal in normal to slightly overweight participants. Whereas, (Giuntini et al., 2015) found a similar dose of 16g reduced intake by 138kcal in normal weight participants.

(Archer et al., 2004) on the other hand found that 24g of inulin reduced food intake by 363.5kcal in overweight men and women. Hess et al. (2011) found a similar reduction of 319kcal with a much smaller dose of 16g in normal weight women. Meanwhile (Karalus et al., 2012) found no effect on satiety or food intake for 10g of inulin in overweight men and women. Whereas in normal weight men and women (Perrigue et al., 2009) found a smaller dose of 6g reduced intake by 89kcal. Whilst there are other methodological variables which may explain some of the equivocal previous results many of these studies with the smaller or non-significant effects included participants who were overweight or obese. Their effectiveness may have been improved had they formulated the preload to individual BMI measures. Only one previous study has attempted to investigate the effects of a BMI scaled fermentable fibre preload, this was a longitudinal study. Genta (2009) investigated the effects of FOS in participants who are obese, they scaled the preload fibre dose (.14g/kg) and found that food intake and weight was significantly reduced over 3 months. The current study demonstrates that a scaled fibre preload is also effective in acute studies.

Limitations

It's difficult to generalise these findings to other fibre preloads as it is unclear whether scaling other fibre preloads would improve the overall effectiveness on appetite and food intake. This effect could potentially be limited to low calorie soluble fibres suspended in a liquid. If the preload is high in energy density it is unlikely that scaling the preload would enhance any effects after the compensatory intake required after the additional preload calories. Further studies are needed to investigate different preload formulations and fibres to explore if scaling the preload improves the efficiency to detect an effect in the preload test meal for other fibre preloads.

We only included women in the current study, the effect observed could potentially be limited to females only. As the systematic review illustrated there could potentially be differences in appetite and food intake regulation between men and women which could potentially impact on the effectiveness to determine the effects of a fibre preload. Further studies would be required to confirm the effects in men.

In conclusion

The importance of the preload formulation has been explored to try the improve the preload study protocol. A scaled fibre preload appeared to improve the overall effectiveness to detect an effect on appetite and food intake in obese women. Participants who are obese compensated for the additional calories in the preload at lunch, this is more likely due to the increase in volume rather than in response to the increase in calories or the increased fibre dose. The reduction in intake we observed at dinner was most likely a result of the increased fibre dose and the effects of fermentation on appetite markers. To elicit the satiety signals required to reduce hunger and food intake a BMI scaled preload would be more appropriate for participants who are obese when the test product in question is a fermentable fibre suspended in a low-calorie liquid. Thus, it might be desirable in future studies incorporating a range of BMIs to adjust the preload to reflect differences in appetite regulation between lean and obese. Improving the design of such preload studies will not only inform the subsequent chapters of this PhD but will help to improve the outcomes and help to advance this field of research further.

Chapter Five

5. Optimising the ad libitum test meal; increasing variety in the ad libitum buffet test meal decreases the sensitivity to detect changes in appetite and food intake after a fibre preload.

5.1 Introduction

Preload studies are often used to validate claims made by food manufacturers; thus food intake methodology is particularly important to functional food research (Gibbons & Blundell, 2019; Hobden et al., 2017). The variable methods adopted in the preload design has led to a large and widely varying literature with limited consensus on the precise methodology (Blundell et al., 2010). Despite the importance of the *ad-libitum* test meal in the preload study design, there is little consensus as to the content or composition of the test meal (Yeomans, 2018).

Previous research suggests that multi-item buffets meals lead to overconsumption during the test-meal compared to limited choice meals (Brondel et al., 2009; Raynor & Epstein, 2001; Wiessing et al., 2012). The potential for the number of food items offered to decrease the likelihood of observing significant changes in hunger and fullness with energy intake through ceiling and floor effects remains to be quantified. The current study aims to directly assess whether the sensitivity of the test meal to detect changes in appetite and food intake induced by a prior preload is altered by the composition of the test-meal itself; dependent on whether the test meal is a limited item meal or multi-item meal.

From the systematic review Chapter 3 the control comparison conditions of n = 42 studies show the composition of the outcome meal used to measure energy or nutrient intake in preload studies varies widely between research studies from 3

food items up to 40. Equally the intake at such test meals varies widely with increasing items from 395.8 kcal (7 item meal) to 1800.9 kcal (18 item meal). This lack of consensus has been considered problematic since variation in this *ad-libitum* meal may potentially influence the primary outcome of the study. Unlike VAS measures of subjective appetite-related sensations which, providing they are administered correctly at regular fixed intervals postprandially, are not influenced by study design. The composition of the outcome meal however has the potential to bias the outcome of the trial.

Although the Multi-item buffet test meal is often used as the outcome measure in food intake studies there has been very little research into the validation of this meal (Forde, 2018). The contents of the test-meal are often overlooked and under reported in research. One of the main issues is that it is difficult to establish valid food selection methodology, therefore this area has not been developed extensively (Blundell et al., 2010). The difficulty depends on the degree of choice offered in terms of both number of food items and variety. It is therefore difficult to determine the optimal test meal composition (Wiessing et al., 2012).

The foods chosen should be appropriate for the meal occasion, such as serving breakfast foods at a breakfast and savoury foods for lunch or dinner (Meiselman, 1996). The use of familiar foods in the buffet test meal may overcome the constraints of using manipulated diets to recreate the real-life feeding situation (Allirot et al., 2012; Gibbons et al., 2019). However, the choice of foods provided in the laboratory is inevitably limited and few reports ever give scientific explanations of why a certain range or selections of foods are chosen for the test meal. Given that several dietary factors can influence food intake in laboratory studies considerable attention should be paid to this aspect of design. Ensuring that the *ad-libitum* outcome meal is

sensitive to manipulations made within the fixed preload test meal is essential in all postprandial appetite studies.

The sensory and nutritional aspects of the meal must also be carefully considered. *ad-libitum* meals tend to provide savoury food items, yet Griffioen-Roose et al. (2009) found there is no difference in the onset of satiation for sweet or savoury ad libitum meals. Forde (2018) suggested the food should have a medium energy density (1–1.5 kcal/g), as small differences in intake of a very energy dense meal may unrealistically increase energy intake reducing the chances of detecting an effect of a preload. Similarly, foods chosen should not be high in a specific macronutrient, as high-fat meals have been found to inflate energy intake and do not generalise to everyday eating patterns (Hopkins et al., 2016).

A strong methodology will exert strong control over the nutritional and sensory aspects of each item. This can be achieved most readily when choices are restricted. Difficulties often arise from the lack of definition of the buffet meal. Many studies profess to offer a buffet test meal yet provide a single course meal with a choice of desserts with very little variety. This is not a buffet meal. Studies also fail to distinguish single food items, often providing a meal with several ingredients but referring to this as a single item. Defining food items is important as several studies suggest that even small sensory differences, such as differences within the colour or texture of foods, can increase consumption through sensory variety (Epstein et al., 2010). Similarly, Brondel et al., 2009 found that the addition of condiments to a meal led to the attenuation of satiety and increased food intake.

There are advantages and disadvantages to both single-item restricted meals and multi-item buffet test meals in appetite research. Nutrient intake in food choice studies is clearly better addressed by presenting a multi-item buffet meal (Blundell et al.,

2010) which allows the participant to make wide choices from foods which differ in energy, energy density and macronutrient content. However, a common concern about the multi-item meal is that it does not reflect the usual eating pattern of most individuals, people seldom face such a variety of foods from which they can freely choose in one sitting (Johnson & Wardle, 2014; Blundell et al., 2010).

The single-course test meal can be used to measure both satiation and energy intake. The single-course meal is the most widely used approach to quantify food consumed in response to a preload. An advantage of the single-course meal is that it enables a clear measure of food intake and is a reliable measure (Gregersen et al., 2008). Conversely the single-course test meal can only measure food intake and not food choice, this also doesn't reflect normal eating patterns where we have choice and variety (Gibbons et al., 2019). Participants are also likely to overestimate the amount of food they would typically choose to consume when served a large single-course *ad-libitum* meal, this is known as the portion sized effect (Diliberti et al., 2004). However, food intake studies must offer large portions of food in excess in order to measure *ab libitum* intake, participants should reach satiation rather than finish the plate as they might habitually.

Multi-item meals are often rated as more palatable than single choice meals. For most foods, increases in flavour pleasantness result in appetising effects which drive short-term overconsumption (Yeomans et al., 2004). Palatability is a major driver of short-term overeating (de Castro et al., 2000; Johnson & Wardle, 2014). However, (Deighton, 2016) found that test-meal palatability was associated with overconsumption but better represented preceding changes in appetite. The highly palatable meal produced energy intakes that were more representative of preceding appetite ratings, but the moderately palatable, meal produced more ecologically valid energy intakes. *ad-libitum* meal selection and design may require a compromise

between sensitivity and ecological validity. The high variety meal, therefore, has the potential to mask the effects on food intake which are induced by a preload, and so decrease the sensitivity of postprandial studies to detect changes in eating behaviour.

It has long been established that increasing the variety within a meal increases the energy consumed (Hetherington, et al., 2006; Bellisle & Le Magnen, 1981; Raynor & Epstein, 2001; Rolls et al., 1981). McCrory et al., 2012 found that exposure to a variety of foods increases intake by as much as 29%. Multi item meals are thought to increase intake through delaying satiation and meal termination (Hetherington et al., 2006). Research investigating the effects of meal variety has tended to concentrate on the physiological and psychological processes that promote meal termination sensory-specific satiety (SSS) (Brondel et al., 2009; Raynor & Epstein, 2001; Rolls et al., 1981; Rolls et al., 1984). Sensory-specific satiety is a phenomenon whereby as the food is eaten, liking for that food decreases, by the end of a meal there is a significant reduction in perceived liking, whilst other foods remain relatively attractive. The single item *ad-libitum* test meal typically has a lower palatability rating, which may further decline as multiple treatments are completed as there is little or no choice, this can lead to rapid onset of sensory specific satiety and is likely to suppress intake relative to a multi-item meal (Rolls et al., 1982).

High hedonic value of many of the foods offered in excess may also induce over consumption in all conditions (ceiling effects) (Espel-Huynh et al., 2018; Norton, et al., 2006), removing the satiating effects of the preload manipulations. Conversely, where little variety is offered in the test meal monotony is likely to occur (Meiselman et al., 2000; Hollie A. Raynor, 2012) Monotony is likely to limit consumption within a study as a whole (floor effect), irrespective of the satiating potential of the preload, resulting in meal termination (Hetherington et al., 2006). This may in turn mask the potentially small effects on appetite which are induced by a preload, and so decrease the

sensitivity of postprandial studies to detect changes in eating behaviour. (Wiessing et al., 2012) attempted to determine whether restricted single-item or multi-item test meals are better able to detect prior changes in hunger and fullness when assessing *ad-libitum* eating behaviour. They found that increasing the variety of an *ad libitum* test meal did not decrease the sensitivity to detect changes in hunger and fullness as participants adjusted their intake accordingly in the multi-item condition.

A poor study design with an unsuitable test meal has the potential to mask the effect of a preload which could be present (Wiessing et al., 2012). Although often suggested, the literature does not provide any clear indication that increasing the number of food items not only increases intake at a single test meal but whether the increased intake is compensated for at the next meal. Furthermore, the potential for the number of food items offered to decrease the likelihood of observing significant changes in hunger and fullness with energy intake through ceiling and floor effects remains to be quantified. Inulin fibre was selected to test this, this fibre has been shown to increase satiety and reduce food intake. Results will help to quantify the optimal preload test meal to improve methodology used in preload studies. This research is important to develop a standardised methodology in laboratory procedures for an agreed standard of working for theory and commercial development.

5.1.1 Hypothesis

We hypothesised that i) There will be an increase in food intake and a reduction in appetite in a high variety buffet meal compared to a low variety buffet meal for normal weight and participants who are obese. ii) Normal weight but not participants who are obese will compensate for the increased intake at the high variety meal. iii) A high variety meal will decrease the chances of observing an effect of a fibre preload on appetite and food intake in participants who are obese.

5.2 Methodology

5.2.1 Ad libitum Lunch

The *ad-libitum* lunch was designed to offer a selection of high and low-fat savoury and sweet food items. The test meals were based on the sensory nutrient relationships of four sensory nutrient food groups; high fat savoury items, low fat savoury items, high fat sweet items and low-fat sweet items. Salad items are not included within these four sensory nutrient food groups but provided in addition to the distinct four groups. This model has been validated as a method to measure food preference and intake and has been used in a variety of studies to test sensory food preference (J. Blundell et al., 2010).

Cold food items appropriate for the meal occasion were served for the buffet lunch meal (Tables 5-1, 5-2 and 5-3). Participants were presented with the buffet items on separate plates on serving trays and instructed to select the items and amount they would like to eat. Each food was presented in excess. The number of items in each meal was decided base on the review of the literature. Five items were the minimum number to be considered a buffet, in the review the lowest intake was found in the 7-item lunch. The ten-item *ad-libitum* buffet was the most common and food intake did not increase further after 18 items. To increase the number proportionately the buffet lunch therefore consisted of 5, 10, and 20 items. Liquid and semi-solid foods were limited at the buffet; milk or juices were not provided to prevent the participants from consuming amounts similar to those consumed habitually, water was however provided at each meal.

Table 5-1 Nutrient and energy profiles of foods for the 5-item lunch

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
High Fat Savoury						
Tesco Mozzarella Grated cheese	serving	100	26	20	1.2	290
Low Fat Savoury						
Kingsmill Soft White Medium 800g	6 slices	180	22.2	6	110.4	582
High Fat Sweet						
Cadburys chocolate mini rolls	3	80.9	3.9	18.6	45.9	360
Low Fat Sweet						
Tesco Jelly Babies	20 sweets	120	6.36	0	96.84	412.8
Salad						
Tomato	serving	80	0	0	0	0
Total			52.34	44.68	167.1	1272

Table 5 -2 Nutrient and energy profiles of foods for the 10-item lunch time buffet.

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
High Fat Savoury						
Flora Margarine	serving	40	0	2.36	0	21.2
Tesco Mozzarella Grated cheese	serving	100	26	20	1.2	290
Low Fat Savoury						
Kingsmill Soft White Medium 800g	4 slices	120	14.8	4	73.6	388
Tesco no added water cooked ham	4 slices	100g	21.4	3.3	1.1	120
High Fat Sweet						
Maryland cookies	serving	100	5.4	22.6	63.8	487
Aero Chocolate Mousse	1 pot	59	2.9	3.6	13.4	98
Low Fat Sweet						
Tesco Jelly Babies	20 sweets	120	6.36	0	96.84	412.8
Apple	1 piece	80	0.24	0.08	9.6	40
Salad						
Cucumber	serving	80	0	0	0	0
Tomato	serving	80	0	0	0	0
Total			77.1	55.94	259.54	1857

Table 5–3 Nutrient and energy profiles of foods for the 20-item lunch time buffet.

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
High Fat Savoury						
Flora Light Margarine	serving	40	0	2.36	0	21.2
Tesco Mozzarella Grated cheese	serving	100	26	20	1.2	290
Tesco Chorizo	5 slices	100	25.4	27.5	0.5	355
Walker's Ready Salted crisps	1 packet	25	1.63	8.5	12.25	132.5
Low Fat Savoury						
Tesco no added water cooked ham	4 slices	100	21.4	3.3	1.1	120
Kingsmill Soft White Medium 800g	4 slices	120	14.8	4	73.5	388
Kingsmill Medium Sliced Wholemeal Bread	4 slices	144	16	2.4	64.4	310
Snack a jacks salt and vinegar	1 packet	22	1.5	1.6	17	89
High Fat Sweet						
Maryland cookies	serving	100	5.4	22.6	63.8	487
Aero Chocolate Mousse	1 pot	59	2.9	3.6	13.4	89
McVities Jaffa Cakes	6 cakes	67.8	3.6	6	51.6	276
Cadburys chocolate mini rolls	3	80.9	4.2	18.6	45.3	375
Low Fat Sweet						
Tesco Jelly Babies	20 sweets	120	6.36	0	96.84	412.8
Ambrosia custard pot	1 pot	150	4.5	3.5	24	150
Apple Slices	1 serving	80	0.2	0.08	9.8	44
Hartley's strawberry Jelly	1 pot	125	0	0	25	100
Salad						
Tomato	serving	80	0.4	0.2	0.7	8
Carrot	serving	80	0.5	0.2	6.3	33.6
Cucumber	serving	80	0	0	0	0
Lettuce	Approx. 1/4	100	0	0	0	0
Total			136.19	121.04	491.69	3599.1

Table 5-4 Nutrient and energy profiles of foods served at the evening meal.

Food	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
Tesco Penn	Serving	250	30	1.75	192.5	883
Tesco Twin Pack Garlic Bread	3 slices	50	3.7	8.1	21.3	175
Del Monte fruit cocktail in juice	1 tin	250	1	0.25	28	122.5
Tesco chunky veg pasta sauce	1 jar	500	9.2	5.2	26	240
Tesco grated mozzarella cheese	Serving	50	13	10	0.7	145
Gu New York Cheesecake	1 pot	80	4	19.4	26.7	300
Total			60.9	44.7	295.2	1865.5

Supper was an *ad-libitum* hot pasta meal with a selection of desserts (Table 5 - 4). An evening snack box was also provided (table 5-5) for participants to consume in the evening. This was to allow for any compensatory intake to be measured after participants had left the laboratory. Participants were instructed to return the snack box with any empty wrappers/waste/uneaten food on their next study day.

Table 5-5 Nutrient and energy profiles of foods provided in the evening snack box.

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
Rivita minis	1 packet	30	2.6	2.2	22.8	113
Tesco marshmallows	1 packet	200	9	0.2	156	670
1 apple or banana	1 piece	80/100	0.2/1.2	Trace/0.3	9.8/23.2	44/105
bar of chocolate	1 bar	66	2	11	29.8	228
Mini Cheddars	1 packet	131	2.7	7.5	12.9	131

All foods and drinks offered in the studies were readily and commercially available to the public. They were prepared in accordance with the manufacturer's instructions, the Guidelines for Human Nutrition Research and the individual standard operating procedures prepared for the equipment or specific food items used. The foods were prepared in the Kissileff Laboratory kitchen which is designed and equipped for the

preparation and serving of food and drink and served in individual booths in the separate eating area.

5.2.2 Statistical Analysis

Number of buffet items

A One-way repeated measures within subject ANOVA was performed for the fixed load condition with the three buffet conditions as the within subject's factor. *Post hoc* analyses correcting for Bonferroni adjustments were carried out to identify where differences lay. Intake at lunch was also analysed in relation to macronutrient content using a repeated measures ANOVA with condition (5-item, 10-item and 20-item adjusted load) as within subject factors.

Changes in ratings of appetite using VAS assessed the nature of any differences in appetite ratings for each buffet meal. Analysis was conducted with a repeated measures ANOVA. The trapezoid rule was used to calculate AUC for each VAS variable and differences in AUC VAS ratings were assessed again using repeated measures ANCOVA with baseline values serving as covariant.

Number of buffet items and Load condition

We ran three separate paired t-tests to analyse the amount of food consumed (in grams and kcal) for the 5-item condition, 10-item condition and 20-item condition in the fixed and adjusted item conditions.

Subjective parameters were analysed for each meal condition using a within-subjects repeated measures ANOVA with condition (5 item fixed vs 5 items adjusted, 10 item fixed vs 10 item adjusted and 20 item fixed vs 20 item adjusted) and time (pre-breakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch, post-lunch, 2 pm, 3 pm, 4 pm, pre-dinner and post dinner; T1–T14) as within-subject factors. T-tests were used

to identify where differences lay. Analysis was then repeated as before and AUC was calculated and analysed using repeated measure ANCOVA.

Satiety Quotients (SQ) were calculated to integrate both the energy content of food ingested during a meal and the associated change in appetite sensations, Green and collaborators developed a SQ as an indicator of the satiating efficiency of food (Green et al., 1997). The SQ is calculated by dividing the change in subjective appetite sensations in response to a meal by the energy content of the meal.

Results

Palatability Buffet Meals

There were significant differences in the buffet meals on scores of taste $f (2, 44) = 32.297$, $p < .001$, palatability $f (2, 44) = 21.526$, $p < .001$ and pleasantness $f (2, 44) = 27.104$, $p < .001$ for the different lunch conditions. Contrasts revealed the 10-item buffet was significantly more palatable $f (1, 22) = 21.271$, $p < .001$, tasty $f (1, 22) = 40.195$, $p < .001$ and pleasant $f (1, 22) = 25.957$, $p < .001$ than the 5-item buffet, the 20-item buffet was significantly more palatable $f (1, 22) = 5.547$, $p = 0.028$, pleasant $f (1.22) = 9.750$, $p = 0.005$ and tasty $f (1, 22) = 8.353$, $p = 0.008$ than both the 5-item buffet and 10-item buffet, The 20-item buffet was significantly more palatable $f (1, 22) = 35.392$, $p < .001$ and pleasant $f (1, 22) = 52.509$, $p < .001$ and tasty $f (1, 22) = 40.025$, $p < .001$ than the 5-item buffet.

There were no significant interactions for buffet meal and weight category.

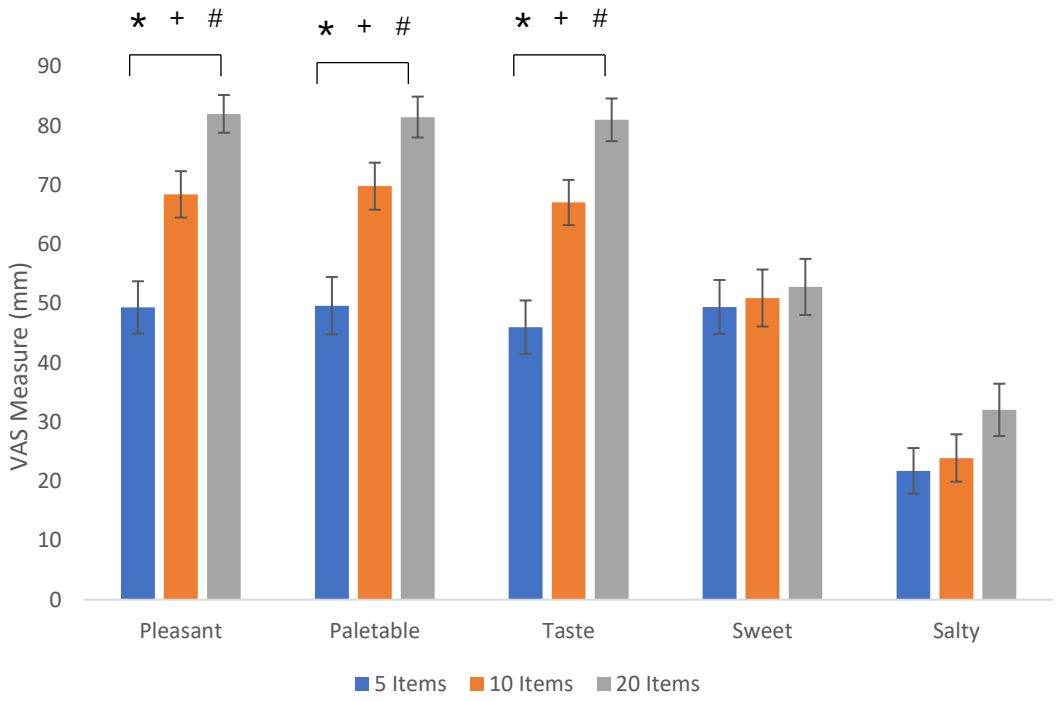

Figure 5-1 Effect of number of items on palatability. Values are mean for 12 normal weight and 12 0bese participants. *P <0.05 5 vs 10 items. +P <0.05 10 vs 20 items. #P <0.05 5 vs 20 items.

All Participants

Intake at Test Meal for 5, 10 and 20 item lunches

Lunch

There was a significant main effect for items on calories consumed at lunch f (2, 44) = 10.752, p < .001). Contrasts revealed a trend for more calories consumed for the 10-item lunch compared to 5-item lunch (86.8kcal, 13.5%) f (1, 22) = 4.249, p = .051). Significantly more calories were consumed for the 20-item lunch compared to 10-item lunch (106.5kcal,14.25%) f (1, 22) = 8.933, p = .007) and significantly more calories were consumed on the 20-item buffet compared to the 5-item buffet (193.3kcal, 25.85%) f (1, 22) = 17.076, p < .001.

A significant main effect for grams of food f (2, 44) = 45.659, p < .001 consumed at lunch time was also found. Contrasts revealed significantly more food (g) was

consumed on the 10-item lunch compared to 5-item lunch (106.5g, 30.5%) f (1, 22) = 28.099, p < 0.001), significantly more was also consumed on the 20-item lunch compared to the 10-item lunch (89.9g, 20.5%) f (1,22) = 23.512, p < .001) and significantly more was consumed on the 20-item lunch compared to the 5-item lunch (196.4g, 44.73%) f (1, 22) = 73.770, p < 0.001.

Dinner

Significantly more food (g) was consumed at dinner for the 10-item condition compared to the 20-item condition (91.3g, 15%) f (2, 44) = 4.941, p = .037. There was no significant effect for number of items on calories consumed at dinner f (2, 44) = 2.172, p = .126.

Total Ad Libitum

There was no significant effect for number of items on total ad libitum calories consumed f (2, 44) = 2.225, p = .120. There was a significant main effect for items on total ad libitum intake (g) f (1.538, 33.842) = 7.807, p = .003. Contrasts revealed significantly more (g) was consumed for the 10-item lunch condition compared to 5-item lunch condition (162.4, 14.76%) f (1, 22) = 9.452, p = .006. Significantly more (g) was consumed for the 20-item lunch compared to the 5-item lunch condition (159.4g, 14.5%) f (1, 22) = 25.397, p < 0.001.

Total

A significant main effect for items on total grams consumed t (2, 44) = 7.704, p = .001. Contrasts revealed participants consumed significantly more total food (g) for the 10-item condition compared to 5-item condition (162.8g, 10.3%) f (1, 22) = 9.347, p = 0.006 and the 20-item condition compared to the 5-item condition (158.9g, 10.1%) f (1, 22) = 24.835, p = < .001. There was no significant effect for total ad libitum calories consumed f (2, 22) = 2.215, p = .121.

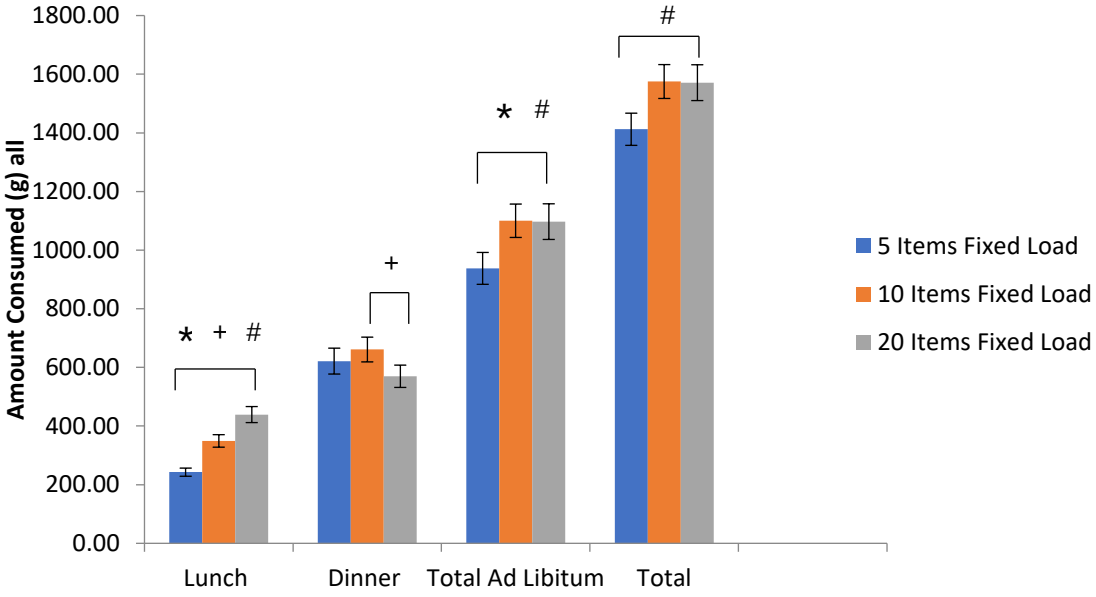

Figure 5-2 Effect of food items at lunch on the ad libitum test meals. Values are mean for 24 participants. *P <.05 5 vs 10 items. +P <.05 10 vs 20 items. #P<.05 5 vs 20 items.

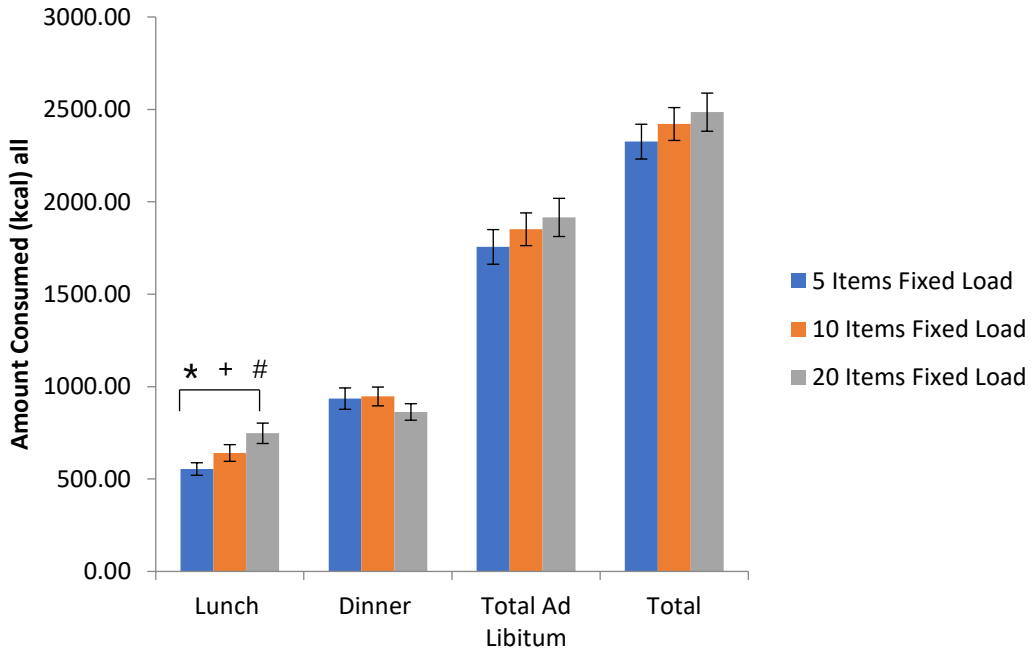

Figure 5-3 Effect of food items at lunch on the ad libitum test meals. Values are mean for 12 normal weight and 12 0bese participants. *P <.05 5 vs 10 items. +P <.05 10 vs 20 items. #P <.05 5 vs 20 items.

Table 5–6 Results summary for food intake for all fixed load conditions for all participants

	5 Item, Fixed load			10 Item, Fixed load			20 Item, Fixed load		
		SE	Std		SE	Std		SE	Std
Lunch									
Gram Intake (g)	242.7*	13.9	68.2	349.2*	21.3	104.5	439.1*	27.4	134.0
Energy Intake (Kcal)	554.1*	33.9	166.0	64.9*	45.2	221.5	747.4*	55.2	27.5
Dinner									
Gram Intake (g)	621.5	44.1	216.1	661.0*	42.3	207.1	569.7	38.1	186.5
Energy Intake (Kcal)	935.0	57.7	282.8	946.8	51.0	249.8	862.8	44.6	218.4
Total Ad Libitum							1097.1	6.8	297.7
Gram Intake (g)	937.7*	54.4	266.5	110.1	57.0	279.2	1097.1*	6.8	297.7
Energy Intake (Kcal)	1755.9	93.7	458.9	1851.2	88.4	433.2	1915.5	103.3	506.0
Total Day									
Gram Intake (g)	1412.2*	54.9	269.1	1575.0	57.7	282.9	1571.1*	61.0	298.9
Energy Intake (Kcal)	2325.6	94.2	461.3	242.8	89.0	436.0	2485.0	103.2	505.7

*P <.05

Table 5-7 Change in food intake grams/Kcal between conditions for all participants and percentage differences.

	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 vs 10 item meal		10 vs 20 item meal		5 vs 20 item meal	
Lunch				Intake	%	Intake	%	Intake	%
Gram Intake (g)	242.70	349.20	439.10	106.50*	3.50	89.90*	2.47	196.40*	44.73
Energy Intake (Kcal)	554.10	64.90	747.40	86.80*	13.54	106.50*	14.25	193.30*	25.86
Dinner									
Gram Intake (g)	621.50	661.00	569.70	39.50	5.98	-91.30*	-16.03	-51.80	-9.09
Energy Intake (Kcal)	935.00	946.80	862.80	11.80	1.25	-84.00	-9.74	-72.20	-8.37
Total Ad Libitum									
Gram Intake (g)	937.70	110.10	1097.10	162.40*	14.76	-3.00	-.27	159.40*	14.53
Energy Intake (Kcal)	1755.90	1851.20	1915.50	95.30	5.15	64.30	3.36	159.60	8.33
Total Day									
Gram Intake (g)	1412.20	1575.00	1571.10	162.80*	1.34	-3.90	-.25	158.90*	1.11
Energy Intake (Kcal)	2325.60	242.80	2485.00	95.20	3.93	64.20	2.58	159.40	6.41

Weight Category analysis for Items

Lunch

There was a significant interaction for items and weight category for lunch calories f (2,44) = 6.473, p = .046. There was no significant interaction for food intake at lunch (grams) f (2, 44) = 1.351, p = .241.

Dinner

A trend for interaction between items and group f (2, 44) = 4.106, p = .057 on energy intake at dinner was observed. There was a significant interaction between load condition and group for food intake (g) f (2, 44) = 4.489, p = .043.

Total *ad-libitum*

There was a significant interaction between the load condition and group for total *ad-libitum* calories consumed f (2,44) = 9.498, p = .039. There was a significant interaction between the items condition and group for total ad libitum food intake (g) f (1, 22) = 7.631, p = .04.

Total Intake

A significant interaction between load condition and group f (2,44) = 5.210, p = .022 on total energy intake (kcal) over the entire study day was observed.

Obese Participants Intake at Test Meal for 5, 10 and 20 item lunches

Lunch

There was a significant main effect for items on calories consumed at lunch f (2, 22) = 4.876, p = .018. Contrasts revealed there were more calories consumed in the 20-item condition than the 10-item condition (115.8kcal, 14.3%) f (1, 11) = 6.635, p = .026 and the 20-item condition compared to the 5-item condition (93.5kcal, 13.4%) f (1, 11) = 6.247, p = .03. There was a significant main effect for items on amount consumed (g) at lunch f (2, 22) = 26.770, p < .001 contrasts revealed more food (g) was consumed in the 10 item condition than the 5 item condition (104.4g, 29.2%) f (1, 11) = 12.607, p = .005, the 20-item condition than the 5-item condition (209.3, 25.8%) f (1, 11) = 37.772, p < .001and the and the 20-item condition than the 10-item condition (115.8g, 14.25%) f (1, 11) = 24.262, p < .001.

Dinner

There was no significant main effect for items on calories consumed at dinner f (2, 22) = 2.239, p = .135 or food intake at dinner (g) f (2, 11) = 2.518, p = .104.

Total *Ad-libitum* Intake

There was no significant main effect for items on total ad libitum calories consumed f (2, 22) = .411, p = .668. There was a significant main effect for items on total g consumed (g) f (2, 22) = 3.921, p = .035. Contrasts revealed total ad libitum (g) intake was higher for the 20-item condition compared to the 5-item condition (144.9g, 13.4%) f (1, 11) =3.921, p = .035 there was a trend for higher total ad libitum intake in the 10-item condition compared to the 5-item condition f (1, 11) = 4.719, p = .053.

Total

There was no main effect for total calories consumed f (2, 22) = .382, p = .687.

There was a significant main effect for items on total amount consumed (g) f (2, 22) = 3.380, p = .037 contrasts revealed participants consumed significantly more total food (g) in the 20-item condition compared to the 5-item condition (143.7g, 9.3%) (f (2, 22) = 7.606, p = .019. There was a trend for greater total intake (g) for the 10-item condition compared to 5-item condition (15.7g, 9.7%) f (2, 22) = 4.702, p = .053

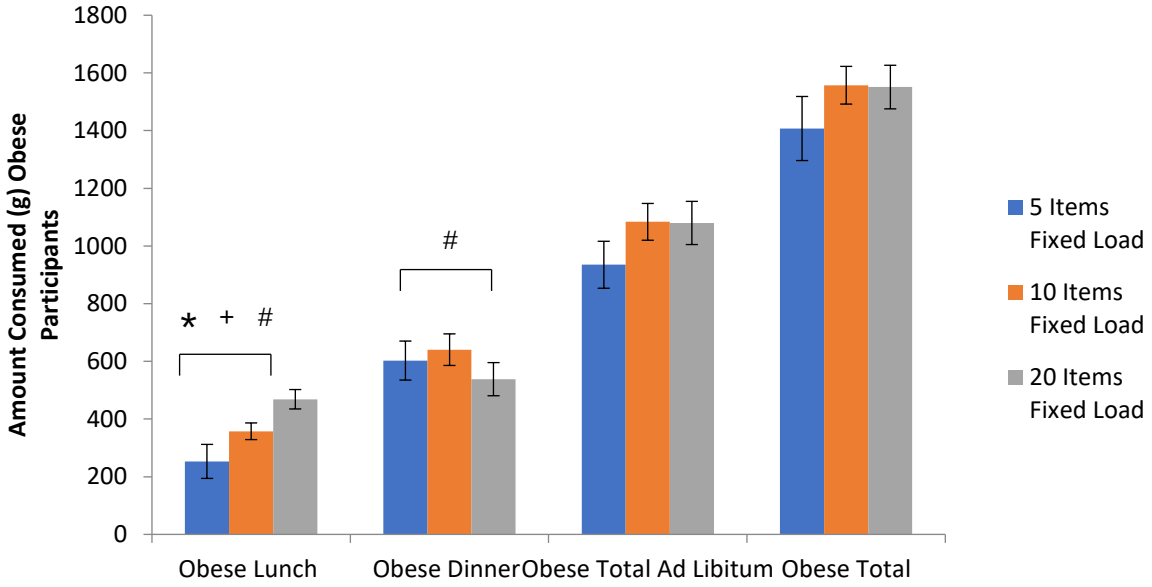

Figure 5 - 4 Effect of food items at lunch on the ad libitum test meals and total intake. Values are mean values for 12 obese participants. *P <.05 5 vs 10 items. +P <.05 10 vs 20 items. # P <.05 5 vs 20 items

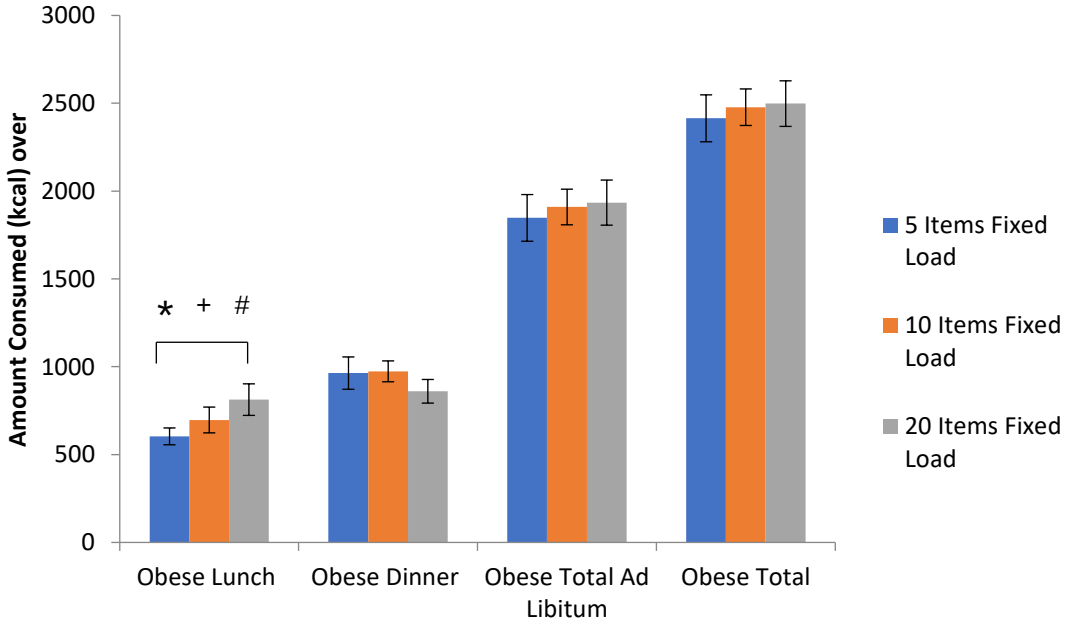

Figure 5-5 Effect of food items at lunch on the ad libitum test meals and total intake. Values are mean values for 12 obese participants. *P <.05 5 vs 10 items. +P <.05 10 vs 20 items. # P <.05 5 vs 20 items.

Table 5-8 Results summary for food intake for all fixed load conditions for *n* = 12 obese participants

	Obese 5 Item Fixed load			Obese 10 Item Fixed load			Obese 20 Item Fixed load		
		SE	Std		SE	Std		SE	Std
Lunch									
Gram Intake (g)	253.2*	59.0	207.6	357.5*	28.8	99.8	468.6*	33.7	116.6
Energy Intake (Kcal)	603.6	47.9	165.9	697.1*	73.6	255.0	812.9*	89.7	31.6
Dinner									
Gram Intake (g)	602.9	67.7	234.5	64.5	54.7	19.0	538.2	57.5	199.2
Energy Intake (Kcal)	963.8	91.9	318.5	973.9	59.4	206.0	86.6	67.2	232.6
Total Ad Libitum									
Gram Intake (g)	934.9	81.4	282.0	1083.7	63.8	221.1	1079.8*	74.9	259.3
Energy Intake (Kcal)	1847.4	132.8	46.1	1909.6	101.5	351.6	1934.6	128.4	444.8
Total Day									
Gram Intake (g)	1407.3	111.1	384.8	1558.0	65.3	226.0	1551.0*	75.6	261.7
Energy Intake (Kcal)	2414.3	133.6	463.0	2478.0	104.0	359.0	2498.0	13.0	449.3

Table 5–9 Change in food intake grams/Kcal between conditions for *n* = 12 obese participants and percentage differences.

	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 vs 10 item meal		10 vs 20 item meal		5 vs 20 item meal	
Lunch				Intake	%	Intake	%	Intake	%
Gram Intake (g)	253.2	357.5	468.6	104.3*	29.17	111.1*	23.71	215.4*	45.97
Energy Intake (Kcal)	603.6	697.1	812.9	93.5	13.41	115.8*	14.25	209.3*	25.75
Dinner									
Gram Intake (g)	602.9	64.5	538.2	37.6	5.87	-102.3	-19.01	-64.7	-12.02
Energy Intake (Kcal)	963.8	973.9	86.6	1.1	1.04	-113.3	-13.17	-103.2	-11.99
Total Ad Libitum									
Gram Intake (g)	934.9	1083.7	1079.8	148.8	13.73	-3.9	-.36	144.9	13.42
Energy Intake (Kcal)	1847.4	1909.6	1934.6	62.2	3.26	25	1.29	87.2	4.51
Total Day									
Gram Intake (g)	1407.3	1558	1551	15.7	9.67	-7	-.45	143.7	9.26*
Energy Intake (Kcal)	2414.3	2478	2498	63.7	2.57	20	.8	83.7	3.35

Normal Weight Intake at Test Meal for 5, 10 and 20 item lunches

Lunch

There was a significant main effect for amount consumed at lunch (kcal) f (2, 22) = 6.394, p = .006; contrasts revealed participants consumed significantly more calories for the 20-item lunch then the 5-item lunch (80kcal,13.7%) f (1, 11) = 18.045, p = .001. There was a significant main effect for amount consumed at lunch (g) t (2, 22) = 19.356, p < .001; contrasts revealed participants consumed more food (g) for the 10 item lunch then the 5 item lunch (108.6g, 31.9%) f (1, 11) = 15.722, p = .002, also during the 20-item lunch than the 10-item lunch (97.3g, 14.3%)f (1, 11) = 5.448, p = .04 and the 20-item lunch then the 5-item lunch (177.3, 26%) f (1, 11) = 36.463, p < .001.

Dinner

There was no significant main effect for items on calories consumed at dinner f (22) = .395, p = .678 or food intake at dinner (g) f (2, 22) = .928, p = .41.

Total Ad libitum Intake

There was no significant main effect for items on total ad libitum calories (2, 22) = 2.019, p = .157. There was a significant main effect for total ad libitum intake (g) f (2, 22) = 3.942, p = .034; contrasts revealed participants consumed significantly more total ad libitum (g) in the 20-food item condition than the 5-item food condition (174g, 15.6%) f (1,11) = 22.2, p = .001. There was a trend for more total ad libitum intake in the 10-item condition than the 5-item condition f (1, 22) = 4.788, p = .051.

Total

There was no significant main effect for total calories consumed f (2, 22) = 2.091, p = .147. There was a significant main effect for total amount consumed (g) t (2, 22) =

3.919, p = .035; contrasts revealed participants consumed more food (g) in the 20-item condition than the 5-item condition (158.9g, 1.1%) f (1, 11) = 22.422, p = .001.

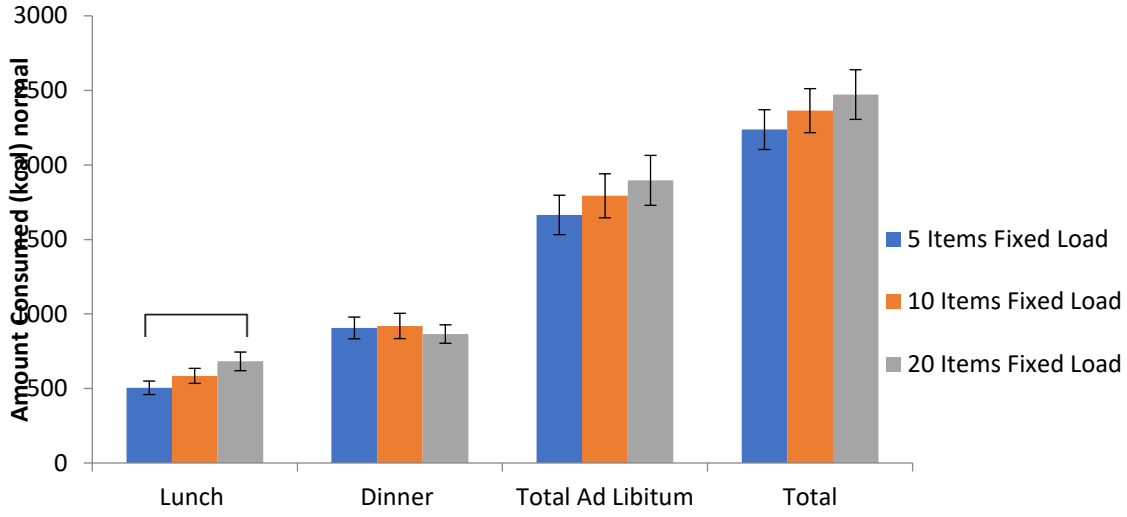

Figure 5 - 6 Effect of food items at lunch on the ad libitum test meals and total intake. Values are mean values for 12 normal weight participants. *P <.05 5 vs 10 items. +P <.05 10 vs 20 items. # P <.05 5 vs 20 items

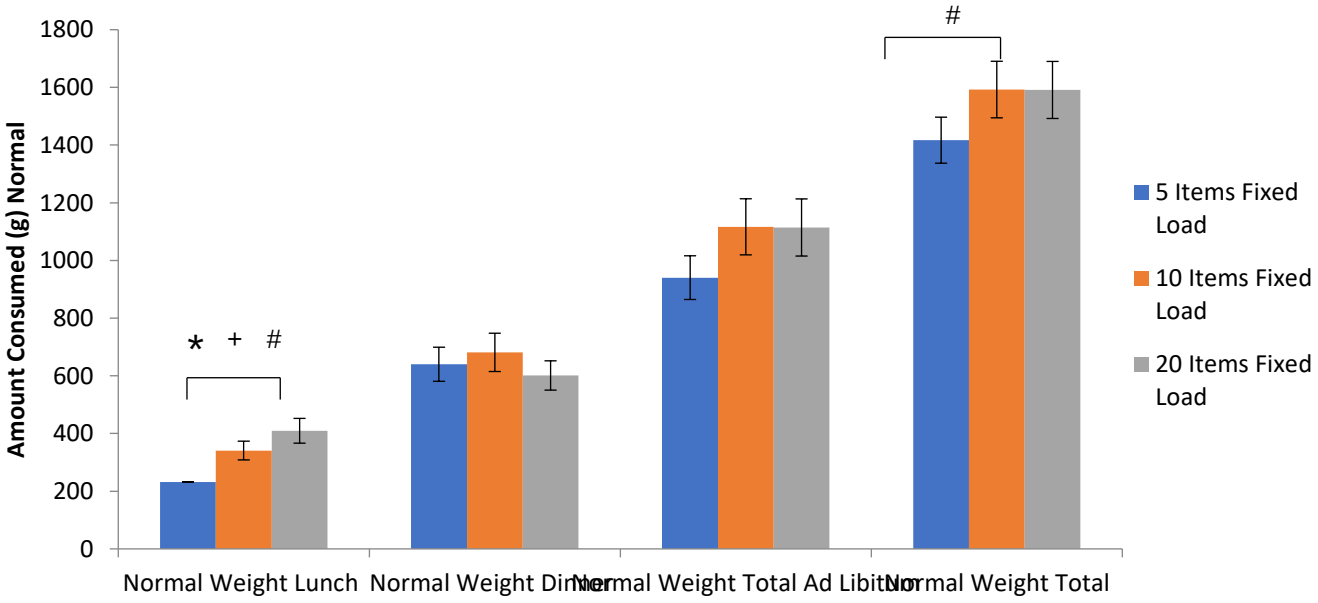

Fig. **5-7** Effect of food items at lunch on the ad libitum test meals and total intake. Values are mean values for 12 normal weight participants. *P <.05 5 vs 10 items. +P <.05 10 vs 20 items. # P <.05 5 vs 20 items.

Table 5 - 10 Food Intake for Normal Weight Participants n = 12

Normal Weight	Normal Weight 5 Item Fixed load			Normal Weight 10 Item Fixed load			Normal Weight 20 Item Fixed load		
		SE	Std		SE	Std		SE	Std
Lunch									
Gram Intake (g)	232.3*	39.8	138.0	34.9*	32.6	112.8	409.6*	42.9	148.5
Energy Intake (Kcal)	504.6	45.4	157.3	584.6*	5.5	174.9	681.9*	62.7	217.3
Dinner									
Gram Intake (g)	64.2	59.1	204.7	681.4	66.4	229.9	601.3	5.7	175.8
Energy Intake (Kcal)	906.2	73.0	252.9	919.6	85.0	294.3	865.0	61.7	213.6
Total Ad Libitum									
Gram Intake (g)	94.4	75.8	262.5	1116.6	97.3	337.0	1114.4*	98.9	342.7
Energy Intake (Kcal)	1664.5	132.4	458.7	1792.9	147.6	511.3	1896.3	167.5	58.3
Total Day									
Gram Intake (g)	1417.1	79.8	276.4	1592.5	98.1	339.8	1591.1*	98.9	342.6
Energy Intake (Kcal)	2237.0	133.4	462.0	2364.1	147.8	511.9	2471.9	166.5	576.6

Table 5 – 11 Change in food intake grams/Kcal between conditions for Normal weight participants and percentage differences.

	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 vs 10 item meal		10 vs 20 item meal		5 vs 20 item meal	
Lunch				Intake	%	Intake	%	Intake	%
Gram Intake (g)	232.3	34.9	409.6	108.60*	31.86	68.70*	16.77	177.30*	43.29
Energy Intake (Kcal)	504.6	584.6	681.9	8.00	13.68	97.30*	14.27	177.30*	26.00
Dinner									
Gram Intake (g)	64.2	681.4	601.3	41.20	6.05	-8.10	-13.32	-38.90	-6.47
Energy Intake (Kcal)	906.2	919.6	865	13.40	1.46	-54.60	-6.31	-41.20	-4.76
Total Ad Libitum									
Gram Intake (g)	94.4	1116.6	1114.4	176.20	15.78	-2.20	-.20	174.00*	15.61
Energy Intake (Kcal)	1664.5	1792.9	1896.3	128.40	7.16	103.40	5.45	231.80	12.22
Total Day									
Gram Intake (g)	1412.20	1575.00	1571.10	162.80	1.34	-3.90	-.25	158.90*	1.11
Energy Intake (Kcal)	2325.60	242.80	2485.00	95.20	3.93	64.20	2.58	159.40	6.41

Items consumed

The number of items participants selected from is presented below. In the limited 5 item meal a maximum of 5 items was consumed in both the fixed and adjusted conditions. For the 10 some participants consumed the maximum 10 items, however in the adjusted load this was only 9. In the 20-item buffet meal a maximum of 15 items were consumed. When we observe the average participants consumed an average of 11 items in the adjusted condition.

Table 5 - 12 Results summary for number of items consumed and total food intake (kcal) in all conditions

	Minimum items consumed	Maximum Consumed	Mean Number of Items Consumed			Minimum total food consumed (kcal)	Maximum total food consumed (kcal)	Mean total food consumed (kcal)		
Condition	Statistic	Statistic	Stat	Std. Error	Std. Deviation	Stat	Statistic	Stat	Std. Error	Std. Deviation
5 Item Fix	3	5	4.29	.13	.62	1627.60	3133.98	2325.61	94.16	461.29
10 Item Fix	4	10	7.67	.26	1.27	1708.34	3493.98	242.77	89.00	436.03
20 Item Fix	7	13	9.33	.35	1.71	1547.45	3391.55	2484.97	103.23	505.72
5 Item Adj	3	5	4.21	.12	.59	1665.47	318.65	218.76	78.63	385.19
10 Item Adj	5	9	7.38	.25	1.21	1647.95	3349.01	2381.43	93.35	457.34
20 Item Adj	7	15	9.96	.44	2.18	1643.88	3313.17	2408.84	104.89	513.87

Macronutrient Content

There was a significant difference in the amount of fat f (1.702, 4.174) = 15.977, p < .001 and protein consumed at lunch f (1.770, 38.397) = 14.161, p < .001; contrasts revealed that there was significantly more fat consumed for the 20-item compared to the 10-item buffet f (1, 22) = 19.846, p < .001 and significantly more for the 20-item buffet compared to 5 f (1, 22) = 2.962, p < .001. Significant differences were also found for the amount of protein consumed on the 5 and 10 item buffets f (1, 22) = 4.556, p = .044, 10 and 20 item buffets f (1, 22) = 12.690, p .002 and the 5 and 20 item buffet f (1,22) = 2.432, p < .001. There were no significant differences in carbohydrate consumption at lunch for any condition.

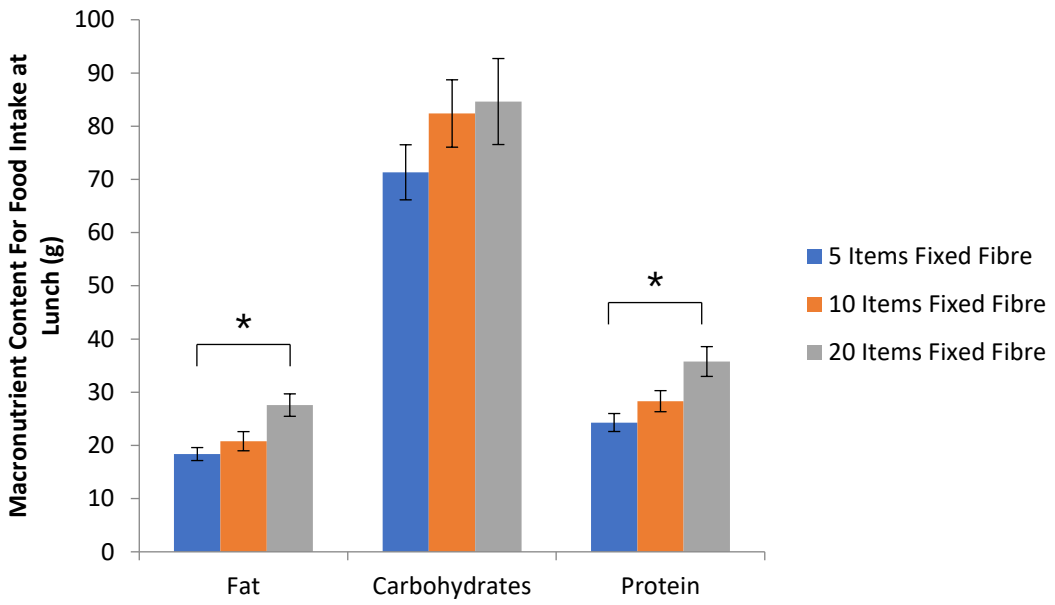

Figure 5 - 8 Macronutrient intake (g) at lunch for the 5 item, 10 item and 20 item lunch. Values are mean for 24 participants.

Figure 5 – 13 Energy density at each test meal all participants (n = 24)

	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load
	All Participants Energy Density kcal/g			Obese Energy Density kcal/g			Normal Weight Energy Density kcal/g		
Lunch	2.28	1.84	1.70	2.38	1.95	1.73	2.17	1.71	1.66
Dinner	1.50	1.43	1.51	1.60	1.52	1.60	1.42	1.35	1.44
Total Ad libitum	1.87	1.68	1.75	1.98	1.76	1.79	1.77	1.61	1.70
Total Intake	1.65	1.54	1.58	1.72	1.59	1.61	1.58	1.48	1.55

Participants consumed a lower energy density in the 20-item lunch meal than the 10-item meal and 5-item meal. Participants who are obese consumed a higher energy density than normal weight participants across all meals. For total intake the lowest energy density was consumed in the 10-item meal for all participants, participants who are obese and normal weight participants. The satiety quotients for the adjusted

condition were consistent with the results indicating the 5-item meal reduced hunger significantly more per gram than the 10-item meal (p=.05) and 20-item meal (p=.025).

Appetite Measures All Participants

A standard time by condition ANOVA analysis of absolute VAS ratings for the 5, 10, and 20-item conditions revealed significant interactions for appetite ratings f (26, 546) = 1.944, p = .004, satisfaction f (26, 546) = 2.030, p = .002, prospective consumption f (26, 546) = 1.882, p = .006 and desire to eat f (26, 546) = 1.882, p = .006 were found. A trend for an interaction was also found for hunger f (26, 546) = 1.392, p = .095 and fullness 26, 546) = 1.498, p = .055

Appetite Score

There was a significant interaction for items and time for appetite ratings f (26, 546) = 1.944, p = .004. T-tests revealed appetite was significantly lower for the 20-item condition compared to the 10-item condition immediately before dinner t (23) = 2.429, p = .023. Appetite was also significantly lower in the 20-item condition compared to the 5-item condition one hour after lunch t (23) =2.867, p = .009 and immediately before dinner t (23) = 3.189, p = .004. There was a trend two hours after lunch t (23) = 1.1998, p = .058 and a trend three hours after lunch t (23) = 1.182, p = .083

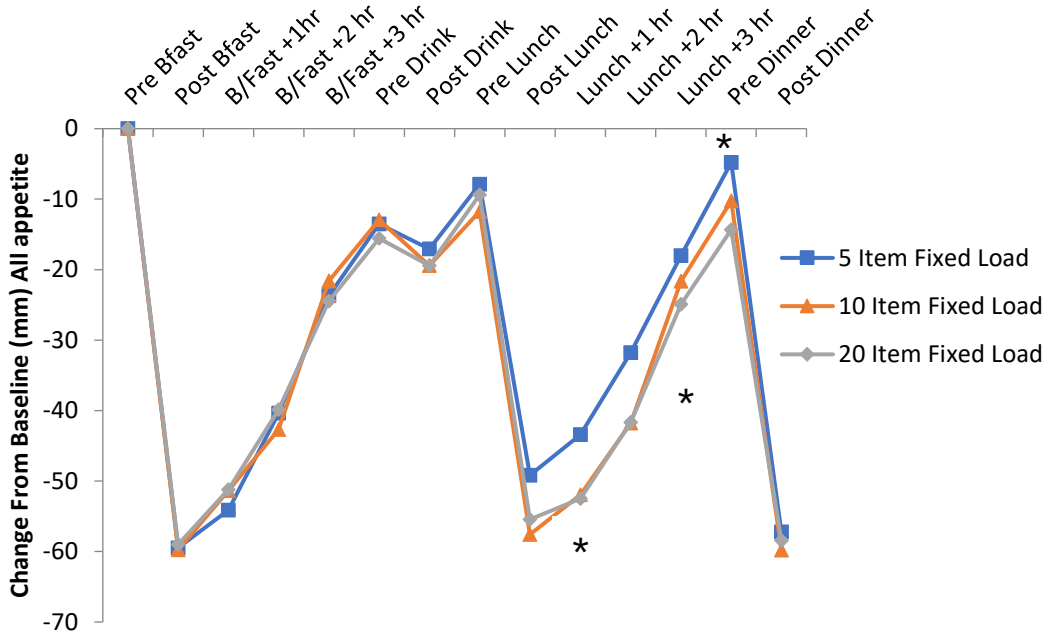

Figure 5 - 9 Visual analogue scale (VAS) ratings for overall appetite score in the 5-item condition, 10 item condition and 20 item condition. Values are presented as changes from baseline score and are means for 24 participants. *P <.05 fixed 5 vs 10 items.

AUC All Participants

There was no main effect of items on appetite AUC f (2, 44) = .70, p = .469, hunger f (2, 44) = .287, p = .752, desire to eat f (2, 44) = .534, p = .590, prospective consumption f (2, 44) = .775, p = .467, fullness (2, 44) = 1.499, p = .234 and satisfaction f (2, 44) = .162, p = .851.

Food Intake Measures

Fibre and Items All Participants

Lunch

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items on food intake at lunch f (2, 46) = 7.324, p =.022. Paired t-tests revealed there was a trend for food intake (kcal) at lunch t (23) = 1.785 p = .088 for the 5-item adjusted condition compared to the 5-item fixed condition, with participants consuming less in the adjusted load condition than the fixed load condition. There were no significant differences for food intake (g) in the 5-item condition.

Dinner

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items on food intake at dinner f (2, 46) = 9.938, p = .041. Paired t-tests revealed there was a trend for dinner calories p = .075 for the 5-item adjusted condition compared to the 5-item fixed with participants consuming less in the adjusted load condition than the fixed load condition. There was no significant difference in food intake in food intake at dinner (g).

Total Ad libitum

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items on total food intake f (2, 46) = 6.358, p = .035. Paired t-tests revealed total ad libitum calories were significantly reduced in the 5-item adjusted condition compared to the 5-item fixed condition t (23) = 2.339 p = .028. There were no significant differences for total ad libitum (grams) intake.

Total Day

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items on total day food intake f (2, 46) = 10.534, p = .027 Total daily calories were significantly reduced in the 5-item adjusted load condition compared to the 5-item fixed t (23) = 2.187, p = .039. There were no significant differences for intake (g).

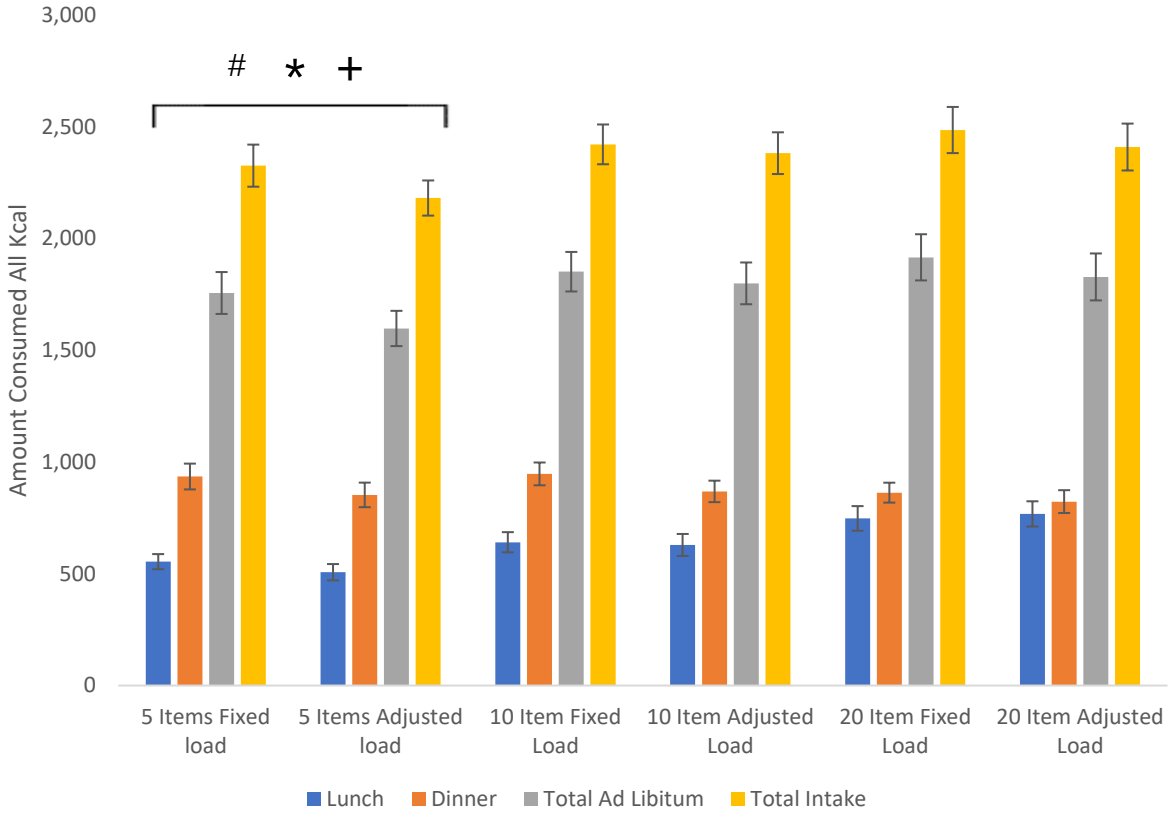

Figure 5 – 10 Effect of load condition on food intake (kcal) at the ad libitum test meals. Values are mean for 24 participants. *P <.05 5 items fixed vs 5 items adjusted lunch. +P <.05 05 5 items fixed vs 5 items adjusted ad libitum intake. #P <.05 05 5 items fixed vs 5 items adjusted total intake.

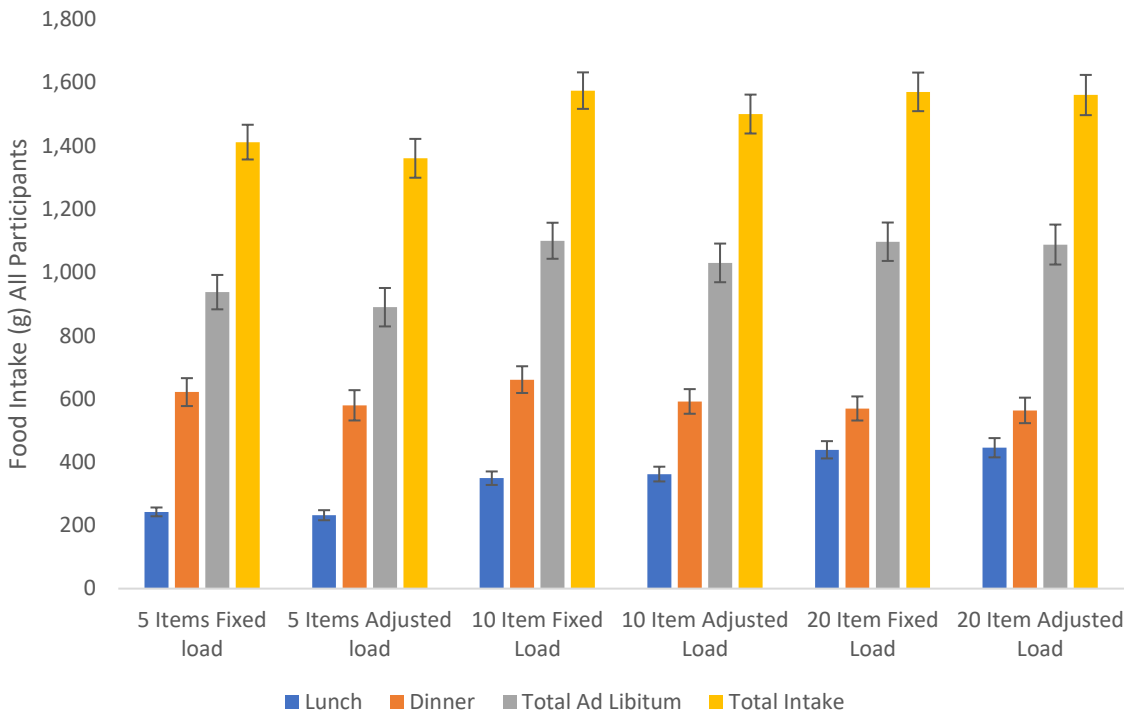

Figure 5 - 11 Effect of load condition on food intake (g) at the ad libitum test meals. Values are mean for 24 participants.

Table 5 - 14 Results summary for food intake for all conditions all participants

	5 Item, Fixed load			10 Item, Fixed load			20 Item, Fixed load			5 Item, Adjusted load		
		SE	Std		SE	Std		SE	Std		SE	Std
Lunch Gram Intake (g)	242.7	13.9	68.2	349.2	21.3	104.5	439.1	27.4	134.0	232.2	15.9	78.0
Energy Intake (Kcal)	554.1	33.9	166.0	64.9	45.2	221.5	747.4	55.2	27.5	506.9	36.5	178.9
Dinner Gram Intake (g)	621.5	44.1	216.1	661.0	42.3	207.1	569.7	38.1	186.5	579.8	47.9	234.8
Energy Intake (Kcal)	935.0	57.7	282.8	946.8	51.0	249.8	862.8	44.6	218.4	852.7	55.0	269.6
Total *ad-libitum* Gram Intake (g)	864.2	54.6	267.4	101.2	56.1	274.7	1008.8	56.3	275.9	811.9	59.0	289.2
Energy Intake (Kcal)	1489.1	83.8	41.7	1587.6	76.4	374.3	161.2	79.0	386.9	1359.6	77.7	38.6
Total Day Gram Intake (g)	1412.2	54.9	269.1	1575	57.7	282.9	1571.1	61.0	298.9	136.9	61.4	30.9
Energy Intake (Kcal)	2325.6	94.2	461.3	242.8	89.0	436.0	2485.0	103.2	505.7	218.8	78.6	385.2

Normal Weight

There were no significant differences on any food intake measures.

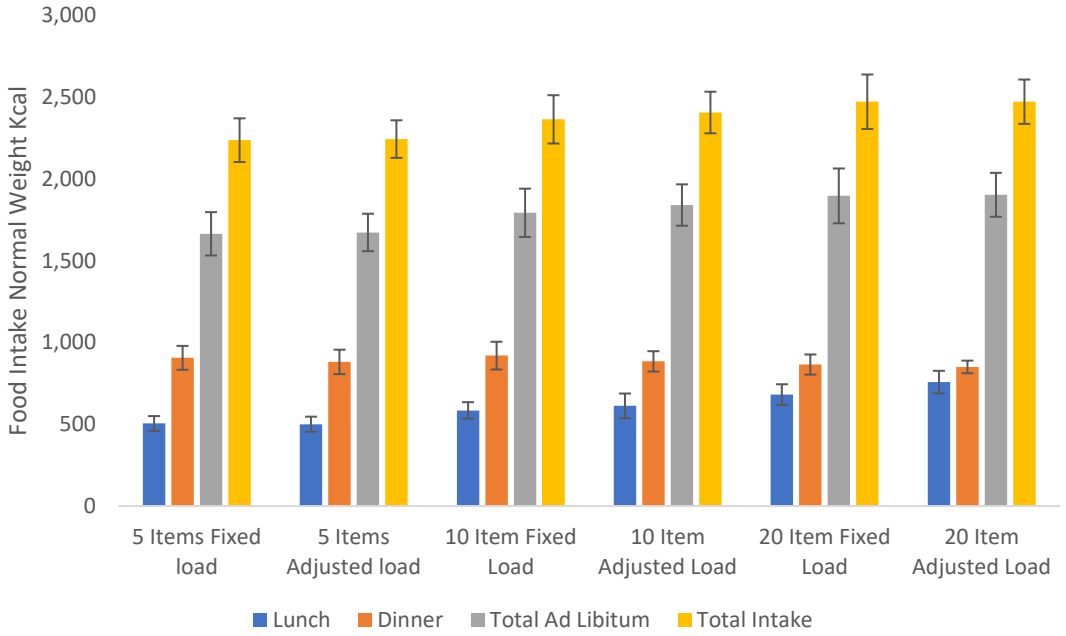

Figure 5 - 12 Effect of load condition on food intake (g) at the ad libitum test meals and evening snack box. Values are mean for 12 normal weight participants.

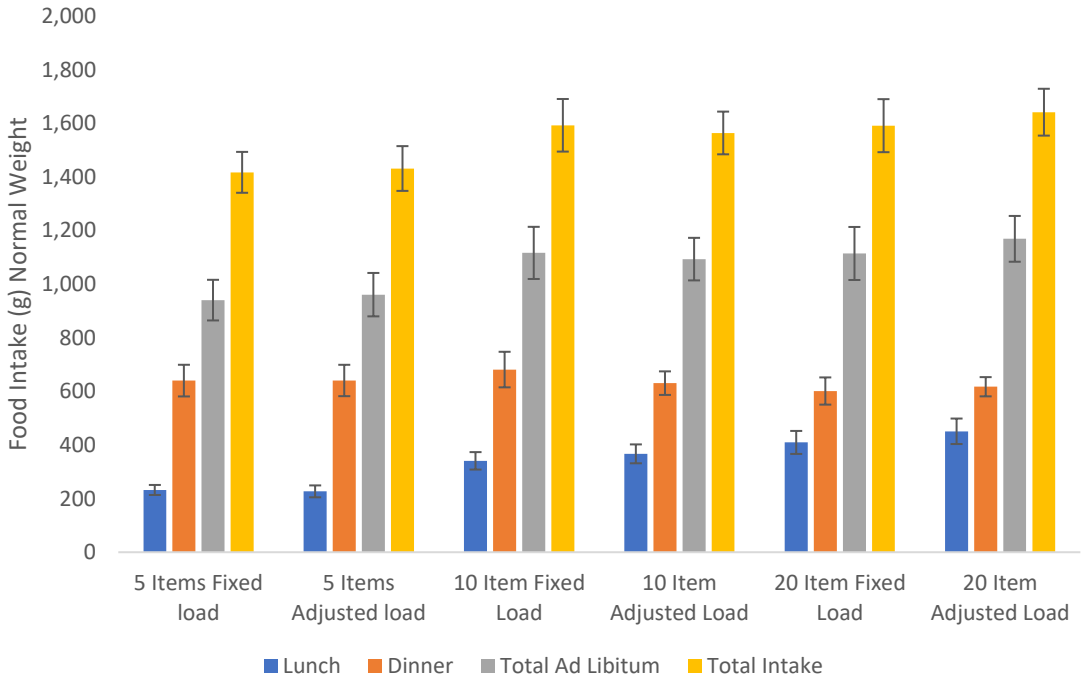

Figure 5 - 13 Effect of load condition on food intake (kcal) at the ad libitum test meals and evening snack box. Values are mean for 12 normal weight participants.

Table 5 - 15 Results summary for food intake for all conditions normal weight

Normal Weight	5 Item, Fixed load			10 Item, Fixed load			20 Item, Fixed load			5 Item, Adjusted load			10 Item, Adjusted load			20 Item, Adjusted load		
		SE	Std		SE	Std		SE	Std		SE	Std		SE	Std		SE	Std
Lunch																		
Protein (g)	23.6	2.4	8.4	25.3	2.2	7.5	32.4	3.1	1.7	23.2	2.5	8.8	29.2	3.6	12.3	31.6	3.3	11.3
Fat (g)	16.9	1.9	6.7	18.9	2.6	8.9	26	2.7	9.5	16.6	1.9	6.5	21.8	4.1	14.1	27.6	3.8	13.2
Carbohydrate (g)	62.9	6.5	22.7	75.7	7.1	24.5	75.3	7.4	25.6	63	6.8	23.5	72	7.2	24.9	91.2	7.4	25.6
Gram Intake (g)	232.3	39.8	138	34.9	32.6	112.8	409.6	42.9	148.5	227.1	22.2	76.8	367	35.3	122.1	451.1	47.5	164.7
Energy Intake (Kcal)	504.6	45.4	157.3	584.6	5.5	174.9	681.9	62.7	217.3	50.6	46.1	159.7	612.4	75.7	262.2	757.8	69	239.2
Dinner																		
Protein (g)	3.5	4.4	15.3	27.6	3	1.5	27.7	2.6	9.2	27.6	2.9	1	27.3	2.7	9.5	27.4	1.6	5.6
Fat (g)	26.9	3.2	1.9	25.9	3.3	11.5	24.7	3	1.4	23.6	2.4	8.5	24.7	3.1	1.7	23.3	2.5	8.5
Carbohydrate (g)	13.8	11.1	38.4	138.8	12.3	42.6	128.3	9.6	33.2	134.7	12.1	41.7	133.4	7.6	26.5	128	6.1	21
Gram Intake (g)	64.2	59.1	204.7	681.4	66.4	229.9	601.3	5.7	175.8	64.6	58.5	202.7	63.7	43.9	152.2	617.3	35.9	124.2
Energy Intake (Kcal)	906.2	73	252.9	919.6	85	294.3	865	61.7	213.6	881.2	74.7	258.7	884.5	62.3	215.8	85.4	38.7	133.9
Total ad-libitum																		
Protein (g)	54.2	5.6	19.5	52.9	4.4	15.4	6	5.1	17.8	5.8	4.9	16.9	56.5	4.7	16.1	59	3.9	13.5
Fat (g)	43.8	4.1	14	44.8	5.5	18.9	5.7	5.2	18	4.2	3.6	12.3	46.5	4.4	15.1	5.9	5.2	18.2
Carbohydrate (g)	193.6	13.3	46	214.6	17.7	61.5	203.6	15.6	53.9	197.8	15.2	52.8	205.3	11.8	4.9	219.2	11.9	41.1
Gram Intake (g)	872.4	71	246.1	1022	93.3	323.2	101.9	89.2	309	867.7	76.3	264.2	997.7	66.2	229.2	1068	72.1	249.9
Energy Intake (Kcal)	141.8	105	363.7	1504	126.9	439.7	1547	114.3	396.1	1382	105.7	366.1	1497	98.5	341.1	1608	94.2	326.2
Total Day																		
Protein (g)	73.4	5.6	19.5	71.7	4.7	16.1	79.6	5.7	19.8	71.3	4.5	15.6	75.5	5	17.2	77.8	4.5	15.4
Fat (g)	63.5	5.6	19.5	63.1	6.7	23.3	69.4	7.1	24.7	58.9	5.5	19.1	68.4	5.8	2.2	7.8	7	24.4
Carbohydrate (g)	318.4	17	58.8	351.1	19.4	67.4	354.8	24	83	332.2	18.1	62.9	346.8	16.8	58.2	353.7	16.1	55.8
Gram Intake (g)	1417	79.8	276.4	1593	98.1	339.8	1591	98.9	342.6	1431	83.5	289.1	1564	79.7	275.9	1641	87.4	302.8
Energy Intake (Kcal)	2237	133.4	462	2364	147.8	511.9	2472	166.5	576.6	2244	114.9	397.9	2406	127	44.5	2472	135.5	469.3

Table 5 – 16 Intake Calculations for Normal Weight Participants including the fibre preload

	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 Item, Adjusted load	10 Item, Adjusted load	20 Item, Adjusted load	5 Items Fixed vs 5 Items Adjusted	10 Items Fixed vs 10 Items Adjusted	20 Items Fixed vs 20 Items Adjusted	5 Items Fixed vs 5 Items Adjusted	10 Items Fixed vs 10 Items Adjusted	20 Items Fixed vs 20 Items Adjusted
Lunch + Preload	Intake	Intake	Intake	Intake	Intake	Intake	Intake	Intake	Intake	% Change	% Change	% Change
Gram Intake (g)	462.30	57.90	639.60	448.80	588.70	672.80	-13.50	17.80	33.20	-3.01	3.02	4.93
Energy Intake (Kcal)	571.80	651.80	749.10	565.40	677.20	822.60	-6.40	25.40	73.50	-1.13	3.75	8.94
Dinner +Preload												
Gram Intake (g)	87.20	911.40	831.30	862.30	852.40	839.00	-7.90	-59.00	7.70	-.92	-6.92	.92
Energy Intake (Kcal)	973.40	986.80	932.20	946.00	949.30	915.20	-27.40	-37.50	-17.00	-2.90	-3.95	-1.86
Total *ad-libitum* **+ Preload**												
Gram Intake (g)	1102.40	1252.30	124.90	1089.40	1219.40	129.10	-13.00	-32.90	49.20	-1.19	-2.70	3.81
Energy Intake (Kcal)	1478.00	1571.50	1614.10	1446.60	1561.70	1673.00	-31.40	-9.80	58.90	-2.17	-.63	3.52
Total Day												
Gram Intake (g)	1417.10	1592.50	1591.10	1431.30	1563.90	1641.40	14.20	-28.60	5.30	.99	-1.83	3.06
Energy Intake (Kcal)	2237.00	2364.10	2471.90	2243.60	2406.10	2472.10	6.60	42.00	.20	.29	1.75	.01

Participants who are obese Food Intake Measures

Lunch

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items on food intake at lunch f (2, 22) = 8.599, p = .03. Paired t-tests revealed there was a significant difference in lunch intake (kcal) in the 5-item fixed condition compared to the 5-item Adjusted condition t (11) = 2.274 p = .044 with participants consuming less in the adjusted load condition than the fixed load condition. After compensatory intake for the preload food intake was reduced by 6.3kcal (9.88%)

Dinner

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items for food intake at dinner kcal f (2, 22) = 6.352, p = .009. Paired t-tests revealed there was a significant difference in food intake at dinner (kcal) in the 5-item fixed condition compared to the 5-item Adjusted condition t (11) = 3.011, p = .012 in the 5-item fixed condition compared to the 5-item adjusted condition with participants consuming less in the adjusted load condition than the fixed load condition. There was a trend for an effect of fibre on food consumed at dinner in the 10-item fixed condition compared to the 10-item Adjusted condition (kcal) p = .072 (91.5kcal, 11.9%) with participants consuming less in the adjusted load condition than the fixed load condition.

Total Ad Libitum

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items for total *ad-libitum* food intake f (2, 22) = 10.644, p = .006. Paired t-tests revealed there was a significant difference in total *ad-libitum* intake

(kcal) for the 5-item fixed vs 5-item adjusted t (11) = 3.509 p = .015 with participants consuming less in the adjusted load condition than the fixed load condition. Food intake was reduced by 20.3kcal (14%) after compensatory intake for the preload. There was a trend for ad libitum intake grams p = .08 with participants consuming less in the adjusted load condition than the fixed load condition. There was a trend for ad Libitum Intake (kcal) p = .053 indicating there was a reduction in intake in the ten-item adjusted condition compared to the 10-item fixed condition (143.3kcal 9%).

Total Intake

A two-way repeated measures ANOVA revealed there was a significant interaction for load condition and items for total food intake f (2, 22) = 7.352, p = .005. Paired t-tests revealed there was a significant difference in total food consumed kcal in the 5-item fixed condition compared to the 5-item Adjusted condition t (11) = 3.167, p = .009 with more calories consumed in the fixed condition compared to the adjusted. Food intake was reduced by 296.3kcal (14%).

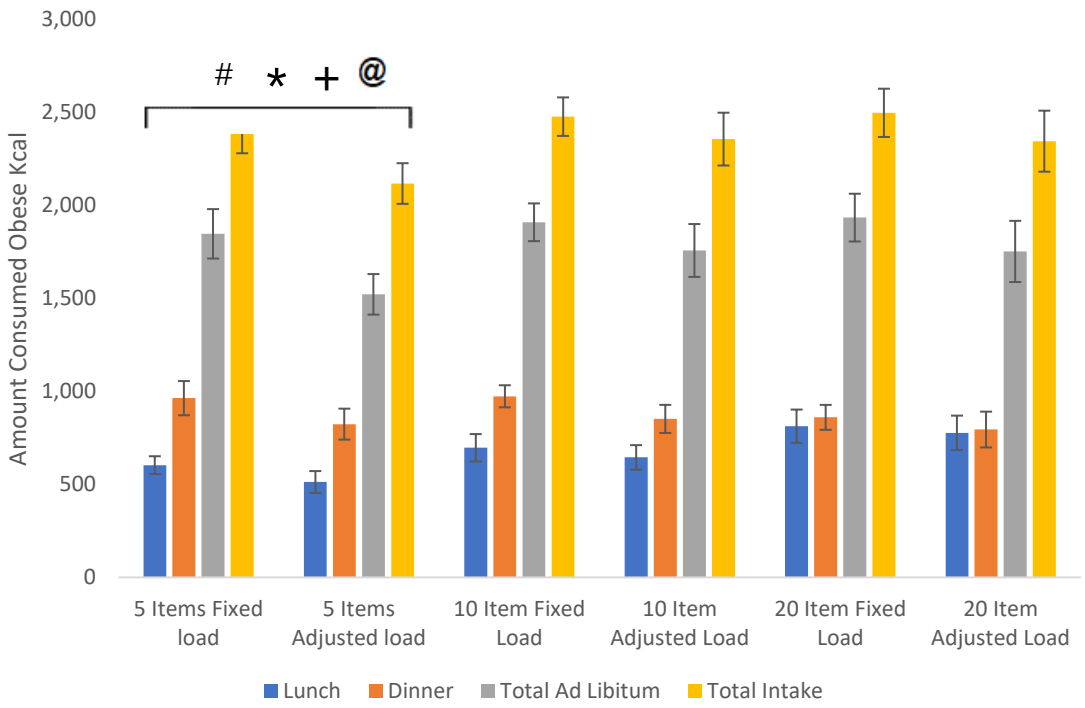

Figure 5 - 14 Effect of food items at lunch on the ad libitum test meals. Values are mean for 12 overweight participants. *P <.05 5 items fixed vs 5 items adjusted lunch. @P <.05 05 5 items fixed vs 5 items adjusted Dinner +P <.05 05 5 items fixed vs 5 items adjusted ad libitum intake. #P <.05 05 5 items fixed vs 5 items adjusted total intake.

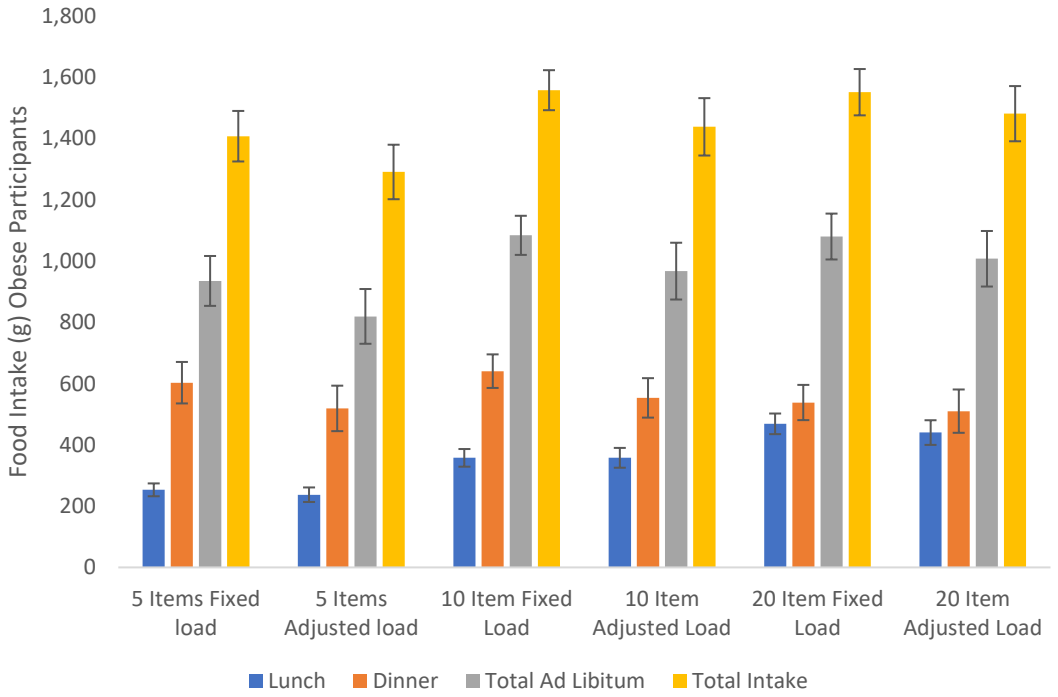

Figure 5 - 15 Effect of food items at lunch on the ad libitum test meals. Values are mean for 12 overweight participants.

Table 5 - 17 Results summary for food intake for all conditions participants who were obese

Overweight	5 Item, Fixed load			10 Item, Fixed load			20 Item, Fixed load			5 Item, Adjusted load			10 Item, Adjusted load			20 Item, Adjusted load		
		SE	Std		SE	Std		SE	Std		SE	Std		SE	Std		SE	Std
Lunch																		
Protein (g)	25	2.4	8.2	31.3	3.3	11.5	39.2	4.7	16.2	24.6	2.7	9.2	31.8	3.2	11.1	35	3.4	11.9
Fat (g)	19.8	1.5	5.1	22.7	2.52	8.7	29.1	3.2	11	17.4	1.4	5	22.4	2.6	9	29.7	3.6	12.5
Carbohydrate (g)	79.8	8	27.8	89	1.5	36.4	94	14.4	49.8	62.9	9.8	34	76.3	9.3	32.3	87.9	12.3	42.5
Gram Intake (g)	253.15	59	207.6	357.5	28.8	99.8	468.6	33.7	116.6	237.3	23.7	82.2	645.3	66	228.5	44.1	4.2	139.3
Energy Intake (Kcal)	603.6	47.9	165.9	697.1	73.6	255	812.9	89.7	31.6	513.2	58.7	203.3	645.3	66	228.5	777.5	92.5	32.5
Dinner																		
Protein (g)	3.4	2.9	9.9	3.9	2.6	9	27.7	2.5	8.8	27.3	3.7	12.7	28.6	2.2	7.8	`26.4	2.9	9.9
Fat (g)	31.1	2.5	8.5	29.1	1.4	4.9	27.9	1.6	5.5	26	2.1	7.2	25.9	2	7.1	24.7	3.3	11.4
Carbohydrate (g)	135.5	15.2	52.5	142	11.9	41.1	12.3	12.7	43.9	116	14.8	51.2	121.8	14.6	5.4	112.7	15.7	54.5
Gram Intake (g)	602.9	67.7	234.5	64.5	54.7	190	538.2	57.5	199.2	518.9	74.2	257.1	553.2	64.4	223.1	510	7.7	244.9
Energy Intake (Kcal)	963.8	91.9	318.5	973.9	59.4	206	86.6	67.2	232.6	824.2	83.3	288.5	852.3	75.5	261.6	795.1	96.3	333.4
Lunch and Dinner																		
Protein (g)	55.4	4.6	15.9	62.2	4.5	15.4	66.9	5.5	19	51.9	5.5	19.2	6.3	5	17.3	61.5	5.3	18.4
Fat (g)	5.9	3.3	11.5	51.8	2.2	7.5	57	2.9	9.9	43.4	2.2	7.7	48.3	3.2	11	54.3	5.9	2.6
Carbohydrate (g)	215.3	22.1	76.7	240	15.4	53.3	214.3	21.2	73.4	179	21.1	73	198.1	21.9	76	20.7	2.2	69.9
Gram Intake (g)	1129.6	117.3	406.2	998	66.5	230	1007	72.8	252.3	756.2	9.5	313.6	91.8	9.8	314.5	950	87.8	304
Energy Intake (Kcal)	1567.4	131.4	455	1671	83.9	291	1674	111	384	1337	118.2	409.6	1497.6	124.6	431.7	1572.6	15.2	52.4
Total Day																		
Protein (g)	73.5	4.5	15.5	79.6	4.6	16	84.2	5.4	18.7	68.7	5.4	18.6	78.2	5.1	17.7	78	5.3	18.4
Fat (g)	71.2	4.3	15	7.2	2.9	1.1	77	3.7	12.8	6.3	2	6.8	67.9	4.5	15.6	7.5	6.4	22.3
Carbohydrate (g)	343.2	23.4	90	352.6	19	65.9	339.5	24.2	83.9	293.6	2.8	72.1	325.8	24.4	84.4	317.4	22.3	77.2
Gram Intake (g)	1407.31	111.1	384.8	1558	65.3	226	1551	75.6	261.7	1291	89	308.2	1437.9	93.6	324.4	148.8	9.1	312
Energy Intake (Kcal)	2414.3	133.6	463	2478	104	359	2498	130	449.3	2118	109.3	378.5	2356.8	142	491.9	2345.6	164.1	568.5

Table 5 – 18 Compensation Calculations for Participants who are obese

	5 Item, Fixed load	10 Item, Fixed load	20 Item, Fixed load	5 Item, Adjusted load	10 Item, Adjusted load	20 Item, Adjusted load	5 Items Fixed vs 5 Items Adjusted	10 Items Fixed vs 10 Items Adjusted	20 Items Fixed vs 20 Items Adjusted	5 Items Fixed vs 5 Items Adjusted	10 Items Fixed vs 10 Items Adjusted	20 Items Fixed vs 20 Items Adjusted
Lunch + Preload	Intake	Intake	Intake	Intake	Intake	Intake	Intake	Intake	Intake	% Change	% Change	% Change
Gram Intake (g)	483.15	587.50	698.60	57.40	978.40*	773.20	87.25	39.90	74.60	15.30	39.95	9.65
Energy Intake (Kcal)	67.80	764.30	88.10	61.50	742.60	874.80	-6.30	-21.70	-5.30	-9.88	-2.92	-.61
Dinner + Preload												
Gram Intake (g)	832.90	87.50	768.20	852.00	886.30	843.10	19.10	15.80	74.90	2.24	1.78	8.88
Energy Intake (Kcal)	1031.00	1041.10	927.80	921.50	949.60	892.40	-109.50	-91.50	-35.40	-11.88	-9.64	-3.97
Total Ad Libitum + Preload												
Gram Intake (g)	1359.60*	1228.00*	1237.00*	1089.30	1243.90	1283.10	-27.30	15.90	46.10	-24.81	1.28	3.59
Energy Intake (Kcal)	1634.60*	1738.20*	1741.20*	1434.30	1594.90	1669.90	-20.30	-143.30	-71.30	-13.97	-8.98	-4.27
Total Day												
Gram Intake (g)	1407.31	1558.00	1551.00	1291.00	1437.90	148.80	-116.31	-12.10	-7.20	-9.01	-8.35	-4.74
Energy Intake (Kcal)	2414.30	2478.00	2498.00	2118.00	2356.80	2345.60	-296.30	-121.20	-152.40	-13.99	-5.14	-6.50

All Participants

Appetite 5-items

There was a trend for appetite p = .061 which indicated appetite was reduced immediately before dinner.

Appetite 10-items

There was also a trend for appetite with reduced appetite f (6.125, 337.452) = 2.016, p = .066 at vas 12 and vas 13.

Appetite 20-Items

There were no significant differences in appetite.

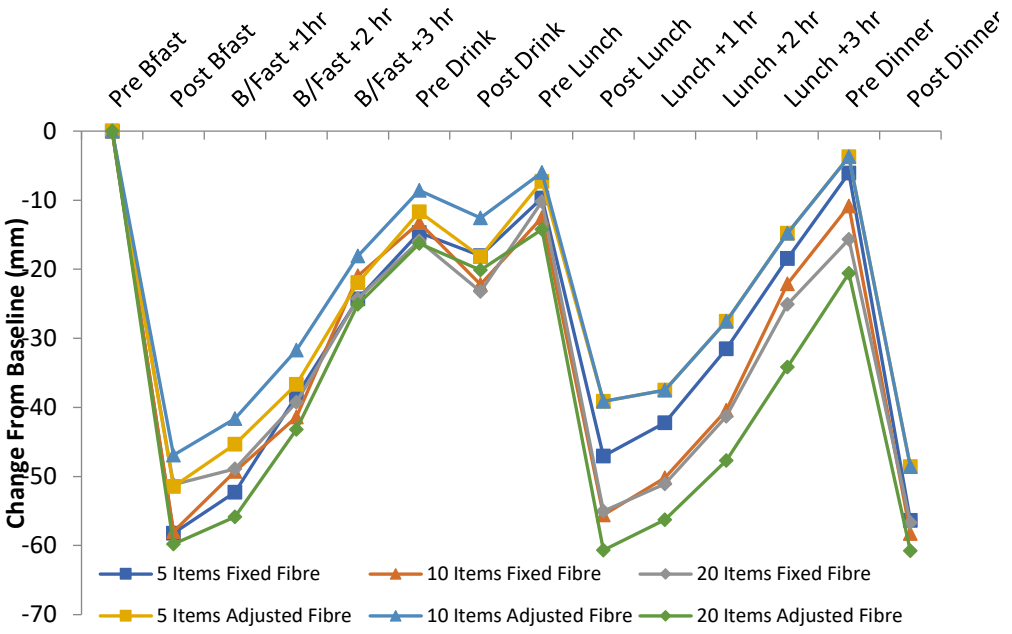

Figure 5 -16 Visual analogue scale (VAS) ratings for appetite scores for all conditions. Values are presented as changes from baseline score and are means for 24 participants.

Normal Weight Participants

Appetite

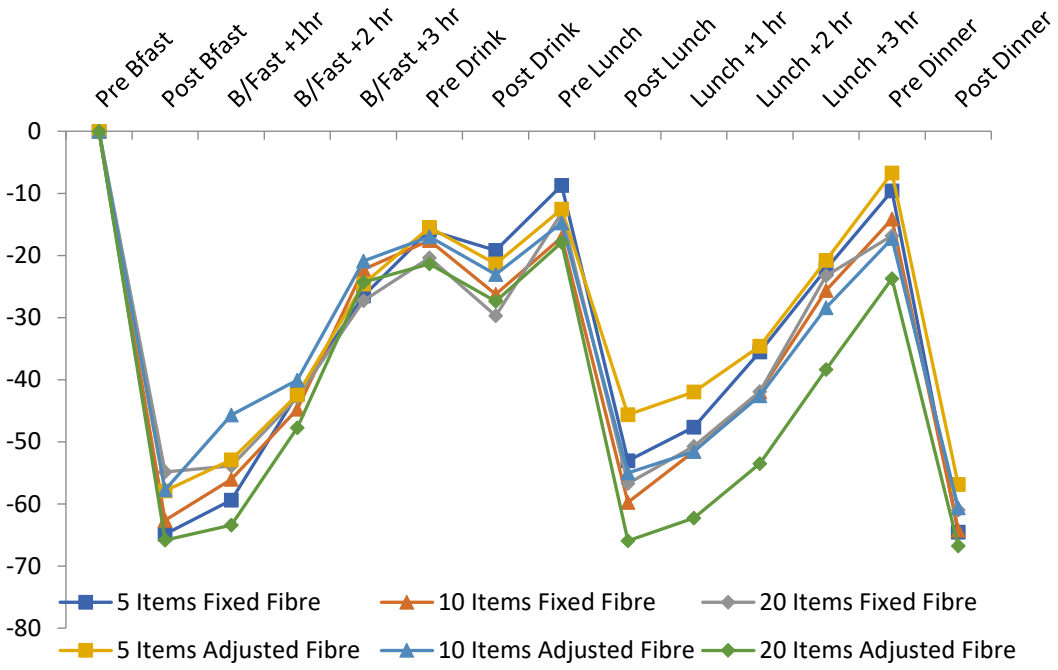

Figure 5 - 17 Visual analogue scale (VAS) ratings for Appetite Scores for the adjusted load and fixed load conditions. Values are presented as changes from baseline score and are means for 12 normal weight participants.

Appetite

Normal Weight Appetite Ratings

There was no significant difference in AUC for any of the conditions.

Participants Who are Obese

Appetite

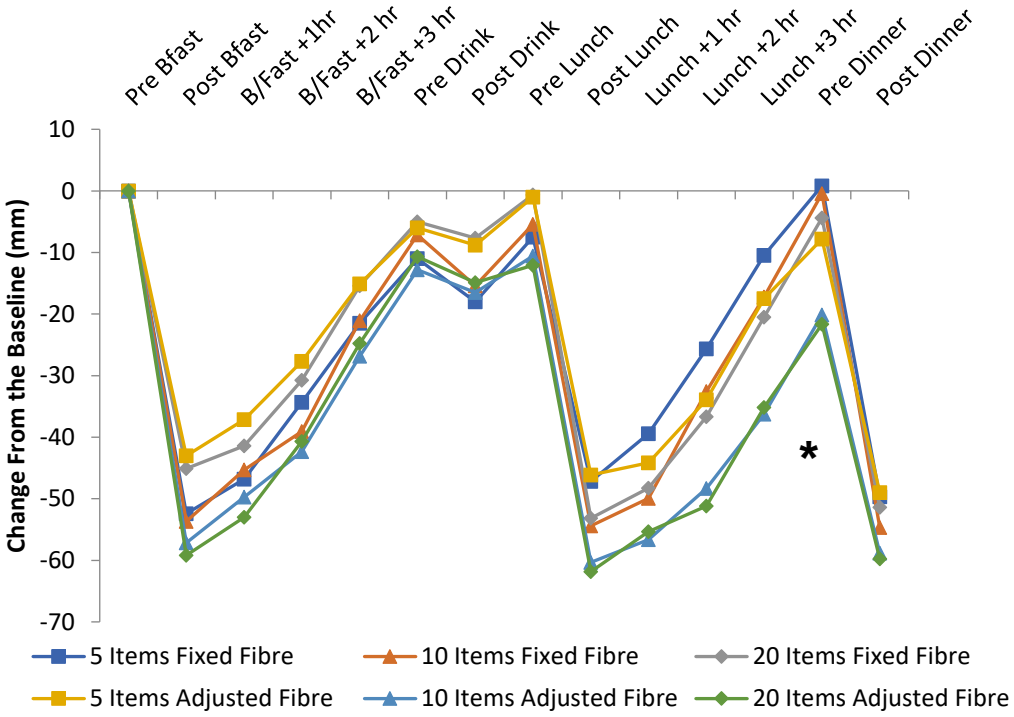

Figure 5 - 18 Visual analogue scale (VAS) ratings for appetite for the adjusted load and fixed load conditions. Values are presented as changes from baseline score and are means for 12 participants who are obese. *P <.05 5 items fixed vs 5 items adjusted. +P <.05 10 items fixed vs 10 items adjusted. #P <.05 20 items fixed vs 20 items adjusted.

5-Item meal Appetite

Appetite and time f (13, 143) = 2.054, p = .02. There was also a significant reduction in appetite immediately before dinner for the adjusted load condition for participants who are obese t (23) = 2.005, p = .007. Consistent with these results there was a significant reduction in AUC p = .014.

5.4 Discussion

This study investigated whether different *ad-libitum* multi-item, buffet-style test meals decrease the sensitivity of the meal to detect changes in appetite and food intake after a fibre preload. Based on previous studies, it was hypothesised that There would be an increase in food intake and a reduction in appetite in a high variety buffet meal compared to a low variety buffet meal for normal weight and participants who are

obese. As predicted food intake significantly increased with increasing number of items for all participants at lunch (242.7g, 349.2g, 431.1g, p=.018; 554.1kcal, 64.9Kcal, 747.4Kcal, p<.001), participants also reported significantly lower appetite in the 10 and 20-item meal conditions immediately after lunch and before dinner, this was consistent with previous research (Raynor & Epstein, 2001; Epstein et al., 2009). Satiety quotients indicated the 5-item meal reduced hunger significantly more per gram than the 10-item meal (p=.05) and 20-item meal (p=.025).

The second hypothesis tested was that normal weight but not participants who are obese will compensate for the increased intake at the high variety meal. Interestingly, there were no significant differences in total food intake (calories) for the obese or normal weight participants for the 3 conditions suggesting participants compensated for the increased calories at lunch later in the day, however there was a significant difference in total food intake (grams). The third hypothesis predicted high variety meal will decrease the chances of observing an effect of a fibre preload on appetite and food intake in participants who are obese. As predicted in participants who are obese the high variety meal did not detect the effects of a fibre preload on appetite or food intake, the limited variety meal however detected an effect of the fibre preload on appetite and food intake. The preload significantly reduced hunger (p=.005) and total food intake (296.3kcal, p=.009) in the 5-item condition. These results highlight how important it is that the *ad libitum* outcome meal is sensitive to manipulations of the preload in postprandial appetite studies (Blundell et al., 2010).

Palatability increased with increasing items, participants found the 20-item meal significantly tastier than both the 10-item meal and the 5-item meal. As previous research has found increasing the variety also increases palatability (Johnson & Wardle, 2014). There is also evidence to suggest that different levels of food variety have a proportional effect on consumption (Epstein et al., 2010). In the current study

doubling the number of items offered in the meal appeared to double the percentage increase in intake for all participants. There was a difference of 86.8kcal in food intake between the 5-item and 10-item lunch, an increase of 15.7%; a difference of 106.5kcal between 10 and 20-item lunch, an increase of 16.6%; and a difference of 193.3kcal between the 5 and 20-item lunch an increase of 34.9%. Our results are consistent with previous research which suggests greater palatability increases intake during in a meal (Sorensen et al., 2003). Palatability can have a considerable effect on meal size our findings aren't dissimilar to one study which found that highly palatable meals were 44% larger than the average meal (de Castro et al., 2000). Appetite measures were consistent with the food intake results indicating a reduction in hunger immediately before dinner in the 20-item condition compared to the 10-item condition, for the 20-item compared to 10-item and, 10-item compared to 5-item.

Palatable foods are thought to influence intake through the activation of hedonic motivational pathways (Egecioglu et al., 2011; Yeomans et al., 2004). Interestingly, palatability has a greater effect on intake in a satiated state than in a hungry state (Yeomans et al., 2001), suggesting that, although homeostatic mechanisms dominate in the hungry state, hedonic mechanisms become more important once homeostatic needs are met. In the current study participants were instructed to eat until they were comfortably full, however the 10 and 20 item meals may have encouraged participants to over consume once their homeostatic needs had been met. This was reflected in a significant reduction in hunger immediately after lunch for both participants who are normal weight and participants who are obese. (Yeomans, 1996) found that exposure to palatable foods reliably increased self-reported appetite, with ratings of hunger increasing during the early stages of a highly palatable meal, this may encourage over consumption and lead to greater feelings of fullness. Studies that measured liking for foods report that ratings for liking are higher when hungry, this indicates some overlap

between the hedonic and homeostatic motivation to eat (Gearhardt et al., 2011) (Finlayson et al., 2007; Finlayson et al., 2008).

Previous research investigating the effects of meal variety concentrated on the physiological and psychological processes that promote meal termination sensory-specific satiety (SSS) (Brondel et al., 2009; Raynor & Epstein, 2001; Rolls et al., 1981; Rolls et al., 1984). In the current study participants consumed on average 9.63 items in the 20 item fixed conditions compared with 7.53 in the 10-item condition. Participants did not consume more than 10 items in the bigger meals. Greater liking for the items added in the 20-item meal may explain the increase in consumption between the 10 item and 20 item meals. This is consistent with more recent research (Hendriks et al., 2019) they demonstrated within meal variety does not necessarily influence sensory specific satiation.

Interestingly, participants were able to adjust their total intake to compensate for the additional calories consumed in the 10 and 20-item lunches, this was apparent in both normal and overweight participants. This is contrary to some studies which found faster recovery of appetite following a more palatable meal making later compensation for increased intake less likely (Hill et al., 1984; Rogers & Blundell, 1990). Previous research suggests that obese individuals are at particular risk of overconsumption where there is a greater variety of food offered (Raynor & Epstein, 2001) and are less likely to compensate after the increased intake (Johnson & Wardle, 2014). Suggesting sensory stimulation from highly palatable food alone is insufficient to increase satiety. There were significant differences in total grams for the 20-item meal compared to the 5-item meal and the 10-item meal compared to the 5-item meal. Therefore, a greater volume was consumed but this was less calorie dense. There were no significant differences in total food intake in both the overweight and obese

condition. Participants compensated for the high variety meals at dinner, although there was still an observed difference in total intake (grams/kcal) across the day.

Detecting the Effects of a Preload

Over consumption induced by the high variety multi-item buffet meal meant participants who are obese did not compensate in response to the fibre preloads. The increase in variety and palatability of the multi-item buffets greatly increased ad libitum food intake (10-item lunch, 20-item lunch). Food intake at lunch increased by 26.1%, 177.3kcal for overweight participants for the 20-item lunch compared to the 5-item lunch this is consistent with (McCrory et al., 2012) who found that exposure to a variety of foods increased intake by roughly 29%. Overweight participants significantly reduced their food intake in the 5-item fibre condition compared to the 5-item control condition for dinner kcal, there was also a trend for dinner grams consumed. The adjusted preload also significantly decreased total ad libitum intake (219.7Kcal, $p = .008$; 101.6g, $p = .032$) and total food intake (187.9 Kcal; $p = .019$; 102.3g, $p = .033$). Increases in flavour pleasantness potentially resulted in appetising effects to drive short-term overconsumption in the high variety meals (Sorensen et al., 2003). Participants who are obese appeared to consume similar amounts in the 10 and 20-item conditions and did not compensate for the fibre preload. It is likely the multi item meals increased intake through delaying satiation and meal termination (Hetherington et al., 2006). The increased items likely swamped any effect of the fibre, weakening the effect on appetite, through overloading the appetite sensations. The high variety meal has the potential to mask the effects on food intake which are induced by a preload, and so decrease the sensitivity of postprandial studies to detect changes in eating behaviour.

Appetite measures were consistent with the food intake results for the 5-item meal indicating a reduction in hunger. There were no significant differences observed in

the 20-item condition and 10-item condition. These results suggest the sensitivity of the test meal to detect changes induced by a prior fibre preload was altered by the composition of the meal, when the number of items at the meal was increased. In the 5-item meal lower ratings of hunger were reported 2 h, 3 h after lunch and before dinner, consistent with reductions in AUC ($p = .014$).

Our findings are contrary to the findings of (Wiessing et al., 2012) they used a high calorie preload to test if 15-item buffet meal was better able to detect changes in appetite than a single item meal. They found that there was no difference between the single item meal and multi-item buffet in terms of detecting an effect on appetite or food intake. The multi-item buffet meal increased variety and consequently palatability. Participants increased their food intake in the multi-item condition (+ 39%) over the 30 min lunch period but were still able to compensate for the high 4 MJ calorie preload similarly to the low .5MJ preload. The compensatory response to the breakfast preloads occurred despite the predictable energy overconsumption induced by the multi-item buffet. Participants were normal weight males; this may suggest a difference due to weight status or gender.

The buffet-style meal adds an additional parameter in food choice and selection, which may not reflect the homeostatic drive to eat but rather is motived by a desire to consume a variety of hedonically appealing foods independent of need state (Berridge, 1996). The number of food items offered decreased the likelihood of observing significant changes in hunger and fullness with energy intake through ceiling effects. However, we did not observe any floor effects in the 5-item condition, participants appeared to eat to satiation and the preload significantly reduced appetite and food intake. Previous studies have found that a limited choice *ad-libitum* test meal typically has a lower palatability rating, which may further decline as multiple treatments are completed as there is little or no choice, this can lead to rapid onset of

sensory specific satiety and is likely to suppress intake relative to a multi-item meal (Brondel et al., 2009). This can be an issue as appetite research is often conducted via a repeated measures design, whereby participants complete up to 6 conditions. It is essential participants liking for the study foods is thoroughly assessed at the screening where a limited item meal is the test meal of choice, if participants dislike the foods on offer, they are unlikely to reach satiation, which would render the results inaccurate (Chapelot, 2013; Hill et al., 1995).

In the current study participants were presented distinct food items on separate plates, rather than presenting a readymade sandwich which may have encouraged participants to consume the entire item. Concerns have been raised about using large single unit foods for the *ad-libitum* test meals, as distinct food items presented as units may be subject to certain bias whereby participants tend to consume the whole item, rather than eating until comfortably full (Geier et al., 2006; Rolls et al., 2004) found that increasing the portion size of a sandwich served as a discrete unit lead to increased energy intake at a single meal without differentially influencing ratings of hunger and satiety.

The limited choice meal included similar buffet items rather than serving a large single course meal. Previous research compared a single course meal to a buffet meal (Wiessing et al., 2012), the differences in the meal types introduces further variables. A criticism of serving large single-course *ad-libitum* meal is that the portion size offered needs to be large for satiation to be reached, rather than plate clearing. Individuals tend to overestimate the amount of food they would typically choose to consume because normal intake is inflated when larger portions are served, this is known as the portion size effect (Zuraikat et al., 2019; Diliberti et al., 2004; Rolls et al., 2004). Serving the food as a limited buffet will reduce the effects of plate finishing, and additionally reduce the effect of variety, particularly in acute studies where food

intake is measured during one test meal and the potential to measure compensatory intake later in the day is limited.

In the current study foods were selected that were appropriate for meal occasion for a western diet. It was important the foods chosen are appropriate for the consumption context under investigation, such as serving breakfast foods at a breakfast *ad-libitum* trial, savoury buffet foods for lunch and a hot evening meal (Cardello & Schutz, 1996). Medium energy density (1–1.5 kcal/g) foods were selected, as small differences in intake of a very energy dense meal may unrealistically overestimate the effect on total energy consumed. For the same reason, the food chosen should not be unusually high in a specific macronutrient, as high-fat meals have been found to inflate energy intake and do not generalise to everyday meal occasions (Green et al., 2000). Participants were screened to ensure chosen foods were familiar, as this may affect true motivation to consume. Cross culturally different meals may be more suitable than others, this must be carefully considered when designing the study, the foods provided must therefore also be culturally acceptable for the meal occasion. The meals provided were culturally suitable. In selecting the meal's predominant sensory properties, most *ad-libitum* test meals tend to include both sweet and savoury items, research shows there is no difference in the onset of satiation for sweet or savoury ad libitum meals (Griffioen-Roose et al., 2009). In the current study a mixture of sweet and savoury items was provided, in equal number, which were increased proportionately for each condition.

Limitations

Although the 3 buffet meals were matched in terms of number of items in each sensory group and items were increased proportionately, increasing the items also increased palatability ratings. Some items provided on the 10 and 20 item buffets were not present on the 5-item buffet, participants may have simply preferred the

foods provided on the 10 and 20 item buffets and consumed more. The quantity of food also increased with increasing items, matching the quantity of food on each buffet meal was not practical, the food offered on each buffet was far greater than participants could consume (there was no plate finishing), but this wasn't matched. Offering a bigger serving to match the quantity on offer on the 20-item buffet was deemed unnecessarily and was not cost effective. It is however difficult to untangle the effects of increasing quantity and palatably from the effects of merely increasing the number of items.

Food intake studies conducted within the laboratory will always be limited by the foods that are presented during the test meal. Conducting appetite studies in a natural setting, where participants choice is not limited to the foods presented would better reflect normal eating behaviour, however the methodological problems associated with this limit the conclusions that can be drawn (Forde, 2018). There is a trade-off between realism and control which the laboratory creates. One test meal will not fit all studies, but the hypothesis under investigation must be carefully considered. Food choice studies will still require a variety of foods to be offered. Assessing liking for study foods on offer where a limited choice meal is offered is paramount.

Allowing participants to leave the laboratory during the inter-meal interval may have affected compliance to the protocol (i.e. no other foods or beverages other than the water provided). Given the time frame we tested over in the laboratory (8h) it was more practical to allow participants to continue their day as usual and attend the laboratory during mealtimes. Allowing participants to leave the laboratory between test meals remains far more accurate than dietary reporting, which cannot be relied upon (Subar et al., 2015).

Although a significant effect was found for increasing items in both normal and overweight participants, due to the design of the study it was not possible to conclude

whether the limited item meal was better able to detect changes of a fibre in a limited item or multi-item meal for normal weight participants.

Conclusion

Increasing the number of items presented within an *ad libitum* test meal significantly increased food consumption, in both overweight and normal weight participants. This overconsumption at the *ad libitum* meal was compensated for in both normal weight and participants who are obese. This highlights the importance of the test meal in acute studies where food intake is only measured during 1 test meal. The multi-item buffet meal encouraged overconsumption in both the control and treatment group compared with restricted choice meals. When designing preload studies to measure food intake a limited item buffet would be most appropriate to assess changes in appetite and food intake. Measuring appetite will always be fraught with methodological issues due to the complex nature of eating behaviour, but such steps can be taken to improve study design. This research is important to develop a standardised methodology in laboratory procedures and helps to move towards quantifying the optimal preload meal design.

Chapter Six

6. Experimental study to investigate the acute effects of inulin, β-glucan in isolation or combination on satiety, glycaemic response and colonic fermentation in females

6.1 Introduction

Research suggests fibre intake increases satiety and reduces food intake (Alfieri, et al.,1995; Howarth et al., 2001; Salleh et al., 2019). However, not all types of fibre produce the same effects (Hervik & Svihus, 2019; Slavin & Green, 2007; Wanders et al., 2011). Populations that report high fibre consumption demonstrate lower rates of obesity (Slavin & Green, 2007; Thompson, et al., 2017). Enhanced satiety may play a key role in this relationship (Slavin & Green, 2007); however, mechanisms of action are not clear, and variations are largely dependent on the physical properties of the fibre type and dose as the systematic review in chapter 3 demonstrated. Most of the physiological benefits of fibre are attributed to two characteristics, viscosity in the small intestine and fermentability in the large intestine (Poutanen et al., 2017). It was unclear if combining fibres with different physio-chemical properties have a synergy effect on appetite and biological markers compared to fibres in isolation. Combining both sensory and physiological measures will help to explore the specific modes of action of β-glucan a viscous fibre, and inulin a fermentable fibre, in isolation and combination as well as explore the health benefits such effects provide in terms of appetite control.

Viscous fibres mediate postprandial glucose response and delay gastric emptying rates via an ability to form viscous mixtures in the GI tract, this leads to a sense of fullness due to greater volume within the stomach (Schroeder, 2013). This in turn could potentially induce feelings of fullness and increase satiety (de Graaf et al., 2004;

Slavin, 2005). Food products containing dietary fibre with a high viscosity, such as β-glucans have shown satiating effects (Beck et al., 2009b). Beyond viscosity, intestinal fermentation of fibres may also influence satiety (Hervik & Svihus, 2019); however, these mechanisms are not completely understood. The fermentation of fibre yields hydrogen, methane and short chain fatty acids (SCFA) (Besten et al., 2013). Breath hydrogen and methane can be measured as a marker of fermentation. SCFAs are products of colonic fermentation, which influence postprandial glucose response by reducing fat competition for glucose disposal (Brighenti et al., 2006). SCFAs may also influence gut hormones and gastric motility (Nilsson et al., 2008). SCFAs have also been hypothesised to promote satiety.

Novel functional fibres are increasingly used in food processing and effectively increase the fibre content of foods. Inulin is one such form of non-viscous, fermentable fibre extracted exclusively from chicory root. Previous studies have demonstrated inulin can increase satiety (Cani et al., 2006), reduce food intake (Whelan et al., 2006) and encourage a healthy digestive system (Kolida, Tuohy, & Gibson, 2002). However, some of the research findings for other studies using inulin-type fibres yield mixed results for their effects on satiety (Karalus et al., 2012). Review articles summarising the research (Roberfroid, 2007; Kelly, 2008) have suggested the variation in the physical properties of the fibre type, dosage and methods could explain the conflicting data.

Inulin is part of a group of fibres called fructans. Inulin-type fructans with different chain lengths are fermented at different rates (Stewart et al., 2008). Chain length influences, not only the fermentation rate, but also the texture and sweetness, the shorter the chain length, the sweeter the taste. Short-chain fructans are rapidly fermented, while longer chains forms are more steadily fermented (Bonnema et al.,

2010). The capacity to be readily fermented may have implications for acute feelings of satiety. The fermentation time of oligofructose is about 5 h whereas the fermentation time of long-chain inulin is about 15 h. Inulin can be hydrolysed via industrial processes to shorten the chain lengths, to be fermented rapidly in the proximal part of the colon, this improves both its functionality and its application in the food industry. It has been demonstrated that consumption of inulin can elicit a rise in breath hydrogen within 2–3 h of consumption (Grysman, Carlson, & Wolever, 2008) and produce elevated levels of serum SCFAs between 3 and 6 h after consumption (Tarini & Wolever, 2010).

Studies examining longer chain FOS and satiety have produced inconsistent results. Cani et al., (2006) found that FOS 8 g twice daily for two weeks enhanced satiety and reduced energy intake in a pilot study with healthy participants (Cani et al., 2006). In contrast, consumption of 8 g of FOS in a meal-replacement bar one to two times a day for two days did not affect appetite rating or energy intake (Peters et al., 2013). A study in overweight adults demonstrated an enhanced satiety response and reductions in energy intake in participants receiving 21 g of FOS daily for 12 weeks (Parnell & Reimer, 2009). Previous research demonstrates short chain inulin would be more suitable to detect an effect in an acute study (Schaafsma & Slavin, 2015).

The importance of dosage has been investigated, with dosage ranging from 9g/day to over 35g/day. Pedersen et al. (2013) found that inulin dosages of 35g or more per day were shown to decrease appetite. (Parnell & Reimer, 2009) found that 21g per day resulted in greater weight loss compared to a placebo. However (Luis et al., 2013) found satiety increased in participants who are obese with a daily dose of just 9g of FOS in enriched cookies. Although a higher dose may increase the effects on satiety and appetite, the potential for GI discomfort also increases with increasing

dose. In animal studies FOS dose often amounts to 10% of total dietary intake (Cani et al., 2004; Cani et al., 2005). This is the equivalent of 40–60 g/d in human terms (Jenkins et al.,1999). If a dose of this size was provided in a human study the GI complications this introduces would become problematic. Human studies indicate that inulin is well tolerated at doses of 15 g/day, amounts exceeding this significantly increase the likelihood of adverse gastro-intestinal symptoms (Grabitske & Slavin, 2009).

Oats and barley contain β-glucan, previous studies have demonstrated both positive (Lumage et al., 2009; Willis et al., 2009) and negative (Peters et al., 2009) effects on satiety and energy intake as the amount and type of β-glucan varies widely between studies. β-glucan influences appetite by not only increasing viscosity in the gastrointestinal tract but also by improving glucose and insulin control (Beck, 2009), a lower insulin and glucose response may increase feelings of fullness (Vitalione, 2009). B-glucans ability to lower postprandial glycemia has been established in numerous studies (Kwong et al., 2013; Makelainen et al., 2007; Qi Wang & Ellis, 2014). Bacteria ferment β-glucans in the intestinal tract, this produces short-chain fatty acids. These may stimulate insulin release from the pancreas and alter glycogen breakdown by the liver and therefore play a role in glucose metabolism (Miyamoto et al., 2018). There is strong evidence β-glucan prolongs cholecystokinin (CCK) elevation after a meal which precipitates satiety signals (Beck, Tosh, Batterham, Tapsell, & Huang, 2009), however, it's unknown whether appetite suppression is the result of increased fullness or an effect of insulin regulation. (Vitalione, 2009).

The evidence suggests that oat β-glucan could have a positive effect on satiety. One study found that consuming a beverage containing 3 g of β-glucans resulted in significantly greater ratings of satiety than a fibre-free beverage (Barone Lumaga et al., 2012). Another study found that overweight men who consumed 7 g/day of β-glucans for 12 weeks experienced a significant increase in satiety (Shimizu et al.,

2008). Pentikäinen et al., (2014) observed that juice containing 4g β-glucan enhanced satiety. The most evident enhancement of satiety was observed when β-glucan amount was doubled to 8g. Enhanced satiety seems to be induced by viscosity development in gastrointestinal conditions. Lyly et al. (2009 & 2010) also found that β-glucan added to beverages increased satiety and decreased hunger compared to a control beverage. Fullness significantly increased, and hunger was significantly reduced more after eating higher viscosity β-glucan oatmeal than ready-to-eat breakfast cereal. Oatmeal has been shown to suppress appetite, increase satiety, and reduce desire to eat, and lowers prospective energy intake (Rebello et al., 2016). Vitaglione et at., 2009) found that a 3% β-glucan-enriched bread significantly reduced hunger and increased feelings of satiety more than a control bread resulting in a 19% reduction of energy intake at an ad libitum lunch.

The importance of dose has been explored, research has suggested that a minimum of 4 to 6 g of β-glucans are needed for appetite suppression (Pentikäinen et al., 2014). The systematic review chapter 3 found that a minimum dose as low as 2.2g/day reduced appetite and food intake. However, another study found that consuming 9 g/day of β-glucans for 24 weeks had no significant effect on appetite or body weight (Pick et al., 1996). Rebello et al., (2016) found that there was no linear dose-response relationship between effect on satiety and the amount of β-glucan. Variability in the processing of oats is thought to alter solubility and viscosity (Tomlin, 1995) which contribute to β-glucan functionality. A review concluded that β-glucans significantly increased satiety and reduced appetite compared with a diet containing no β-glucans, although it didn't always translate into decreased food intake in short-term studies.

In an acute setting, fibre properties, such as viscosity, may play a greater role in satiety than fermentation. Examination of the effect of soluble fibres on satiety in the form of beverages shows a greater satiety response as viscosity of the fibres increased (Lyly et al., 2009). Beverage viscosity has been inversely related to

postprandial hunger (Mattes & Rothacker, 2001). Viscous fibres create gastric distention and delay gastric emptying (Ménard et al., 2018), leading to a sense of fullness due to greater volume within the stomach (Spetter, de Graaf, Mars, Viergever, & Smeets, 2014). Viscous fibres often show satiating effects in the hours immediately after consumption. (Vitaglione et al., 2009) found that β-glucans have shown satiating effects 3 h after a preload containing β-glucan compared to a control. Solubility and viscosity are the most likely mechanisms of satiety; however, *β-glucan* can also be fermented in the large intestine. It is uncertain which of these two β-glucan characteristics viscosity or fermentability most influences satiety-related hormones, it could be both, by different mechanisms (Khoury et al., 2012).

It is unclear if combining fibres with different physio-chemical properties have a synergy effect. Only one study to date has tested the combined effect of β-glucan and FOS, a type of fructo-polysaccharide with similar properties to inulin, on appetite ratings and food intake (H. P. Peters et al., 2009). Participants consumed a meal-replacement bar at 09:00, an ad libitum lunch at 13:00 and a second test bar at 19:00. The control bar contained 0.3 g β-glucan, and the 3 equicaloric test bars contained an additional 0.9 g β-glucan (from barley), 8 g FOS, or 0.9 g β-glucan + 8 g FOS. The addition of β-glucan, FOS, or a combination of did not affect appetite ratings or food intake. Efficacy may have improved if the content of β-glucan was greater, or if the second test bar was provided earlier in the test session to allow fermentation to take place.

To assess the impact of a combination of fibres on appetite and food intake, we propose to assess the effects of inulin and β-glucan in isolation/combination on underlying regulatory mechanisms, namely glycaemic response, colonic fermentation and satiety in an acute study. The effects of the fibres will be measured over six hours in the laboratory, two fixed load meals will also be served during this period. Oatwell® β-glucan and Metamucil inulin were selected as the study products.

Oatwell® is natural oat bran and contains high concentrations of valuable β-glucans and soluble fibres which are found naturally within oats. Oatwell is commercially available and can be purchased as an individual ingredient in the UK. Studies with the specific outcome of appetite control have shown that bioactive oat β-glucan can promote these positive outcomes with a daily intake of 3-4g. Oatwell β-glucan can also be incorporated into beverages. Metamucil® inulin has been used in previous ethically approved studies including the studies presented in chapter 4 and 5.

This study will add to existing human research for combining fibres with different physio-chemical properties. The potential synergistic effects of such fibres on glycaemic response, colonic fermentation and satiety remains to be explored. Combining both sensory and physiological measures will help to explore the specific modes of action of β-glucan and inulin in isolation and combination as well as explore the health and consumer benefits such effects provide in terms of appetite control.

6.1.1 Hypothesis

We hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) and glycaemic response after a preload with inulin or β-glucan in isolation compared to a control, this decrease will be further enhanced after a preload with inulin and β-glucan in combination. ii) There will be an increase in colonic fermentation after a preload with inulin or β-glucan in isolation compared to a control, this increase will be further enhanced after a preload with inulin and β -glucan in combination.

6.2 Methods

6.2.1 Participants

Fifteen healthy women aged 18–64, with a body mass index (BMI) between 20–30 kg/m^2 (10 normal weight, 5 overweight) completed the study.

6.2.2 Study design

This was single blind within subject randomised crossover study which tested the effects of β-glucan, inulin or a combination there of on glycaemic response, colonic fermentation, appetite and food intake. Participants consumed a BMI scaled preloads of inulin, β-glucan or a combination of β-glucan with inulin and a control. Visual analogue scales (VAS) measured appetite ratings over 8 hours, hydrogen breath test was measured over 6 h and glycaemic response was measured over 3.5 h. Participants completed appetited ratings for 8 h.

Participants were allocated to receive each of the four conditions in randomised order using a counterbalanced sequence. Each testing period was separated by a week washout period. At the end of four laboratory visits each participant received each of the conditions. The sample size (15 female participants normal weight to overweight) has been calculated on the basis of the previous research and using the G-power program. Randomisation to the study was conducted by means of Latin squares.

6.2.3 Materials and Tools/Methods of Measurement

6.2.3.1 Hydrogen breath test

Participants completed hydrogen breath test measures before, immediately after, and 6 hours post-preload across four test days to assess carbohydrate fermentation. A portable desktop monitor, the GastroCH4ECK Gastrolyser was utilised to measure

both hydrogen (H_2) and methane (CH_4) levels in expired breath samples in response to appropriate substrates. The basis for breath testing in these circumstances is that intestinal bacteria break down carbohydrates to produce the gases. The sole source of the gases is bacterial fermentation of carbohydrate in the gut, so estimation of hydrogen in breath samples can be used to study the passage of carbohydrates through the gut.

6.2.3.2 Glycaemic Response

Participants underwent finger prick glucose measures before, immediately after and over the course of 3 and a half hours post-preload across four test days to assess insulin response. The meters used required a small blood sample and are designed to ensure minimal pain and tissue damage. A single-use needle, housed in a single-use lancet stick, was used to prick the tip of participant's finger and a drop of blood placed on a test strip. The strip is then inserted into the glucose monitor to obtain a reading. To prevent infection risk from potentially contaminated blood appropriate PPE was worn. Additionally, test strips containing participants' blood and other clinical waste were discarded in clinical waste bags. Furthermore, to prevent needle stick injury, all sharps were disposed of in sharps bins.

6.2.4 Study procedure/standardised Instructions

On each day preceding the study day participants were asked to keep their food intake and activity levels similar and to record these in a diary from 5 pm until they retired for the night. They were asked not to consume any alcohol and not to eat or drink anything except water from 12 midnight until they attended the study centre the following morning.

On each test day participants attended the Kissileff Laboratory between 08:30 and 9:00 hours for breakfast. A fixed lunch meal was also be served between 12:00 and 12:30 hours.

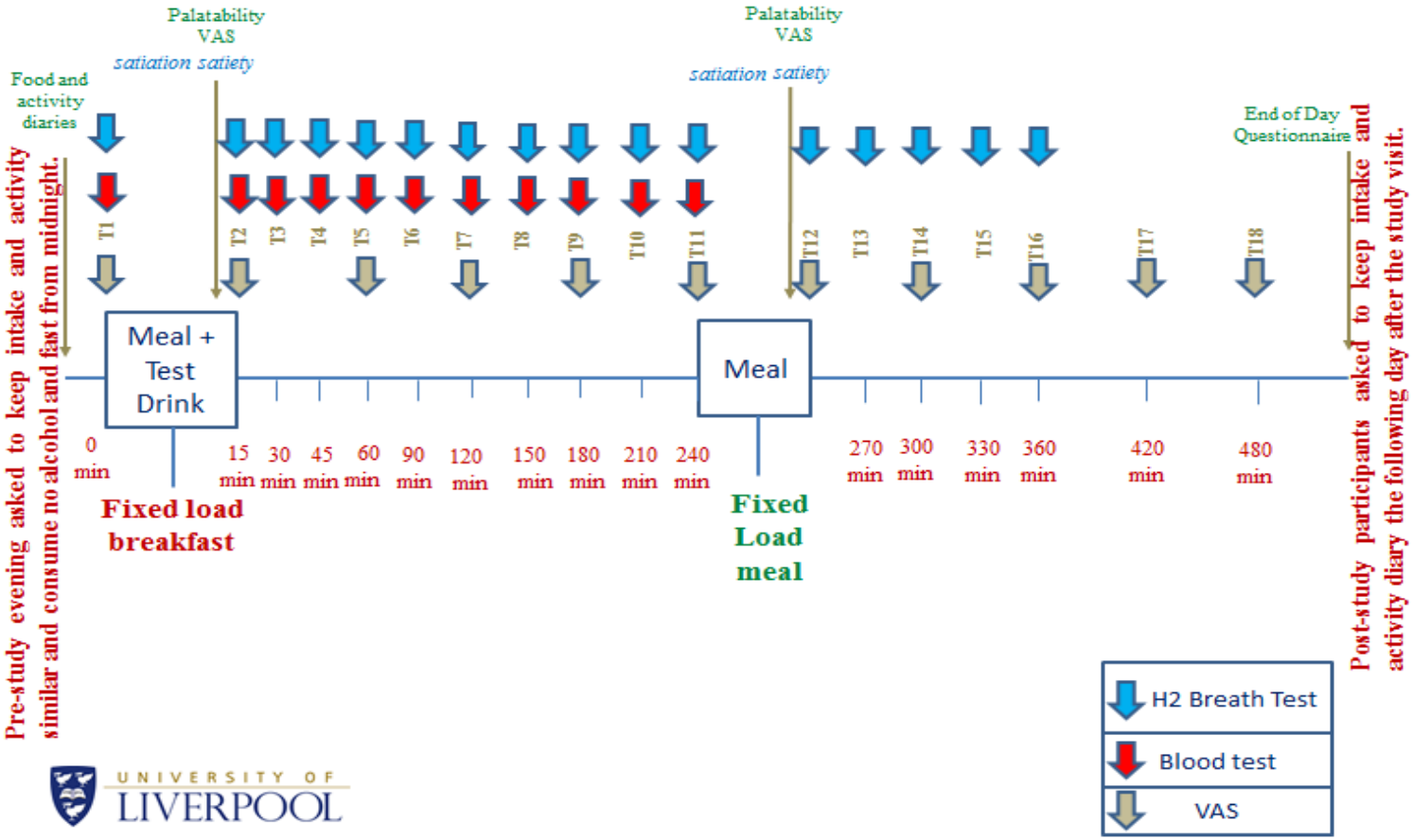

Figure 6 - 1 Test Day Outline

1. At ~8.30am participants attended the study centre. On arrival participants were seated in an experimental booth (meals were consumed in isolation so that social influence does not affect food selection and intake). The evening food intake and activity diary was collected.
2. Participants completed several VAS to rate their appetite sensations (hunger, fullness, desire to eat, prospective consumption) and thirst.
3. A hydrogen breath test and finger prick blood sample were then taken.
4. Participants were then asked to consume all of a BMI scaled fixed-load breakfast and a preload smoothie drink containing inulin/β-glucan in isolation/combination or a control smoothie, in a 15 min period.
5. After 15 minutes participants completed a VAS to rate their appetite sensations. A hydrogen breath test and finger prick blood sample were taken.
6. Hydrogen breath tests and finger prick blood sampling were taken every 15 minutes for an hour after breakfast and then every half an hour up until 240 minutes from the start of the study. VAS assessed appetite sensations at hourly intervals over the same testing period.
7. After the VAS, hydrogen breath test and finger prick blood sample at 240 minutes, a fixed load lunch was served. Participants were instructed to consume all the lunch, signalling when they have finished via the buzzer provided. Upon finishing their meal, participants rated pleasantness of the food.
8. Hydrogen breath test and VAS were taken immediately after lunch.
9. Hydrogen breath tests continued to be taken every 30 minutes until 360 minutes from the start of the testing period and VAS continued to assess appetite sensations every hour until 360 minutes from the start of the testing period.

10. Participants left the laboratory but completed two further VAS measures after leaving the laboratory at 420 minutes and 480 minutes from the start of the testing period.

Participants completed an end of day questionnaire at the end of each study day.

Participants remained in the laboratory for the duration of the study, consuming only the foods provided. The foods provided are familiar everyday items available from Tesco stores. *ad-libitum* water was provided throughout the testing session. The same procedure was followed for the four conditions with the β-glucan/Inulin/inulin+β-glucan/control in random order with a one-week washout period in-between. Lunchtime was fixed at precisely 4 hours after breakfast. The meal was served in individual booths in the test study centre.

6.2.5 Test Foods

Breakfast and standard Preloads

A fixed load breakfast was provided; the precise energy content of which depended on individual participant energy needs (25% energy needs relative to body weight – 10kg increments). The breakfast consisted of toast with margarine and the test smoothie.

The test smoothie was carefully formulated in order to disguise the taste and the texture of the β-glucan fibre. Combining each fibre in a fruit smoothie helped to conceal any differences in texture, this also masked the taste. Extensive taste tests took place in order to check that the 4 test drinks did not differ in taste or on any other sensory dimension. To reduce the potential for olfactory differences to impact on the β-glucan drinks, the smoothies were also presented in a cup with a lid and were consumed through a straw. Researchers were satisfied that after taste tests the drink provided did not differ on any measure and had an acceptable level of taste.

Table 6 - 1 Foods served at breakfast.

Composition of the BMI scaled Smoothie	
Ingredient	**Amount (g/kg/m2)**
Inulin	0.66
B-glucan	0.22
Blue berries	3.33
Sweetener	0.1
Asda raspberry and blueberry juice	7.6
Lactose Free Milk	4.26
Food	
Warburtons Bread	5.3
Flora Margarine	0.4

Fixed Load Lunch

The fixed load lunch meal was based on the daily intake requirements for a normal to overweight female and was scaled appropriately for BMI. A fixed load meal was selected to minimise the impact on the post meal hydrogen breath tests. The fixed load meal was fixed at precisely 4 hours after breakfast. Ham bread rolls and crisps were provided. Participants were instructed to consume the entire meal. Juices or milk were not provided as accompaniments to prevent impacting on the hydrogen breath test; however, water was provided throughout the day.

6.2.6Statistical analysis

Analysis was performed using SPSS for Windows Version 24. Data conformed to the requirements for parametric analysis therefore Analysis of Variance (ANOVA) was used. If the assumptions of sphericity were violated, Greenhouse Geisser correction was employed. All tests were two tailed, and a P value < 0.05 was considered

significant. Data was analysed to assess the effect of condition on appetite and bio-markers.

Appetite Ratings

Changes in ratings of appetite such as hunger, fullness, prospective consumption, and desire to eat were assessed. These parameters rated on the VAS were analysed using within-subject ANOVA for repeated measures with condition (β-glucan and inulin combined, β-glucan, inulin and control) and time (pre-breakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch, post-lunch, 2 pm, 3 pm, 4 pm, 5pm) as within-subject factors. If a time-by-condition interaction effect was found significant, paired t-tests were conducted at each rating time between conditions. An appetite score was also calculated using the formula ((hunger + desire to eat + prospective appetite) + (100 - fullness) + (100 - satiety))/5 for each condition in order to reduce variance in the appetite data. The trapezoid rule was used to calculate area under the curve (AUC) in accordance with the recommendations of Blundell, for each VAS variable and differences in AUC VAS ratings were assessed using repeated measures ANCOVA with baseline values serving as covariant.

Biological Markers

Breath hydrogen and methane measures were analysed using within-subject ANOVA for repeated measures with condition (β-glucan and inulin combined, β-glucan, inulin and control) and time (pre-breakfast, post-breakfast, 9.30 am, 9.45 am, 10 am, 10.30 am, 11 am, 11.30 am, 12 pm, 12.30 pm, pre-lunch, post-lunch, 2.30 pm, 3 pm) as within-subject factors. If a time-by-condition interaction effect was found significant, paired t-tests were conducted at each rating time between conditions. Plasma blood glucose was also analysed using within-subject ANOVA for repeated measures with condition (β-glucan and inulin combined, β-glucan, inulin and control) and time (pre-

breakfast, post-breakfast, 9.30 am, 9.45 am, 10 am, 10.30 am, 11 am, 11.30 am, 12 pm, 12.30 pm and pre-lunch) as within-subject factors. The trapezoid rule was used to calculate area under the curve (AUC), for each biomarker variable and differences in AUC for biomarkers were assessed using repeated measures ANCOVA with baseline values serving as covariate.

6.3 Results

Participants

In total, 26 participants were screened, 16 were recruited and 15 completed the study. One participant withdrew due to reasons unrelated to the study. The screening measures, demographic (age) and anthropometric (weight, height, BMI) characteristics of the completing participants are shown in Table 1.

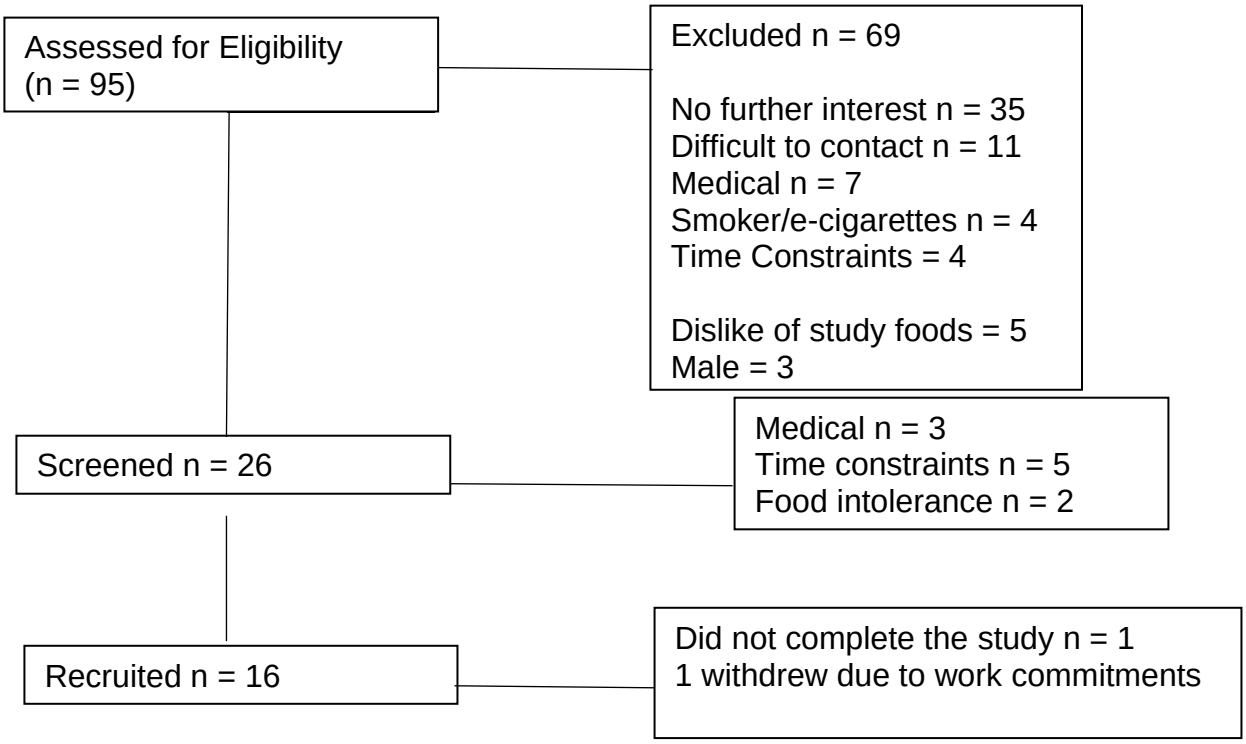

Figure 6 - 2 Total enquiries, participants screened and recruited into the study

Table 6 - 2 Baseline characteristics of participants who completed the study

	(*n* =15*)*		
	Mean	SE *a*	Std *c*
Age (years)	24.53	1.26	4.88
Height (m)	1.66	.18	.68
Weight (kg)	63.89	2.112	8.21
BMI (kg/m^2)	23.18	.55	2.13
DEBQ *b*) restraint score)	2.12	.11	.51

a Standard error. *b* Dutch eating behaviour questionnaire. *c* Standard deviation

Study Product

The preload drink provided a normal weight or overweight fixed load. The load provided 0.66g/kg/m^2 inulin, 0.786g/kg/m^2 of β-glucan, 3.64 g/kg/m^2 blue berries, 8.01g/kg/m^2 berry juice and 4.26g/kg/m^2 lactose free milk. An average of 22.5kg/m^2 for normal weight participants and 27.5kg/m^2 for overweight participants was taken to calculate the load for normal weight and overweight participants (table 2).

Table 6 - 3 Composition of the BMI scaled drink.

Ingredient	Amount (g/kg/m^2)
Inulin	0.66
B-glucan	0.79
Blue berries	3.64
Berry juice	8.01
Lactose Free Milk	4.26

Table 6 - 4 Composition of the preload drinks for each weight category.

	Normal Weight				Overweight			
	B-glucan and Inulin	B-glucan	Inulin	Control	B-glucan and Inulin	B-glucan	Inulin	Control
Fibre								
Gram Intake (g)	15 (inulin) 17.7 (β-glucan)	17.7	15	0	18.15 (inulin) 21.62 (β-glucan)	21.62	18.15	0
Energy Intake (Kcal)	112.9	48.5	64.7	0	137.44	59.24	78.2	0
Berry Juice								
Gram Intake (g)	180.3	180.3	180.3	180.3	220.28	220.28	220.28	220.28
Energy Intake (Kcal)	45.1	45.1	45.1	45.1	55	55	55	55
Blueberries								
Gram Intake (g)	82	82	82	82	100.1	100.1	100.1	100.1
Energy Intake (Kcal)	39.36	39.36	39.36	39.36	48	48	48	48
Milk								
Gram Intake (g)	95.85	95.85	95.85	95.85	117.15	117.15	117.15	117.15
Energy Intake (Kcal)	38.34	38.34	38.34	38.34	46.86	46.86	46.86	46.86
Total Preload								
Gram Intake (g)	390.85	375.85	373.15	358.15	477.3	459.15	455.68	437.525
Energy Intake (Kcal)	235.7	171.3	187.5	122.8	287.3	209.1	228.06	149.86

Palatability

There were no significant differences in taste f(1.916, 26.825) = 1.026, p = .369, palatability f(3, 45) = .227, p = .877, pleasantness f (3,4) = 1.060, p = .367, saltiness f (1.837, 25.723) = 1.497, p = .242 or sweetness f (3, 45) = .615, p = .609 for the drink in all conditions.

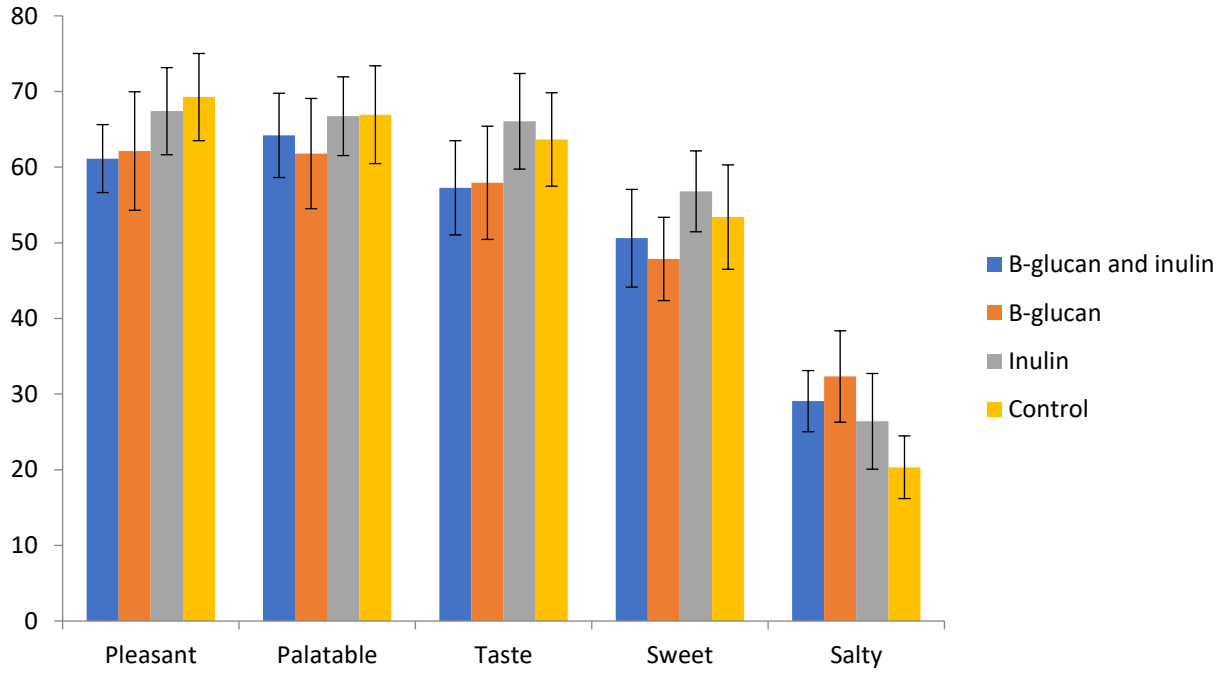

Figure 6 - 3 Effect of condition on the different dimensions of taste for the preload drink. Values are mean for 15 participants.

Appetite Score

There was an overall significant interaction between fibre condition and time for the four conditions f (27, 378) = 1.660, *p* = .022.

Appetite Score Inulin, β-glucan and Combined Fibre vs Control

Post Breakfast

A within subject ANOVA revealed that there was a significant effect of fibre on appetite post breakfast f (3, 45) = 5.009, p = .005, η_p^2 = 246. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .002 compared to the control condition.

Breakfast +1 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite breakfast + 1 h f (3, 45) = 5.487, p = .003, η_p^2 = 282. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .003, compared to the control condition. Appetite was also significantly reduced p = .042 in the combined fibre condition compared to the β-glucan fibre in isolation.

Breakfast +2 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite breakfast + 2 h f (3, 45) = 5.186, p = .004, η_p^2 = .270. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .001 compared to the control condition.

Breakfast +3 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite breakfast + 3 h f (3, 45) = 3.336, p = .027, η_p^2 = .214. Pairwise comparisons revealed

that appetite was significantly reduced in the inulin and β-glucan condition p = .001 compared to the control condition.

Pre-Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on appetite pre-lunch f (3, 45) = 8.030, p = .006, η_p^2 = .364. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = 034 compared to the control condition.

Post Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on appetite post-lunch t f (3, 45) = 4.797, p = .016, η_p^2 = .455. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .002, compared to the control condition.

Lunch + 1 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 1 h f (3, 45) = 5.150, p = .004, n = .269. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .003 compared to the control condition.

Lunch + 2 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 2 h f (3, 45) = 15.368, p < .001, η_p^2 = .523. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .004,

compared to the control condition. Appetite was also significantly reduced p = .037 in the combined fibre condition compared to the β-glucan fibre in isolation.

Lunch +3 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 3 h f (3, 45) = 14.172, p < .001, η_p^2 = .475. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p < .001 compared to the control condition. Appetite was also significantly reduced p = .011 in the combined fibre condition compared to the β-glucan fibre in isolation.

Lunch +4 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 4 h f (3, 45) = 7.938, p = .003, η_p^2 = .275. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p < .001. compared to the control condition. Appetite was also significantly reduced in the combined fibre condition compared to the β-glucan fibre p = .014 in isolation and the inulin fibre in isolation p = .035.

Inulin and β-glucan in isolation did not significantly reduce appetite relative to the control. There was no significant difference between appetite scores in the combined fibre condition and inulin in isolation.

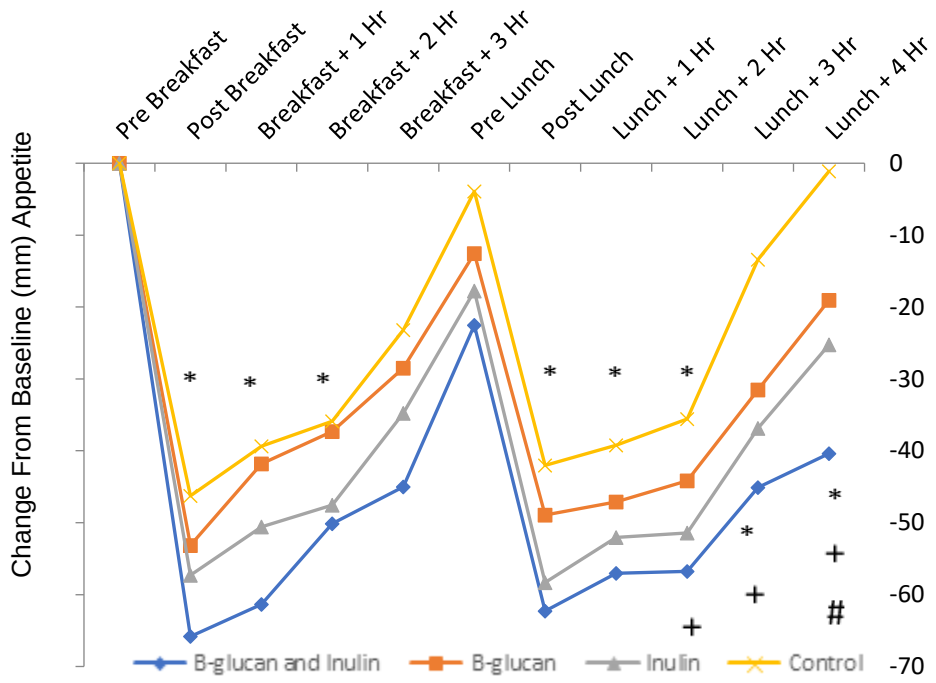

Figure 6 – 4 Visual analogue scale (VAS) ratings for appetite. Values are presented as changes from baseline score and are means for 15 participants. *P < 0.05 Inulin and B-glucan combined vs. control. +P < 0.05 Inulin and B-glucan combined vs B-glucan in isolation. #P < 0.05 Inulin and B-glucan combined vs inulin in isolation.

Biological Markers

Hydrogen Breath Production

There was a significant interaction between the four fibre conditions and time f (39, 546) = 16.803, p < .000 in hydrogen breath production.

Hydrogen Breath Production ANOVAs

Breakfast +3 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production breakfast + 3 hours f (3, 45) = 6.560, p = .001, η_p^2 = = .319. Pairwise comparisons revealed that breath hydrogen production was significantly

increased in the inulin and β-glucan combined condition p = .015 compared to the control condition. Breath hydrogen production was also significantly increased p = .035 in the β-glucan condition compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the inulin fibre in isolation p = .026.

Breakfast +3.5 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production 3.5 hours post breakfast f (3, 45) = 7.942, p < .001, η_p^2 = .361. Pairwise comparisons revealed that breath hydrogen production was significantly increased in the inulin and β-glucan combined condition p = .008 compared to the control condition. Breath hydrogen production was also significantly increased p = .025 in the β-glucan condition compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the β-glucan fibre in isolation p = .011 and the inulin fibre in isolation p = .015.

Pre-Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production pre-lunch f (3, 45) = 9.758, p < .001, η_p^2 = .411. Pairwise comparisons revealed that breath hydrogen production was significantly increased in the inulin and β-glucan combined condition p = .002 compared to the control condition. Breath hydrogen production was also significantly increased p = .018 in the β-glucan condition compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the β-glucan fibre in isolation p = .008 and the inulin fibre in isolation p = .043.

Post Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production post lunch $f (3, 45) = 8.695$, $p < .001$, $\eta_p^2 = .383$. Pairwise comparisons revealed that breath hydrogen production was significantly increased in the inulin and β-glucan combined condition $p = .0101$ compared to the control condition. Breath hydrogen production was also significantly increased $p = .019$ in the β-glucan condition compared to the control. The inulin condition also significantly increased breath hydrogen production $p = .014$ compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the β-glucan fibre in isolation $p = .001$ and the inulin fibre in isolation $p = .026$.

Lunch +.5 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production lunch + .5 hours $f (3, 45) = 19.566$, $p < .001$, $\eta_p^2 = .583$. Pairwise comparisons revealed that breath hydrogen production was significantly increased in the inulin and β-glucan combined condition $p < .001$ compared to the control condition. Breath hydrogen production was also significantly increased $p = .002$ in the β-glucan condition compared to the control. The inulin condition also significantly increased breath hydrogen production $p = .001$ compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the β-glucan fibre in isolation $p = .011$ and the inulin fibre in isolation $p = .01$.

Lunch +1 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production lunch + 1 hour f (3, 45) = 30.576, $p < .001$, $\eta_p^2 = .686$. Pairwise comparisons revealed that breath hydrogen production was significantly increased in the inulin and β-glucan combined condition $p > .001$ compared to the control condition. Breath hydrogen production was also significantly increased $p = .003$ in the β-glucan condition compared to the control. The inulin condition also significantly increased breath hydrogen production $p < .001$ compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the β-glucan fibre in isolation $p < .001$ and the inulin fibre in isolation $p < .001$.

Lunch + 1.5 Hour

A within subject ANOVA revealed that there was a significant effect of fibre on breath hydrogen production lunch + 1.5 hours f (3, 45) = 22.195, $p < .001$, $\eta_p^2 = .613$. Pairwise comparisons revealed that breath hydrogen production was significantly increased in the inulin and β-glucan combined condition $p = < .001$ compared to the control condition. Breath hydrogen production was also significantly increased $p = .002$ in the β-glucan condition compared to the control. The inulin condition also significantly increased breath hydrogen production $p = .001$ compared to the control. There was also a significant increase in breath hydrogen production in the combined fibre condition compared to the β-glucan fibre in isolation $p < .001$ and the inulin fibre in isolation $p = .005$.

Consistent with these observations, significant effects of condition on AUC breath hydrogen were identified, the inulin and β-glucan combined condition ($p < .001$) and

the inulin condition (p = .003) resulted in a higher AUC compared to the control condition. There was also a significant effect on AUC for the combined fibres compared to the fibres in isolation with the combined fibres resulting in higher AUC compared to both the inulin (p = .021) fibre and β-glucan (p = .007) fibres in isolation. There was also an insignificant trend for AUC breath hydrogen for the β-glucan condition compared to the control condition (p = .064).

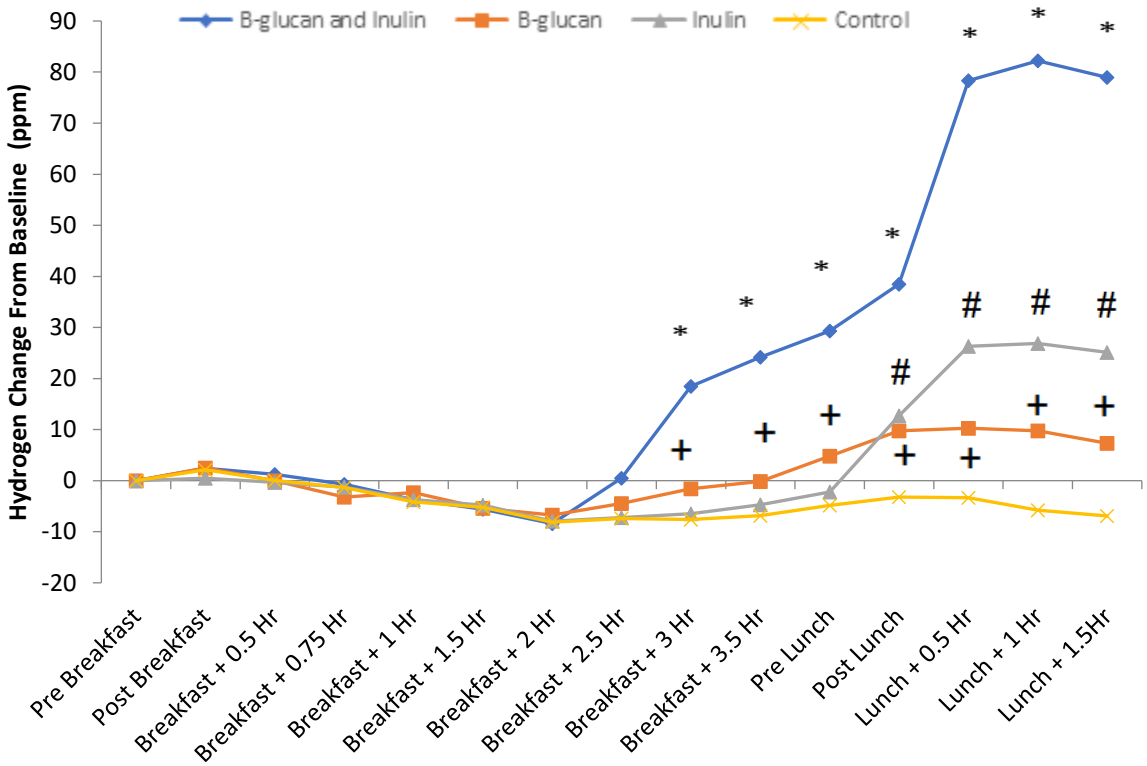

Figure 6 - 5 Breath Hydrogen production (ppm). Values are presented as changes from baseline score and are means for 15 participants. *P < 0.05 Inulin and B-glucan combined vs. control. +P < .05 B-glucan vs. control. #p < .05 inulin vs control.

Plasma Blood Glucose

There was a significant overall interaction for fibre condition and time for plasma blood glucose f (27, 378) = 3.630, p < .000.

Plasma Blood Glucose for the fibres in isolation and the control condition

Post Breakfast

A within subject ANOVA revealed that there was a significant effect of fibre on plasma glucose concentrations post breakfast f (3, 45) = 8.607, p = .004, η_p^2 = .381. Pairwise comparisons revealed that plasma glucose concentrations were significantly reduced in the inulin and β-glucan combined condition (p < .001) compared to the control condition. Plasma glucose concentrations were also significantly reduced (p = .05) in the combined fibre condition compared to the inulin fibre in isolation.

Breakfast + 3 Hours

A within subject ANOVA revealed that there was a significant effect of fibre on plasma glucose concentrations breakfast + 3 h f (3, 45) = 5.252, p = .023, η_p^2 = .273. Pairwise comparisons revealed that plasma glucose concentrations were significantly increased in the combined fibre condition (p = .007) compared to the inulin fibre in isolation.

Breakfast + 3.5 Hours

A within subject ANOVA revealed that there was a significant effect of fibre on plasma glucose concentrations breakfast + 3.5 h f (3, 45) = 8.486, p = .003, η_p^2 = .374. Pairwise comparisons revealed that plasma glucose concentrations were significantly increased in the combined fibre condition (p = .003) compared to the inulin fibre in isolation. There was also a non-significant trend (p = .043) 3.5 hours post breakfast for an increase in plasma glucose concentration for the combined β-glucan and inulin condition compared to the control.

Pre-Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on plasma glucose concentrations pre-lunch f (3, 45) = 5.537, p = .001, η_p^2 = .383. Pairwise comparisons revealed that plasma glucose concentrations were significantly increased in the inulin and β-glucan combined condition p = .007 compared to the control condition. Plasma glucose concentrations were also significantly increased in the combined fibre condition compared to the inulin fibre in isolation p = .002 and the β-glucan fibre in isolation p = .008.

There was no significant difference in plasma glucose concentrations for the β-glucan condition compared to the control condition or the β-glucan condition compared to the combined β-glucan and inulin condition There was a significant difference in AUC plasma glucose concentrations (p=.044) for the inulin and β-glucan combined condition resulted in a lower AUC compared to the inulin condition. There were a no significant differences in peak blood glucose in any condition compared to the control condition or the combined β-glucan and inulin condition.

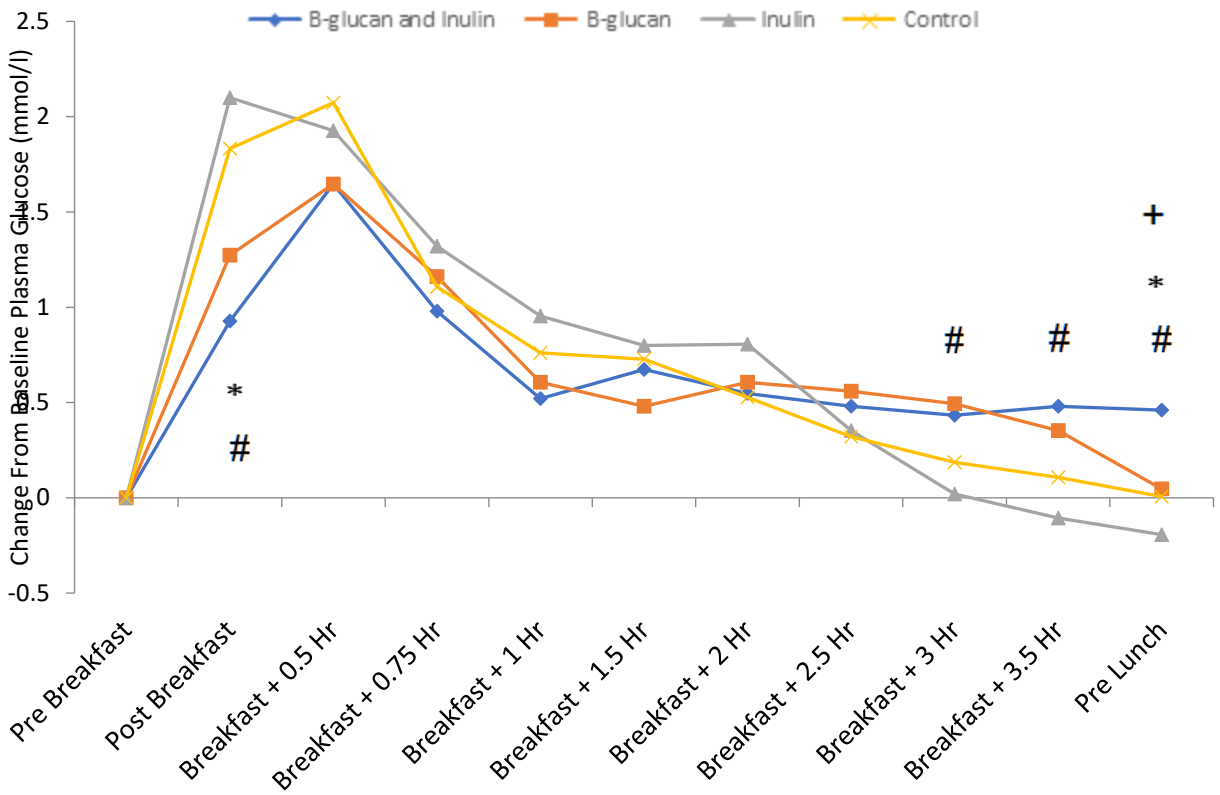

Figure 6 - 6 Plasma Glucose concentration (mmol/L). Values are presented as changes from baseline score and are means for 15 participants. *P < 0.05 Inulin and B-glucan combined vs. control. +P < 0.05 Inulin and B-glucan combined vs B-glucan in isolation. #P < 0.05 Inulin and B-glucan combined vs inulin in isolation.

6.4 Discussion

The current study set out to explore how different fibres in isolation or combination may influence appetite through their different modes of action. We found that when inulin and β-glucan were combined they significantly reduced hunger and appetite. This was consistent with a significant reduction in glycaemic response immediately after breakfast until lunch and a significant increase in H_2, a marker for fermentation immediately before lunch. The fibres in isolation were less effective at reducing appetite and indicated a trend with reductions in appetite 7 and 8 hours after the fibre. Surprisingly β-glucan did not significantly reduce appetite in the hours immediately after the preload, as we may have expected with a viscous fibre present, however an

effect on glycaemic response post breakfast was present, though this was not statistically significant. Both fibres in isolation indicated increased H_2 breath production 4 hours after the preload a marker of colonic fermentation this was consistent with the trend for a reduction in appetite 7 and 8 hours after the preload. H_2 breath production was also significantly higher than both the inulin and β-glucan fibres in isolation 3.5 h after the preload onwards. The fibres in combination significantly reduced appetite 3 and 4 hours after lunch compared to the β-glucan condition and 4 hours after lunch compared to the inulin fibre in isolation, where there was no significant effect on appetite detected for the β-glucan or inulin fibre in isolation compared to the control. Surprisingly glycaemic response was also significantly reduced in the combined fibre condition compared to the control, when no effects for β-glucan in isolation were found. Glycaemic response was also significantly reduced pre-lunch in the combined fibre condition compared to the fibres in isolation. These results suggest there was a additive effect on glycaemic response 4 hours after the preload, h2 breath production 3.5 hours after the preload and appetite 8 hours after the preload.

Only one previous study to date has combined oat β-glucan and inulin Peters et al (2009), contrary to the current study results they found that the addition of β-glucan, FOS, or a combination thereof did not affect appetite ratings or food intake. This study tested the effects of a meal replacement bar at 09:00 and 19:00 containing 0.9g of β-glucan and 8g of FOS. The low dose of β-glucan, preload matrix and timings may have explained the lack of effect. Efficiency could have improved if the content of β-glucan was greater, or if the second test bar was provided earlier in the test session to allow fermentation to take place. The current study provided the preload containing β-glucan and inulin in a smoothie at breakfast. The liquid preload may have been more effective than the solid preloads used in the previous study, (Wanders et al., 2011) found that fibres were more satiating when provided as a liquid compared to

solids. In the present study a larger fibre dose was used, which was provided in a single dose at breakfast, this may explain the differing results. The current study included oat β-glucan as opposed to barley β-glucan, therefore there could have been variation in the physio-chemical properties, fermentation rate or viscosity. Viscosity depends upon the solubility as well as the molecular mass of the fibre and is an important determinant of the physiologic response (Rebello et al., 2016). It is also possible variations in the fibre processing differed to the current study as the fibre processing and characteristics interact with the human GI tract to influence the physiological effects (Poutanen et al., 2017).

β-glucan

There was no effect on appetite in the immediate post-ingestive period following the β-glucan breakfast preload. This was surprising as beverage viscosity has been inversely related to postprandial hunger (Mattes & Rothacker, 2001). The smoothies were matched on dimensions of taste and texture. Previous results for the immediate effect of viscous fibres have suggested the sensory differences between the test preload and control preload may enhance some of the effects found. The 4 test preloads were formulated to have the same 'thick' texture, the oro-sensory exposure to the drinks would not have differed between the preloads. Oro-sensory exposure to food is thought to trigger anticipatory responses (Yeomans et al., 2005), and these associations are likely to influence explicit expectations about the effect a food or drink will have on appetite (McCrickerd & Forde, 2016), including how filling a food is likely to be (expected satiation) and the extent to which it will stave off hunger until the next meal (expected satiety).

Studies indicate that drinks with a thick consistency suppress hunger to a greater extent than equi-caloric flavour matched thin versions (Zijlstra et al., 2009), sensory

characteristics of a beverage interact with its post-ingestive effects to influence satiety. Yeomans and Chambers (2011) reported that when participants consumed a low-energy drink with thick and creamy sensory characteristics participants ate more at the test meal than after the low-energy version without the enhanced sensory context. The sensory characteristics predicted the delivery of nutrients, generating expectations that these drinks would be filling, which acted to enhance the experience of satiety. Therefore, the control drink may have had an effect on appetite sensations, reducing the observed effects in of the fibre condition.

In the current study it was anticipated that the viscous fibre may have mediated postprandial glucose response and delay gastric emptying rates via an ability to form viscous mixtures in the GI tract to induce feelings of fullness and increase satiety (de Graaf & Hulshof, 1996). Although there was a reduction in postprandial glucose response immediately after the preload, this wasn't statistically significant. We didn't observe any significant differences in appetite in the immediate post prandial period up until lunch. The similar preload formulation in the control smoothie potentially interacted with the post-ingestive effects to influence satiety in the control smoothie.

Previous research found that the effects of viscosity play a greater role than fermentation, this did not appear to be the case in the current study. (Pentikainen et al., 2014) found that 4g of β-glucan in a drink significantly reduced appetite over 180 minutes. (Lyly et al., 2009) also found that the effects of soluble fibres on satiety in the form of beverages shows a greater satiety response as viscosity of the fibres increased. However, the satiating effect of a particular food is highly dependent on the early experience of consumption. Early cognitive and sensory appraisal of a food's quality and quantity and the later post-ingestive and post-absorptive

physiological signals are generated by ingestion of nutrients (Van Kleef et al., 2012). These signals are combined and fed back through the peripheral nervous system and a number of brain centres, including areas associated with homeostasis, but also learning, reward and memory (Toepel et al., 2015) which together generate the experience of satiation and satiety.

There was a significant difference in hydrogen breath production for the β-glucan fibre in isolation compared to the control 3 hours after the preload, this was consistent with the findings from (Queenan et al., 2007). Breath hydrogen production remained significantly higher than the control until the end of the 6-hour testing session. Consistent with this there was a significant reduction in desire to eat compared to the control 4 hours post lunch and a trend for a reduction in both hunger and appetite 7 hours and 8 hours after the preload. The effects of β-glucan appeared to be in response to the fermentation rather than the acute effects of the viscosity. Inulin appeared to take longer to ferment compared to the β-glucan, the H_2 had significantly risen 4 hours after the preload. Breath hydrogen production remained significantly higher than the control until the end of the 6-hour testing session in all three fibre conditions. B-glucan produced H_2 at 3 hrs but indicated lower H_2 production, it's unclear whether H_2 production correlates with satiety hormone production (Cani et al., 2009), however in the current study the reduction in appetite measures at 7 and 8 hours would suggest this. Inulin appeared to produce more H_2 than β-glucan, but it took slightly longer for H_2 production to begin. Similarly, Queenan (2007) found that inulin is fermented more rapidly between 0 and 4 hours and reaches a peak at 8 hours fermentation continues over 24 hours, whereas β-glucan shows signs of fermentation at 4 hours but ferments more steadily between 8 and 24 hours (Queenan et al., 2007).

Inulin

Inulin significantly reduced appetite immediately after breakfast, this was consistent with the peak glycaemic response. This was a surprising result given inulin's physio-chemical properties. However, consistent with our findings (Hess et al., 2011) found that 15g of inulin added to a hot chocolate drink significantly reduced appetite shortly after ingestion. Although the inulin smoothie in the current study did not significantly differ on any dimension of taste, there was a difference observed in the sweetness. Evidence suggests that the sweet taste signalling mechanisms identified in the oral cavity operate in the gastrointestinal system and may influence the development of satiety (Low et al., 2014). The cephalic-phase of appetite regulation anticipates the ingestion of food and responses are then generated in many parts of the gastrointestinal tract (Smeets et al., 2010). Such anticipatory signals are mainly determined by sight, smell and taste. This could potentially the significant difference in desire to eat 1-hour post breakfast, 3 hours post breakfast.

The inulin condition did not stabilise the glycaemic response and fell below the control line before lunch time. This is contrary to (Lightowler., 2018) who found that inulin was an effective strategy to reduce postprandial blood glucose response to foods. Although there was no significant difference in hunger or appetite for the inulin fibre in isolation there was a reduction in hunger and appetite 7 hours and 8 hours after the preload. This reduction is likely to be due to fermentation and is consistent with the findings of (Heap et al., 2016) who found no significant differences in AUC hunger but found an effect on appetite 6 hours post inulin dose. The hydrogen breath tests were consistent with (Slavin et al., 2007) they found that inulin begins to be fermented four hours post prandially and peaks within 8 hours.

While breath hydrogen excretion indicated that β-glucan and inulin had started to undergo fermentation, this does not mean that the fibre had undergone complete fermentation at the time of satiety measurements (through 360 min). Rather, fermentation may have continued for several more hours and may have increased satiety after the test period or even the following day. For example, Alles et al. (1996) found that chronic consumption of 15 g/d of a FOS significantly increased 24 h integrated excretion of breath hydrogen and fermentation continued over time. Coupled with our findings, this suggests that if colonic fermentation does impact satiety the effects may be delayed until several hours later.

Our results are corroborated by several studies which found an effect for inulin on appetite with a longer study duration of 6 h or more for example (Lee et al., 2016) measured appetite over 8 hours after an inulin preload, they found that appetite was reduced after 6 hours. The length of the test session may not have been sufficiently long enough to influence appetite acutely. This could be explained due to the chain length of the inulin used. Although we used a short chained inulin increased satiety may have continued beyond the test period as the chain length of FOS impacts its rate of fermentation (Stewart et al., 2008). Short-chain FOS are rapidly fermented, while longer chains forms are more steadily fermented. The inulin's capacity to be fermented may have implications for acute feelings of satiety later in the day.

Synergy Effect

β-glucan and Inulin exerted a similar effect in isolation compared to the fibres in combination although at a lower magnitude and often not statistically significant, suggesting the fibres in isolation produces weaker effects on satiety. Combining inulin and β-glucan enhanced these effects indicating that the combination produced the strongest effect. Often the effects of combinations of ingredients are no greater than

their respective components, and it is entirely possible that the effects of two different fibres could counteract one another to have no effect combined.

Surprisingly the combined fibre condition appeared to reduce hunger earlier in the day compared to the fibres in isolation. When the fibres were combined hunger and appetite was significantly reduced for the combined fibre condition post breakfast, 1 h post breakfast, 3 h post breakfast, post lunch, 1 h post lunch, 2 h post lunch, 3 h post lunch and 4 h post lunch, AUC was also significant. There was also a significant reduction in appetite for the combined condition compared to the β-glucan fibre in isolation 2 h post lunch, 3 h post lunch and 4 h post lunch compared to the inulin fibre in isolation, when there was no significant effect found on appetite for either fibre in isolation relative to the control. This suggests there could be a additive effect on appetite 4 hours post-lunch. It is worth noting the preload in the combined condition was much higher in calories, the initial reduction in appetite in the post-ingestive period could have potentially been due to the increase in mass and calorie content in the smoothie rather than a direct effect of the fibres themselves. This is plausible as calories in beverages are difficult to detect and elicit a weaker satiety response than solids (Campbell, & Mattes, 2007; Tieken et al., 2007).

There was also a significant reduction in glycaemic response for the combined fibre condition post breakfast and pre-lunch, the combined fibre condition compared to inulin in isolation and pre-lunch for the combined fibre condition and the β-glucan in isolation. There was no significant effect for either fibre in isolation compared to the control. This also suggests there was a additive effect compared to the fibres in isolation pre-lunch for glycaemic response. Plasma glucose did not return to the baseline after the 4-hour testing period and remained significantly higher in the combined fibre condition pre-lunch. An increase in H_2 production 3.5 h post breakfast

onwards was also observed compared to both fibres in isolation, with the increase exceeding the combined effects of the fibres in isolation, this indicates an additive effect on fermentation. Hunger ratings were consistent with the reduced glycaemic response before lunch and a large rise in H_2 production pre-lunch, it is unlikely the changes in hunger observed at this timepoint (4 hours post prandial) were related to the differences in the preload calories and mass but rather the synergistic physiological effects of the combined fibres.

The slightly different modes of action may explain the significantly reduced hunger and appetite throughout the test period in the combined fibre condition. The effects of the combined fibres enhanced those effects. Although the reductions in appetite in the immediate post-ingestive period did not appear to be due to the viscosity of β-glucan. In the combined fibre condition the β-glucan reduced glycaemic response which placed blood glucose above the control. Previous research found that the β-glucan may influence appetite by not only increasing viscosity in the gastrointestinal tract but also by improving glucose and insulin control (Beck, 2009), a lower insulin and glucose response may increase feelings of fullness (Vitalione, 2009). β-glucan appears to prolong cholecystokinin (CCK) elevation after a meal, which results in prolonged satiety (Vitalione, 2009). The blunted glucose response after β-glucan was similar to the findings of (Vitaglione et al., 2009), however, our appetite results did not show a decrease in hunger at 120 minutes after the preload as they did. The source of β-glucan in the current study was oats as opposed to barley this could explain our differing results.

Reductions after the test lunch may have been due to the fermentable action of both fibres. Fermentation of fibres are thought to influence satiety (Hervik & Svihus, 2019) through production of SCFAs, products of colonic fermentation, and influence postprandial glucose response by reducing fat competition for glucose disposal

(Brighenti et al., 2006). Fermentation of SCFAs influence gut hormones and gastric motility (Nilsson et al., 2008) they also influence postprandial glucose response by reducing fat competition for glucose disposal (Brighenti et al., 2006). A bigger dose in the isolated fibre condition may have had a larger effect on appetite and biological markers however the dosage we used was carefully considered and it was decided that a higher dose could have significantly increased the likelihood of adverse GI symptoms (Grabitske & Slavin, 2009). In the current study the isolated and combined fibres were well tolerated with no significant negative GI symptoms reported during the 24-hour test window or in the subsequent days after.

Limitations

There were several limitations to the study. Participants were not fasted during the test day but consumed 2 fixed meals. Had we tested participants in a fasted state the potential for the meals to confound the biological markers results would have been removed, however, it was deemed unethical for participants to fast during the entire 8h test session, after the standard 12-hour overnight fast. The increase in breath hydrogen excretion (indicative of fermentation) was interesting; however, repeated measures of breath hydrogen excretion until 8 h in line with the appetite assessment may have provided greater insight into the relationship between fermentation and satiety response.

The test smoothies were carefully formulated, and taste tests were conducted prior to the study. A smoothie was selected as this helped to mask the consistency of the viscous fibre in 2 of the conditions. The preload was formulated to minimise any differences in palatability, increasing palatability has been linked to increasing satiation and/or satiety (Sorensen et al., 2003). Though there were no significant differences on any dimension of palatability the smoothie's containing inulin displayed a slightly sweeter taste. There was also a slight variation in mass (grams) and calorie

content of the smoothies due to the different fibres. The difference in calories and mass may have explained the reduction in hunger for the combined fibre preload in the hours immediately after the preload.

We were unable to characterise the viscosity of the β-glucan fibre. *In vivo* measurements of β-glucan viscosity might have provided a better understanding of the satiating effects of the specific β-glucan fibre under investigation particularly in the post ingestive period before gastric emptying will have taken place. These measurements are however difficult and expensive to obtain.

Our study population was also relatively lean which may have influenced our findings. The effects of fibre on energy regulation do seem to be more apparent in normal weight participants (Howarth et al., 2001). We did not include men in our study, gender differences have been detected in previous studies, in one study FOS reduced food intake in women, but increased food intake in men (Cornier et al., 2010). Our findings and conclusions are therefore limited to women.

Conclusion

This study adds to existing human research for combining fibres with different physio-chemical properties. Although β-glucan did not affect appetite earlier in the day as we anticipated, it demonstrated signs of fermentation before the inulin fibre. The fibres in combination had a additive effect, they reduced appetite significantly more and earlier in the day than each fibre in isolation. Together these results suggest there could be a additive effect for inulin and β-glucan on appetite, glycaemic response and colonic fermentation. The effects of the fibres in combination appear to be greater than the sum of the effects of the fibres in isolation. It remains to be explored if the same effects are observed on food intake. It would therefore be useful to investigate

whether the effect could be observed on energy intake to significantly reduce food intake more than each fibre in isolation.

Chapter Seven

7. Combing oat β-glucan and inulin in an acute study; the Effects on Appetite and Food intake.

7.1 Introduction

In the previous study (chapter 6) it was demonstrated that a combination of fibres had a significant effect on biological markers and significantly reduced appetite. The previous study measured the effects of the fibres on appetite and biological markers with fixed meals provided at set time points. Measuring *ad-libitum* food intake and biological markers within the same study presents a methodological challenge as it is difficult to untangle the effects of the fibre from the effects of the *ad-libitum* foods consumed. A follow up study chapter 7 extends the study presented in chapter 6 using the same preload drinks to test the effects of the fibre combinations on appetite and food intake in an acute study. The test day will be extended to measured appetite and food intake over 8 hours in the laboratory to optimise the methodology to test the effects of the fermentable fibre. In the Systematic review chapter 3, study duration was identified as a key methodological variable, with optimal study duration being 8 hours or more for fermentable fibres. The current will explore if combining a viscous fibre β-glucan with a fermentable fibre inulin can also reduce food intake in an acute study.

The mechanisms underpinning appetite expression are a key target for suppressing hunger and controlling energy intake (Fiszman & Tarrega, 2017). Within-meal satiation and post-meal satiety are generated by the chemical, physical and sensory characteristics of the foods consumed. B-glucan has effects on early post-meal satiety through their high viscosity and bulking effects (Rebello et al., 2016). Whilst inulin increases colonic fermentation and the production of Short Chain Fatty Acids (SCFA), the effects of inulin are usually observed over a longer timeframe (>4

hours) (Salmean, 2017). It is unclear whether these different physico-chemical properties can be exploited and potentially have a synergy effect on food intake. Some previous studies have found a dissociation between satiety and food intake, demonstrating an effect for fibre on appetite but no effect on food intake (Heap et al., 2016; Zaremba et al., 2018), whilst others only find an effect on energy intake (Hess et al., 2011). The complex nature of appetite control, with the interplay between hedonic and homeostatic drivers of food intake, often mean that the relationship between satiety and food intake is not a straightforward one (Saper, Chou, & Elmquist, 2002).

Food products containing dietary fibre with a high viscosity, such as β-glucans have shown satiating effects (Beck et al., 2009). Previous studies have explored the mechanisms underpinning the physiological effects of viscous fibres such as β-glucan and how this can lead to an increase in satiety and food intake. Viscous fibres mediate postprandial glucose response and delay gastric emptying rates via an ability to form viscous mixtures in the GI tract, this leads to a sense of fullness due to greater volume within the stomach (Schroeder et al., 2013). This acts to delay nutrient absorption, slowing the delivery of glucose into the bloodstream and reducing the need for insulin. These fibres' abilities to lower postprandial glycemia is established in numerous studies (AbuMweis et al., 2016; Solah et al., 2016; Steinert, Raederstorff, & Wolever, 2016; Zhang, Luo, & Zhang, 2017) and the previous chapter of this thesis.

Intestinal fermentation of fibres may also influence satiety and food intake (Poutanen et al., 2017); however, these mechanisms are not completely explained. The fermentation of fibre yields hydrogen, methane and SCFA. In the previous study breath hydrogen and methane were measured as a marker of fermentation. SCFA have been hypothesised to promote satiety and reduce food intake. SCFAs influence postprandial glucose response by reducing fat competition for glucose disposal (Furio

Brighenti et al., 2006). They may also stimulate insulin release from the pancreas and alter glycogen breakdown by the liver and therefore protect against insulin resistance. Fermentation of SCFAs may also influence gut hormones and gastric motility (Covasa et al., 2019). This, in turn, could potentially induce feelings of fullness, increase satiety (Burcelin, 2016; Byrne et al., 2015; Frost et al., 2014) and reduce food intake (Chambers et al., 2015). Effects on food intake are typically only reported after repeated exposure with a sufficient dose (Salmean, 2017; Verhoef, Meyer, & Westerterp, 2011) or a duration sufficient for complete fermentation of the fibre to occur (Stewart et al., 2008).

The effects of inulin and β-glucan in isolation on satiety have been explored. Many studies have explored the effect of inulin on appetite with mixed results, some studies have demonstrated Inulin can increase satiety (Cani et al., 2006), However, other studies found no effects on satiety (Karalus et al., 2012). B-glucan has also shown promising results for an effect on appetite (Matia-Merino, & Huffman, 2015). Robello et al. (2016) found that consuming a beverage containing 2.5 g of β-glucans resulted in significantly greater ratings of satiety than a fibre-free beverage. Pentikainen et al. (2014) observed that juice containing 4g β-glucan enhanced satiety. The most evident enhancement of satiety was observed when β-glucan amount was doubled, with a total dose of 8g in juice rather than a biscuit. However, Peters et al. (2009) found no effect on appetite for β-glucan for 0.8g twice a day. Review articles summarising the research (Roberfroid, 2007; Kelly, 2008; Slavin & Green, 2007; Hervik & Svihus, 2019; Wanders et al., 2011) have suggested the variation in the physical properties of the fibre type, dosage and methods could explain the conflicting data.

Equally the results for the effects of inulin on food intake are equivocal. Karalus et al. (2012) reported that 10 g of inulin type fructans did not affect food intake or satiety sensations compared to controls in acute settings. However, Harrold, Hughes, and

O'Shiel (2013) found that when normal to slightly overweight women consumed a 15g inulin-based soluble fermentable fibre 15min before an *ab-libitum* lunch, food intake and energy intake were significantly reduced (31.9 g, 9.1%; 80 kcal, 11.7%) compared with a no fibre control. Salmean (2017) also found that 16 g/d of inulin-type fructans (ITFs) in 330 ml water significantly reduced ratings for hunger, desire to eat, and prospective food consumption and significantly increased ratings for satisfaction and fullness. Subsequently, the fibre group consumed 21% less kcal from food at lunch (453 ± 47 kcal) compared to controls (571 ± 39 kcal). When looking at a higher dose, 21 g/day of ITFs for 12 weeks resulted in reduced self-reported energy intake coupled with a positive impact on plasma satiety hormones (Parnell Reimer 2009). Cani et al. (2006) reported that 16 g/day of ITFs enhanced satiety and reduced energy consumption, in an acute study. Interestingly Hess et al. (2011) found that using a smaller dose of inulin type fructans, 5–8 g/day in a beverage, failed to elicit any impact on satiety sensations, but found a significant reduction in food intake in women. This study highlights those physiological processes underpinning appetite may override the subjective feelings to exert an effect on food intake. In a review, Korczak and Slavin (2018) found that fructooligosaccharides, oligofructose, and inulin, provided in low doses (<10 g/day), generally do not affect measures of human appetite including satiety or food intake and should not be recommended as a fibre with sole satiating power.

The effects of B-glucan on food intake are equally inconsistent. Beck et al. (2009b) found that consumption of 2.2g of β-glucan in overweight and individuals who are obese increased subjective satiety and subsequent meal intake decreased by greater than 95kcal with higher β-glucan dose of 5g. Kim et al., (2006) found 2g β-glucan had only a marginal effect on VAS ratings and no effect on energy intake. Energy intake was measured after only 2 h which may explain the negative findings. Zaremba et al. (2018) found that 4g of β-glucan at breakfast reduced GLP-1, plasma insulin and

blood glucose and increased satiety but did not reduce intake in healthy participants. Similarly, Rebello et al. (2015) compared the effects of 2 oat-based breakfast cereals on appetite, satiety, and food intake. Oat B-glucan reduced hunger and energy intake at lunch by 85kcal compared to the control breakfast. However Hartvigsen et al. (2014) found that a β-glucan bread induced lower initial glycaemic response, increased satiety but did not significantly reduce energy intake 270 minutes later at the test meal compared to a control bread. Meanwhile (Vitaglione et al., 2009) found that a 3% β-glucan-enriched bread significantly reduced hunger and increased fullness and satiety more than a control bread, energy intake at lunch was significantly reduced by 19%. Though the literature is equivocal as to whether β-glucan reduced energy intake where an acute effect has been observed the reduction in calories ranges between 86kcal (Rebello, et al., 2016) and 267kcal (Beck et al., 2009) with a dose of 4g or more. One study found that 3g of β-glucan reduced intake by 670kcal over 24 hours (Lumaga et al., 2012).

Unfortunately, in these studies' food intake is often among secondary objectives. The evidence for inulin and β-glucan reducing food intake, are not conclusive, results vary with the type of dietary fibre and administration protocols. Whilst study data suggests that inulin and β-glucan may be of benefit by reducing caloric intake, compensation for treatment-induced reductions in food intake need to be examined. B-glucan contains 3.2 kcal per g whereas inulin contains 4.3kcal/g kcal, these fibres in isolation or combined will add considerable additional calories. It is not always clear from the study data whether the reduction in calories is considered after any compensatory intake for fibre preload. It is possible any reduction in calories where energy intake has not been measured across the entire day could be due to compensation for the additional calories in the test fibre condition.

Enhanced synergy occurs when the combined effect of the fibre is greater than the sum of those individual effects. In recent years there has been an interest in exploring the potential synergy effects of different fibres. However, only one published study to date has investigated the effects of combining β-glucan and FOS, a type of fructo-polysaccharide with similar properties to inulin, on appetite ratings and food intake (Peters et al., 2009). Participants consumed a meal-replacement bar at 09.00, an ad libitum lunch at 13.00 and a second test bar at 19.00. The control bar contained 0.3 g β-glucan, and the 3 equicaloric test bars contained an additional 0.9 g β-glucan (from barley), 8 g FOS, or 0.9 g β-glucan plus 8 g FOS. Interestingly they found no effect for β-glucan, FOS, or a combination of for appetite ratings or food intake. The efficacy may have improved if the content of β-glucan was greater, or if the second test bar was provided earlier in the test session to allow fermentation to take place.

In the previous study the effects of inulin and β-glucan were assessed in isolation/combination on underlying regulatory mechanisms, glycaemic response, colonic fermentation and satiety. The effects of the fibres were measured over six hours in the laboratory. It was demonstrated that combining fibres with different physio-properties can have an enhanced effect as there was a significant increase in glycaemic response, colonic fermentation and satiety when the fibres were combined compared to when they were offered in isolation.

We propose to assess the effects of inulin and β-glucan in isolation and combination on satiety and food intake in an acute study. The effects of the fibres will be measured over eight hours in the laboratory. Two ad libitum meals will be served during this period. Oatwell® β-glucan and Bioglan® inulin were selected as the study products. This study will follow on from our previous study exploring the effects of combining

fibres with different physio-chemical properties on biological markers. The potential synergistic effects of such fibres on food intake remains to be explored. Combining both sensory and food intake measures will help to explore the consumer benefits of β-glucan and inulin in isolation and combination in terms of appetite control and food intake.

7.1.1 Hypothesis

We hypothesised that i) There will be a decrease in appetite ratings (sense of hunger or appetite) after a preload with inulin or β-glucan in isolation compared to a control, this decrease will be further enhanced after a preload with inulin and β-glucan in combination. ii) There will be a decrease in food intake after a preload with inulin or β-glucan in isolation compared to a control, this decrease will be further enhanced after a preload with inulin and β -glucan in combination.

7.2 Methods

7.2.1 Participants

Eighteen healthy women aged 18–64, with a body mass index (BMI) between 20–30 kg/m^2 completed the study.

7.2.2 Study design

This was a single blind within subject randomised crossover study which tested the effects of β-glucan, inulin or a combination there of on appetite and food intake. Participants consumed BMI scaled preloads of inulin, β-glucan or a combination of β-glucan with inulin and a control. Visual analogue scales (VAS) were measured over 8 hours and food intake was measured during two ad libitum meals served at lunch and dinner time. Participants also completed appetite ratings and a food diary for the remainder of the day after their study visit. Participants received an evening snack

box to take home to allow food intake to be measured for the remainder of the day. Participants were allocated to receive each of the four conditions in a randomised order using a counterbalanced sequence. Each testing period was separated by a week washout period. At the end of four laboratory visits each participant completed each of the conditions. The sample size (18 female participants normal weight to overweight BMI) was calculated on the basis of the previous research and a power calculation conducted using G-Power. Randomisation to the study was conducted by means of Latin squares.

7.2.3 Study procedure/standardised Instructions

On each day preceding the study day participants were asked to keep their food intake and activity levels similar and to record these in a diary from 5 pm until they retired for the night. They were asked not to consume any alcohol and not to eat or drink anything except water from 12 midnight until they attended the study centre the following morning.

On each test day participants attended the Kissileff Laboratory between 08:30 and 9:30 hours for breakfast, an ad libitum lunch meal was served between 12:30 and 13:30 hours and an ad libitum dinner was served between 16:30 and 17:30.

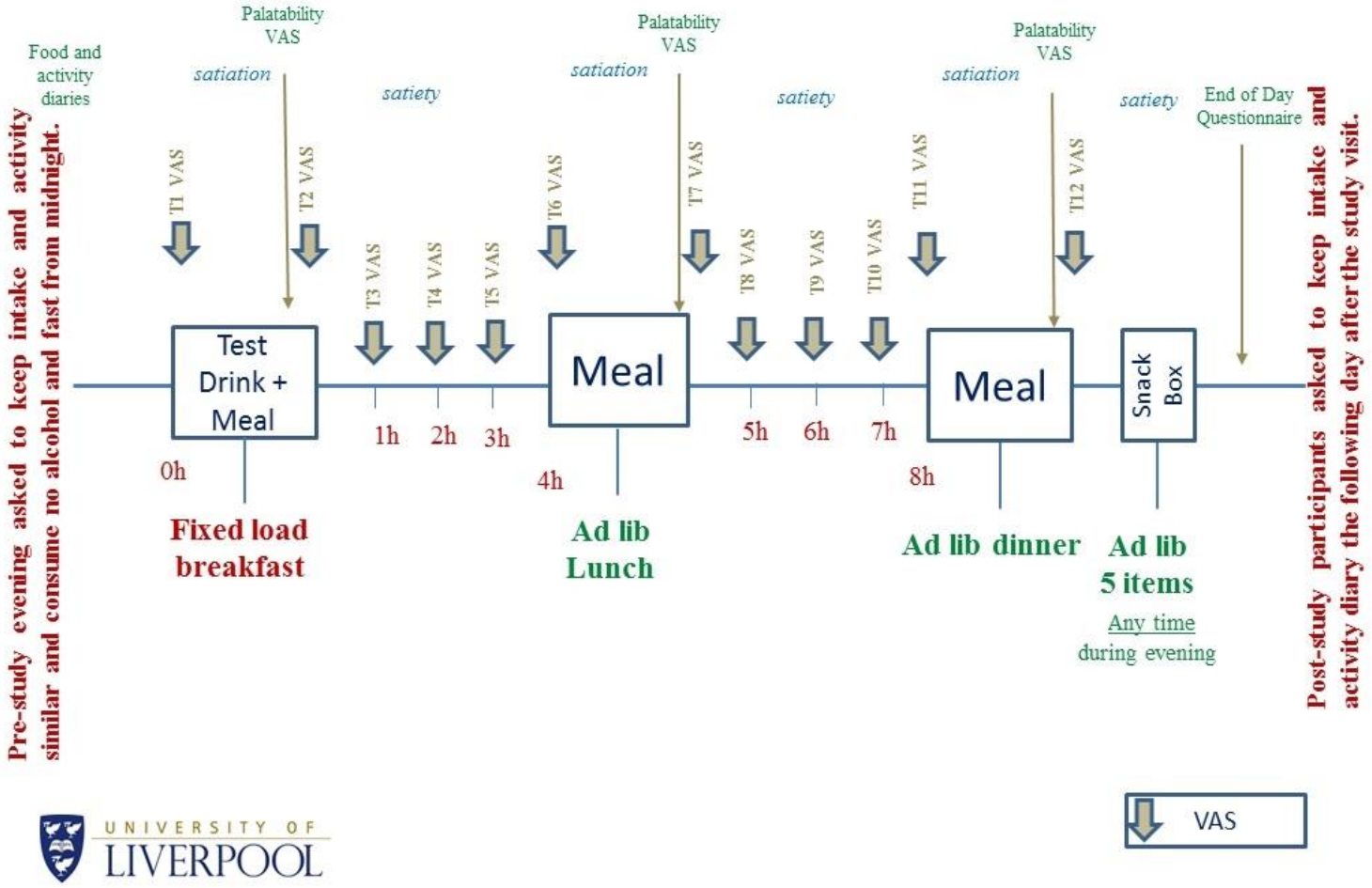

Figure 7 - 1 Protocol diagram outlining the study day.

Test Day Outline

1. At ~8.30 am participants attended the study centre. On arrival participants were seated in an experimental booth (meals were consumed in isolation so that social influence did not affect food selection and intake). Evening food intake and activity diary were collected.
2. Participants completed several VAS to rate their appetite sensations (hunger, fullness, desire to eat and prospective consumption) and thirst.
3. Participants were then asked to consume all of a BMI scaled fixed-load breakfast and a preload smoothie drink containing inulin/β-glucan in isolation/combination or a control smoothie.
4. Upon finishing their meal, participants were again asked to rate their appetite sensations and pleasantness of the food.
5. Participants were then free to leave the study centre once the researcher has provided some further questionnaires to complete hourly. (Participants were instructed to abstain from eating or drinking between breakfast and lunch except water that was provided by the study).
6. Four hours after breakfast participants returned to the study centre to rate their appetite sensations (hunger, fullness, desire to eat, prospective consumption) and thirst. Between meal VAS was collected.
7 A 5-item *ad libitum* lunch was served. All items were served in moderate excess with the intent that participants would not consume the entirety of any single item. Participants were instructed to eat as much as they desired from the choice of foods and water offered, taking as long as they wished (up to half an hour), signalling when they have finished.
9. Upon finishing their meal, participants were asked to rate their appetite sensations and pleasantness of the food. The researcher provided some further questionnaires to complete hourly until the next meal.

10. Participants returned for supper four hours later and were served a hot *ad-libitum* evening meal. Between meal VAS was also collected. Participants were asked to consume as much as they like, taking as long as they wish (up to half an hour), signalling when they had finished.
11. The study day was then complete, and participants were provided with an evening snack box, they were instructed to eat only from the snack box for the remainder of the day. Participants were asked to complete a diary to record food intake for the remainder of the 24h period and an end of day questionnaire to assess overall appetite experiences of the study day. Arrangements were made to return the snack box containing any uneaten snacks/empty wrappers at the next visit.

Participants were free to leave the study centre between each meal but were instructed to consume only the foods provided during the test day. The foods provided were familiar everyday items. *ad-libitum* water was provided throughout the testing session. The same procedure was followed for the four conditions with the B-glucan/inulin/inulin β-glucan/ control in random order with a one-week washout period in-between.

7.2.4 Preload Smoothie

The preload smoothie was formulated to covertly contain either one or a combination of the study fibres. We selected two readily available fibres supplied by a high street health store, Oatwell® β-glucans and Bioglan® inulin. Each fibre was combined in a kitchen blender with frozen blueberries and lactose free milk. Oatwell is natural oat bran and contains high concentrations of valuable β-glucans, soluble fibres, which are found naturally within oats. Oatwell is commercially available and can be purchased as an individual ingredient in the UK. Studies with the specific outcome of appetite

control have shown that bioactive oat β-glucan can promote these positive outcomes with a daily intake of 3-4g. Oatwell β-glucan can also be incorporated into beverages.

Bioglan® is a commercially available fibre, it is consumed as part of the standard European diet. It is also available to purchase as an individual ingredient in a variety of stores in the UK. Bioglan® was selected as it is 100% natural inulin fibre, is flavour-free and can be easily incorporated into beverages.

Taste tests took place in order to check that the 4 test drinks did not differ in taste or on any sensory dimension. To reduce the potential for olfactory differences to impact on the β-glucan drinks, the smoothies were also presented in a cup with a lid and were consumed through a straw. Combining each fibre in a fruit smoothie also helped to conceal any differences in texture. Researchers were satisfied after the taste tests that the drink provided did not differ on any measure and had an acceptable level of taste.

Table 7 - 1 Composition of the BMI scaled Smoothie.

Ingredient	**Amount (g/kg/m2)**
Inulin	0.66
B-glucan	0.22
Blue berries	3.33
Sweetener	0.1
Asda raspberry and blueberry juice	7.6
Lactose Free Milk	4.26

7.2.5 Test Foods

Breakfast

A fixed load breakfast was provided; the precise energy content of which depended on individual participant energy needs (25% energy needs relative to body weight – 10kg increments). The breakfast consisted of toast with margarine and a smoothie.

Table 7 - 2 Foods served at breakfast.

Food Type	Weight of item (g)	Protein (g)	Fat (g)	CHO (g)	Kcal
Cornflakes	30	2.1	0.3	2.5	113
Flora	10	0.5	4.5	0.5	41
Semi-skimmed Milk	125	4.5	2.25	6	62.5
Warbutons Toastie Small loaf (2.5 slices)	72	2.2	0.36	32.5	175.7
Total		9.3	7.41	41.5	392.2

***Ad-libitum* meals**

The *ad-libitum* meals (lunch and supper) were designed to offer a selection of high and low-fat savoury and sweet food items. The ad libitum lunch was tested in chapter 4 and the 5-item meal was identified as the most effective meal for detecting changes in appetite and food intake. Participants were presented with the cold food buffet items on separate plates and instructed to select the items and amount they would like to eat. Each food was presented in excess. Selected foods were weighed, and weighed leftovers were considered in intake calculations. Liquid and semi-solid foods were limited at the buffet: juices or milk were not provided to prevent the participants from consuming amounts similar to those consumed habitually, instead 500ml of chilled water was provided at each meal.

Supper was *ad-libitum* hot pasta meal with a selection of desserts (Table 7-4). Participants were served the pasta meal in a large serving bowl with other food items presented on separate plates. The portion size was decided using previous published research from the Kisseliff Laboratory (Harrold et al., 2012; Harrold et al., 2014), we were satisfied that participants were provided with food in excess to allow participants to reach satiation, rather than consume all foods on offer. Lunchtime was fixed at precisely 4 hours after breakfast and dinner 4 hours after lunch. All meals were served in individual booths in the test study centre.

Table 7 - 3 Nutrient and energy profiles of foods for the 5-item lunch time buffet.

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
High Fat Savoury						
Medium Cheddar Cheese	serving	100	24.9	32.2	2.1	416
Low Fat Savoury						
Hovis rolls	4	247.6	24.4	11.2	98.4	612
High Fat Sweet						
Cadburys Buttons	serving	80	6.6	19.7	49.2	402
Low Fat Sweet						
Jelly Babies	20 sweets	120	6.36	0	96.84	412.8
Salad						
Beef Tomato	serving	80	0	0	0	0
			62.26	63.1	246.54	1842.8

Supper was an *ad-libitum* hot pasta meal with a selection of desserts (Table 7 - 4).

Table 7 - 4 Nutrient and energy profiles of foods for the *ad–libitum* supper

Food	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
Tesco Penn	Serving	250	30	1.2	192.5	816
Tesco Garlic Bread	3 slices	50	3.7	8.1	21.3	175
Tesco chunky veg pasta sauce	1 jar	500	9.2	5.2	26	240
Tescos grated mozzarella cheese	Serving	50	13	10	0.7	145
Peaches in Fruit Juice	Serving	400	2	0	44	200
Magnum Chocolate ice cream	1 bar	100	4	16.4	26.7	280
			62.26	63.1	246.54	1842.8

An evening snack box was provided (table 7-5) for participants to consume in the evening. This was to allow for any compensatory intake to be measured after participants had left the laboratory. Participants were instructed to return the snack box with any empty wrappers/waste/uneaten food on their next study day.

Table 7 - 5 Nutrient and energy profiles of foods provided in the evening snack box.

Food Item	No of Items	Amount (g in serving)	Protein (g in serving)	Fat (g in serving)	CHO (g in serving)	Kcal in serving
Rivita minis	1 packet	22	1.5	1.6	17	89
Tescos marshmallows	1 packet	200	9	0.2	156	670
1 apple	1 piece	80	0.2	Trace	9.8	44
bar of chocolate	1 bar	66	2	11	29.8	228
Mini Cheddars	1 packet	131	2.7	7.5	12.9	131
Total			15.4	20.3	225.5	1210

All foods and drinks offered in the studies are readily and commercially available to the public. They were prepared in accordance with the manufacturer's instructions, the Guidelines for Human Nutrition Research and the individual standard operating procedures prepared for the equipment or specific food items used. The food was

prepared in the Kissileff Laboratory kitchen which is designed and equipped for the preparation and serving of food and drink and served in the separate eating area.

7.2.6 Statistical Analysis

Appetite Ratings

Changes in ratings of appetite such as hunger, fullness, prospective consumption, and desire to eat were assessed. These parameters rated on the VAS were analysed using within-subject ANOVA for repeated measures with condition (β-glucan and inulin combined, β-glucan, inulin and control) and time (pre-breakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch, post-lunch, 2 pm, 3 pm, 4 pm, 5pm) as within-subject factors. If a time-by-condition interaction effect was found significant, paired t-tests were conducted at each rating time between conditions. An appetite score was also calculated using the formula ((hunger + desire to eat + prospective appetite) + (100 - fullness) + (100 - satiety))/5 for each condition in order to reduce variance in the appetite data. The trapezoid rule was used to calculate area under the curve (AUC) in accordance with the recommendations of Blundell, for each VAS variable and differences in AUC. VAS ratings were assessed using repeated measures ANCOVA with baseline values serving as covariant. Analysis was repeated for the daytime split's pre-breakfast, post-breakfast, 10 am, 11 am, 12 pm, pre-lunch T1-T5 and post-lunch, 2 pm, 3 pm, 4 pm, 5pm T6 – T11.

Food Intake

We ran three separate paired t-tests to analyse the amount of food consumed (in grams and kcal) for the inulin and β-glucan combined condition, the inulin condition and B-glucan condition with the control condition. Food intake measures were compared at lunch, dinner, lunch and dinner combined, total ad libitum intake and total intake. Intake including the preload at each meal/combined meal in kcal/grams

was also calculated as well as percentage difference after the compensatory intake after the preload.

7.3 Results

Participants

In total, 31 participants were screened, 21 were recruited and 18 completed the study. One participant was withdrawn due to not adhering to the study protocol. Two participants withdrew due to reasons unrelated to the study. The screening measures, demographic (age) and anthropometric (weight, height, BMI) characteristics of the completing participants are shown in Table 1.

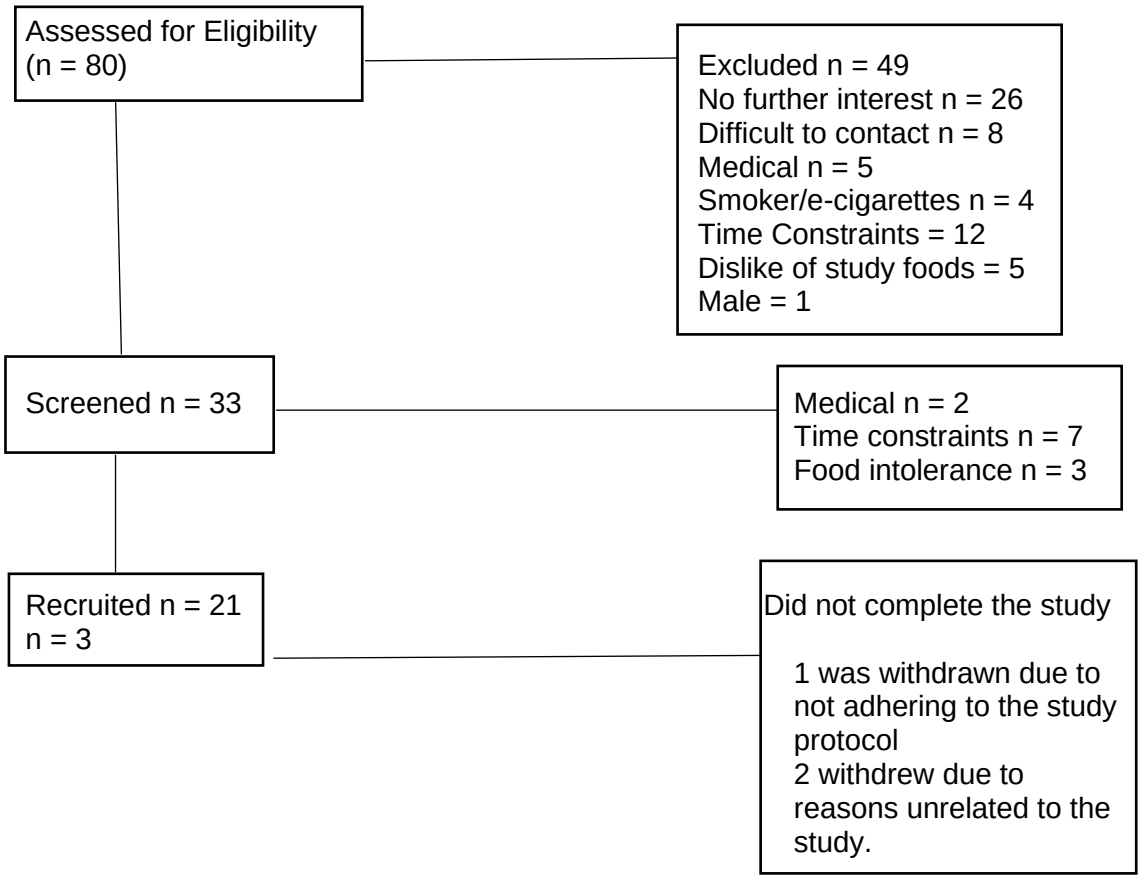

Figure 7 - 2 Total enquiries, participants screened and recruited into the study

Table 7 – 6 Baseline characteristics of participants who completed the study

	(*n* =18*)*		
	Mean	SE *a*	Std *c*
Age (years)	26.41	1.65	6.72
Height (m)	1.67	.23	.79
Weight (kg)	67.2	2.7	9.38
BMI (kg m2)	24.1	.87	3.41
DEBQ *b*) restraint score)	2.19	.21	.63

a Standard error. *b* Dutch eating behaviour questionnaire. *c* Standard deviation

Study Product

The preload drink provided a normal weight or overweight fixed load. The load provided 0.66g/kg/m^2 inulin, 0.786g/kg/m^2 of β-glucan, 3.64 g/kg/m^2 blue berries, 8.01g/kg/m^2 berry juice and 4.26g/kg/m^2 lactose free milk. An average of 22.5kg/m^2 for normal weight participants and 27.5kg/m^2 for overweight participants was taken to calculate the load for normal weight and overweight participants (table 2).

Table 7 - 7 Composition of the BMI scaled drink.

Ingredient	Amount (g/kg/m^2)
Inulin	0.66
B-glucan	0.79
Blue berries	3.64
Berry juice	8.01
Lactose Free Milk	4.26

Table 7 - 8 Composition of the preload drinks for each weight category.

	Normal Weight				Overweight			
	B-glucan and Inulin	B-glucan	Inulin	Control	B-glucan and Inulin	B-glucan	Inulin	Control
Fibre								
Gram Intake (g)	15 (inulin) 17.7 (B-glucan)	17.7	15	0	18.15 (inulin) 21.62 (B-glucan)	21.62	18.15	0
Energy Intake (Kcal)	112.9	48.5	64.7	0	137.44	59.24	78.2	0
Berry Juice								
Gram Intake (g)	180.3	180.3	180.3	180.3	220.28	220.28	220.28	220.28
Energy Intake (Kcal)	45.1	45.1	45.1	45.1	55	55	55	55
Blueberries								
Gram Intake (g)	82	82	82	82	100.1	100.1	100.1	100.1
Energy Intake (Kcal)	39.36	39.36	39.36	39.36	48	48	48	48
Milk								
Gram Intake (g)	95.85	95.85	95.85	95.85	117.15	117.15	117.15	117.15
Energy Intake (Kcal)	38.34	38.34	38.34	38.34	46.86	46.86	46.86	46.86
Total Preload								
Gram Intake (g)	390.85	375.85	373.15	358.15	477.3	459.15	455.68	437.525
Energy Intake (Kcal)	235.7	171.3	187.5	122.8	287.3	209.1	228.06	149.86

Palatability

There were no significant differences in taste f(1.916, 26.825) = 1.026, p = .369, palatability f(3, 45) = .227, p = .877, pleasantness f (3,42) = 1.060, p = .367, saltiness f (1.837, 25.723) = 1.497, p = .242 or sweetness f (3, 45) = .615, p = .609 for the drink in all conditions.

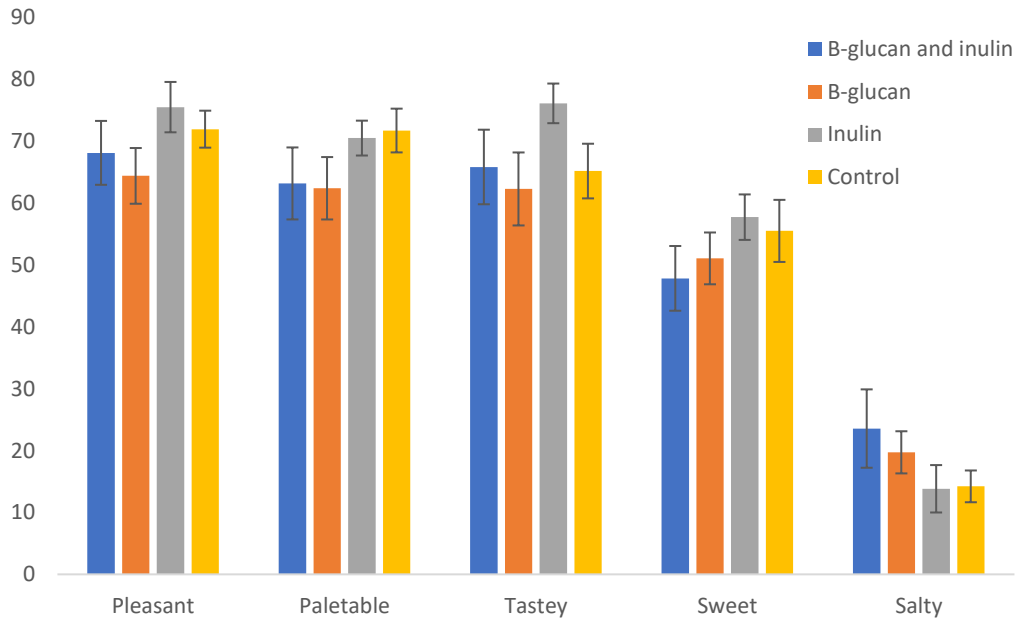

Figure 7 – 3 Effect of condition on the different dimensions of taste for the preload drink. Values are mean for n = 18 participants.

Food intake

Lunch Food Intake (kcal)

A within subject ANOVA revealed that there was a significant effect of fibre on lunch food intake (kcal) F (3, 51) = 4.489, p = .049, η_p^2 = .209. Pairwise comparisons revealed that lunch food intake was reduced for the β-glucan and inulin combined condition compared to the control (p = .032) with a reduction of 67.5 kcal (9.7%), after compensatory intake for the preload participants consumed 45.4kcal, 5.6% more in the combined fibre condition. There were no significant differences in lunch food

intake for the β-glucan in isolation and inulin in isolation conditions compared to the control condition. There was also no significant difference in total food intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Lunch Food Intake (grams)

There was no significant difference in lunch intake (grams) p = .131.

Dinner Intake (kcal)

A within subject ANOVA revealed that there was a significant effect of fibre on dinner food intake (kcal) F (3, 51) = 9.376, p = .001, η_p^2 = .355. Pairwise comparisons revealed that dinner intake was reduced in the the β-glucan and inulin combined condition compared to the the control (p < .000) (182.8kcal, 19.3%, after compensatory intake for the preload food intake was reduced by 69.9kcal, 6.5%) Dinner food intake was also reduced in the β-glucan condition compared to the control (p = .001) (with a reduction of 127.1kcal, 13.4%, after compensatory intake for the preload food intake was reduced by 78.6kcal 97kcal 3.9%). The inulin condition significantly reduced dinner food intake compared to the control (p =.004) (110kcal, 11.6%, after compensation for the preload food intake was reduced by 45.3kcal 4.2%). There was hno significant difference in dinner intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Dinner Intake (g)

A within subject ANOVA revealed that there was a significant effect of fibre on dinner food intake (grams) F (3, 51) = 8.930, p < .001, η_p^2 = .344. Pairwise comparisons revealed that dinner intake was reduced in the β-glucan and inulin combined condition compared to the control (p < .001) (with a reduction of 109.5g 17.7%, after

compensatory intake for the preload intake was reduced by 76.8g 7.9%). Dinner food intake was also reduced in the β-glucan condition compared to the control ($p < .001$) (by 91g, 14.7% After compensatory intake food intake was reduced by 73.3g 7.5%). The inulin condition dinner food intake was significantly reduced compared to the control ($p < .000$) (with a reduction of 52.3g, 8.5%, after compensatory intake food intake was reduced by 37.3g, 3.8%). There was no significant difference in dinner intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Lunch and Dinner (Kcal)

A within subject ANOVA revealed that there was a significant effect of fibre on lunch and dinner food intake (kcal) $F (3, 51) = 7.632$, $p < .001$, $\eta_p^2 = .290$. Pairwise comparisons revealed that dinner intake was reduced in the β-glucan and inulin combined condition compared to the control ($p < .001$) (with a calorie reduction of 250.3 kcal (15.2%) in the combined fibre condition, after compensatory intake food intake was reduced by 137.4kcal (7.8%). There was also a reduction in lunch and dinner food intake in the β-glucan condition compared to the control ($p =.014$) with a calorie reduction of 54.5kcal (9.4%), after compensatory intake for the preload food intake was reduced by 106kcal (6%). Food intake was significantly reduced in the inulin condition compared to the control ($p = .023$) (with a calorie reduction of 121.16 (4%) in the inulin condition compared to the control, after compensatory intake food intake was reduced by 56.5kcal 3.2%). There was no significant difference in lunch and dinner intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Lunch and Dinner (g)

A within subject ANOVA revealed that there was a significant effect of fibre on lunch and dinner food intake (grams) $F (3, 51) = 6.938$, $p < .001$, $\eta_p^2 = .290$. Pairwise

comparisons revealed that lunch and dinner intake (g) was reduced in the β-glucan and inulin combined condition compared to the control p = .001, (with a reduction of, 132.7g 15.2%, after compensatory intake was reduced by 99.5kcal (8.1%) in the combined fibre condition compared to the control. There was also a reduction in lunch and dinner food intake in the β-glucan condition compared to the control (p = .006), with a reduction of 92.1g (10.6%) in the β-glucan condition. After compensatory intake food intake was reduced by 74.4g (6.1%). Food intake was significantly reduced in the inulin condition compared to the control (p = .001) with a reduction of 51.1g (5.9%), after compensatory intake food intake was reduced by 36.11g (2.9%).. There was no significant difference in dinner intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Total *Ad-Libitum* Intake (kcal)

A within subject ANOVA revealed that there was a significant effect of fibre on total *ad-libitum* food intake (kcal) $F (3, 51) = 8.832$, $p < .001$, $\eta_p^2 = .370$. Pairwise comparisons revealed that *ad-libitum* intake was reduced in the β-glucan and inulin combined condition compared to the control p < .001 (with a reduction of 347.7kcal 16.49%, after compensatory intake food intake was reduced by 234.8kcal (10.5%). There was also a reduction in total *ad-libitum* food intake in the β-glucan condition compared to the control p < .001, (with a reduction of 203.9kcal (9.7%), after compensatory intake food intake was reduced by 155.4kcal (7%)). Food intake was significantly reduced in the inulin condition compared to the control p = .003 with a reduction of 188.1kcal (8.9%) in the inulin condition, after compensatory intake food intake was reduced by 123.4kcal (5.5%). There was no significant difference in dinner intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Total *ad-libitum* Intake (g)

A within subject ANOVA revealed that there was a significant effect of fibre on total *ad-libitum* food intake (g) $F (3, 51) = 7.642$, $p < .001$, $\eta_p^2 = .280$. Pairwise comparisons revealed that *ad-libitum* intake was significantly reduced in the β-glucan and inulin combined condition compared to the control p = < .001 with a reduction of 195.5g (18.9%), after compensatory intake food intake was reduced by 162.8g (11.7%). There was also a reduction in total *ad-libitum* food intake (g) in the β-glucan condition compared to the control p = .003 with a reduction of 132.5g (12.8%) compared to the control, after compensatory intake food intake was reduced by 114.8g (8.2%). Food intake was significantly reduced in the inulin condition compared to the control p = .003 with a reduction of 93g (9%), after compensatory intake food intake was reduced by 78g (5.6%).). There was no significant difference in total ad libitum intake (g) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Total Food Intake kcal

A within subject ANOVA revealed that there was a significant effect of fibre on total food intake (kcal) $F (3, 51) = 5.072$, $P = .001$, $\eta_p^2 = .38$. Pairwise comparisons revealed that total food intake was reduced for the β-glucan and inulin combined condition and the control ($p < .000$) with a reduction of 197.2kcal (7.8%), total food intake was also significantly reduced in the β-glucan condition compared to the control ($p = .02$) with a reduction of 97kcal (3.9%) and in the inulin condition compared to the control ($p = .04$) with a reduction of 111.93kcal (4.4%). There was however no significant difference in total food intake (kcal) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Total intake (g)

A within subject ANOVA revealed that there was a significant effect of fibre on total food intake (grams) $F (3, 51) = 6.074$, $P = .001$, $\eta_p^2 = .41$. Pairwise comparisons revealed that total food intake was reduced for the β-glucan and inulin combined condition compared to the control ($p < .000$) with a reduction of 165.9g (13.6%), total food intake (g) was also reduced in the β-glucan condition compared to the control ($p = .02$) with a reduction of 165.9g (13.6%) in the β-glucan condition. Food intake was reduced in the inulin condition compared to the control ($p = .04$) with a reduction of 65.5g (5.4%). There was no significant difference in total food intake (grams) for inulin in isolation and β-glucan in isolation compared to the β-glucan inulin combined condition.

Table 7 - 9 Summary of food intake findings for n=18 participants

	Inulin and B-glucan			B-glucan			Inulin			Control		
		SE	Std		SE	Std		SE	Std		SE	Std
Lunch												
Gram Intake (g)	230.59	17.6	74.66	252.64	11.32	48.01	254.97	15.26	64.75	253.74	14.75	62.59
Energy Intake (Kcal)	628.78*	46.17	195.88	668.85	43.24	183.46	685.06	55.69	236.27	696.25	46.33	196.55
Dinner												
Gram Intake (g)	508.37*	30.52	129.48	526.86*	40.14	170.31	565.53*	40.07	170.02	617.87	32.2	136.6
Energy Intake (Kcal)	766.11*	54.54	231.41	821.81*	35.82	151.97	838.92*	51	216.37	948.89	35.45	150.42
Lunch and Dinner												
Gram Intake (g)	738.9*6	38.74	164.35	779.5*	44.77	189.94	820.5*	47.22	200.33	871.61	39.28	166.66
Energy Intake (Kcal)	1394.89*	78	330.93	1490.65*	54.08	229.43	1523.98*	85.91	364.5	1645.14	66.12	280.54
Total Ad Libitum												
Gram Intake (g)	839.61*	48.26	204.75	902.71*	47.26	200.49	942.14*	67.22	285.19	1035.16	53.34	226.29
Energy Intake (Kcal)	1760.93*	122.96	521.67	1904.79*	122.35	519.09	1920.56*	150.39	638.05	2108.65	127.26	539.93
Total Day												
Gram Intake (g)	1050.29*	47.06	199.67	1113.66*	47.18	200.18	1150.71*	65.6	278.33	1216.23	52.74	223.77
Energy Intake (Kcal)	2330.69*	119.14	505.48	2430.3*	121.63	516.04	2415.96*	148.45	629.84	2527.9	130.56	553.9

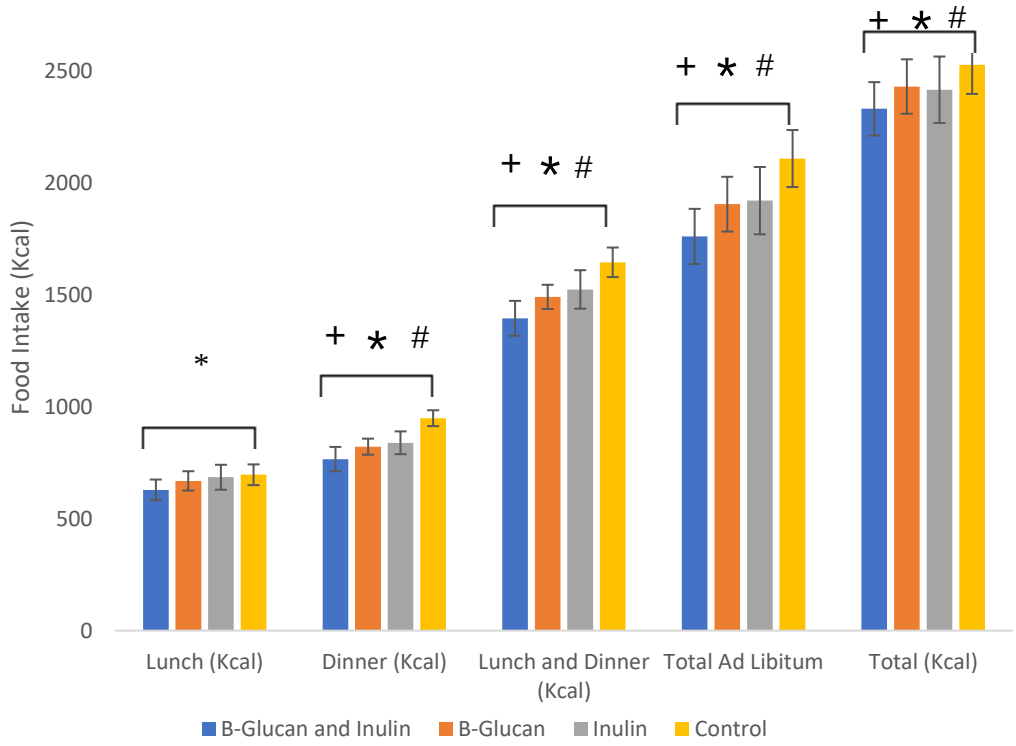

Figure 7 - 4 Effect of treatment on food intake (kcal). Values are means for 18 participants. +P <.05 β-glucan and inulin *P <.05 β-glucan. # P <.05 inulin.

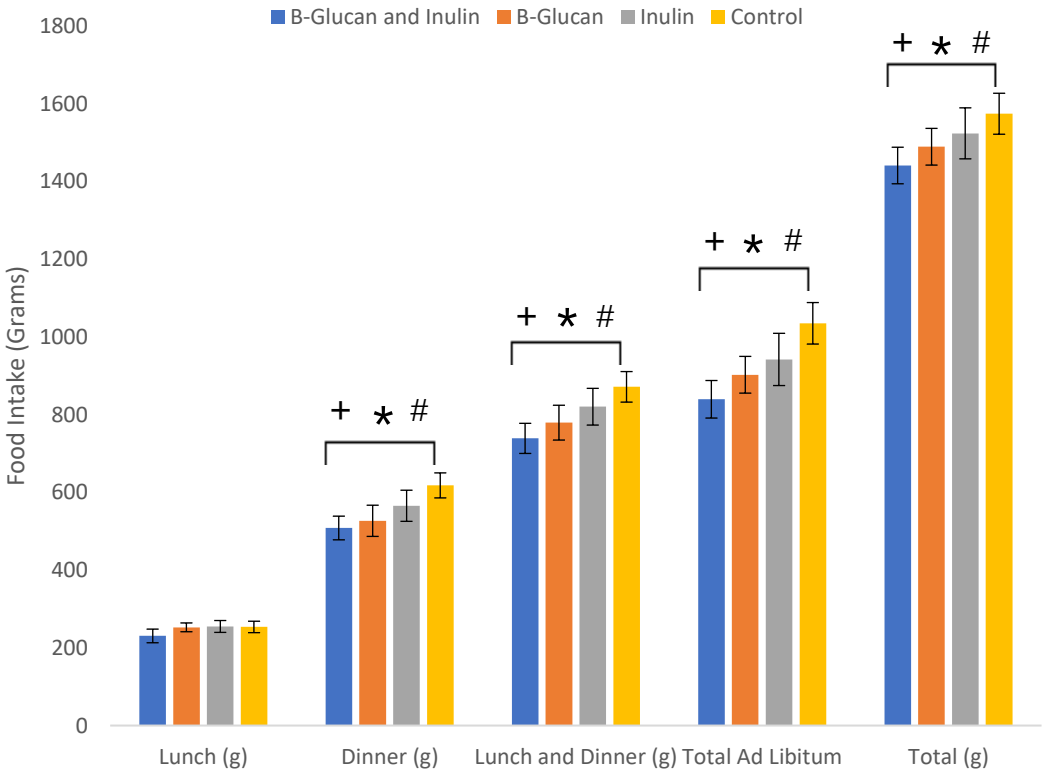

Figure 7 - 5 Effect of treatment on food intake (grams). Values are means for 18 participants. +P <.05 β-glucan and inulin *P <.05 β-glucan. # P <.05 inulin.

Total *Ad Libitum* Intake Kcal/Grams

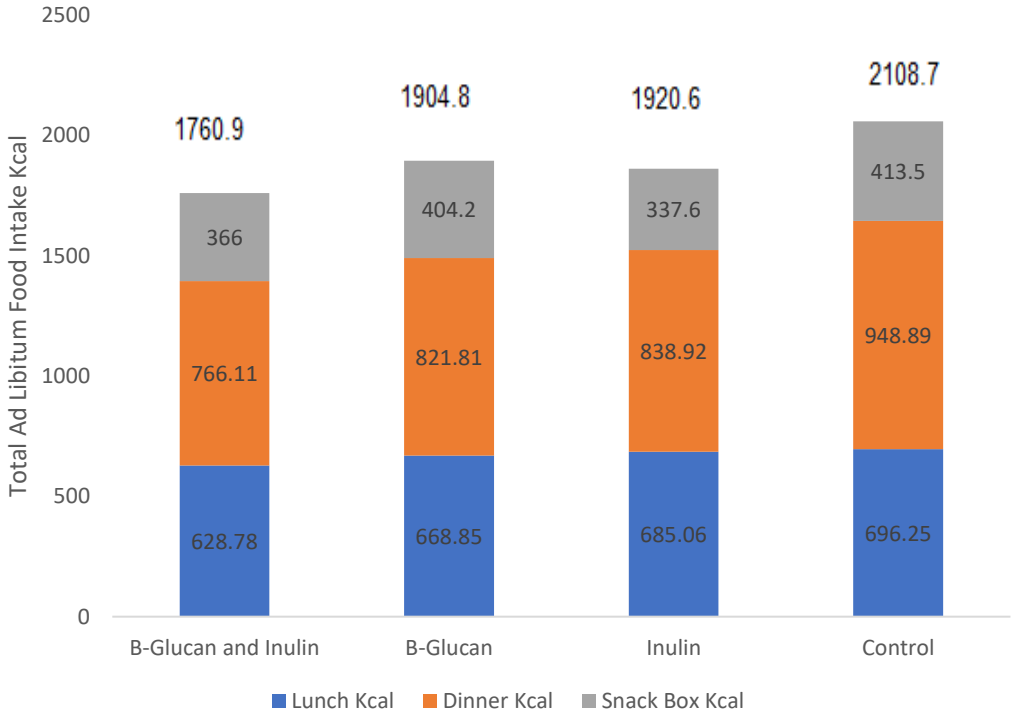

Figure 7 – 6 Effect of treatment on ad libitum intake for each meal (kcal). Values are means for 18 participants. +P <.05 β-glucan and inulin *P <.05 β-glucan. # P <.05 inulin.

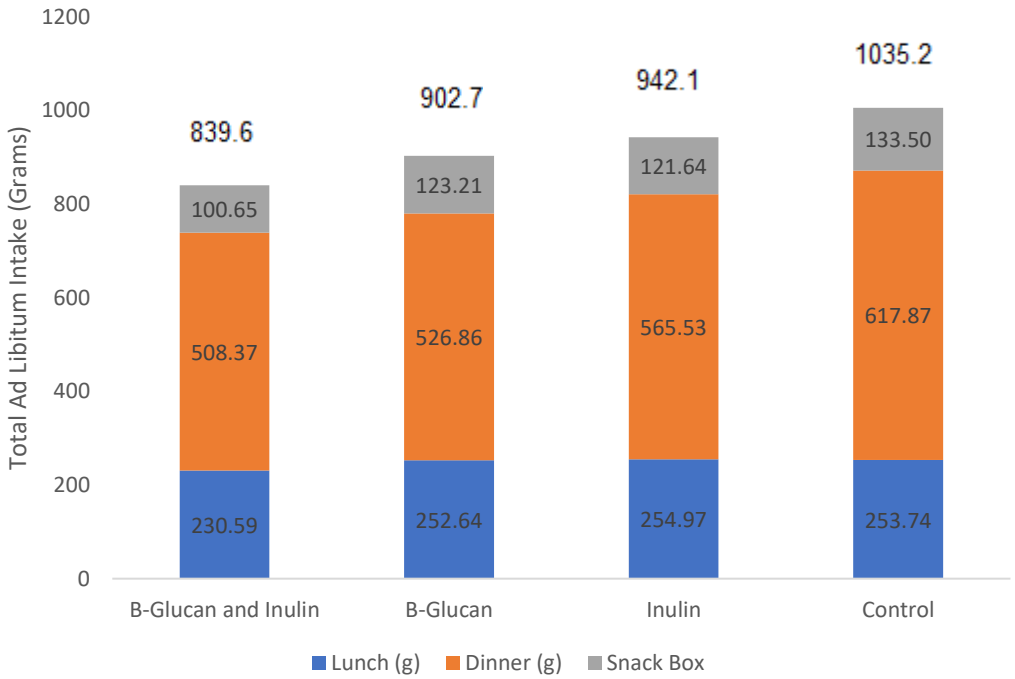

Figure 7 - 7 Effect of treatment on ad libitum intake for each meal (grams). Values are means for 18 participants. +P <.05 β-glucan and inulin *P <.05 β-glucan. # P <.05 inulin.

Compensatory Intake

Compensatory intake was calculated for participants (grams and Kcal) for the preload at lunch, dinner, and total ad libitum. Participants did not compensate for the preload at lunch time and consumed more kcal and grams in the combined condition, β-glucan condition and inulin condition. Participants compensated for the calories in the preload at dinner, they consumed fewer calories in the combined condition, β-glucan condition and inulin condition compared to the preload after compensating for the preload. Similarly, the preload was fully compensated for in the total ad libitum intake and total intake for the combined condition, β-glucan and inulin conditions.

Table 7 - 10 Difference in food intake for each condition compared to the control condition.

	Inulin and β-glucan	β-glucan	Inulin	Control	Inulin and β-glucan	β-glucan	Inulin	Inulin and β-glucan	β-glucan	Inulin
	Food Intake				Difference in Food intake			% Difference		
Lunch										
Gram Intake (g)	230.59	252.64	254.97	253.74	23.15	1.10	-1.23	9.12	0.43	-0.48
Energy Intake (Kcal)	628.78*	668.85	685.06	696.25	67.47	27.41	11.19	9.69	3.94	1.61
Dinner										
Gram Intake (g)	508.37*	526.86*	565.53*	617.87	109.50	91.01	52.34	17.72	14.73	8.47
Energy Intake (Kcal)	766.11*	821.81*	838.92*	948.89	182.78	127.08	109.96	19.26	13.39	11.59
Lunch and Dinner										
Gram Intake (g)	738.96*	779.50*	820.50*	871.61	132.65	92.11	51.11	15.22	10.57	5.86
Energy Intake (Kcal)	1394.89*	1490.65*	1523.98*	1645.14	250.25	154.49	121.16	15.21	9.39	7.36
Total Ad Libitum										
Gram Intake (g)	839.61*	902.71*	942.14*	1035.16	195.54	132.45	93.02	18.89	12.79	8.99
Energy Intake (Kcal)	1760.93*	1904.79*	1920.56*	2108.65	347.73	203.87	188.09	16.49	9.67	8.92
Total Day										
Gram Intake (g)	1441.14*	1489.51*	1523.861*	1574.38	133.2	84.9	50.51	8.46	5.39	3.21
Energy Intake (Kcal)	2330.69*	2430.30*	2415.96*	2527.90	197.21	97.60	111.93	7.80	3.86	4.43

Table 7 - 11 Difference in food intake including the preload

	Inulin and β-glucan	β-glucan	Inulin	Control	Inulin and β-glucan	β-glucan	Inulin	Inulin and β-glucan	β-glucan	Inulin
					Difference in Intake including Preload			Difference in Intake including preload %		
Lunch + Preload										
Gram Intake (g)	621.44	628.49	628.12	611.89	-9.55	-16.60	-16.23	-1.56	-2.71	-2.65
Energy Intake (Kcal)	864.48	840.15	872.56	819.05	-45.43	-21.09	-53.51	-5.55	-2.58	-6.53
Dinner + Preload										
Gram Intake (g)	899.22	902.71	938.68	976.02	-76.80	-73.31	-37.34	-7.87	-7.51	-3.83
Energy Intake (Kcal)	1001.81	993.11	1026.42	1071.69	-69.88	-78.58	-45.26	-6.52	-7.33	-4.22
Lunch and Dinner + Preload										
Gram Intake (g)	1129.81	1155.35	1193.65	1229.76	-99.95	-74.41	-36.11	-8.13	-6.05	-2.94
Energy Intake (Kcal)	1630.59	1661.95	1711.48	1767.94	-137.35	-105.99	-56.46	-7.77	-5.99	-3.19
Total Ad Libitum + Preload										
Gram Intake (g)	1230.46	1278.56	1315.29	1393.31	-162.84	-114.8	-78.02	-11.69	-8.24	-5.60
Energy Intake (Kcal)	1996.63	2076.09	2108.06	2231.45	234.83	-155.4	-123.39	-10.52	-6.96	-5.53
Total Day										
Gram Intake (g)	1441.14	1489.51	1523.861	1574.38	-133.2	-84.9	-50.51	-8.46	-5.39	-3.21
Energy Intake (Kcal)	2330.69	2430.30	2415.96	2527.90	-197.21	-97.60	-111.93	-7.80	-3.86	-4.43

Total Food intake kcal/grams

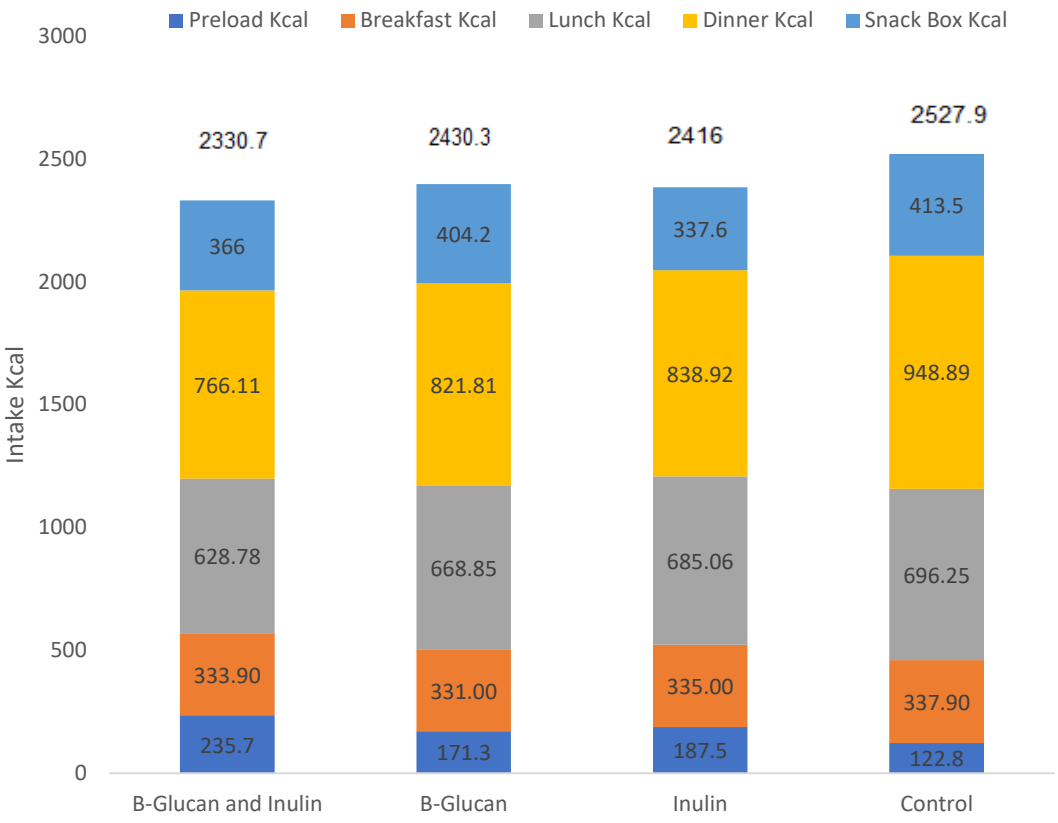

Figure 7 - 8 Effect of treatment on total food intake (kcal). Values are means for 18 participants.

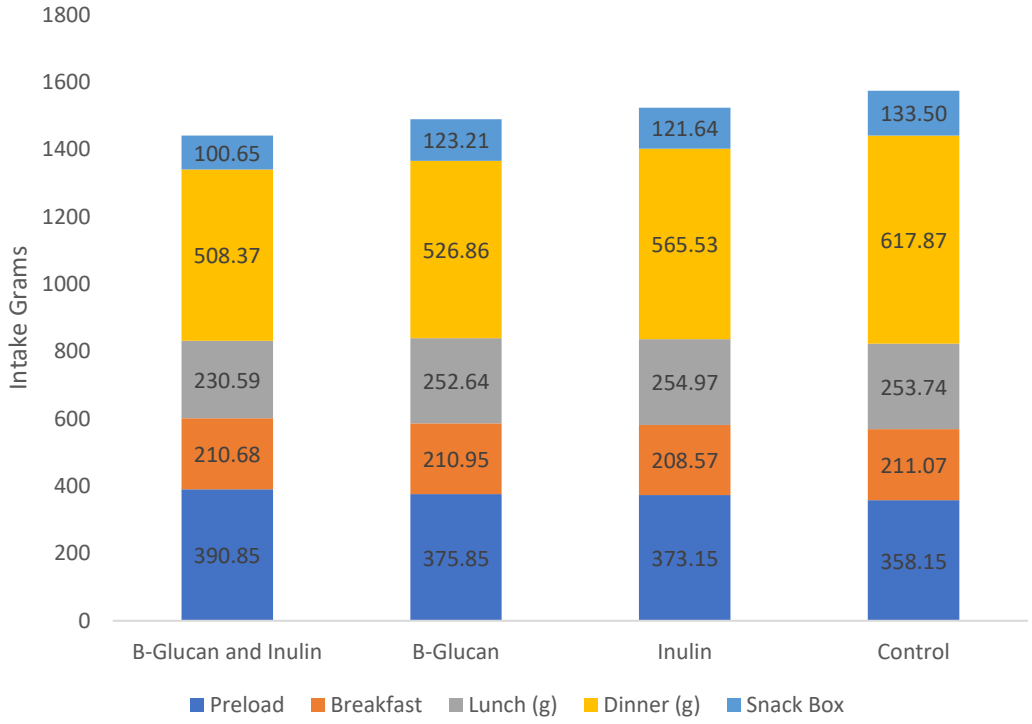

Figure 7 - 9 Effect of treatment on total food intake (grams). Values are means for 18 participants.

Total food and drink intake (grams)

A within subject ANOVA revealed that there was a significant effect of fibre on total food and drink intake (grams) F (3, 51) = 19.093, p < .001, η_p^2 = .529. Pairwise comparisons revealed that total food and drink intake was reduced in the β-glucan and inulin combined condition compared to the control (p < .001, with a reduction of 158.2g (5.9%) in the combined fibre condition. Total food and drink intake was also reduced in the β-glucan condition compared to the control p = .022, with a reduction of 110.8g (4.2%) in the β-glucan condition. The inulin condition total food and drink intake was significantly reduced compared to the control p = .037 with a reduction of 61.5g (2.3%) in the inulin condition. The combined fibre condition also significantly reduced total food and drink intake compared to the inulin in isolation condition p< .001 with a reduction of 96.8g 3.7%.

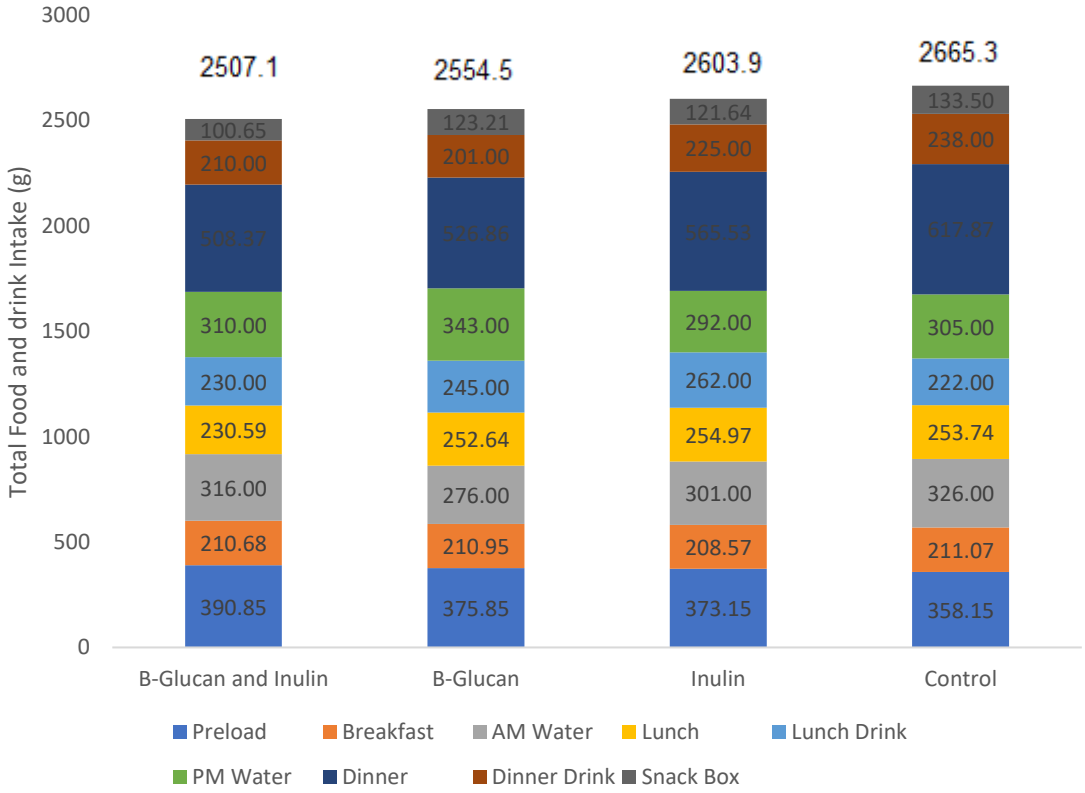

Figure 7 - 10 Effect of treatment on total food and drink intake (grams). Values are means for 18 participants.

Total Ad-Libitum food and drink Intake (Grams)

A within subject ANOVA revealed that there was a significant effect of fibre on total *ad-libitum* food and drink intake (grams) $F (3, 51) = 21.250$, $p < .001$, $\eta_p^2 = .556$. Pairwise comparisons revealed that total *ad-libitum* food and drink intake was reduced in the β-glucan and inulin combined condition compared to the control $p < .001$ with a reduction of 190.5g (9.1%) in the combined fibre condition. Total food and drink intake was also reduced in the β-glucan condition compared to the control $p = .003$ with a reduction of 128.4g (6.1%) in the β-glucan condition. The inulin condition total food and drink intake was significantly reduced compared to the control $p = .007$ with a reduction of 73g (3.5%). in the inulin condition. The combined fibre condition also significantly reduced total food and drink intake compared to the inulin in isolation condition $p< .001$ with a reduction of 116.5g 5.8%.

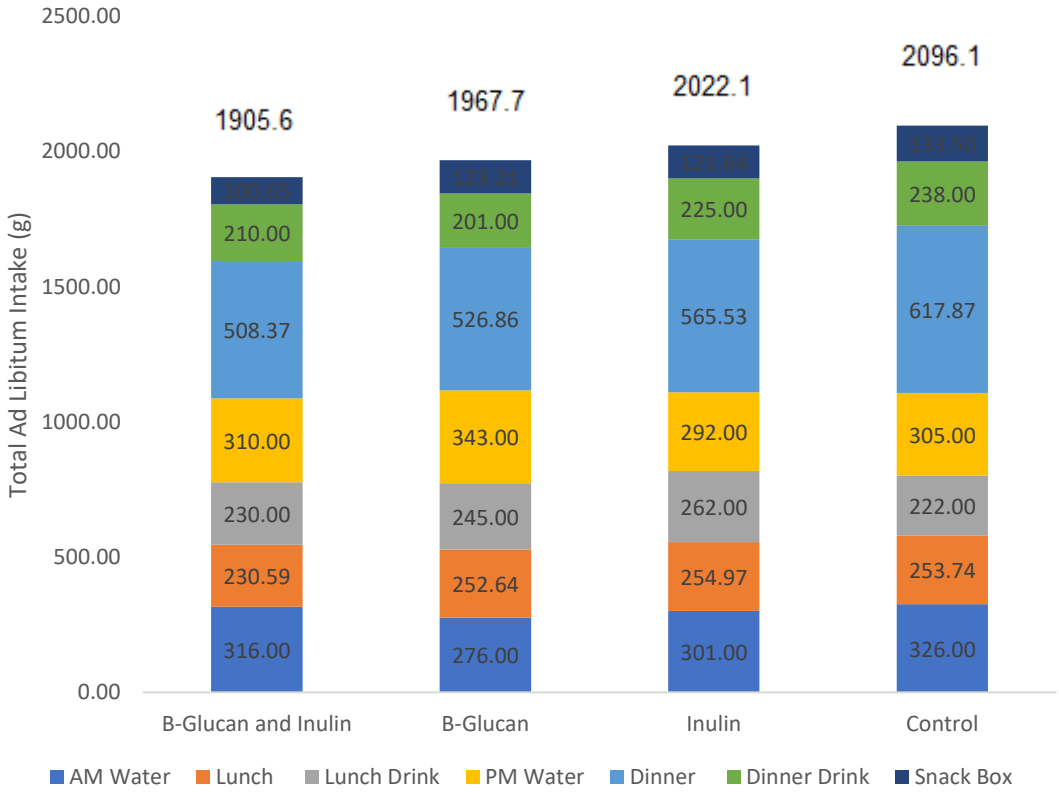

Figure 7 - 11 Effect of treatment on total ad libitum food and drink intake (grams). Values are means for 18 participants.

Table 7 – 12 Energy Density for each Condition

	Inulin and β-glucan	β-glucan	Inulin	Control
Lunch	2.73	2.65	2.69	2.74
Dinner	1.51	1.56	1.48	1.54
Lunch and Dinner	1.89	1.91	1.86	1.89
Total Ad Libitum	2.10	2.11	2.04	2.04
Total Day	2.22	2.18	2.10	2.08

Participants consumed a similar energy for each condition.

Appetite Score

Pre-breakfast-Post dinner (t1-t11) All Fibres

Using a standard time by condition ANOVA analysis of absolute VAS ratings there was a significant interaction for appetite score and time for the four fibre conditions f (30, 480) = 4.124, p < .001.

Post Breakfast

A within subject ANOVA revealed that there was a significant effect of fibre on appetite post breakfast f (3, 51) = 4.339, p = .009, η_p^2 .213. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .003 compared to the control condition. Appetite was also significantly reduced p = .011 in the combined fibre condition compared to the β-glucan fibre in isolation.

Breakfast + 1 h

A within subject ANOVA revealed that there was a significant effect of fibre on appetite breakfast + 1 h f (3, 51) = 6.509, p = .001, η_p^2 .289. Pairwise comparisons revealed that appetite was significantly reduced in the combined inulin and β-glucan condition p = .002 compared to the control condition.

Breakfast + 2 h

A within subject ANOVA revealed that there was a significant effect of fibre on appetite breakfast + 2 h f (3, 51) = 5.301, p = .004, η_p^2 .271. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan combined condition p =.014 compared to the control condition.

Pre-Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on appetite pre-lunch f (3, 51) = 7.421, p = .003, η_p^2 .341. Pairwise comparisons revealed that appetite was significantly reduced p = .001 in the combined fibre condition compared to the β-glucan fibre in isolation.

Post Lunch

A within subject ANOVA revealed that there was a significant effect of fibre on appetite post lunch f (3, 51) = 7.938, p = .003, η_p^2 .275. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .002 compared to the control condition. Appetite was also significantly reduced p < .001 in the combined fibre condition compared to the β-glucan fibre in isolation.

Lunch + 1

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 1 h f (3, 51) = 8.453, p = .001, η_p^2 .342. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p < .001compared to the control condition. Appetite was also significantly reduced p < .001 in the combined fibre condition compared to the β-glucan fibre in isolation.

Lunch + 2

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 2 h f (3, 51) = 10.509, p = .001, η_p^2 .310. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p < .001 compared to the control condition. Appetite was also significantly reduced p < .001 in the combined fibre condition compared to the β-glucan fibre in isolation.

Pre-Dinner

A within subject ANOVA revealed that there was a significant effect of fibre on appetite lunch + 3 h f (3, 51) = 14.104, p < .001, η_p^2 .368. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p < .001 compared to the control condition. Appetite was also significantly reduced p < .001 in the combined fibre condition compared to the β-glucan fibre in isolation.

Post-Dinner

A within subject ANOVA revealed that there was a significant effect of fibre on appetite post dinner f (3, 51) = 16.129, p = .001, η_p^2 .289. Pairwise comparisons revealed that appetite was significantly reduced in the inulin and β-glucan condition p = .003 compared to the control condition. Appetite was also significantly reduced p < .001 in the combined fibre condition compared to the β-glucan fibre in isolation. There was non-significant a trend for a reduction in appetite in the combined fibre condition compared to the inulin in isolation p = .068.

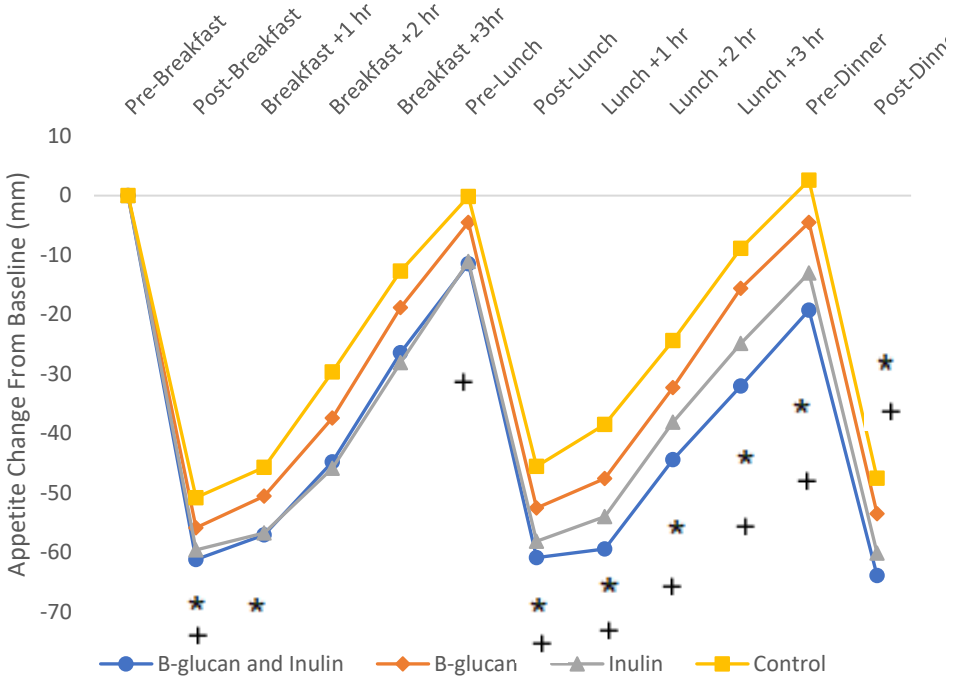

Figure 7 – 12 Visual analogue scale (VAS) ratings for appetite. Values are presented as changes from baseline score and are means for 18 participants. +P < .05 B-glucan vs. control. #p < .05 inulin vs control, *p < .05 inulin and β-glucan combined vs control. +P < 0.05 Inulin and B-glucan combined vs B-glucan in isolation. #P < 0.05 Inulin and B-glucan combined vs inulin in isolation.

7.4 Discussion

In the previous study we assessed the effects of inulin and β-glucan in isolation/combination on underlying regulatory mechanisms; glycaemic response, colonic fermentation and satiety. The effects of the fibres were measured over six hours in the laboratory. We demonstrated combining fibres with different physio-properties had a additive effect on biological markers and appetite as there was a significant reduction in glycaemic response 4 h post preload relative to the fibres in isolation, when there was no effect found for isolated fibres. There was also a significant increase in h2 breath production 3.5 h post preload indicating colonic fermentation in the combined fibre condition relative to the fibres in isolation with the increase exceeding the combined effects of the fibres in isolation. Appetite measures

were also significantly reduced 8 h post preload relative to the fibres in isolation when there was no effect found for either fibre in isolation relative to the control.

The current study aimed to examine the effect of inulin and β-glucan in isolation/combination, administered prior to a fixed load breakfast on appetite and energy intake with an *ad-libitum* lunch and dinner test meal. The inulin and β-glucan in isolation produced clear effects on total food intake compared to the control condition with a reduction in food intake (β-glucan 97kcal, 3.9%; inulin 111.93kcal 4.4%), suggesting they strengthen within meal satiation. However, in combination caloric intake was not significantly further suppressed beyond the combined reduction of each fibre in isolation across the study day (combined fibre condition 197.2kcal 7.8%). A significant difference in total food and drink intake (96.8g 3.7%) and total *ad-libitum* food and drink intake (116.5g 5.8%) for the combined fibre condition compared to the fibres in isolation was observed, though this reduction was not greater than the combined reduction of the fibres in isolation. Changes in subjective appetite ratings did become distinct in the combined fibre condition and significantly supressed appetite compared to both the control condition and β-glucan fibre conditions (post lunch-post dinner), however only a trend was observed for combined fibre compared to the inulin fibre in isolation four hours post breakfast. There was therefore no additive or synergy effect on appetite or food intake.

The combined fibre condition induced a decrease in energy intake compared to the control condition demonstrating clear effects on within-meal satiation during the dinner test meal. B-glucan and inulin exerted a similar effect although at a lower magnitude, suggesting the fibres in isolation produce weaker effects on satiation. Combining inulin and β-glucan although enhanced the effects relative to the fibres in isolation, but did not exceed the combined isolated effects. Often the effects of combinations of ingredients are no greater than their effects in isolation, and it is

entirely possible that a additive effect on appetite and biological markers as was found in chapter 6 does not equate to a similar effect on food intake (Salleh et al., 2019).

There was a significant difference in calorie intake for the lunch time *ad-libitum* meal, after compensatory intake had been taken into consideration calories at lunch time increased. The treatment conditions were not standardised in terms of their energy content, specifically the energy content of combined fibre condition differed by 112.9 calories and was not matched with the control. The compensatory energy intake required for overcompensation was considerable, over the 240 minutes. Almiron-Roig et al., 2013 found that the intrameal interval may be critical in determining the extent of energy compensation, this interval is dependent on preload attributes such as physical form and energy density.

Appetite measures were not consistent with the reduction in food intake (kcal) before compensation at lunch as there was no significant differences in appetite measures immediately before lunch for the fibre conditions in isolation or combined. There were, however, significant differences in appetite immediately after the preload. This could potentially be due to the difference in calorie content. Though some studies suggest calories are difficult to detect in a liquid, others suggest they can still impact on appetite. Almiron-Roig & Drewnowski, (2003) compared the effects of 4 equal volumes of orange, milk, and water, they found that the drinks containing calories were associated with higher fullness and reduced hunger rating and desire to eat compared to the water as soon as 20 minutes after consumption, but had no effect on food intake 2 hours later. These results are consistent with the current study as there was an immediate effect in the post-ingestive period (20 minute and 1 hour) on appetite but no effect on food intake 4 hours later.

The two fibres in isolation reduced total food intake by similar amounts. β-glucan reduced intake by 97.6kcal (3.9%) while inulin reduced intake by 111.9kcal (4.4%). The findings for β-glucan were consistent with (Beck et al., 2009b) they tested seven male and seven female (BMI 25-36 kg/m^2) and found β-glucan increased subjective satiety at a dose of 2.2g. Subsequent meal intake decreased by 95kcal with higher β-glucan dose of 5g. Our results for food intake for the inulin fibre in isolation meanwhile were consistent with the results of (Cani et al., 2006) they reported that 16g a day of oligofructose an inulin type fructan led to enhanced satiety and reduced energy consumption of 120kcal 5.3%, in an acute study. The reduction in intake was consistent with a reduction in hunger for the inulin fibre and combined fibre condition after lunch through to pre-dinner, however β-glucan in isolation did not reach statistical significance for appetite measures. These findings are also consistent with the large increase H_2 breath production in the previous study for the combined fibre condition. This suggests the fermentation may have increased satiety hormones after lunch at 240 minutes onwards.

An additive effect for appetite and biomarkers was found in the previous study; however, this was not reflected in the current study. Although a synergistic effect was not found in terms of enhancing the effectiveness of the fibres in combination above the sum of their ability to reduce food intake individually, there is a benefit of combining different fibres. An additive or synergy effect on food intake may have been apparent had we supplemented and tested over a longer period of time. It may be intuitive to believe the effect of this combination was entirely predictable; however, the additive effects of ingredients cannot be assumed. Often the effects of combinations of ingredients are no greater than their respective components, and it is entirely possible and no less likely that the ingredients may counter act each other's effects.

The current study also demonstrated beneficial short-term effects of inulin on appetite and food intake. These extended beyond caloric compensation for the breakfast smoothie for the dinner calorie intake, total *ad-libitum* calorie intake and total calorie intake. It can take several hours for fermentable carbohydrates to reach the colon and sometimes up to 24 hours to fully digest, where SCFA are produced by the microflora (den Besten et al., 2013). This could not account for the effects of inulin on hunger observed in this study as we observed a reduction in appetite less than 4 hours. However, in the previous study we demonstrated that consumption of inulin can elicit a rise in breath hydrogen within 2–3 h of consumption and produce elevated levels of serum SCFA between 4 and 6 h after consumption (Tarini & Wolever, 2010). The reduced glycaemic response observed in the previous study may also explain the findings, we observed a lower glycaemic response 2 hours after the preload. It remains unclear if post absorptive signals generated by the fermentation of inulin or the reduced glycaemic response could plausibly account for the observed reduction in hunger so soon after the inulin preload.

As the previous study found the effects of β-glucan appear to be in response to the fermentation and glycaemic control rather than the acute effects of the viscosity. Previous research found that the effects of viscosity play a greater role than fermentation (Poutanen et al., 2017), this did not appear to be the case in this study as there were no significant differences in appetite measures across the test day. In an acute setting, fibre properties such as viscosity are thought to play a greater role in satiety than fermentation. (Lyly et al., 2009) found that the effects of soluble fibres on satiety in the form of beverages shows a greater satiety response as viscosity of the fibres increased. Beverage viscosity has also been inversely related to postprandial hunger (Mattes & Rothacker, 2001). Some of the previous findings for viscosity could be inflated by differences in sensory properties between the control and treatment conditions (Barone Lumaga et al., 2012). The sensory properties for

each drink were matched as closely as possible to the control drink to remove this confounding variable.

In the current study there were no significant differences in palatability or any dimension of taste. As we anticipated the inulin smoothie was rated as slightly sweeter than the other smoothies, but this difference was not statistically significant. Previous studies may have found a greater effect for viscous fibres as the control comparison was a non-viscous drink and not matched on dimensions of texture. Previous research has demonstrated slight changes in the texture of a drink can lead to increased satiety (McCrickerd et al., 2012). In the current the study the sensory properties of the drinks were matched between the different conditions so that each smoothie was thick in texture this could have potentially masked any increases in satiety the viscous fibres brought about after the drink was consumed. The viscosity of the liquid may be important in determining hunger response. Mattes and Rothacker (2001) reported significantly greater and more prolonged reductions in hunger with thicker shakes compared with thinner shakes. All other aspects of nutrient composition of the shakes were held constant in these studies. Marciani et al. (2001) compared solutions of different viscosity on satiety. Twelve healthy participants ingested 500 ml of a low- or high-viscosity test meal, either containing energy or being a non-nutrient control. They found that the viscous non-nutrient condition had similar effects to the non-viscous energy containing drink. This highlights the effects of viscosity on satiety.

No adverse events relating to the study product were reported by participants during the full study period or at post-study debriefing. The study products were developed, and the maximum dose selected were carefully considered following manufacturers guidelines and previous literature. The study products were not reported to

significantly increase GI symptoms or nausea during study days indicating that they were well tolerated. These data suggest that the reductions in energy intake observed were due to the selective actions of the fibres present.

Limitations

The treatment conditions were not standardised in terms of their energy content, the energy for each fibre drink was not matched with the control carbohydrate source due to the difficulty in trying to also match the sensory characteristics. It was important that the fibre drinks did not differ on any dimension of palatability or taste, to achieve this the fibre was suspended in a sweet smoothie, this masked both the taste and texture particularly of the β-glucan, however we could not match the calorie content. Caloric compensation indicated the effects of the fibre were above those of the increased calorie content of the fibre preload. It was difficult to develop a suitable product with similar sensory characteristics but without distinct differences in calorie content when the fibre itself contained calories (Almiron-Roig et al., 2013). We could have increased the load of the preload in the control condition to match the calorie content in the fibre conditions however, this would have had a greater impact on results as previous research has shown volume rather than calorie content has a bigger impact on satiety in liquid preloads (Rolls & Roe, 2002).

The participants were homogeneous, ideally, we would have recruited more overweight participants, however, due to difficulties with recruitment this was not achieved. Given the differences in appetite control between normal and overweight participants and particularly the difference in fermentation rate and gastric distension we may have found slightly different results had we included a bigger proportion of overweight participants.

Conclusion

Although we did not detect a synergy effect for food intake or appetite, the current study suggests combining inulin and β-glucan in a smoothie preload significantly reduced food intake beyond the compensation needed for the preload drink. Providing the fibre in a beverage and as a component of a meal adds to the generality and potential practical application of the product we formulated. Enhanced satiety foods directly promote reduced food intake and also aid compliance with healthy eating and weight management strategies. It is however worth noting that the reduction in intake across a 24 h test period does not infer that there would be a reduction in food intake in a longitudinal study. Given the mechanisms underpinning fermentation of both inulin and β-glucan (changes in gut microbiota and subsequent alterations to SCFA release) the current design is not optimal for demonstrating their long-term appetite-altering potential. This study highlights the importance of time course when comparing the physiological effects on food intake. This study serves as an acute proof of concept study for combining fibres with different physiological properties in a smoothie, the long-term effects would need to be investigated to see if there is an effect on long term appetite control and food intake.

Chapter 8

8. The Impact of fibre related Nutrition and Health Claims on choice and perception in functional beverages.

8.1 Introduction

Obesity action plans have highlighted the need to encourage and support individuals to make healthier choices, particularly in relation to food, eating, and diet. Claims on foods can help consumers make healthier food choices. Increasing demand for healthier food products has led to a surge of interest in formulating products with functional ingredients. Fibre is one such functional ingredient which has substantiated health claims, for a variety of functions. These functions relate to improving health, risk reduction, satiety and weight management. Studies specifically focusing on consumer reactions and understanding health claims are lacking. There are a magnitude of potential predictors of the acceptability and consumption of drinks with fibre related health claims including demographic factors, nutrition knowledge, health motivation, dietary goals and specific health claim knowledge.

Obesity Epidemic

The growing prevalence of obesity worldwide has led to an urgent need to determine the factors that cause obesity to try to tackle the problem. A variety of approaches have been developed, in recent years there has been pressure placed on the food industry to help to try to tackle the issue. The food industry plays a key role in trying to reduce obesity by improving food formulation to develop healthy products and encouraging individuals to make healthier food choices through better labelling and nutrition information.

Food Labelling; Front of Pack Information

Providing the consumer with as much information as possible to communicate and encourage them to make healthier decisions through food labelling is important. Consumers' interest in healthy eating could be increased by adopting appropriate communication strategies on food packaging (Hung et al., 2019). Information from nutrition claims, health claims and front-of-pack labels can help inform consumers about the health value of food products this in turn may help individuals to achieve a healthy, balanced diet (Tony Benson et al., 2018). There are several types of nutrition and health claims found on food labels in addition to the simple listing of the nutrients present in a food product.

Nutrition and Health Claims

A nutrition claim simply states the presence of a nutrient e.g., "high in fibre" it does not link this to any specific health benefit or risk reduction. A health claim similar to a nutrition claim but further states, suggests or implies that health benefits can result from consuming a given food, for instance that a food can "reduce post prandial glycaemic response."

Health Claims Issues

Health claims are commonly exploited to market products to consumers (Annunziata et al., 2014). Food companies often want to increase consumption of their highly processed foods such as refined breakfast cereals and fruit drinks, these products are fortified with nutrients but are often also high in sugars (Prada et al., 2021). Health claims are less accessible to fresh foods such as fruit and vegetables which are of much higher nutritional value. Fresh foods have fewer marketing resources available; these foods generally are not packaged and so are less able to display health claims. It could be argued that a diet containing whole foods without any processed food,

would be far more beneficial than one containing fortified processed foods with health claims (Liu, 2003). Whole foods provide better nutrients and a healthier diet; however, the food industry will seek to encourage consumers to eat more of their products whether they are healthy or not. Demand for processed foods remains high as they are often convenient and cheap (Rao et al., 2013). Providing products with added health benefits is a positive step, but the potential to encourage consumers to consume these heavily marketed processed foods over healthier whole foods remains an issue (Martinho, 2020).

Health Claims – The Halo Effect

Care must be taken to avoid overestimating the healthfulness of products carrying health claims. Although the claims made on food products require empirical evidence to be substantiated by EFSA often foods with a nutrition or health claim are only marginally healthier than similar products without a claim (Kaur et al., 2016). Claims can often lead consumers to ignore the product's nutrient profile instead making global inferences about how healthy a product is based on the specific claim (Talati et al., 2017). Consumers may choose a product with a high in fibre nutrient claim but ignore the high sugar content, this creates positivity bias as the product provides positive information. The mere presence of a health claim has been found to positively bias consumers' perceptions of a product carrying claim. Consumers may incorrectly judge an unhealthy product as healthier than they would if no claim was present, which has the potential to encourage consumers to increase their consumption of these foods if they overestimate their nutritional value (Talati et al., 2016). This is known as the health halo effect.

Studies that have assessed the positivity bias of nutrient claims in unhealthy foods have reported a positivity bias in terms of decreased calorie estimation (Talati et al.,

2017) and increased consumption (Kaur et al., 2017). However, some report no difference between claim and no claim conditions in terms of perceived healthiness (Maubach et al., 2014). The research on health claims is more limited than that for nutrient claims. One study found evidence of more positive evaluations resulting from general level health claims (Maubach et al., 2014), while others found that a higher level claim on unhealthy products led to increased perceptions of healthiness and willingness to try (Gastón et al., 2009), willingness to pay (Barreiro-Hurle et al 2010) or mixed results across different variables (Kozup et al., 2003).

Health Claims Negative Effects

Foods containing claims can not only lead to overstating the positive effects but can also lead to negative preconceptions. Health claims carry a message of increased healthiness, but do not necessarily make the product more appealing to the consumer. Weight loss claims can often suggest products are low calorie and not as filling, consumers may question value for money resulting in different purchasing choices. There is also the link between healthy foods reducing perceived taste, consumers may avoid foods which are marketed as healthy due to their preconceptions about the taste. Some consumers, found the health image of the carrier products is more important than just the wording of the claim (Lähteenmäki, 2013).

Consumer Perceptions

How a product carrying a health claim is perceived will differ between individuals. Demographic variables, including gender (Carels et al., 2007), age (Ares & Gambaro, 2007), socioeconomic status (Darmon & Drewnowski, 2008), and education (Hiza et al., 2013) have been found to play an important role in predicting the acceptability of products carrying health claims. Beyond demographics psychological variables

including general measures for nutritional knowledge (Wardle et al., 2000), health related motivates and dietary goals as well as specific health claim knowledge and motivation. Familiarity or prior experience with the food is often overlooked as a predicter but plays a key role in predicting acceptability of products carrying health claims (Spence et al., 2016).

Gender

Studies about functional foods, nutrition and health claims have considered the effects of gender. Some studies suggest that women have a higher preference for functional foods (Bower et al., 2003; Lähteenmäki, 2013) and products with health claims (de Jong, Ocké, Branderhorst, & Friele, 2003), and respond more positively to nutrition and health claims (Urala et al., 2003). Conversely, other studies did not demonstrate major gender effects on consumer attitude towards functional foods (Lyly et al., 2007) or foods carrying healthy claims (Verbeke, 2005). It has been suggested that the effect of gender depends on the combination of product, the considered nutrient and the claimed health benefit (Ares & Gambaro, 2007). Men might be more interested in cholesterol-lowering benefits, whereas women might be more responsive to bone health promises or weight management claims (Lähteenmäki, 2013).

Age

Different age groups react differently in terms of perceived healthiness of different carrier products (Ares & Gambaro, 2007). Older people have been shown to be more interested in claims relating to disease/health in response to increased chronic illnesses that require changes in diet (Miller & Cassady, 2012). As metabolism often slows with advancing age, maintaining a healthy weight becomes more difficult which may lead to more dieting behaviours and seeking products containing weight management claims. Equally younger people who take their general health for

granted having little experience of illness/disease could focus more on products carrying weight/appetite related claims as they may be more preoccupied with self-image, rather than health.

Changes in later life may also impact how nutrition information on food labels is processed. In addition to increased chronic illnesses that require changes in diet (Miller & Cassady, 2012), there are age-related changes that may alter how information is processed and decisions are made. With increasing age, for example, working memory declines, the reduction in maintenance and manipulation of information in short-term memory may make it more difficult to activate and maintain information long enough to interpret its relevance. Older adults may compensate for challenges such as these through a reliance on prior knowledge or motivation (Soederberg et al., 2011).

Education

Education is significantly associated with healthy eating. Educated people are more likely to consume the recommended intake of healthy food, for example fruits and vegetables and to eat less fat (Finger et al., 2013). Education has also been so shown to improve how well health claims can be processed. Those with a higher level of education are more likely to understand the health claim and less likely to overestimate the claimed effect. Having a higher level of education can also significantly influence food purchases (Worsley, 2002), indicating that level of education can pose a substantial obstacle to promoting healthy eating. The effects on health claim processing, food choice and diet could, however, be due to a higher level of nutritional knowledge (Viola et al., 2016).

Nutrition Knowledge

Several studies about consumer acceptance of foods with health claims have concluded that other factors, such as attitudes, knowledge and familiarity, rather than demographics explain the consumption of such foods. Nutrition knowledge has an important effect on eating behaviour. Evidence suggests that prior nutrition knowledge is important for nutrition information processing (Miller et al., 2011) as well as a variety of health and diet decisions (Anthony Worsley, 2002). This positive correlation between knowledge and nutritional behaviour is described in many studies (Spronk et al., 2014). Knowledge of nutrition is related to food choice (Tepper et al., 1997), accuracy of label use (Miller & Cassady, 2015) and perceptions of food healthfulness (Crites & Aikman, 2005).

Lack of general nutrition knowledge can limit consumers' ability to understand or evaluate health claims, leading to lower perceived benefit or credibility (Ares et al., 2008). Howlett, Burton, and Kozup (2008) found that, misinterpretation of nutrient information occurred when prior knowledge levels were low. Those with low nutritional knowledge are also more likely to experience positivity bias towards unhealthy food products containing a health claim than those with high nutritional knowledge. Miller and Cassady, (2015) found that individuals with high nutritional knowledge often rely on prior knowledge to make decisions about the healthfulness of a product. Higher levels of nutrition knowledge were also linked to less trust in health claims (Lalor et al., 2009).

Consumer health claim knowledge is associated with a correct use of health-related information (Lalor, 2011) as knowledge is related to the ability to process health claims (Miller & Cassady, 2015; Moorman & Matulich, 1993) which can influence the extent of information use (Drichoutis et al., 2005; Grunert et al., 2010). Health claims usage

is mainly related to nutrition knowledge, whereas understanding of nutrition information on food labels is mainly related to interest in healthy eating (Grunert et al., 2010) and correct use of health-related information (Benson et al., 2018). Knowledge is related to the ability to process health claims (Lähteenmäki, 2013), but highly knowledgeable consumers may be less motivated to process the information, because they may think that they already have a sufficient degree of knowledge (Maclnnis et al., 1991; Burton & Andrews, 1996).

Motivation

Even though essential nutritional knowledge is required, knowledge alone is unlikely to be sufficient to encourage individuals to make healthy food choices (Anthony Worsley, 2002). Motivational factors associated with healthy eating may be necessary for compliance with what individuals already know is important for health (Brug, 2008). Marika Lyly et al. (2007) found that health motivation is particularly important for products with low palatability, as higher level of motivation might be needed to compensate for the taste. Those motivated to lose weight are more likely to accept lower calorie alternative that is less palatable than someone who is not motivated to lose weight. As long as consumers perceive trade-offs between taste and health, interest in healthy eating will be limited unless individuals are motivated (Grunert et al., 2010).

Consumers with different health motivation might react differently to health claims. Studies suggested that the interaction of motivation and ability influences consumers' health behaviours (Hung et al., 2017) as the highest level of health information processing is the result of not only high ability but also high motivation (Maheswaran & Sternthal, 1990). In relation to health claims, the need for information about food, diet and health is driven by interest in health and nutritional aspects of

food, as consumers who believe in the importance of healthy eating tend to be more engaged in health promoting behaviours such as reading health-related information (Wardle & Steptoe, 1991).

Dietary Modification

Individuals who modify their diets to include or exclude certain foods or nutrients may be more goal-directed in their approach to food selection which may impact their decision making based on food labels. Dietary modification includes any self-identified dietary modification strategy that represents a change or modification relative to a perceived norm. Dietary modification of this sort could include food restrictions recommended by a health professional due to a chronic condition (e.g., to reduce sugar due to diabetes), food allergies (nuts, wheat, dairy), values (vegetarian, vegan, pescatarian, religion) or general health (decreased saturated fat, increased fibre, decreased salt).

Such modifications can positively impact health behaviours (Miller et al., 2011).

Previous research has suggested individuals who report having dietary restrictions are goal-oriented in their approach to food choice which influences how they process nutrition information on food packages. Specifically, goal-oriented individuals may have a greater ability to use the cognitive resources they have to more fully engage in the decision-making process. In this sense, goals may motivate individuals to make more informed decisions therefore, these individuals might be more inclined to apply the nutrition knowledge or motivation that they possess to the task at hand.

Familiarity with Products

Familiarity with a food product is one of the most common predictors of food choice. Consumers often chose to ignore information on front of packs instead choosing to

buy products out if habit and familiarity (Grunert et al., 2010). Whether a consumer has prior experience, or the product is considered a trusted brand by the consumer, they are more like to purchase foods (Benson et al., 2018). Verbeke et al., (2009) found that psychological factors such as consumers' familiarity with foods carrying claims and belief in the claims were the most consistent predictors on perceptions of the products. In another study, familiarity with the functional product and claim type boosted product ratings, however all product appeal decreased when participants perceived the functional foods as a marketing ploy (Hodgkins et al., 2019).

Health claims did not significantly influence drink choice; however, participants chose the "maintains blood sugar" drink slightly more than the "fuller for longer" drink compared to the control, participants chose the "high in fibre" drink less than the control drink. Personal factors did not predict drink choice. However, health claims did significantly affect perceptions of the drinks and personal factors predicted those perceptions.

Hypothesis

The current study aimed to gain insight into the effects of nutrition and health claims on purchasing intent and perceptions of a fibre drink. It was hypothesised: -

(i) Participants will choose a fibre drink with a health claim present significantly more than a drink without a health claim present.
(ii) Personal factors (demographics, nutritional/health claim knowledge, motivation to eat) will positively predict drink choice.
(iii) The presence of a health claim will positively affect participants perceptions (willingness to buy, taste, heath, weight management, fullness) of a drink.
(iv) Personal factors (demographics, nutritional/health claim knowledge, motivation to eat) will predict how participants perceive a drink with a health claims present.

To test the hypothesis participants will take part in an online task. Smoothies and juices were selected to match the drinks used in the previous studies (Chapters 4-7) and enable realistic variations in the products with different health claims.

8.2 Materials and Methods

8.2.1 Sampling

An opportunity sample was used. The questionnaire was shared on various media platforms such as the university website, local groups as well as larger social media platforms and websites.

8.2.2 Ethical Approval

Ethical approval was obtained from the University of Liverpool Research Ethics Committee. Respondents provided informed consent prior to completing the survey and did not receive an incentive or payment for their participation.

Procedure

An internet-based questionnaire was developed using the resources available at www.qualtrics.com. Once participants had given their consent, participants were asked to provide their age, gender, weight (in kg or stones), and height (in m or feet), level of education and whether they were following a dietary modification strategy. They were randomly allocated to one of four conditions; (1) a no claim control condition (n = 52), in which the drink presented carried a neutral “New Flavour” message, (2) a “Feel Fuller for Longer” condition (n = 52), in which the drink displayed a health claim, (3) a “Helps to maintain blood sugar levels” condition (n = 50) in which a health claim was presented, (4) a “high in fibre” condition (n = 53) in which a nutrition claim was presented. The aim here was to test whether the presence of a health or

nutrition claim would influence participant's choice and perception of a drink. On completion, participants were debriefed and thanked for their time.

8.2.3 Questionnaire

Following a review of the literature, validated items were selected to measure factors potentially thought to impact the perception of health claims. These psychological items were included alongside other relevant sociodemographic and dietary modification strategies to form the study questionnaire.

8.2.3.1Sociodemographic Measures

Age, gender, and highest level of education were collected.

BMI

Body Mass Index (BMI) was calculated using self-reported height and weight (weight in kilograms divided by square of height in metres). The World Health Organisation cut-offs were used to classify respondents to underweight (<18.50), normal weight (18.50–24.99), and overweight (>25) obese, morbidly obese.

Current hunger and thirst

Current hunger and thirst status were measured using a VAS scale with 0 indicating not at all hungry/thirsty and 100 indicating extremely hungry/thirsty.

Psychological Measures

Nutrition Knowledge

Nutrition knowledge was assessed using the Nutrition Knowledge Questionnaire (Parmenter & Wardle, 1999; Kliemann et al., 2016). The questionnaire has four sections we selected two sections which were most relevant to the hypothesis. The

first covering general nutrient knowledge and second which covered the relationships between diet and disease and beliefs, as well as knowledge of any diseases associated with eating too much or too little of various types of food. Cronbach's alpha was used assess the internal consistency of each subscale. Nunnally and Bernstein (1994) suggests α = .70 as a lower acceptable bound for alpha. Cronbach's alpha revealed high internal consistency for the current data (α = .863).

Health Claim Knowledge

Objective health claim knowledge was measured using five items. These items were adapted from previous research (Benson et al., 2018; Hung et al., 2017) "The claim 'iron contributes to normal cognitive function' in other words means…" and "The claim 'omega-3 fatty acids help to maintain a healthy cardiovascular system' in other words means…".. All items had four possible answers, of which one answer was correct. Participants' objective knowledge score was the number of correct answers provided, ranging from 0 to 5, with a higher score indicating greater objective knowledge. Cronbach's alpha revealed high internal consistency for the current data (α = .802).

Motivation to eat

The Food Choice Questionnaire (Steptoe et al., 1995) was used to measure motivation to eat this instrument comprises items that represent food attributes, intrinsic and extrinsic, which may motivate consumers in choosing foods. Each item permit to grade the relevance of the food choice on any given day, through a 4-point scale (1 = not important, 2 = little important, 3 = moderately important, 4 = very important). The questionnaire measures motivational dimensions, each of which includes three to six items. These dimensions are: Health, Convenience, Sensory appeal, Natural content, Weight control, Familiarity, and Environmental Concerns. The questionnaire has shown adequate internal consistency with Cronbach's

coefficients ranging from .72 and .86 for the various factors identified, and adequate validity. Cronbach's alpha for the current data revealed good to high internal consistency for the scales ranging from α = .781 (environmental concerns) to α = .905 (health).

Motivation to process health claim messages

Three items adapted from previous research (Benson, 2018; Moorman, 1990) were used to measure motivation to process nutrition and health claims, for example, "I pay attention to nutrition and health claims on food". Items used a scale from 1 (strongly disagree) to 5 (strongly agree). Scores could therefore range from 3 to 15, with a higher score indicating greater motivation. Benson (2018) found that internal consistency for the scale was excellent (α = .92), similar to the original scale consistency of α = .94 (Moorman, 1990). Cronbach's alpha revealed high internal consistency for the current data (α = .827).

Dietary Modification Strategy

Dietary modifications can be set by the individual or recommendation from a health professional in response to potential or actual health conditions (Miller et al., 2012). They may involve following a specific weight loss or weight management diet or avoiding specific foods due to food allergies, intolerances, personal preferences or cultural beliefs. In the present study, we wanted to include dietary goals regardless of their source, whether the individual has chosen to follow, or they were recommended by a health professional. Both could be equally important for food choice. Participants were asked to respond to the question: -

"Do you have any diet-related restrictions?"

Participants were provided with 4 options as well as an open-ended answer to cover any dietary modification strategies not mentioned.

No

Yes, I am currently following a weight loss/weight maintenance diet

Yes, due to a medical condition e.g., diabetes or IBS

Yes, due to a specific food allergy or intolerance e.g., gluten or lactose intolerant

Yes, due to personal/cultural beliefs/preferences e.g., vegetarianism

Other……..

8.2.4 Study Products

To examine the impact of health claims on selection, drinks images were adapted from real products currently on sale (see figure 8-1). In total, 10 drinks were used (Appendix 11), a mixture of smoothies and juice drinks currently on the market in the UK. These products were selected based on their similarity in flavours/product size. In addition, as each product was required to carry each of the selected claims, it was important that each product-claim combination had ecological validity and was realistic.

Real products which are currently available in the UK were selected to create a realistic selection task. Additional nutrition information was not provided as the current study aimed to test the effects of the claim on each specific drink in isolation. Research has shown that consumers often don't use nutrition information to make purchasing decisions about food products, instead attending to front of pack information such as health claims (Benn et al., 2015). The current study aimed to explore how participants perceive and accept the drinks in the absence of any other food label or nutrition information.

In total, there were 10 different possible drink/claim combinations (See Appendix 11). Participants were randomised to one of 4 conditions, one claim per condition.

Ten different types of drink were presented with or without the claim present and participants were asked to choose which drink they would choose at that moment in time (See Appendix 11 for sample task).

Figure 8-1 Example of the drinks packaging with claims used in survey.

Health Claims

Claims relevant to fibre were tested. We selected a nutrition claim, a health-related claim and a satiety related claim. We wanted the claims to be realistic therefore we consulted the EFSA guidelines for accepted claims and their suggested wording. We selected claims which would be easy for all participants to understand, without any complex phrases such as “glycaemic response” or “satiety” which participants may not be familiar with.

We selected the following nutrition/health claims

Nutrition Claim – “High in Fibre”

Functional Health Claim “Helps to maintain blood sugar levels”

Functional Claim specific to Satiety “helps you to feel fuller for longer”

Figure 8-2 Nutrition claim for fibre label

In addition, the absence of a claim was also investigated. A control condition without a label present was also included. In order to keep the images consistent a neutral “new flavour” message was also added to avoid participants simply selecting the images without the message present in the no claim condition.

Figure 8-3 Control condition label

The nutrition claim ‘high in fibre’ was chosen due to the prevalence of high fibre claims in the UK and Ireland, this is something consumers would be familiar with. “Helps to maintain blood sugar levels”’ was chosen as this is a substantiated claim for both inulin and β-glucan fibre. In our previous laboratory study, we also found that a fruit smoothie containing inulin and β-glucan significantly reduced glycaemic response. These claims were more suitable for the drinks chosen and are most relevant to our previous findings. ‘Helps you to feel fuller longer’ is an EFSA substantiated health claim, this has been previously used on products within the UK and Ireland. Furthermore, previous research which investigated satiety claims (claim only in the absence of a product) suggested that future research should examine the impact of claims in the context of product packaging (Bilman et al., 2012).

8.2.5 Task 1 Drink Choice Task

Participants were presented with a series of 10 pairs of different no claim/claim combinations and are asked to choose which drink they would purchase if they were priced equally. Online shopping for groceries has been growing in recent years with as many as 45% of consumers using online shopping in 2019, using a virtual online choice task has high ecological validity as it isn't dissimilar to shopping online. Consumers shopping online will make decisions about which foods to buy simply by looking at images of products rather than interacting or attending to other on pack information (Benn et al., 2015).

Drink Perceptions Task

For each of the 20 products presented in task 1, participants were also asked to rate each drink on five parameters using a sliding scale from 0-100: Taste, health, filling, aids weight loss and how likely to buy. For example, "Please rate the following item on taste?"

8.2.6 Statistical Analysis

All data was analysed using IBM SPSS Statistics v25 (SPSS Inc., Chicago, IL, USA). Descriptive statistics (means (M), standard deviation (SD)) were used to explore the data. A series of Poisson Regressions were conducted to explore the effects of the health claims on choice and the perceptions on choice. Poisson regression is used to predict a dependent variable that consists of count data given one or more independent variables. The data on the choice task included count data where there was only one of two choices, a drink with a claim or a drink without a claim. Count data is different to the data measured in other well-known types of regression (e.g., linear regression require dependent variables that are measured on a "continuous"

scale). Poisson regression also requires data that must be zero or greater, it cannot consist of minus values. Poisson regression should ideally only be performed when the mean count is a small value (e.g., less than 10), there were 10 trials in the choice task which suggested poisson regression would be most appropriate. The independent variables, also needs to be measured on a continuous, ordinal or nominal/dichotomous scale. For example, the perception task measuring “how tasty” on a scale from 0 to 100. There also should also be independence of observations to use a poisson regression. This means that each observation is independent of the other observations; for example, in the choice task one observation on the task did not provide information on another to influence participant’s choice. A poisson regression was therefore the most appropriate method to analyse the choice task data.

Analysis of Variance (ANOVAs) were used to assess if there were any differences on perceptions of taste, health, willingness to buy or how filling the product was perceived to be for the claim/no claim items. This was explored further for each condition using t-test correcting for multiple comparisons using Bonferroni adjustment. A series of hierarchical multiple regression analyses were used to understand how the claims affected choice and to see if there are any significant predictors (demographics, nutrition knowledge, motivation, dietary modification strategies or health claim knowledge/motivation) for how the products carrying the 3 health claims are perceived.

Results

8.1. Participants

In total, 207 participants aged 19–76 years old (M = 40.7, SD = 10.9) completed the survey (see Table 8-1 for sociodemographic details), 173 females and 34 males. In terms of education, 79.7% were educated to at least university undergraduate level. Nutritional knowledge was quite high with a mean score of 45.5 (79.8%). Health claim knowledge was relatively high with a mean score of 4 from a maximum of 5. Individuals had moderate motivation to process, with a mean motivation score of 7.3 (minimum possible 3, maximum possible 15). Participants were most motivated to eat by taste followed by health; they were least motivated by familiar foods. In terms of dietary goals 86 (41.5%) of participants were following a specific diet, of these 38 (18.4%) were following a weight loss/weight maintenance diet, 121 (58.5%) were not following a specific diet.

Table 8-1 Characteristics of the Sample

		Total Participants		Control Condition		fuller for Longer		Glycaemic Control		High in fibre	
		n	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
	Total	207	100	52	25.1	52	25.1	50	24.2	53	25.6
Gender	Male	34	14	8	12.8	9	13.6	7	11.4	10	18.9
	Female	183	86	46	87.2	43	86.4	43	88.6	43	81.1
Education	None	0	0	0	0	0	0	0	0	0	0
	Secondary school	8	3.4	1	2.6	0	0	2	4.5	4	8.1
	College	16	6.3	4	7.7	4	6.8	2	4.5	6	10.8
	Associates/ Vocational Degree	21	10.6	3	5.1	6	11.4	6	11.4	7	13.5
	Undergraduate	78	36.7	21	41.0	18	34.1	17	34.1	21	40.5
	Masters	65	32.4	16	30.8	21	40.9	18	36.4	10	18.9
	Doctorate	19	10.6	7	12.8	4	6.8	5	9.1	4	8.1
Dietary Goals	No Diet	128	58.5	21	41.0	33	63.6	36	72.7	37	70.3
	weight loss/weight maintenance diet	33	18.4	15	28.2	5	9.1	6	11.4	8	16.2
	medical condition	18	9.2	8	17.9	5	9.1	3	6.8	1	2.7
	food allergy or intolerance	14	5.3	1	2.6	6	11.4	3	6.8	3	5.4
	personal/cultural beliefs	13	7.7	5	10.3	4	6.8	1	2.3	3	5.4
	Other	2	1	1	0	0	0	0	0	1	0
		M	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***	***M***	***SD***
Age		40.7	10.9	39.3	12.3	43.2	11.4	40.9	9.7	39.6	10.5
BMI		29.3	31.7	28.9	8.3	26.6	6.0	35.2	46.7	26.5	7.1
Height		1.6	0.8	1.6	0.1	1.6	0.1	1.7	0.1	1.6	0.1
Weight		79.1	89.4	77.3	22.0	70.2	13.8	98.1	139.8	70.8	17.8
Choice Task	Health Claim Selected	5.3	4.0	5.3	4.0	5.8	4.4	6.3	3.6	3.7	4.0
	Control Selected	4.7	4.0	4.7	4.0	4.2	4.4	3.7	3.6	6.3	4.0
Nutritional Knowledge		45.5	7.0	45.3	7.2	45.2	7.2	47.3	5.6	44.9	9.4
Motivation to Eat	Weight	2.3	0.7	2.3	0.7	2.2	0.6	2.4	0.7	2.4	0.9
	Health	2.9	0.7	2.8	0.7	3.0	0.6	3.0	0.6	2.7	0.7
	Natural	2.5	0.9	2.5	0.9	2.6	0.8	2.5	0.9	2.3	1.0
	Taste	3.3	0.6	3.3	0.8	3.3	0.6	3.5	0.5	3.3	0.5
	Familiar	1.8	0.8	1.7	0.9	1.8	0.8	1.5	0.6	1.9	0.8
	Convenient	2.7	0.7	2.7	0.9	2.7	0.7	2.6	0.8	2.8	0.6
	Environment	2.4	0.9								
Objective Health Claim knowledge		4.0	1.1	4.0	1.3	4.1	1.0	4.0	1.2	3.9	1.2
Motivation to process Health Claims		7.3	2.5	6.4	2.4	7.4	2.5	7.1	2.7	8.1	2.2

Choice data

Claim Type

Data were not consistent with Poisson distribution ($p < .0001$) but were also not consistent with any other tested (normal, exponential, or uniform). Further, Goodness-of-fit showed data were very over-dispersed when using both Fixed Value and Pearson Chi Square scale parameter methods and a robust covariance matrix estimator. Thus, Negative Binomial regression with log-link function and a Pearson Chi-Square estimated parameter value was used instead.

The omnibus test showed that the alternative model was a better fit than the intercept-only null model (Likelihood ratio $\chi^2(3) = 8.94$, $p = .03$), and a test of model effects showed statistical significance between conditions (claim types) at the standard alpha level (Wald $\chi^2(3) = 9.47$, $p = .02$). Compared to selection for the 'New Flavour' control claim (over a 'no label' control image), labelled products (compared to no label products) containing the 'Fuller Longer' (6.4%) and the 'Blood Sugar' (20.2%) messages were selected more, although were not statistically significantly different from 'New Flavour' product selection ($p = .73$ and $p = .29$, respectively). Conversely, products showing the 'Fibre' claim were chosen 30% less, although again this difference was not statistically significant ($p = .057$).

Predictors of claim type

Next, Negative Binomial regressions were performed in succession with each claim type compared to the 'New Flavour' baseline. For each regression, participants' perceptions of product taste, healthfulness, filling-ness, weight-loss value, and their nutritional knowledge were entered as covariates in the model (main effects considered, only).

For the ‘Fuller Longer’, ‘Blood Sugar’, and ‘Fibre’ claims, the Omnibus tests showed statistically non-significant test statistics, suggesting that the alternative models were not a better fit than the intercept-only null models (Likelihood ratio $\chi^2(5) = 5.38$, $p = .37$; Likelihood ratio $\chi^2(5) = 6.31$, $p = .28$; Likelihood ratio $\chi^2(5) = 4.81$, $p = .44$, respectively).

Negative Binomial regressions were performed in succession with each claim type compared to the ‘New Flavour’ baseline. For each regression participants scores for dietary modification goal, nutritional knowledge, eating for weight, eating for health, eating natural foods, eating for familiarity, eating for convenience, knowledge of food claims and motivated by food claims were entered as covariates in the model (main effects considered only).

For the ‘Fuller Longer’, ‘Blood Sugar’, and ‘Fibre’ claims, the Omnibus tests showed statistically non-significant test statistics (Likelihood ratio $\chi^2(5) = 7.64$, $p = .57$; Likelihood ratio $\chi^2(5) = 9.95$, $p = .354$; Likelihood ratio $\chi^2(5) = 12.52$, $p = .185$, respectively).

Table 8.2 Frequency of choice in each experimental condition.

	No Claim *n* =52		Fuller For Longer *n* = 52		Glycaemic Response *n* = 50		High in Fibre *n* = 53	
	Control	Claim	Control	Claim	Control	Claim	Control	Claim
Trial 1	22	30	24	28	18	32	29	24
Trial 2	30	22	22	30	21	29	37	16
Trial 3	18	34	27	25	17	33	29	24
Trial 4	25	27	23	29	22	28	37	16
Trial 5	25	27	20	32	20	30	36	17
Trial 6	22	30	21	31	19	31	34	19
Trial 7	25	27	18	34	22	28	39	14
Trial 8	24	28	20	32	15	35	29	24
Trial 9	25	27	25	27	14	36	34	19
Trial 10	28	24	18	34	19	31	31	22
Total	244	276	218	302	187	313	335	195
Mean	4.7	5.3	4.2	5.8	3.7	6.3	6.3	3.7

Perception ANOVAS

A series of ANOVAS were conducted to see whether there were any significant differences in willingness to buy, perceptions of weight management, filling, health and taste for a drink carrying a nutrition/health claim compared to a no claim control drink.

Willingness to Buy

There was an interaction between willingness to buy and claim condition f (3,160) = 4.365, p = .006. For Paired t-tests revealed that participants were significantly more willing to buy the claim carrying drink in the fuller for longer condition t (43) = -2.347, p =.024, and the controls blood sugar condition t (43) = -2.560, p = .014 There was no significant difference in willingness to buy in the in the high in fibre condition.

Taste

There were no significant differences in taste perceptions between the drinks carrying a high in fibre, controls blood sugar or feel fuller for longer condition compared to a no claim condition control.

Filling

There was a main effect for how filling participants perceived the drink f (1, 206) = 38.632, p = .042. There was also a significant interaction for condition and how filling the drink was perceived f (1,206) = 31.830, p > .001. Paired t-tests revealed that participants perceived the claim drink significantly more filling in the maintains blood sugar condition, t (43) = -5.744, p >.001 and high in fibre condition t (52) = -3.310, p = .002 conditions compared to the no claim control condition. There was no significant difference in how filling participants perceived the claim drink compared to the control for the fuller for longer claim.

Health

There was a significant interaction between health perception and claim condition f (3,206) = 3.924, p = .010. Paired t-tests revealed that participants perceived the claim drink significantly healthier in the blood sugar condition t (52) = -3.119, p = .003 compared to the no claim control, but not in either the fuller for longer or added fibre conditions compared to the control condition.

Weight

There was a significant interaction for perceived weight loss scores f (1,206) = 14.027, p >.001. Paired t-tests revealed that participants perceived the claim drink as significantly more able to aid weight management in the blood sugar condition p (52) = -3.290, p=.002 and the added fibre condition t (52) = -2.090 compared to the control condition, p = .044 but not in the fuller for longer condition.

Hierarchical Regressions

A series of hierarchical regressions were conducted for each health claim. Hierarchical regression analysis was conducted to analyse the effect of personal factors on perceptions of a drink carrying a “controls blood sugar”, “high in fibre” and a “feel fuller for longer” claim. The hierarchical regressions (Table 8-3) were used to examine the effects of psychological factors after controlling for physiological, sociodemographic factors on 5 measures of perception; willingness to buy, taste, health, filling and weight management. The first step of the regression consisted of hunger and thirst, demographics age, education, BMI and gender was added as the second step and psychological variables as the third step. The overall models for the controls blood sugar, high in fibre and fuller for longer conditions are presented below.

Hierarchical Regression "Controls Blood Sugar"

The overall models for the controls blood sugar condition were significant for aids weight loss, willingness to buy, perceptions of health and perceptions of taste. The overall model for perceptions of fullness were not significant.

Controls blood sugar Claim - Aids weight loss

The overall regression model predicted approximately 66% of the variance in weight loss perceptions ($R2 = .657$, $F (16, 33) = 2.557$, $p = .017$. Hunger and thirst predicted approximately 9% of the variance in perceptions of weight management. After controlling for hunger and thirst step 2 predicted approximately 25% of variance in aids weight loss perceptions, although only age significantly predicating aids weight loss perceptions, older participants had lower perceptions of weight loss. After controlling for age and education, step 3 predicted approximately 32% of variance in aids weight loss perceptions, although only eating to stay healthy significantly predicating weight loss perceptions, with lower motivation to eat healthy being associated with higher perceptions of weight loss.

Controls Blood Sugar Claim - Willingness to Buy

The overall regression model predicted approximately 63% of the variance in willingness to buy ($R2 = .629$, $F (16, 35) = 3.919$, $p < .001$ Hunger and thirst predicted approximately 5% of the variance in willingness to buy. After controlling for hunger and thirst step 2 predicted approximately 27% of variance aids weight loss perceptions, although only age significantly predicating willingness to buy with older participants being associated with less willingness to buy. After controlling for age and education, step 3 predicted approximately 31% of variance in willingness to buy, being motivated to eat familiar foods significantly predicated aids willingness to buy, with

greater motivation to eat familiar foods being associated with greater willingness to buy.

Controls Blood Sugar Claim - Perceptions of health

The overall regression model predicted approximately 61% of the variance in perceptions of health (R2 = .606, F (16, 35) = 2.860, p =. 009. Hunger and thirst predicted approximately 10% of the variance in perceptions of health although only hunger was a significant predictor with greater perceptions of health in people who were hungry. After controlling for hunger and thirst step 2 predicted approximately 14% of variance for perceptions of health. After controlling for age and education, step 3 predicted approximately 37% of variance in perceptions of health, although only being motivated to eat familiar foods significantly predicated perceptions of health, with being motivated to eat familiar foods being associated with greater perceptions of health.

Controls Blood Sugar Claim - Perceptions of Taste

The overall regression model predicted approximately 52% of the taste perceptions (R2 = .515, F (16, 35) = 2.408, p =.023. Hunger and thirst predicted approximately 1% of the variance in perceptions of taste. After controlling for hunger and thirst step 2 predicted approximately 22% of variance in taste perceptions, although only age significantly predicating taste perceptions with older participants being associated with lower perceptions of taste. After controlling for age and education, step 3 predicted approximately 29% of variance in taste perceptions, although only being motivated to eat familiar foods was significant with greater motivation to eat familiar foods being associated with greater perceptions of taste.

Table 8-3 Explained adjusted R^2 Change, *F*-Change and standardized coefficients (β) for each regression for perceptions after the addition of each step in the maintains blood sugar condition.

	Willingness to buy Rating	Taste Rating	How filling Rating	How Healthy Rating	Helpful for weight loss Rating
	β	β	β	β	β
Step 1: Physiological					
R^2 Change	0.053	0.001	0.021	0.098	0.09
***F*-Change**	F (2, 47) = .1.152	F (2, 47) = .026	F (2, 47) = .431	F (2, 47) = 2.238	F (2, 47) = 2.030
Hunger	0.232	0.01	0.109	0.308*	0.303
Thirst	-0.007	0.033	-0.115	-0.135	-0.017
Step 2: Demographic					
R^2 Change	0.266*	0.220*	0.147	0.142	0.252*
***F*-Change**	F (4, 43) = 4.480	F (4, 43) = 5.501*	F (4, 43) = 3.440	F (4, 43) = 2.125	F (4, 43) = 7.476*
Age	-0.748*	-0.628*	-0.482*	-0.358	-0.556*
Education	-0.109	-0.076	0.026	0.05	0.153
BMI	0.028	-0.019	-0.205	-0.156	-0.056
Gender	0.069	-0.015	-0.083	0.078	0.154
Step 3: Psychological					
R^2 Change	0.310*	0.292*	0.293	0.366	0.315*
***F*-Change**	F (10, 33) = 2.397	F (10, 33) = 1.973*	F (10, 33) = .293	F (10, 33) = 2.735*	F (10, 33) = 3.064*
Nutrition Knowledge	0.156	0.123	0.247	0.129	0.062
Eating to control weight	-0.25	-0.231	-0.112	-0.192	0.219
Eating to stay healthy	-0.237	-0.136	-0.013	-0.236	-0.321*
Eating foods that are natural	0.056	-0.044	0.173	0.144	0.241
Eating for taste	-0.023	0.005	-0.066	0.033	-0.023
Eating Familiar foods	0.467*	0.490*	0.224	0.530*	0.467*
Eating for convenience	0.09	-0.17	-0.161	-0.031	0.121
Knowledge of health claims	0.078	0.143	0.198	0.198	0.288
Claim motivated	-0.029	0.054	0.248	0.095	0.144
Diet	-0.014	0.018	0.151	0.077	0.19

Hierarchical Regression – "Feel Fuller for longer"

A hierarchical regression analysis was conducted to analyse the effect of personal factors on perceptions of a drink carrying a "feel fuller for longer" claim. The overall models for the feel fuller for longer condition were significant for aids weight loss, willingness to buy, perceptions of health and perceptions of taste. The overall model for perceptions of fullness and taste were not significant.

Feel Fuller for Longer Claim - Likely to aid weight loss

The overall regression model predicted approximately 51% of the variance in aids weight loss perceptions (R2 = .514, F (16, 32) = 2.557, p =.017. Hunger and thirst predicted approximately 12% of the variance in perceptions of weight management although only thirst was a significant predictor with higher thirst in people who had higher perceptions of weight loss. After controlling for hunger and thirst step 2 predicted approximately 15% of variance aids weight loss perceptions, although only gender was significant, women had higher perceptions of weight loss. After controlling for age and education, step 3 predicted approximately 24% of variance in aids weight loss perceptions, although only being motivated to eat for health, motivated to eat to lose weight and eating familiar foods significantly predicated aids weight loss perceptions. Greater motivation to eat to lose weight and greater motivations to eat for health was associated with greater perceptions weight loss. Being motivated to eat familiar foods was also associated with greater perceptions of weight loss.

Feel Fuller for Longer Claim - Willingness to Buy

The overall regression model predicted approximately 49% of the variance willingness to buy (R2 = .485, F (16, 32) = 2.098, p =.048 Hunger and thirst predicted approximately 7% of the variance in willingness to buy. After controlling for hunger

and thirst step 2 predicted approximately 10% of the variance in willingness to buy. Only gender was significant with women having higher ratings of willingness to buy. After controlling for age and education, step 3 predicted approximately 31% of variance in willingness to buy, although only eating to control weight was significant. Greater eating to control weight was associated with greater willingness to buy.

Feel Fuller for Longer Claim - Perceptions of health

The overall regression model predicted approximately 61% of the variance in how healthy participants perceived the drink to be in the fuller for longer condition (R2 = .614, F (16, 32) = 2.213, p =.026. Hunger and thirst predicted approximately 12% of the variance in perceptions of health of the claim carrying drink. After controlling for hunger and thirst step 2 predicted approximately 13% of variance in perceptions of health, although only gender significantly predicated perceptions of health with women having greater perceptions of health. After controlling for age and education, step 3 predicted approximately 37% of variance in health perceptions, although only higher ratings in eating to control weight were significant, with eating to control weight being associated with greater perceptions of health.

Table 8-4 Explained adjusted R^2 Change, F-Change and standardized coefficients (β) for each regression for perceptions after the addition of each step in the Feel Fuller for Longer condition.

	Willingness to buy Rating	**Taste Rating**	**How filling Rating**	**How Healthy Rating**	**Helpful for weight loss Rating**
	β	**β**	**β**	**β**	**β**
Step 1: Physiological					
R^2 Change	0.074	0.067	0.096	0.118	0.117*
***F*-Change**	F (2, 49) = 1.603	F (2, 49) = 1.438	F (2, 49) = 2.120	F (2, 49) = 2.032	F (2, 49) = 2.637
Hunger	-0.001	-0.105	-0.033	-0.003	-0.064
Thirst	0.277	0.269	0.317*	0.344	0.354*
Step 2: Demographic					
R^2 Change	0.102*	0.078	0.076	0.125*	1.513*
***F*-Change**	F (4, 45) = 2.354	F (4, 45) = 1.739	F (4, 45) = 1.754	F (4, 45) = .092	F (4, 45) = .065
Age	−0.004	-0.075	-0.071	-0.122	-0.066
Education	−0.03	0.25	0.142	0.114	0.097
BMI	-0.86	-0.76	-0.247	-0.048	-0.021
Gender	0.598*	0.447*	0.562*	0.523*	0.661*
Step 3: Psychological					
R^2 Change	0.308*	0.216	0.207	0.371	2.437 *
***F*-Change**	F (10, 35) = 1.928	F (10, 35) = 1.090	F (10, 35) = 1.072	F (10, 35) = .201	F (10, 35) = .352
Nutrition Knowledge	-0.0273	-0.388	-0.239	-0.1	-0.066
Eating to control weight	0.314*	0.003	-0.061	0.352*	0.373*
Eating to stay healthy	0.217	0.342	0.424	0.334	0.37*
Eating foods that are natural	-0.033	-0.208	-0.068	0.3	-0.079
Eating for taste	-0.088	0.009	-0.069	-0.112	-0.152
Eating Familiar foods	0.082	0.004	0.109	0.09	0.335*
Eating for convenience	0.218	-0.128	0.025	0.195	0.021
Knowledge of health claims	-0.039	-0.046	-0.107	-0.079	-0.002
Claim motivated	-0.05	0.331	0.22	0.068	0.06
Diet	0.787	-0.056	0.328	-0.062	-0.056

Hierarchical Regression – "**High in Fibre"**

A hierarchical regression analysis was conducted to analyse the effect of personal factors on perceptions of a drink carrying a "high in fibre" claim (Table 8–5). The overall models for the high in fibre condition was significant for perceptions of taste. The overall models for perceptions of weight loss, willingness to buy, health and fullness were not significant.

High in fibre Claim - perceptions of taste

The overall regression model predicted approximately 61% of the variance in taste perceptions (R2 = .607, F (16, 36) = 2.375, p =.029. Hunger and thirst predicted approximately 8% of the variance in perceptions of taste although only hunger was a significant predictor with higher perceptions of taste in people who were hungry. After controlling for hunger and thirst step 2 predicted approximately 13% of variance in taste perceptions, although only BMI significantly predicated taste perceptions with participants with a higher BMI perceiving the drink to be tastier. After controlling for age and education, step 3 predicted approximately 39% of variance in taste, although only eating to stay healthy significantly predicated taste, with participants motivated by eating to stay healthy having greater perceptions of taste.

Table 8-5 Explained adjusted R^2 Change, *F*-Change and standardized coefficients (β) for each regression for perceptions after the addition of each step in the high in fibre condition.

	Willingness to buy Rating	Taste Rating	How filling Rating	How Healthy Rating	Helpful for weight loss Rating
	β	β	β	β	β
Step 1: Physiological					
R^2 Change	0.263	0.081	0.151	0.153	0.035
***F*-Change**	F (2, 49) = .6.063	F (2, 49) = .081	F (2, 49) = 3.027	F (2, 49) = 3.063	.612F (2, 49) = .612
Hunger	0.399*	0.178*	0.303	0.288	0.174
Thirst	0.175	0.346	0.132	0.154	0.021
Step 2: Demographic					
R^2 Change	0.083	0.132*	0.008	0.075	0.032
***F*-Change**	F (4, 45) = 2.028	F (4, 45) = .213	F (4, 45) = .160	F (4, 45) = 1.543	F (4, 45) = .543
Age	-0.211	-0.125	-0.078	-0.23	-0.125
Education	0.2	0.118	0.049	0.151	0.13
BMI	-0.008	0.309*	0.062	0.133	0.081
Gender	0.325*	0.131	0.101	0.155	0.115
Step 3: Psychological					
R^2 Change	0.199	0.394	0.185	0.118	0.245
***F*-Change**	F (10, 38) = 1.115	F (10, 38) = .317	F (10, 38) = .719	F (10, 38) = .462	F (10, 38) = .910
Nutrition Knowledge	0.159	0.018	-0.117	-0.099	-0.087
Eating to control weight	0.176	-0.099	-0.069	0.117	0.121
Eating to stay healthy	0.161	0.688*	0.3	0.355	0.408
Eating foods that are natural	0.053	-0.229	-0.166	-0.216	-0.462
Eating for taste	-0.172	0.077	0.1	-0.102	-0.318
Eating Familiar foods	0.213	0.078	0.03	0.02	0.118
Eating for convenience	0.068	-0.001	-0.284	-0.077	-0.16
Knowledge of health claims	0.01	-0.06	0.011	0.05	0.043
Claim motivated	-0.007	0.256	-0.097	0.181	0.113
Diet	0.244	0.183	0.349	0.116	0.262

Discussion

The present study was undertaken to increase our understanding of how fibre related claims on drinks may influence choice and perception. This is the first study to test fibre related claims in the context of a drink. Three specific claims were explored; a nutrition claim "high in fibre" and two health related claims, one relating to satiety "feel fuller for longer" and a glycaemic response claim "helps control blood sugar levels". Overall, the health claims did not significantly influence choice in any of the 3 claim conditions however participants selected the fuller for longer claim drink 6.4% more than the "new flavour" control, the "controls blood sugar" claim was chosen 20.2% more than the new flavour claim whilst the "high in fibre claim" was chosen 30% less than the new flavour claim. Personal factors including dietary modification goals, nutritional knowledge, eating motivation, health claim knowledge and health claim motivation did not influence choice in any of the 3 conditions.

The results suggest that health claims do not significantly influence choice in the context of a drink. This is contrary to a systematic review that found health claims influenced food choice (Kaur et al., 2017). The added fibre claim appeared to reduce choice. The "added fibre" claim is a nutrition claim, nutrition claims simply state the contents of a specific nutrient, they tend to be less effective influencing food choice than general health claims which link the claim to a specific function (e.g., helps maintain blood sugars) (Hodgkins et al., 2019). The "feel fuller for longer" and "controls blood sugar" claim were general level health claims, they increased choice but again this wasn't significant. Previous research suggests the "fuller longer" claims aren't as effective as specific health claims as consumers are often sceptical about their effectiveness (Griffioen-Roose et al., 2013), the results were consistent with the "maintains blood sugar" claim appearing to influence choice more than the fuller for longer claim. The context of the carrier product may also influence choice (Profeta,

2019). High in fibre claims may work on other products (Kaur et al., 2017), but not in a beverage. Drinks are not generally considered to be high fibre items as opposed to foods containing grains such as cereals or breads. Drinks are often considered to be less satiating than whole foods (McCrickerd et al., 2014). Our findings are contrary to previous studies that found health claims can lead to positivity bias, whereby consumers ignore the product's nutrient profile instead making global inferences about how healthy a product is based on the specific claim (Talati et al., 2017). However, our findings were consistent with Talati et al., (2016) who found that health claims did not increase positivity bias, participants made judgements based on the product and not solely the claim.

Perception of Drinks with a Claim

Despite the lack of an effect on drink choice, participant's perception of the drinks differed between the control and claim drink. The presence of a nutrition/health claim increased perceptions of weight management and how filling the drink was, this was consistent with (Benson et al., 2018). There were also interactions for health and willingness to buy in the claim condition, however there was no effect on taste. Products carrying health claims especially those related to weight management and fibre are often perceived as less tasty (Anguah et al., 2017). Consumers may avoid foods which are marketed as healthy due to their preconceptions about the taste. However, in the current study this didn't appear to be the case as participants didn't perceive the claim carrying drink as less tasty, this suggests the image of the carrier products is more important than just the wording of the claim (Lähteenmäki, 2013).

Perception of Drinks with a Specific Claim

Perceptions were explored further for each separate health claim. Participants were more willing to buy the drink in the "feel fuller for longer" condition, however this did

not translate into choosing the drink in the choice task. They also did not perceive the drink as more filling. Previous research has found that “fuller for longer” claims increase perceptions of fullness (Griffioen-Roose et al., 2013); De Ridder et al., 2017 found that the effects on appetite are often overestimated. However, (Bilman et al., 2012) found that consumers had an accurate understanding of satiety claims, they recognised the limited effects of such products and didn’t overestimate their effectiveness. Many consumers are unaware claims such as “fuller for longer” must be fully substantiated (Annunziata et al., 2014). The European Food Safety Authority (EFSA) states that claims relating to satiety or fullness should be supported by scientific evidence from human intervention studies (EFSA 2012). Claims relating to satiety are often viewed as marketing gimmicks rather than substantiated by authority (Griffioen-Roose et al., 2013).

Surprisingly the drink in the “high in fibre” condition was perceived as more filling and was perceived as more likely to help aid weight management than the control drink. Although participants perceptions of the claim drinks increased, participants chose the high fibre drink 30% less. Equally the “controls blood sugar condition” drink was perceived as significantly more filling, healthier, was more likely to aid weight management and participants were more willing to buy. Numerous studies have found an impact of claims on perceived healthiness (Dean et al., 2012; Gravel et al., 2012; Lähteenmäki et al., 2010; Wang et al.,2016). Previous research suggests that to be influenced by health claims consumers also need to have a positive attitude towards functional food products (Dean et al., 2012). Participants perceptions did not appear to affect their scores on the choice task which suggests there is an element of incongruence between their perceptions and choice. The differences in foods used in studies may explain different findings. It has been suggested that the inherent healthiness of the product carrying the claim has a stronger affect on perceived healthiness than the claim (Bech-Larsen & Grunert, 2003). The differences in foods

and types of claims used across studies highlights the heterogeneity. Factors other than claims may also have a greater influence on consumer perceptions of the products, such as prior knowledge and motivations (Miller & Cassady, 2015).

Personal factors Predict Perception

Fuller for Longer

Perceptions of the claim drink were explored further to see if personal factors could predict how participants perceived the claim drinks. For the “fuller for longer” condition participants who were motivated to eat to control weight and who were motivated to eat to stay healthy perceived the claim drink as more likely to aid weight management. Higher scores in eating to control weight also predicted higher scores in perceptions of health. Consumers with different health motivation might react more positively towards health claims. Studies suggested that the interaction of motivation and ability influences consumers’ health behaviours (Hung et al., 2017) as the highest level of health information processing is the result of not only high ability but also high motivation (Maheswaran & Sternthal, 1990).

Controls Blood Sugar

For the “controls blood sugar” condition those motived to eat familiar foods perceived the blood sugar claim drink as tastier, healthier, more likely to help aid weight management, they were also more willing to buy the drink. Familiarity with a food product is one of the most common predictors of food choice. Consumers often chose to ignore information on front of packs instead choosing to buy products out if habit and familiarity (Grunert et al., 2010). Whether a consumer has prior experience, or the product is considered a trusted brand by the consumer, they are more like to purchase foods (Benson et al., 2018). Verbeke et al. (2009) found that psychological

factors such as consumers' familiarity with foods carrying claims and belief in the claims were the most consistent predictors on perceptions of the products.

Older people perceived the health claims less healthy, less tasty and less likely to help them to lose weight in the blood sugar claim. This is contrary to previous findings that found that older people have been shown to be more interested in claims relating to disease/health in response to increased chronic illnesses that require changes in diet (Miller & Cassady, 2012). The findings in the current study were potentially biased as the participants who completed the questionnaire were a self-selecting sample who may have only completed the questionnaire as they have an existing interest in diet and health, which may have made them more sceptical to the claims. Alternately they may have been in better health due to their interest in diet/health and therefore had less interest in the claims.

High in Fibre

Surprisingly eating to stay healthy predicted higher ratings of taste in the "high in fibre" condition. Lyly et al. (2007) found that health motivation is particularly important for products with low palatability, as higher level of motivation might be needed to compensate for the taste. Those motivated to lose weight are more likely to accept lower calorie alternative that is less palatable than someone who is not motivated to lose weight. As long as consumers perceive trade-offs between taste and health, interest in healthy eating will be limited unless individuals are motivated (Grunert et al., 2010).

Dietary goals and weight

Dietary goals did not influence choice or perception. This is contrary to previous research that suggests that motivation and ability are key factors determining consumers' food choice (Brug, 2008). Motivation depends on personal relevance. Consumers who already experience a health-related problem are most receptive to products addressing that specific health condition (Contini et al., 2015). In the context of satiety-enhancing foods, it is clear that consumers already experiencing overweight problems (or are concerned about weight maintenance) will be more receptive to satiety claims (Painchaud et al., 2016). Nutritional knowledge may enhance responsiveness to products with a health claim (Gastón et al., 2008), posing a challenge to convince those consumers least involved and knowledgeable about nutrition and health links.

Knowledge

Higher nutritional knowledge and health claim knowledge did not predict choice or increasing perceptions of health for the drinks. Consumer health claim knowledge is associated with a correct use of health-related information (Lalor, 2011) as knowledge is related to the ability to process health claims (Miller & Cassady, 2015; Moorman & Matulich, 1993) which can influence the extent of information use (Drichoutis et al., 2005; Grunert et al., 2010). Nutritional knowledge was quite high across the sample with an average score of 80% this may have had an impact on results. Miller and Cassady (2015) found that individuals with high nutritional knowledge often rely on prior knowledge to make decisions about the healthfulness of a product. Higher levels of nutrition knowledge were also linked to less trust in health claims (Lalor et al., 2009). Knowledge is related to the ability to process health claims (Lähteenmäki, 2013), but highly knowledgeable consumers may be less motivated to process the information. Benson (2018) found that adults, who vary in knowledge, motivation, and dietary modification status, process health claims to make accurate decisions

regarding food healthfulness. However, consistent with our results, even where participants perceived the claim drinks as healthier or more filling this didn't appear to influence their choice.

Limitations

The effects of the 2 drinks were tested in isolation, although the task was similar to online shopping it is difficult to generalise the results to a real shopping situation where there is more than 1 item presented at a time. The task therefore lacked ecological validity. The products used in the task were branded and currently on sale in the UK. Participants may have had previous experience with the products therefore participants may have been rating the drinks on actual taste rather than their perception in the presence of the claim. This may have an impact in both the ratings and choice task. However, novel faux unbranded products would have created problems as participants may have rated such products low due to unfamiliarity. Participants may have perceived the specific drinks selected for the task as unhealthy rating the drink lower in both conditions irrelevant of the claim present.

Conclusion

Nutrition and health claims affected perceptions of the drinks but had little impact on choice. Consumer characteristics such as motivation to eat for weight management or health may moderate the impact of health claims on perceptions and but has no effect on choice. Providing the consumer with as much information as possible to communicate and encourage them to make healthier decisions through food labelling is important. Consumers' interest in healthy eating could be increased by adopting appropriate communication strategies on food packaging (Hung et al., 2019). Information from nutrition claims and health claims can help inform consumers about the health value of food products this in turn may help individuals to achieve a healthy, balanced diet (Tony Benson et al., 2018). However, health claims alone aren't

enough to influence food choice. Better communication about how these products are regulated will instil greater consumer confidence in health claims on drinks and may improve consumer acceptability.

Chapter 9

9. Synthesis of research findings

In order to fully demonstrate the contribution of this thesis to our understanding of acute fibre exposure and its effects on appetite and food intake through biological, psychological and behavioural mechanisms, it is necessary to consider the findings discussed above within the wider context of appetite and food intake research. The aims and key findings of the work described in chapters 3-8 of this thesis are described below. There were four key area that were focused on, the preload study design, the specific modes of action of different fibres, combing fibres with different physio-chemical properties to reduce appetite and food intake, and consumer perceptions. Firstly, a systematic review was conducted to identify any gaps in the literature. Each research chapter informs the next. This chapter collates the research findings and describes how the original contributions that arise from this thesis integrate with the existing literature to aid our understanding of appetite control. Consideration is given to the strengths and limitations of the current work and the implications and potential for future research.

The rapidly rising rates of obesity over the last several decades have been attributed to a complex interplay between biological, psychological and environmental determinants of eating behaviour (Butland et al., 2007; Vandenbroeck et al., 2007). A variety of approaches have been developed to try to tackle obesity from diets and pharmacological weight loss agents to more extreme forms of surgery. Due to the ineffectiveness and associated adverse side effects there has been an increased interest in functional foods. Fibre has been identified as a functional ingredient due to its physiochemical properties to reduce appetite and food intake (Alviña & Araya, 2016). Although research suggests fibre plays a key role in appetite regulation results are often equivocal with little consensus on their effects (Howarth et al., 2001; Slavin,

2005; Wanders et al., 2011) This thesis adds to the literature to help to understand some of the physiological processes of the metabolism as well as psychological and social processes involved in appetite and food intake.

9.1 Overview of Aims

The literature for fibre and appetite was summarised to provide a current view of how important the move towards functional foods is in the battle against obesity and to identify areas which warrant further investigation. A key aim of this thesis was to **i) Identify the specific fibre types/doses that reliably increase satiation, satiety and reduce food intake**. This aim was addressed by Chapters 3 and is discussed in section 9.2.1. The equivocal data for an effect of fibre on appetite and food intake suggested the methodology may play a part in the variable data. The current methods used to investigate the acute effects of fibre on appetite were investigated to see if the methodology could be improved, to impact on the design of the subsequent studies and potential outcomes within this thesis. Attention was focused on two key areas of the preload design which had relatively little or no attention in the existing literature the preload formulation and outcome test meal. A key aim of this thesis was to **ii) Assess the optimal study design through scaling a preload according to BMI.** This aim was addressed by Chapters 4 and are discussed in section 9.2.2. Scaling the preload according to BMI was explored to test if a scaled preload was more effective at reducing appetite and food intake in an acute study, to incorporate into the optimal study design. Another key aim was to iii) **Identify the optimal number of ad libitum test meal items to detect an effect on appetite and food intake.** This aim was addressed in Chapter 5 and discussed in section 9.2.3. There has been little attention paid to the *ad-libitum* test meal, often with very little explanation for the outcome meal chosen. The importance of the *ad libitum* test meal must not be underestimated and must be sensitive to detect an effect on appetite and food intake.

Specific fibre types/doses that increase satiation and satiety were identified and the effects of enrichment with fibres with different physical properties on satiation and post meal satiety were explored. **A further key aim was to iv) Assess the influence of meal enrichment with fibres of different physical properties on satiation and post meal satiety. To explore the relative contribution of proximal psychological and distal gut/neuroendocrine factors to prandial/post prandial behaviour to identify probable mechanisms of ingredient effects on appetite.** This aim was addressed by chapter 6 and 7 and is discussed in section 9.2.4. With this changes in appetite in response to a fibre preload were explored during an acute study, to identify the probable mechanisms of ingredient effects on appetite**.** Reliable biomarkers were utilised to determine the efficiency of different fibres for appetite control products and combined these with subjective measures of appetite and objective measures of intake. **Another aim was to v) Explore the effects of combining fibres with different physiochemical properties on appetite and food intake.** This aim was addressed in chapters 6 and 7 and is discussed in section 9.2.5. Fibres with different physiochemical properties were combined to see if they improve the outcome relative to fibres in isolation. Another key aim was to **vi) Explore consumer perceptions of products carrying fibre related health claims.** This aim was addressed in chapter 8 and is discussed in section 9.2.6. Consumer perceptions of fibre related health claims on drinks products were assessed to see if health claims predict choice and acceptability.

9.2 Overview of the main findings

The systematic review of acute studies showed that dietary fibres relevantly reduced appetite in 61.9% of comparisons and reduced appetite on average by 13%. Food intake was reduced in 57.6% of comparisons by an average of 83.1kcal (8.5%). More viscous fibres appeared to increase satiety more, whereas fermentable fibres were more effective at reducing food intake. (Chapter 3). After scaling the preload for BMI,

the participants who are obese reduced appetite, feelings of hunger and reduced total food intake in the adjusted load condition above the compensation required for the increase in preload calories (chapter 4). Furthermore, in an intervention study it was shown that food intake at the test meal significantly increased with increasing number of items for all participants at lunch. However, there were no significant differences in total food intake for the obese or normal weight participants as they compensated for the increased calories. A limited variety meal detected an effect of the fibre preload on appetite and food intake in participants who are obese. The preload significantly reduced hunger and total food intake in the limited item condition (chapter 5).

It was demonstrated viscous fibre did not affect satiety in the immediate post-ingestive period, the effects on satiation and satiety may be mediated by orosensory exposure. There was a reduction in appetite after 7h for both β-glucan and inulin in isolation, consistent with these findings there was an increase in H_2 breath production, suggesting colonic fermentation (chapter 6). Combining fibres with different physio-properties reduced glycaemic response, increased colonic fermentation and satiety, this was significantly enhanced compared to when the fibres (β-glucan and inulin) were offered in isolation. (Chapter 6 and 7). Inulin and β-glucan in isolation reduced total food intake compared to the control condition, suggesting they strengthen within meal satiation. In combination calorie intake was not significantly further suppressed beyond the combined reduction of each fibre in isolation, but changes in subjective appetite ratings did become distinct in the combined fibre condition. This suggests although appetite and food intake were not suppressed further there could still be merit in combining fibres as the fibres combined reduced appetite and food intake consistently across the day (chapter 7). Health claims did not significantly influence drink choice, however, participants chose the “maintains blood sugar” drink slightly

more than the “fuller for longer” drink compared to the control, participants chose the “high in fibre” drink less than the control drink. Personal factors did not predict drink choice. Health claims did significantly affect perceptions of the drinks and personal factors predicted those perceptions.

9.2.1 Identify the specific fibre types/doses that reliably increase satiation, satiety and food intake.

The research field was lacking a systematic review focusing on fibres in a liquid and semi-solid matrix. A systematic review was conducted to identify gaps in the current literature to focus the research. In a previous review, it was suggested that fibres are more satiating in liquid form compared to solids. This provided new insights into the variable data for fibre, satiety and food intake and identified key areas where further research may help to improve the methodology. It was found that fibres were effective in reducing appetite in 61.9% of studies and food intake in 57.6% of studies. These effect rates were much higher than previous reviews which included a variety of food matrices (Clark & Slavin, 2013; Wanders et al., 2011). In the current review clinical populations were excluded to try to exclude the heterogeneous data. In chapter 3, studies that tested up to 24 hours were included, previous reviews had excluded studies testing over 240 minutes, this was considered an arbitrary timeframe. Research has suggested that some fibres may take slightly longer to have an effect (Hervik & Svihus, 2019), for example some fermentable fibres take up to 6 hours to influence appetite or food intake (Holscher, 2017) this was confirmed in the experimental studies chapters 6 and 7. Including studies that tested over 240 minutes adds to the previous reviews to gain a clearer picture of the acute effects of fibre on appetite and food intake.

Our findings challenge the previous reviews which found that viscous fibres were more effective at reducing appetite and food intake (Wanders et al., 2011; Clark et al., 2013). In chapter 3 the most effective fibres overall were those fibres which displayed both viscous and fermentable properties. Viscous fibres were more effective at reducing appetite, whereas fermentable fibres appeared to be better at reducing food intake, though the effect size was similar for both fibres. Including studies with a longer duration may have improved the effect rate as fermentable fibres take longer to influence appetite and food intake. Equally, fermentable fibres could be more effective in a liquid or semisolid matrix. This review also reconfirms the disassociation between food intake and appetite in the literature (Yeomans, 2018). Some fibres had a significant effect on food intake with no significant differences in appetite found. This confirms the need to test using multiple measures to gain the greatest insight into appetite and food intake.

Key methodological aspects which could help to improve future research were identified. These were preload formulation, fibre dose, study duration, and outcome test meal. Liquid preloads are possibly more effective than solid preloads as they are easier to covertly manipulate (Almiron-Roig et al., 2013), with very little sensory difference and reduced risk of prior experience of the satiating impact of the product. Fibre dose varied across fibre groups, increasing fibre dose appeared to be more effective across the studies. Eight studies were tested over 24 hours with a 100% effect rate. This may suggest measuring over a longer period allows more time for fermentable fibres to have an effect and allow more time for compensatory intake to take place in the post ingestive period. Of the three studies which included participants who are obese there were no significant effects found for appetite or food intake. Previous research has suggested obese individuals are less responsive to manipulations as appetite regulation differs in obese as they exhibit weaker satiety signals (Lean et al., 2018), they are more susceptible to hedonic hunger (Gabriela

Ribeiro et al., 2018) and eating in the absence of hunger (Perez-Morales et al., 2014). No study to date had investigated whether scaling a preload for BMI could improve study outcomes for obese individuals in an acute fibre study. This was an area that needed to be addressed. Another area with very little consensus was the outcome test meal; previous studies show a large variation and little consensus to the contents. In chapter 3 test meals with the largest variety (33 buffet items) found no effect on appetite or food intake. This was identified as a second key area which warranted further investigation.

Previous research has suggested combining fibres could potentially reduce appetite and food intake (Poutanen et al., 2017). Only a few studies to date have been conducted to explore the combined effect of different fibres, some with promising results (Harrold et al., 2013), whilst others not so (Peters et al., 2009). In chapter 3, potential fibre combinations were identified examining the most effective fibres and biggest effect sizes in the systematic review. The most effective fibre b-glucan was selected, given the number of significant comparisons and effect rates. This is contrary to previous review findings which found guar gum was most effective (Wanders et al., 2011). The current review focused on liquid and semisolid preloads; studies demonstrate b-glucan is more effective in a liquid matrix. Given the different modes of action and average study duration, inulin was identified as having the biggest effect size on food intake, whilst effects on appetite were consistent. These fibres were identified as a good combination as they have slightly different modes of action and could be combined easily due to their solubility, the slightly sweet taste of inulin also had the potential to improve palatability of such combinations. Conducting this systematic review helped to inform the empirical studies in this thesis to identify new directions to begin to address the equivocal research.

9.2.2 Adjusting an inulin fibre preload for BMI significantly reduces total food intake and hunger in participants who are obese.

A second key aim addressed by this thesis was to explore whether scaling the preload for BMI in an acute prebiotic study could potentially improve the overall effectiveness to reduce appetite and food intake in participants who are obese. It was identified in the systematic review, in chapter 3, that the results for the effects of fibre on appetite and food intake were equivocal often for the same fibre. This was the first study of this kind to look at individually scaling the preload for BMI. The preload study design is often adopted as the method of choice to test the short-term effects of a preload and is widely used of to substantiate health claims; however, it is fraught with methodological issues. Studies often include participants who are normal weight and overweight/obese in the same study; this is thought to help with generalisation, yet the differences in appetite regulation for individuals who are normal weight and individuals who are obese isn't addressed in the study designs.

Chapter 4 describes the findings, whereby scaling a preload for BMI in obese women reduced appetite, feelings of hunger and reduced food total intake compared to a fixed load preload. Specifically, participants who are obese compensated for the additional calories in the preload at lunch, this is more likely due to the increase in volume rather than in response to the increase in calories or fibre dose for the adjusted load preload. The reduction in intake observed at dinner was most likely a result of the increase in fibre dose itself and the effects of fermentation on appetite markers. As anticipated, there were no significant differences for normal weight participants, the level of scaling was appropriate for both normal weight and participants who are obese suggesting that there could be a case for individual scaling of a preload for BMI in acute preload studies.

There isn't currently a directly comparable study that has successfully tested scaling the preload for BMI. Only one study to date has attempted to investigate the effects of a BMI scaled fermentable fibre preload, this was a longitudinal study. Genta (2009) investigated the effects of FOS in participants who are obese, they scaled the preload fibre dose (0.14g/kg, 0.21g/kg) in 2 separate conditions. Although they found that food intake and weight was significantly reduced over 3 months in the low dose condition, participants in the high fibre dose condition were withdrawn from the study due to adverse GI symptoms. Contrary to these findings, chapter 4 found that a 0.66g/kg dose of inulin was well tolerated in an acute study; this highlights the importance of considering the fibre type and dose and that fibre dose cannot merely be increased exponentially. Care must be taken in preload studies to administer the optimal dose, without increasing unpleasant side effects. It is unclear whether the higher dose in chapters 4 and 5 could potentially cause GI symptoms over time, this would need to be explored further in a longitudinal study.

Post-ingestive Effects Volume

The volume of the fibre preload was increased, to scale the preload according to BMI. This was something which had not been done before with a fibre preload. Increasing the volume avoided any sensory difference in taste, previous studies have demonstrated that a small difference in taste can enhance satiety (Low et al., 2014). Inulin displays a slightly sweet taste which can influence appetite, this was demonstrated in chapter 6 when the inulin preload decreased hunger in the immediate post ingestive period. Increasing the load did however also increase the calorie content due to the fibre present. Scaling the preload in such a way had not been tested. Previous studies had suggested individuals who are obese/overweight have weaker post-ingestive satiety signals (Epstein, 1996; Batterham et al., 2003). Our study challenged this assumption as the fibre preload elicited a post-ingestive response on food intake 30 minutes later. After compensation for the preload

participants who are obese reduced their food intake, however this wasn't statistically significant. Rolls et al (2010) on the other hand found that obese women are less sensitive than lean women to covert variations in the energy content of orally ingested preloads (Rolls & Roe, 2002). Equally, Parretti et al. (2015) found that a fibre preload had a significant effect on intake in the participants who are obese after just 30 minutes, however participants did not sufficiently compensate for the preload calories. Almiron-Roig et al. (2003) also challenged the current study as they found liquid preloads fail to trigger physiological satiety mechanisms so that compensation for energy consumed as beverages is imprecise and incomplete.

The results in chapter 4 suggest that the volume of a liquid preload has a greater influence on satiety than the energy content. The larger preload volume is thought to increase stomach distension and lead to a feeling of fullness. It was expected that the participants who are obese would not compensate for the increase in calories and that they would not respond to the small increase in gastric load. Previous research suggests obese individuals have a decreased ability to respond to post-ingestive satiety signals, gastric emptying can be impaired with delayed interaction of nutrients with the intestine resulting in decreased GLP-1 and PYY secretion (Wang et al., 2015). Hellstrom (2013) also produced evidence that obese individuals are less responsive to internal physiological cues indicative of hunger or satiety and that overweight people require more calories and more volume to feel fully satiated. Chapter 4 challenges this as the participants who are obese appeared to maintain their ability to respond to covert changes in volume.

Combing Measures

Chapter 4 also adds to the research as it highlighted the importance of measuring both appetite and food intake in an acute study. There was a disassociation for appetite after lunch, with no effect found. A longer time frame could be required for

the post-ingestive effects of the fibre preload on appetite to be observed (Poutanen et al., 2017). It is commonly assumed that appetitive sensations serve to link energy need with energy intake, but this is not reliably observed (Mattes, 2006). The present data reconfirms this, as despite the lack of response in appetite measures to the preload, there was a difference in the compensatory dietary response they elicited. Our findings serve to highlight the need to combine measures of satiation and satiety.

Fermentable Fibre Dose

A further finding from chapter 4 was that appetite and food intake was significantly reduced above the compensation required for the preload. The suggests that the scaled fibre dose is more appropriate for participants who are obese, their food intake was reduced relative to normal weight participants. Previous studies have demonstrated that participants who are obese may require a bigger fibre dose than normal weight participants to detect an effect (Archer et al., 2004). Karalus et al., (2012) found no effect on satiety or food intake for 10g of inulin in overweight men and women. Whereas, Perrigue et al., (2009) found a smaller dose of 6g reduced intake by 89kcal in normal weight men and women. The effects on appetite and food intake at dinner were more likely due to the fermentable fibre. This may also suggest that the time elapsed was more appropriate to test the effects of scaling a fermentable fibre preload, dinner was served 4.5 hours after the preload. These results were consistent with previous studies which demonstrated inulin increases satiety and reduced food intake after 240 minutes (Hess et al., 2011).The findings at dinner were consistent with a significant increase in H_2 breath production for inulin after 7 hours in chapter 6. It may be postulated that the increase in SCFAs as a result of the increased dose may have increased post-ingestive satiety signals, due to an increase in satiety hormones. Studies indicate inulin undergoes fermentation after 4 hours, however other studies suggest this may continue for several hours, increasing SCFA production and satiety hormones, potentially reducing appetite and food intake over

a longer period. This could explain why a further reduction in intake was observed after participants left the laboratory. When total intake for the participants who are obese was examined, there was significant reduction in total food intake. These results highlight the need to test over the entire study day to measure compensatory effects on food intake. These findings also reconfirmed the need to test for second meal effects. In chapter 3 the acute studies presented the preload either on arrival or after a short time interval of up to 60 minutes before presenting a test meal at 240 minutes, this timeframe could be too short to observe any significant effects.

Compensation

A further finding from chapter 4 was that the participants who are obese fully compensated for difference in the preload at dinner as well as total ad libitum calorie intake. The *ad-libitum* meals were served at 30 minutes and 270 minutes post preload. The significant reduction in intake and significant overcompensation took place at dinner. This is contrary to previous research that has shown that obese individuals do not compensate for calories as fully as normal weight participants. In the present study it was established that the participants who are obese did overcompensate for the calories in the preload, but this was after 270 minutes, it isn't clear whether normal weight participants would have compensated at an earlier time point as the differences in the preload for normal weight participants were not large enough to detect a difference. Insufficient energy compensation, both in the short and the long term, has been associated with increased energy intakes and positive energy balance, leading to obesity (Almiron-Roig et al., 2003). Gadah et al. (2016) highlighted that study design may affect estimated energy compensation. A high calorie preload may reduce any compensatory effects (Warwick & Weingarten, 1994), whilst a higher macronutrient composition may artificially inflate the satiety effects. Taking the results from the systematic review chapter 3, it appears easier to detect an effect of a fibre on food intake in normal weight participants in an acute study. Our

results may also suggest participants who are obese may take longer to compensate. This could explain the insignificant effects in the systematic review for obese individuals, the time frame may not have been long enough. Normal weight participants are more able to compensate after calories in a preload than participants who are obese, this confirms the previous findings from (Almiron-Roig et al., 2013).

De Graaf and Hulshof (1996) suggested the energy loads of the manipulations appear to be particularly critical. Hence, the energy differences of preloads within studies may have been responsible for yielding negative results with respect to energy compensation in some studies (Pribic et al., 2017) as highlighted in chapter 3. It was confirmed the scaled preload was effective, in chapter 7, when the BMI scaled smoothie was tested in an acute study over 8 hours with inulin and β-glucan fibre. The effects detected for inulin in isolation were however smaller than those detected in chapter 4, it could be postulated this was due to the extra calories in the smoothie. Appetite and food intake were reduced at a later timepoint and with a smaller effect size. This highlights the importance of preload formulation when designing an acute study.

9.2.3 The Effects of Food Variety on Appetite and Food Intake in Normal Weight and Obese Females.

In accordance with the stated aim in this thesis iii) **Identify the optimal number of ad libitum test meal items to detect an effect on appetite and food intake.** Chapter 5 attempted to quantify the optimal number of items to detect an effect of a fibre preload. There was little consensus as to the form, content and nutritional composition of the *ad libitum* test meal. This is the first study of this kind to attempt to establish a standardised test meal. The main findings were that overweight participants significantly reduced their food intake and appetite in the 5-item fibre condition compared to the 5-item control condition. The high variety meals were less

sensitive to detecting the effects of a fibre preload on food intake and appetite. Over consumption induced by the high variety multi-item buffet meal meant participants who are obese did not compensate in response to the fibre preloads. These results suggest the sensitivity of the test meal to detect changes induced by a prior fibre preload was altered by the composition of the test meal, when the number of items was increased. These results also suggest the test meal appears to be even more critical in an acute study with only one test meal. A poor study design with an unsuitable test meal has the potential to mask the effect of a preload which could be present (Wiessing et al., 2012). Standardising the number of items in the *ad-libitum* test meal advances this field of research as it allows for appropriate comparisons to be made across studies that investigate the same fibre types and may ultimately help identify fibres with the greatest satiating effect.

Another hypothesis tested, in chapter 5, was whether increasing items in a buffet meal increased consumption and whether participants compensated for increased intake at the next meal. This is the first time second meal effects for normal weight and participants who are obese have been tested. It has long been established that increasing the variety within a meal increases the energy consumed (Bellisle et al.,1981; Rolls et al., 1981), however the effects on the second meal are not something that has been explored. Multi-item meals are thought to increase intake through delaying satiation and meal termination. Participants who are obese consume more calories habitually (Brondel et al., 2007), but they may also be more susceptible to over consuming when a variety of foods are offered (Johnson & Wardle, 2014). Food intake at lunch increased by 26.1%, 177.3kcal for overweight participants for the 20 item lunch compared to the 5 item lunch, this is consistent with (McCrory et al., 2012) who found that exposure to a variety of foods increased intake by roughly 29%.

Interestingly in chapter 5, normal weight and participants who are obese compensated for the additional calories consumed in the 10 and 20 item lunches at the second meal. This is contrary to some studies which found faster recovery of appetite following a more palatable meal making later compensation for increased intake less likely (Hill et al., 1984; Rogers & Blundell, 1990). Previous research also suggests that individuals who are obese are at particular risk of overconsumption where there is a greater variety of food offered (H. A. Raynor & Epstein, 2001), the findings in chapter 5 support this, however participants were not less likely to compensate after the increased intake as Johnson & Wardle (2014) found. These findings highlight not only the importance of food variety through number of items offers but also the importance of the second test meal to gain further insights into food intake behaviour.

Only one study to date has investigated variety in the *ad-libitum* test meal. The findings in chapter 5 are contrary to the findings of Wiessing et al. (2012) they used a high calorie preload to test if 15 item buffet meal was better able to detect changes in appetite than a single item meal. They found that there was no difference between the single item meal and multi-item buffet in terms of detecting an effect on appetite or food intake. In chapter 5, the multi-item buffet meal increased variety and consequently palatability. Participants increased their intake in the multi-item condition EI (+ 39%) over the 30 min lunch period but were still able to compensate for the high 4 MJ calorie preload similarly to the low 0.5MJ preload. It was notable that the compensatory response to the breakfast preloads occurred despite the predictable energy overconsumption induced by the multi-item buffet. This study compared a single isocaloric meal to a buffet meal. These meals are fundamentally different. In chapter 5 the effects of variety were directly compared using the same types of food, just increasing the number of items on offer.

This was the first study to include similar buffet items in the limited item meal rather than serving a large single isocaloric course meal as in the Wiessing et al. (2012) study. The differences in the meal types introduces further variables. The portion size offered in the large single-course *ad-libitum* meal needs to be large enough for satiation to be reached, rather than plate clearing. Individuals tend to overestimate the amount of food they would typically consume as normal intake is inflated when larger portions are served, leading to the portion size effect (Zuraikat et al., 2019; Diliberti et al., 2004;, Rolls et al., 2004). In chapter 5 serving the food as a limited buffet reduced the effects of plate clearing, whilst reducing the effects of variety; this methodology has the potential to improve the accuracy of measuring satiation in the laboratory.

With reference to the satiety cascade, it is evident that sensory and hedonic factors have an important role in meal termination. It is well documented that the palatability of a food has a positive effect on the amount eaten in both normal weight and individuals who are obese (Brondel et al., 2007; Johnson, 2014). Consistent with this, in chapter 5 as palatability increased with increasing items, participants found the 20-item meal significantly tastier than both the 10-item meal and the 5-item meal. Increases in flavour pleasantness appeared to result in appetising effects to drive short-term overconsumption in the high variety meals (Sorensen et al., 2003). Participants who are obese consumed similar amounts in the 10 and 20 item conditions and did not compensate after the fibre preload. It is likely the multi-item meals increased intake through delaying satiation and meal termination (Hetherington et al., 2006). The effects of the fibre preload on appetite and food intake were reduced in the high variety meals. This also suggests the increased items likely swamped any effect of the fibre, weakening the effect on appetite, through overloading the appetite sensations and so decreased the sensitivity to detect changes in eating behaviour.

This finding is far reaching in terms of food intake research and could explain the equivocal results found in chapter 3.

Palatable foods are thought to influence intake through the activation of hedonic motivational pathways (Egecioglu et al., 2011; Yeomans et al., 2004). Interestingly palatability has a greater effect on intake in a satiated state than in a hungry state (Yeomans et al., 2001), suggesting that although homeostatic mechanisms dominate in the hungry state, hedonic mechanisms become more important once homeostatic needs are met. In chapter 5 participants were instructed to eat until they were comfortably full, however the 10 and 20 item meals may have encouraged participants to overconsume once their homeostatic needs had been met, thereby delaying satiation. This was reflected in a significant reduction in hunger immediately after lunch compared to the 5 item lunch for both normal and participants who are obese. Yeomans (1996) found that exposure to palatable foods reliably increased self-reported appetite, with ratings of hunger actually increasing during the early stages of a highly palatable meal, this may encourage over consumption and lead to greater feelings of fullness, our results appeared to be consistent. Studies that measured liking for foods, report that ratings for liking are higher when hungry, this indicates some overlap between the hedonic and homeostatic motivation to eat (Gearhardt et al., 2011; Finlayson et al., 2007; Finlayson et al., 2008).

In chapter 5 participants surprisingly didn't consume more than 10 individual items in the 20-item condition. Previous research investigating the effects of meal variety have centred around the physiological and psychological processes that promote meal termination sensory-specific satiety (SSS) (Brondel et al., 2009; Raynor & Epstein, 2001, Rolls et al., 1981; Rolls et al., 1984). (Hetherington et al., 2006; Brondel et al., 2009; Rolls, 2007). The increase in intake from 10 to 20 items was more likely to be explained by food liking than SSS. The delay in meal termination was possibly due

to an increase in liking as the 20-item meal was rated higher for palatability. This highlights the importance of food liking when designing the test meal. It is essential participants are screened for food liking before participating. Our results for food liking are consistent with research that demonstrates within meal variety does not necessarily influence sensory specific satiation (Hendriks et al., 2019).

Chapter 5 highlights that food liking is even more essential where the test meal on offer is a limited item meal, if participants dislike the foods on offer, they are unlikely to reach satiation, which would render the results inaccurate. Given the significant reduction in both appetite and food intake in the 5-item adjusted meal this would suggest participants reached satiation in the 5-item meal rather than SSS terminating the meal. The limited item meal is not without its limitations, and these must be carefully considered when designing future food intake studies. Where multiple treatments are completed with little or no choice, this can lead to rapid onset of sensory specific satiety and is likely to suppress intake relative to a multi-item meal (Brondel et al., 2009). In chapter 4 participants ate to satiation, food liking was thoroughly assessed at the screening, with participants excluded if they disliked a single item on the limited item meal, this is something recommend for future studies.

Inconsistencies when investigating dietary fibre as an appetite suppressant in the acute study setting may continue to give rise to conflicting findings if non-standardised eating protocols continue to be adopted (Gibbons et al., 2019). The goal of any appetite-suppressing food is to reduce energy intake; yet as highlighted in chapter 3, although subjective appetite feelings may imply satiation, this does not always translate into a reduction in food intake. The test meal parameters should be considered more frequently. Standardising the *ad libitum* test meal as evidenced in chapter 5 will allow for appropriate comparisons to be made across studies that investigate the same fibre types and may ultimately help identify fibres with the

greatest satiating effect. Because, hypothetically, acute satiating effects seen in the short term could translate into longer-term effects, standardisation of the above parameters should help to identify efficient satiating fibres to be investigated in the long term. This approach may strengthen the research for the role of fibre in appetite and food intake regulation.

9.2.4 Identify Probable Mechanisms

In accordance with the aim stated in this thesis iii) **explore the influence of different fibres in the preload on satiation and post meal satiety and to decipher to contribution of proximal psychological and distal factors gut factor on prandial/post prandial behaviour, to identify probable mechanisms of such effects.** This aim was addressed in chapters 6 and 7, initially, the effects of the fibres in isolation will be discussed. In chapter 3 more viscous fibres in a liquid or semi solid matrix were more effective at reducing appetite, whereas fermentable fibres were slightly more effective at reducing food intake in an acute study.

Chapter 3 identified soluble fibres which warranted further investigation; b-glucan a viscous fibre which can also be fermented in the colon and inulin a fermentable fibre. Incorporating the results from chapters 4 and 5 to optimise the methodology the different modes of action of each fibre in isolation and combination were explored. Appetite measures and biological markers were first explored in chapter 6. A second study (chapter 7) followed a similar protocol to measure the impact of these fibres on food intake. This is the first time to our knowledge that biomarkers and food intake have been explored in parallel in this way, these data provide a more comprehensive assessment. Conducting 2 separate studies also allowed the effects on biomarkers to be tested over a longer time period in the laboratory, to explore the different modes of action of each fibre in isolation and combination.

Proximal psychological

There is a temptation to ignore sensory and cognitive measures of satiety in favour of more advanced physiological measures, however it was demonstrated in chapters 4, 5, 6 and 7 the importance of considering the cephalic phase of eating as well as the metabolic effects. The preload drink was formulated for each study, carefully considering the sensory aspects to match each drink with a control drink. In chapter 6 and 7 surprisingly there was no effect on appetite in the immediate post-ingestive period following the β-glucan breakfast preload. This was surprising as beverage viscosity has been inversely related to postprandial hunger (Mattes & Rothacker, 2001). The smoothies were matched on dimensions of taste and texture. Previous results for the immediate effect of viscous fibres have suggested the sensory differences between the test preload and control preload may enhance some of the effects found (Chambers et al., 2015).

Texture

In chapters 6 and 7, 4 test preloads were formulated to have the same 'thick' texture, the orosensory exposure to the drinks would not have differed between the preloads. Orosensory exposure to food is thought to trigger anticipatory responses (Yeomans et al., 2005), and these associations are likely to influence explicit expectations about the effect a food or drink will have on appetite (K. McCrickerd & Forde, 2016), including how filling a food is likely to be (expected satiation) and the extent to which it will stave off hunger until the next meal (expected satiety). Studies indicate that drinks with a thick consistency suppress hunger to a greater extent than equicaloric flavour matched thin versions (Zijlstra et al., 2009). The sensory characteristics of a beverage interact with its post-ingestive effects to influence satiety. Yeomans and Chambers (2011) reported that when participants consumed a low-energy drink with thick and creamy sensory characteristics participants ate less at the test meal than after the low-energy version, without the enhanced sensory context. The sensory

characteristics predicted the delivery of nutrients, generating expectations that these drinks would be filling, which acted to enhance the experience of satiety. It was anticipated the viscous fibre would reduce appetite in the immediate post ingestive period however, there was no significant difference in appetite. In chapters 6 and 7, the control drink may have increased sensory signals via the increased viscosity, in both the test drink and control drink, to mediate satiety in both conditions, which may have reduced the observed effects of the fibre present. Sensory aspects of the preload may override any physiological effects. This highlights the importance of formulating the test drinks and also suggests texture in drinks could be used as a functional characteristic to enhance satiety.

Palatability

A further finding, in chapter 6 and chapter 7, was that Inulin significantly reduced appetite immediately after breakfast, this was consistent with the peak glycaemic response. This was a surprising result given inulin's physiochemical properties. However, consistent with our findings Hess et al., (2011) found that 15g of inulin added to a hot chocolate drink significantly reduced appetite shortly after ingestion. Although the inulin smoothie, in chapters 6 and 7, did not significantly differ on any dimension of taste there was a difference observed in the sweetness. Evidence suggests that the sweet taste signalling mechanisms identified in the oral cavity operate in the gastrointestinal system and may influence the development of satiety (Low et al., 2014). The cephalic-phase of appetite regulation anticipates the ingestion of food and responses are then generated in many parts of the gastrointestinal tract (Smeets et al., 2010). This could explain the findings in chapters 6 and 7, as there was a significant difference in desire to eat 1-hour post breakfast in the inulin condition.

These results demonstrate that the sensation of satiety is dependent on more than just the metabolic effects of nutrients in the gut. These early satiety signals will integrate with post-ingestive and post-absorptive signals to determine satiety. Blundell and Halford (1994) conceptualised this in the satiety cascade model, they proposed that even before food arrives in the gut, cognitive and sensory signals generated by the sight and smell of food, and by the orosensory experience of food in the oral cavity will influence not only how much is eaten at that eating episode (satiation) but also in the period after consumption. Pre-ingestive sensory and cognitive signals signify the imminent arrival of a nutrient load, the body's rapid response to this information is to physiologically prepare for the efficient digestion, absorption and metabolism of nutrients (Chambers, 2016). This highlights the importance of formulating the test and control preload in acute studies, even small sensory differences in the preload formulation can influence the results. Equally, when formulating satiety enhancing products the taste and texture are essential to enhance post ingestive effects.

Nutrient and hormonal signals

In Chapter 6 it was anticipated the viscous fibre would mediate postprandial glucose response and delay gastric emptying rates, via an ability to form viscous mixtures in the GI tract, to induce feelings of fullness and increase satiety (de Graaf & Hulshof, 1996). Although there was a reduction in postprandial glucose response immediately after the preload, this wasn't statistically significant, however peak blood glucose concentration was reduced, and blood glucose remained stable until before lunch. Despite this, there were no significant differences observed in appetite in the immediate post prandial period up until lunch. This suggests there was a physiological effect, yet this didn't induce feelings of fullness or increased satiety. This is consistent with the results of Liu et al. (2012) they found that despite a reduction in glycaemic response there was no effect found on appetite. The inulin condition did not stabilise the glycaemic response and blood glucose fell below the control line

before lunch time. This is contrary to Lightowler et al. (2018) who found that inulin was an effective strategy to reduce postprandial blood glucose response. This may suggest that the study had some limitations that should be addressed in future designs. The similar preload formulation in the control smoothie potentially interacted with the post-ingestive effects to influence satiety in the control smoothie. Equally, the structural and functional differences of the fibre could have affected the results for the fibres in isolation.

Colonic and metabolic signals

The fermentation rates of both fibres in isolation were distinct and indicated an effect after 4 hours. In chapter 7 there was a significant reduction in hunger for inulin just 3 hours post preload, this was a surprising result as it was anticipated the bioactivity of the fermentable fibre would take longer to have an effect, as the systematic review in chapter 3 found. Queenan (2007) found that inulin is fermented more rapidly between 0 and 4 hours and reaches a peak at 8 hours and fermentation continues over 24 hours, whereas β-glucan shows signs of fermentation at 4 hours but ferments more steadily between 8 and 24 hours (Queenan et al., 2007). For β-glucan a reduction in appetite was not observed until 7 and 8 hours post preload. Our results are however consistent with the fermentation rates of inulin and β-glucan. In chapter 6 there was a large increase in H_2 breath production at 4 hours for inulin whereas β-glucan the increase was much more subtle. This suggests that β-glucan may require longer for its fermentation properties to have an effect on appetite. This highlights the need to test over a longer period and supports the second test meal when measuring food intake.

The results for food intake (Chapter 7) were consistent with the reduction in appetite, there was a significant reduction in food intake at dinner for both inulin and β-glucan. β-glucan reduced intake by 97.6kcal (3.9%) while inulin reduced intake by 111.9kcal

(4.4%), this was consistent with our findings in the systematic review chapter 3. The findings for β-glucan were also consistent with Beck et al. (2009); they tested seven male and seven female (BMI 25-36 kg/m) and found β-glucan increased subjective satiety at a dose of 2.2 g. Subsequent meal intake decreased by 95kcal with higher β-glucan dose of 5g. Our results for food intake for the Inulin fibre in isolation, meanwhile, were consistent with the results of Cani et al. (2006).They reported that 16g a day of oligofructose, an inulin type fructan, led to enhanced satiety and reduced energy consumption of 120kcal 5.3%, in an acute study. The significant reduction in food intake is likely to be due to the fermentable properties of each fibre. It should be noted that the effects on intake were observed eight hours after the experimental manipulation suggesting bioactivity took eight hours to manifest. This would suggest satiety effects which may involve distal small intestine/colonic mechanisms (Mattea et al., 2018). Post absorptive signals generated by the colonic fermentation of inulin and β-glucan could account for the later observed satiety effects. Inter-meal intervals of 6 h or more may be more suitable for fermentable fibres that are suggested to influence appetite processes via the production of short-chain fatty acids produced as a result of colonic microbial fermentation and the subsequent release of GLP-1 and PYY (Adam et al., 2014). Chapter 7 supports the need to observe second meal effects. The fibre may take longer to have an effect or may modulate not only the first subsequent meal after consumption, but also later meals on the same or even subsequent day (Ibrugger et al., 2014).

Chapters 6 and 7 have highlighted several key points which will aid future research. Using a variety of measures has provided greater insight into the mechanistic study of fibres, with different physiochemical properties, to dissect the impact of individual ingredients in explaining the observed time course of effects on appetite and food intake. Although no immediate post-ingestive effects for β-glucan were found, as

anticipated, these results highlight how such potential confounding factors of manipulating liquid preloads must be considered. Previous research suggests liquids are easier to manipulate however this study has demonstrated how small differences in the preload formulation can have a large impact on the outcome variables. Chapter 6 and 7 demonstrates that regulation of appetite works in concert with oral, gastric, intestinal, and post-absorptive mechanisms.

9.2.5 Synergy Effects

In accordance with the stated aims of this thesis, in chapters 6 and 7, the effects of inulin and β-glucan were explored further to see if there were any potential synergistic effects on appetite and food intake. This is the first time a study has examined the acute effects of a combination of fibres on intake and appetite, using best practice approaches, with a combination of measures taken across the day. Exploring the fibres using a variety of measures has provided a clearer picture of the mechanisms underpinning the changes observed with the fibres in combination. In vitro studies had shown that combining fibres had a positive effect on fermentation and production of SCFAs (Lecerf et al., 2012), however, it wasn't clear if these findings would be found in a human study. In a systematic review Salleh et al., (2019) identified synergy effects a key area of research that warrants further investigation. Methodology from chapters 4 and 5 was incorporated to investigate the effects.

In chapter 6 there was a additive effect on biomarkers and appetite with a significant reduction in appetite 8 h post preload, 3.5 h post preload for hydrogen breath production and 4 h post preload for glycaemic response in the combined fibre condition relative to the fibres in isolation. There were no effects found for the fibres in isolation compared to the control at the same time points. Chapter 7 however, there was no additive or synergy effects on appetite or food intake. β-glucan and Inulin

exerted a similar effect in isolation compared to the fibres in combination, although at a lower magnitude and often not statistically significant, suggesting the fibres in isolation produce weaker effects on satiety. Combining inulin and β-glucan enhanced these effects in chapter 6 indicating that the combination produced the strongest effect on appetite and biological markers. Often the effects of combinations of ingredients are no greater than their respective components. A synergy effect on food intake may have been apparent had a low-calorie preload been formulated. There was a considerable number of calories in the combined fibre preload which required a large overcompensation, equally had the fibre been tested over a longer period of time there may have been a synergy effect on food intake. It may be intuitive to believe the additive effect of this combination was entirely predictable; however, the additive effects of ingredients cannot be assumed, particularly where they are provided in a formulated smoothie.

Surprisingly, the combined fibres appeared to reduce hunger earlier in the day in the combined fibre condition compared to the fibres in isolation. The initial reduction in appetite in the post-ingestive period could have potentially been due to the increase in mass and calorie content in the smoothie rather than a direct effect of the fibres themselves. It is plausible though, as calories in beverages are difficult to detect and elicit a weaker satiety response than solids (Campbell, & Mattes, 2007; Tieken et al., 2007).

Only one previous study to date has combined β-glucan and inulin to observe an effect on appetite and food intake (Peters et al., 2009), our findings are contrary to this study. Peters et al (2009) found that the addition of β-glucan 1.8g, FOS 16g, or a combination thereof in a meal replacement bar did not affect appetite ratings or food intake. The low dose of β-glucan, preload matrix and timings may have explained the lack of effect. In chapters 6 and 7, a larger fibre dose in a liquid preload was served

at breakfast, as opposed to a solid preload served at 3 time points. In Chapter 3 fibres were more satiating when provided as a liquid. A larger fibre dose provided in a single dose at breakfast may have enhanced the effects. Further, chapters 6 and 7 included oat β-glucan as opposed to barley β-glucan, therefore there could have been variation in the physio-chemical properties. Viscosity depends upon the solubility as well as the molecular weight of the fibre and is an important determinant of the physiologic response (Rebello et al., 2016). It is also possible variations in the fibre processing differed to the current study as the fibre processing and characteristics interact with the human GI tract to influence the physiological effects (Poutanen et al., 2017). Key weaknesses were identified in the previous study to help design the studies in chapters 6 and 7.

This is the first time the synergy effect for inulin and β-glucan has been explored with a combination of biological, psychological and behavioural measures. In chapters 6 and 7 the slightly different modes of action may explain the significantly reduced hunger and appetite throughout the test period in the combined fibre condition. There was also a significant reduction in glycaemic response for the combined fibre condition post breakfast and pre-lunch, where there was no effect found for the fibres in isolation. The combined fibres significantly reduced glycaemic response 4 h after the preload compared to both the inulin fibre in isolation and the β-glucan fibre in isolation. This suggests there was also an additive effect on glycaemic response in the combined fibre condition compared to the fibres in isolation. Plasma glucose did not return to the baseline after the 4-hour testing period and remained significantly higher in the combined fibre condition pre-lunch. A significant increase in H_2 production 3.5 h post breakfast was also observed compared to both fibres in isolation, greater than the combined effects of the fibres in isolation, which indicates a additive effect on fermentation. Hunger ratings were consistent with the reduced glycaemic response before lunch and a large rise in H_2 production pre-lunch, it is

unlikely the changes in hunger observed at this timepoint (4 hours post prandial) were related to the differences in the preload calories and mass, but rather the physiological effects of the combined fibres. Reductions after the test lunch may have been due to the fermentable action of both fibres. Fermentation of fibres are thought to influence satiety (Hervik & Svihus, 2019) through production of SCFAs, products of colonic fermentation, influence postprandial glucose response by reducing fat competition for glucose disposal (Brighenti et al., 2006). Fermentation SCFAs influence gut hormones and gastric motility (Nilsson et al., 2008) they also influence postprandial glucose response by reducing fat competition for glucose disposal (Brighenti et al., 2006).

Although the studies in chapters 6 and 7 are not sufficient to support satiety claims as evidence of prolonged efficacy is required, they do provide valuable proof-of-concept data. When developing products targeted to impact on appetite, the usual approach is to initially examine for acute appetite suppressing effects before examining for enduring effects and weight management potential. Providing the smoothie with a meal has demonstrated the preload is effective when consumed with other foods, as Blundell stated this is an important test of real-life application. Measuring biomarkers for a longer period has gleaned more inciteful results as the bioactivity of the preload is measured over a longer period of time. Understanding the mechanisms involved has enhanced our knowledge of different fibres and could lead to the development of more effective functional beverages using this fibre combination, the methodology also paves the way to test other fibre combinations.

9.2.6 Consumer perceptions of drinks carrying fibre related health claims.

A further key aim of this thesis was to explore consumer perceptions of drinks carrying fibre related nutrition/health claims. In chapter 8, a questionnaire study was conducted to explore the effects of fibre related health/nutrition claims. Incorporating

the findings from chapter 6 and 7 a “fuller longer”, “maintains blood sugar levels” and “high in fibre” claim was incorporated on a variety of different smoothies and juice drinks to test how drinks carrying these claims might be perceived by the general public. This was the first study to look at perceptions of nutrition/health claims specific to fibre on a drink. Health claims have not been extensively researched so this research was conducted to address the gaps in knowledge regarding functional beverages, consumer choice and perception. In chapter 8 results suggested that overall claims did not influence choice, however they did influence perception. This is contrary to the findings of Kaur et al., 2017 who conducted a systematic review and found that health claims influenced food choice. Previous research suggests that there are lots of personal factors that affect food choice (Leng et al., 2017). Consistent with these findings personal relevance of the claim was key with claims specific to fibre. This study adds to the literature as it demonstrates that when the carrier product is a functional beverage, claims may not be as effective as when placed on other food products.

Participants chose the fibre claim 30% less than the control drink, it isn’t clear whether participants didn’t believe the claim in the content of a drink or whether the level of health claim itself had an impact on choice. The general level health claims were slightly more effective with fuller for longer claim chosen 6.4% more and the maintains blood sugar levels 20.2%. The blood sugar claim was most effective, however there was no significant effect of claim on choice. Surprisingly despite the low score on the choice task the “high in fibre” condition was perceived as more filling, healthier and more likely to help aid weight management than the control drink. Claims had no effect on perceptions of taste, this is contrary to previous findings which found products with health claims are perceived as less tasty (Vadiveloo, Morwitz, & Chandon, 2013).

The “fuller for longer” claim was the least effective of the 3 claims tested, participants did not perceive the drink as more filling. Beverages are not commonly associated with satiety; this perception may explain the results. Personal factors had a large impact on how well products carrying health claims are perceived. Those who were motivated to eat to control weight and to stay healthy perceived the claim drink as more likely to aid weight management. Higher scores in eating to control weight also predicted higher scores in perceptions of health. Motivation and ability are key factors determining consumers’ systematic processing of the claim message (Brug, 2008). Consumers with different health motivation may react differently to health claims. Studies suggested that the interaction of motivation and ability influences consumers’ health behaviours (Hung et al., 2017) as the highest level of health information processing is the result, of not only high ability, but also high motivation (Maheswaran & Sternthal, 1990). A consistent finding is that consumers who already experience a health-related problem are most receptive to products addressing that specific health condition (Hung et al., 2017).

Another key finding in chapter 8 was that nutritional knowledge had no effect on choice or perception, this was a surprising result given consumer health claim knowledge is associated with a correct use of health-related information (Lalor, 2011) and is related to the ability to process health claims (Miller & Cassady, 2015; Moorman & Matulich, 1993). In chapter 8 nutritional knowledge was high across the sample, this may have affected the results. Nutritional knowledge is thought to enhance responsiveness to products with a health claim (Benson et al., 2019), however Miller and Cassady (2015) found that individuals with high nutritional knowledge often rely on prior knowledge to make decisions about the healthfulness of a product. Higher levels of nutrition knowledge were also linked to less trust in health claims (Lalor et al., 2009). The high nutritional knowledge in chapter 8 may explain the lack of effect on choice, participants high level of knowledge may have led to less trust in the claims.

Knowledge is related to the ability to process health claims (Lähteenmäki, 2013), but highly knowledgeable consumers may be less motivated to process the information.

This was the first study to look at nutrition and health claims specific to fibre in a functional beverage. Drinks purporting to be healthy have received negative press in recent years with concerns over the sugar content in these so called “healthy drinks”. Claims relating to satiety such as “Fuller for longer” are often perceived as a gimmick, used by manufacturers to market products, rather than a substantiated claim (Griffioen-Roose et al., 2013). Trust in health claims, coupled with the negative connotations associated with functional beverages, may explain the lack of effect observed in chapter 8. However, despite this those who are motivated to eat for weight and health perceived the products as more likely to help them lose weight. Work is required to change these negative connotations before functional beverages are widely accepted. Those motivated to eat for weight and health could be a key market to target. A further study focused on individuals motivated to eat for health and weight management may demonstrate different results in the choice task. There needs to be a marketing incentive for the food industry to reformulate existing products to add functional ingredients. This research, despite the lack of an effect on overall choice, suggests there is the potential market for such products, but personal motivation is key.

9.3 Strengths

There are several key strengths in this thesis which enhances the results to improve the overall conclusions that can be drawn. The research described in this thesis comprises a broad range of study designs. A systematic review was performed (chapter 3), four intervention studies (chapters 4, 5, 6 and 7) and a questionnaire study (chapter 6). Moreover, the outcome measures varied per study; satiation was studied (chapter 4, 5 and 7), satiety (chapter 4, 5, 6 and 7), energy intake (chapter 4,

5 and 7), biological markers; hydrogen breath and glycaemic response (chapter 6) and consumer choice and perceptions (chapter 8). To our knowledge, the literature review was the first review that systematically and quantitatively summarised intervention studies relevant to the effects of dietary fibre classes on satiety and energy intake in liquids and semi solids. The review generated new hypothesis which were tested in the subsequent studies.

Combined Methodology

Not only were designs combined across studies, each study combined a variety of methods to gain the greatest insights into the effects of the preload on appetite and food intake. Intake measures alone give only a very crude assessment of appetite, the clearest answers may be seen when multiple measures are taken and used in combination (Yeomans, 2018). As the results demonstrate, testing the effects of the fibre preload on appetite and biomarkers in a separate study (chapter 7), created the opportunity to measure biomarkers over a longer period without food *ad libitum* intake interfering with measures. Not only this, serum blood glucose concentrations are often collected in tandem with food intake and satiety ratings using sequential blood draws at time points that overlap with subjective appetite ratings (Forde, 2018). The very nature of drawing blood at regular intervals could impact on appetite and food intake measures, conducting two separate studies removes such confounding factors.

Optimal preload design

In chapter 3 two key methodological issues in the literature were identified. In chapter 4 we explored scaling a preload drink for BMI in an acute preload study. A BMI scaled preload improved the outcome, reduced appetite and food intake in participants who are obese. In chapters 6 and 7, the scaled preload methodology was successfully utilised in two acute studies. Although highlighted in several review studies, the preload test meal is yet to be validated. The preload test meal can vary on many

dimensions in terms of variety, palatability and volume. In chapter 5 the optimal *ad-libitum* buffet test meal was explored to try to improve the chances of detecting and effect of a fibre on appetite and food intake in an acute study. First, a literature review was conducted to assess how the test meal varies across studies. After assessing the data, 3 meals were tested as part of a 5-item limited variety buffet meal, a 10-item medium variety buffet meal and a 20-item high variety buffet meal. The optimal number of items to detect an effect on appetite and food intake was verified. The limited item meal was more sensitive to detect an effect. This test meal was included in chapter 7.

Longer Test Session

It was identified in chapter 3 that an inter-meal interval of 6 h or more may be more suitable for the effects of fermentable fibres to be observed, this was confirmed in the experimental studies. Fermentable fibres influence appetite processes via the production of short-chain fatty acids, produced by colonic microbial fermentation, and the subsequent release of GLP-1 and PYY (Rahat-Rozenbloom et al., 2017). Second meal effects were tested in a subsequent meal and the remainder of the test day in chapters 4, 5 and 7. Some papers suggest fermentable fibre may modulate not only the first subsequent meal after consumption but also later meals (Ibrugger et al., 2014; Poutanen, 2017). Testing over a longer time interval has gained better insights into the mechanisms underpinning the effects of fibre on appetite and food intake. In chapters 6 and 7 the fibres in isolation had little or no effect on appetite or food intake after 240 minutes, yet there was a significant reduction in dinner food intake 8 h after the preload.

Real Application

In chapters 6 and 7 the fibre preload was served as a component part of the breakfast meal. Serving the preload with other foods provided a more realistic measure of the

effects of preload. Previous research has suggested the effect of a preload on satiety cannot be considered, based on the effect of one component in isolation, as the effect observed may change when the preload is consumed with other foods (Blundell et al., 2010). The effect of a preload is dependent on what it is ingested together with (Hervik & Svihus, 2019). The preload in chapter 6 and 7 was effective when presented with a meal.

9.4 Limitations

There are some limitations to the research described in this thesis, which should be considered when interpreting the findings and when designing studies to address the further research questions raised by this work. The current thesis yields several methodological limitations that should be addressed. There were limitations inherent to measuring appetite in the laboratory, but also some study specific limitations.

Limitations inherent to Measuring Appetite

Measuring appetite and food intake in the laboratory environment lacks ecological validity. In chapters 4, 5, 6, and 7 participants consumed each test meal in isolation, in the laboratory without any other social/environmental influences. The meals were also fixed at specific times, eating patterns in the natural environment are not so arbitrary. Quite often the onset of an eating occasion will be in response to hunger and physiological processes (Yeomans, 2018). Presenting food at such fixed time points invites participants to eat when they would not necessarily want to, in opposition to feelings of hunger. The fixed meals during test days were served at roughly 9am, 1pm and 5pm. These timings could be at variance with regular eating patterns of participants. However, these very limitations give the level of experimental control needed to elucidate the complex factors controlling appetite and food intake.

Data recorded under ‘real’ conditions might be expected to be more representative. Yet it would be difficult to partial out the confounding variables in a naturalistic setting, which lacks accuracy and control. Laboratory studies need to be considered with some caution, but arguably the best approach to elucidate mechanisms for a proof of concept study is to test under controlled laboratory conditions and then use longer-term studies to further check the validity of the laboratory findings and improve the generalisation of the results (Gibbons & Blundell, 2019). The effects found in chapters 6 and 7 would therefore require further investigation to investigate if the effects are observed outside the laboratory.

Although the preloads in the experimental studies were covertly manipulated participants were aware that their intake was being measured. Ethically (and practically) it would be impossible to administer an intake test without informed consent. This raises critical questions about data interpretation and the conclusions that can be drawn from laboratory food intake studies. If participants were aware their intake is being monitored, this knowledge could potentially influence behaviour. Research has shown females may be particularly susceptible to reducing their energy intake because of elevated self-presentation concerns. In the current thesis concealing that eating behaviour was being measured and observed may have had an impact on results. Although the experimenter was not present when the participants were eating, the participants were aware that their intake was being measured. In a systematic review Robinson et al., (2015) raised concerns over the ecological validity of laboratory feeding methodologies where participants are aware food intake is the measure under investigation. They suggested the use of a cover story to mitigate the impact of heightened participant awareness in the laboratory may improve the conclusions drawn from eating behaviour research. Future research should consider participant awareness and try to strike a balance between

participant's fully informed consent and reducing the impact of participant's awareness of their eating behaviours being observed.

Limitations Specific to this Thesis

The experimental studies described in Chapters 3,4,6, and 7, were a female-only sample, and thus findings provide limited applicability to male populations. Nonetheless, as these were preliminary studies, it was necessary to minimise the effects of individual variation. Indeed, gender differences have been observed with regards to appetite and food intake (Hess et al., 2011), further to this the systematic review in chapter 3 found no effect in the all-male comparisons.

The study sample sizes were based on previous studies, power calculations were used to confirm that each study had enough statistical power, however, a larger sample size could have improved the results. Time constraints due to the number of full test days required for each individual participant with an acceptable washout period meant that more participants could not be tested. The within-subject design removes individual variability in the data but is more difficult to recruit participants who are willing to commit to full days in the laboratory. Effort was made to recruit participants from outside the university population to reduce bias in terms of age and education level. Recruitment was restricted by geographical area due to the constraints and demands of the laboratory studies. Although we recruited outside the university, it may still be difficult to generalise these results to the rest of the population.

In chapter 6 participants had to remain in the laboratory for the full 6 hours of the test session, however participants were free to leave the laboratory during the intrameal interval in chapters 4, 5, and 7. It cannot be confirmed participants complied to the protocol (i.e., no other foods or beverages) when they left the research unit. This could

potentially explain the varied results found for appetite in chapter 6 and 7 as there was a n additive effect found in chapter 6 but this wasn't apparent in chapter 7. Chapter 6 participants remained in the laboratory; therefore, it was possible to confirm that participants followed the strict protocol. Allowing participants to leave the laboratory assisted with participant retention as they could continue their day as usual between test meal sessions. Food and activity diaries were used to as a tool to check that participants adhered to the overnight fast, however it cannot be confirmed all participants adhered to this.

The questionnaire study tested the effects of the health claims in isolation without other products present to choose from. Whether this would be ecologically valid compared to a real shopping task is unclear, however this method was comparable to an online shopping experience and has been used in previous studies. Well, known branded drinks were included in the questionnaire study, participant's prior experiences may have confounded results on both choice and perception tasks. However, had we used faux products the unfamiliarity of the products may have also had an impact on both choice and perception.

9.5 Future Directions

As the findings of this thesis show, despite significant research into fibre, satiety and food intake, there is still a portion of conflicting data on the effects of fibre. This work has added substantially to this area of research to 1) improve the methodology of future research studies and 2) explore the potential to exploit fibres with different physio-chemical characteristics to improve the effectiveness of fibres to develop functional beverages and 3) Explore consumer perceptions of health claims on beverages. More research is needed to continue to advance this field further.

Additional work is also needed to explore the optimal test meal design. Food liking is a key aspect for measuring food intake. Future research designs may offer participants a choice of foods and present the limited item buffet with those foods. This approach would not only reflect usual eating patterns more closely, but it would also reduce the impact of individual differences in food preferences, whilst removing the confounding effects of presenting a variety of items. Food liking appears to play a key role in appetite control. An individual will eat to satiation if they like the foods. Further investigation is needed to explore the contents of the test meal further, an individual may overeat their "favourite food" however if their second food choice is presented this might provide the right amount of liking to reach satiation, without encouraging over consumption. This idea could be developed further to provide a more realistic eating experience. Future research may look to offer participants a menu to select their meal from. This would maintain the high level of control needed in the laboratory yet remove any potential for the limited food items presented to reduce intake, but also provide the realistic element of choice which individuals have in the free-living environment.

In chapter 6 it was demonstrated that combining fibres had an additive effect on appetite and biological markers but had no enhanced effects beyond the fibres in isolation on food intake after compensation for the preload (chapter 7), however it is not clear whether the results found were limited to acute studies. Whether such products could be developed to use over a longer period is unclear. More work is needed specifically with participants who are obese to explore the differences in appetite control to see if the combination of ingredients reduces appetite and food intake over a longer period.

Further research is needed to look at the different physiochemical properties, to identify the most effective products to reduce appetite and food intake. The limited

research on synergy effects of fibre is promising but further investigation is needed to look at other fibre combinations particularly in beverages. Modifying products via industrial processes may improve the versatility of some fibres to be combined in a drink to provide the most effective combinations to reduce appetite and food intake. Work is needed to formulate palatable low-calorie drinks with added fibre, the effects observed in chapter 7, could potentially be enhanced in a low-calorie drink, this would require input on an industrial scale.

9.6 Implications

The implications of this research are far reaching in the context of increasing prevalence of overweight and obesity in societies worldwide. Enhancing the satiating capacity of foods may help people control energy intake and weight to help reduce obesity rates, improve health, and alleviate the strain on public services. The implications could be considered on several levels, they benefit research, the food industry, the consumer, and public health. This thesis adds to the literature to help to understand some of the physiological processes of the entire metabolism as well as psychological and social processes involved in appetite and food intake. This research has not only investigated the satiety capacity of ingredients but also explored how such benefits could be communicated to consumers to help them make better food choices to control their energy intake.

This was the first study to try to validate the *ad-libitum* test meal to allow comparisons to be made across studies that investigate the effects of fibre on appetite and food intake. Ultimately this methodology may help identify fibres with the greatest satiating effect. Prior to embarking on such studies (which require considerable financial investment), it is important to identify the most useful and valid research tools. This thesis contributes to that process. Improving methodology may improve the outcomes to drive forward new product development. Selecting the optimal fibre in

the food matrix is critical for food manufacturers; this thesis identified a fibre combination that reduces appetite and food intake. Although these acute studies are not sufficient to support satiety claims, they do provide valuable proof-of-concept data to be explored further in a longitudinal design. Improving the methodology could therefore benefit research, industry, and eventually the consumer through new and improved product development.

In chapter 8 the effect of fibre related health claims on drinks were investigated. It was important to test not only the internal effects of fibre on satiety and food intake but to test how the general population react to external cues relating to fibre. Appetite is a complex interaction of physiological and non-physiological mechanisms (Bilman et al., 2017). Actual food choice is the result of a complex interaction between internal satiety signals, other food benefits, and environmental cues such as health claims. Satiety enhancing product features need to be convincingly and responsibly communicated to consumers. Chapter 8 investigated how such claims might influence choice. This research has key implications for the food industry identifying a target market for such satiety/health enhancing products, but also has implications for the consumers who could potentially benefit from such products.

Food manufacturers as profit seeking enterprises, consider the cost and the potential effect of reformulation of existing products on consumer acceptability and product sales. There would be little benefit to the food industry reformulating existing products covertly unless the government mandated it. The expanding market for functional foods and beverages suggests the food industry could benefit from food reformulation and overtly communicating this to consumers. The food and Drink Federation (FDF) launched the Action on Fibre initiative in 2021 to help bridge the gap between fibre intakes and government dietary recommendations. Action on fibre members include 28 world leading house-hold brands who have committed to making pledges to

increase the fibre content of their products. The members have pledged to encourage and support people to eat more fibre through not only reformulation but to use the power of marketing, communications, and health claims to help create a better understanding about the benefits of fibre. The uptake for such initiatives would suggest that the food industry understands the importance of reformulation and see commercial benefit in communicating such improvements to consumers.

The research also has implications for public health and obesity. Approximately 14 million adults in the EU were overweight or obese in 2018, with this figure rising by close to half a million every year. There are numerous health consequences, for example incidences of type II diabetes and fatty liver (associated with excessive weight) putting strain on public health services. If the effects detected in the food intake study, chapter 7, could be replicated in a longitudinal study outside the laboratory, the 197kcal reduction over the course of a year would equate to a weight loss of over 9.3kg. The reduction in appetite observed could also help individuals to adhere to weight management strategies, by lessening the effect of sensations of hunger on motivation and mood. This thesis has increased understanding of the regulation of appetite and food choice, long term the outcomes from this research could potentially lead to healthier food consumption, this could undoubtedly contribute to a wider societal benefit to curb overconsumption of nutrient poor foods and halt the global rise in obesity.

9.7 Final summary

In summary, this thesis contributes to our understanding of the acute effects of different fibres on appetite, food intake, and biological markers. Probable mechanisms of fibre effects on appetite were identified and evaluated the relative contribution of proximal psychological and distal gut factors to prandial and post prandial behaviour. Further to this we have explored combining fibres with different

physiochemical properties. This work has also provided the most comprehensive analysis to date of the key methodological components used to validate the effects of fibre on appetite and food intake. Further, as behavioural responses determine the health impact of such products, this research has not only investigated the satiety capacity of ingredients but also explored how such benefits could be communicated to consumers to help them make better food choices to control their energy intake.

When considered within the context of obesity, fibre enhanced drinks could directly promote reduced food intake and aid compliance with healthy eating and weight management strategies. The contribution of factors other than the metabolic effects of nutrients to post-consumption feelings of satiety should not be underestimated. To move fibre and satiety research forward more enduring studies are required taking an integrated approach to move beyond proof of concept. If all stakeholders work together taking this integrated approach to satiety, to optimise the development of and persuasively promote satiating food choices, this could help to develop a marketable product to assist in the control of appetite.

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APPENDIX 1

Screening Procedure		√
Information Check	Received Participant Info. Explain precise details of study (6-week study,6 visits; breakfast, fibre drink, lunch, supper and snack box, VAS, no exercise study days, no alcohol day before study day; weighing at the screening visit, like study foods; eat all breakfast) Understand and willing to follow protocol? Check participant has bank account.	
Consent Form	2 copies to be signed and dated. 1 for participant 1 for file	
Height	Measured according to SOP	
Weight	Measured according to SOP	
BMI	Work out BMI. Range must be 18.5 – 24.99 or 30-34.99 kg/m^2 BMI =	
Age	Exclude if <18 or >65 years	
Medical Questionnaire	NB No GI or IBS. Regular digestive upsets >one/week with no known causes.	
Study Foods	Exclude if >25% study foods/meal disliked.	
DEBQ	Score restraint scale	
Dieting History	Check not dieting within last month	
Suitable for Study	Yes No. Reason:	
Give food and activity diary with instructions as follows: complete from 5.00 pm, similar regime, no strenuous exercise, no alcohol, no food after 12 midnight, only water between 12 and breakfast in lab. No exercise on study days. Apply or banana for snack box.		
Confirm arrangements overleaf by email/letter		

APPENDIX 2

UNIVERSITY OF LIVERPOOL

ARE YOU INTERESTED IN APPETITE RESEARCH?

VOLUNTEERS WANTED TO PARTICIPATE IN A STUDY INVESTIGATING THE ACUTE EFFECT OF A FIBRE ON APPETITE AND FOOD INTAKE

We are looking for healthy females who are:

- **Normal Weight or Overweight**
- **Age 18 - 65**
- **Willing to take part in a 4 week study**
- **Able to visit the University for 3 meals on 4 separate study days**
- **Able to eat most everyday foods**
- **Non-smokers**
- **No food allergies or intolerances**

Reasonable reimbursement for time and effort will be provided.

If you are interested please contact Catherine Slevin

on: 07597178762

or email: cslevin@liv.ac.uk

APPENDIX 3

UNIVERSITY OF LIVERPOOL

Consent Form

Title of Research Project: Experimental study to investigate the acute effect of a fibre on appetite and food intake.

Researcher(s): Catherine Slevin

Please initial box

1. I confirm that I have read and have understood the information sheet dated April 2017 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. ☐
3. I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish. ☐
4. I agree to my GP being informed of my participation in the study if required. ☐
5. I agree to take part in the above study. ☐

Participant Name	Date	Signature
Researcher	Date	Signature

The contact details of lead Researcher (Principal Investigator) are:

Dr Joanne Harrold
Kissileff Ingestive Behaviour Laboratory,
Department of Experimental Psychology,
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APPENDIX 4

Medical History Questionnaire

Participant Initials **Participant Number**

This questionnaire is designed to establish your suitability for this research project. The questionnaire will not be used as part of the study data but will be kept separately and securely for your well being during the study.

Some of the questions ask about personal information. If you do not wish to answer please let the researcher know. All information taken is confidential.

Please answer as honestly as possible.

1. Are you taking or using any medicine or any other drug, either from your doctor or on your own accord (excluding contraceptives)? **Yes / No**
   If so, please list the items below:
   _____

2. Will your use of this medication alter during the course of the study?

   Yes / No

3. Are there any foods you don't eat?

   Yes / No
   If so, please state what and why.
   _____

4. Are you allergic to anything that you are aware of? **Yes / No**

5. The following foods have been known to cause allergies. Have you ever consumed these foods **AND** had an allergic reaction to them?

	Previously Consumed	Allergic Reaction
Peanuts	**Yes / No**	**Yes / No**
Nuts	**Yes / No**	**Yes / No**
Dairy produce	**Yes / No**	**Yes / No**
Seeds	**Yes / No**	**Yes / No**
Eggs	**Yes / No**	**Yes / No**
Fish	**Yes / No**	**Yes / No**
Shellfish	**Yes / No**	**Yes / No**
Soy(a)	**Yes / No**	**Yes / No**
Celery	**Yes / No**	**Yes / No**
Mustard	**Yes / No**	**Yes / No**
Strawberries	**Yes / No**	**Yes / No**

Cherries	**Yes / No**	**Yes / No**

	Previously Consumed	Allergic Reaction
Kiwifruit	**Yes / No**	**Yes / No**
Pulses	**Yes / No**	**Yes / No**
Foods containing **sulphur dioxide/sulphites sulphites** (eg soft drinks, white wine, dried fruits	**Yes / No**	**Yes / No**
Foods containing **lupin** (eg, seeded bread, pastries)	**Yes / No**	**Yes / No**
Foods containing **gluten** (eg wheat, rye, barley, oats)	**Yes / No**	**Yes / No**
Foods containing **lactose** (eg milk, cheese, ice-cream)	**Yes / No**	**Yes / No**
Foods containing **salicylates** (eg dried plums, dates, figs, mushrooms)	**Yes / No**	**Yes / No**

6. Are there any foods which make your mouth, lips or throat tingle?

Yes / No

7. Have you ever suffered from anaphylaxis or anaphylactic shock?

Yes / No

8. Did you suffer from severe childhood allergies?

Yes / No

9. Do you have any chest or breathing problems?

Yes / No

10. Do you have asthma?

Yes / No

If so, is it controlled or uncontrolled?

_____How?_____

11. Do you have or have you had any heart or blood

Yes / No
pressure problems?

12. Do you have any stomach, bowel or digestive problems? **Yes / No**

13. Do you regularly suffer from digestive upsets? **Yes / No**

14. Do you suffer from fainting attacks, fits or seizures? **Yes / No**

15. Are you diabetic? **Yes / No**

16. Do you have Coeliac Disease? **Yes / No**

17. Have you ever suffered from hepatitis, jaundice, liver

Yes / No
or kidney disease?

18. Are you currently receiving any other medical treatment? **Yes / No**

19. Have you had Bariatric surgery for weight control

    Yes / No
    or any other reason?

20. Are you pregnant, likely to become pregnant or breast

    Yes / No
    feeding?

21. Do you smoke? **Yes / No**

22. Have you recently given up smoking? **Yes / No**

    If so, when? _____

23. Do you drink alcohol?

    Yes / No
    If yes, how many units per week.

    _____

24. Do you regularly take social drugs? **Yes / No**

25. Is there anything else concerning your health, you think

    Yes / No
    we should know about?

    _____

26. Have you ever been on a diet? **Yes / No**

27. Are you currently on a diet? **Yes / No**

    Why?

    To lose weight
    To control weight
    Healthy eating
    Medical reason
    Other:

    Please specify _____

28. What exercise do you do in a typical week?

_____

29. Have you lost a significant amount of weight within the last year?

Yes / No

30. Have you significantly increased your level of physical activity over the past 2-4 weeks?

Yes / No

If yes please specify how _____

31. Do you intend to increase your physical activity levels during the study?

Yes / No

If yes please specify how _____

For Office use only

Date Screened	All questions answered	Yes / No
Researcher	**Suitable for study**	**Yes / No**

APPENDIX 5

DIETING HISTORY

This section is designed to establish your previous dieting history. For some questions you may find you cannot remember all the details, please give approximate answers. Any questions you do not wish to answer please leave a blank.

Below are listed several types of weight loss treatments available to most people. Please tick all the weight loss programmes you have tried.

G.P. DIET	☐	WEIGHT WATCHERS	☐
JAW WIRING	☐	LOW FAT DIET	☐
COMMUNITY DIETICIAN	☐	EXERCISE	☐
WEIGHT LOSS CLINIC	☐	ACUPUNCTURE	☐
SLIMMERS WORLD	☐	HIGH PROTEIN DIET	☐
MAGAZINE DIET	☐	CALORIE COUNTING	☐
HOSPITAL DIETICIAN	☐	HIGH FIBRE DIET	☐
HYPNOSIS	☐	CONSULTANT PHYSICIAN	☐

OTHERS:_____

For each weight loss programme you have tried please try to answer the following questions. You may not remember all the details, please use approximate answers where necessary, by putting a ? before the answer to indicate an approximate. If you cannot remember at all, please leave a blank.

For example

WEIGHT LOSS PROGRAMME:	
DATE TRIED:	WEIGHT BEFORE STARTED:
WHY DID YOU START THIS PROGRAMME?	
LENGTH OF COMPLIANCE TO PROGRAMME:	
WEIGHT LOSS:	WEIGHT LOSS MAINTAINED:
LEVEL OF DIFFICULTY:	
LEVEL OF MOTIVATION:	WHY DID YOU STOP THIS PROGRAMME?

Programme 1.

WEIGHT LOSS PROGRAMME:

DATE TRIED: WEIGHT BEFORE STARTED:

WHY DID YOU START THIS PROGRAMME?

LENGTH OF COMPLIANCE TO PROGRAMME:

WEIGHT LOSS: WEIGHT LOSS MAINTAINED:

LEVEL OF DIFFICULTY:

LEVEL OF MOTIVATION: WHY DID YOU STOP THIS PROGRAMME?

Programme 2.

WEIGHT LOSS PROGRAMME:

DATE TRIED: WEIGHT BEFORE STARTED:

WHY DID YOU START THIS PROGRAMME?

LENGTH OF COMPLIANCE TO PROGRAMME:

WEIGHT LOSS: WEIGHT LOSS MAINTAINED:

LEVEL OF DIFFICULTY:

LEVEL OF MOTIVATION: WHY DID YOU STOP THIS PROGRAMME?

APPENNDIX 6
DEBQ-R.

Participant ID _____ **Date** _____

Please tick the box that applies best to each of the numbered statements

	Never	Seldom	Sometimes	Often	Very Often
1. If you have put on weight do you eat less than you usually do?					
2. Do you try to eat less at mealtimes than you would like to eat?					
3. How often do you refuse food or drink offered to you because you are concerned about your weight?					
4. Do you watch exactly what you eat?					
5. Do you deliberately eat foods that are slimming?					
6. When you have eaten too much, do you eat less than usual the following day?					
7. Do you deliberately eat less in order not to become heavier?					
8. How often do you try not to eat between meals because you are watching your weight?					
9. How often in the evenings do you try not to eat because you are watching your weight?					
10. Do you take your weight into account with what you eat?					

APPENDIX 7

Example of Study Foods

The following foods will be offered in the study. Please tick to confirm whether or not you are able to eat each item and whether or not you are allergic or intolerant to it. If you are happy to consume all of these foods please sign in the space provided at the bottom of this sheet, otherwise contact the researcher to discuss the study foods. You may still be able to participate.

Breakfast

You will be asked to consume the following breakfast foods. Please indicate if you are able and willing to eat these by ticking the appropriate boxes.

	Like	Dislike	Allergic/Intolerant
Blue berry Smoothie			
Warburtons white bread Flora original Margarine			
Cornflakes			
Warburtons Toasty Bread Flora Margarine			
Semi Skimmed Milk			

Lunch

Please indicate with a √ how you feel about the following foods which will be offered at a buffet lunch.

Item	Like	Dislike	Indifferent	Allergic/Intolerant
Cheddar Cheese				
Seeded Rolls				
Cadburys Buttons				
Jelly Babies				
Tomato				

Item	Like	Dislike	Indifferent	Allergic/Intolerant
Penn Pasta				
Garlic Bread				
veg pasta sauce				
mozzarella cheese				
Magnum Ice Cream				
Peaches				

I confirm that I have no known allergies or intolerance to any of the above foods unless otherwise indicated above.

I confirm that all the above data is correct to my knowledge

Participant _____ Researcher _____ Date _____

Participant's name printed (i.e. in clear capital letters) _____

APPEDIX 8

GI Questionnaire

Experimental study to investigate the effect of fibre on appetite in women.

OFFICE USE ONLY: DAY:

PARTICIPANT: DATE:

CONDITION: 1 2 3 4 5 6 TIME:

INSTRUCTIONS FOR PARTICIPANTS:
Please read each question and then circle the number that best represents how you are feeling in relation to that particular sensation at this moment.

EXAMPLE:

How **TIRED** do you feel **at this moment?**

Not tired 1 2 (3) 4 5 Very tired

PLEASE ANSWER THE FOLLOWING QUESTIONS:

How **BLOATED** have you felt today?

1 2 3 4 5

How **COMFORTABLE** have you felt today?

1 2 3 4 5

How **FLATULENT** have you felt today?

1 2 3 4 5

How **TIGHT** has your stomach felt today?

1 2 3 4 5

How much **ABDOMINAL DISCOMFORT** (e.g. stomach cramps) have you felt today?

1 2 3 4 5

THANK YOU

APPENDIX 9

Participant Number _____ **Date**

_____

Daily Food and Activity Diary

PLEASE COMPLETE THIS DIETARY RECORD FOR THE EVENING BEFORE YOU ATTEND THE LABORATORY.

Use ONE section per eating or drinking episode. Please write down everything you eat and drink from 5.00pm until first thing the morning of the study. Please give as much information about the foods you eat as possible. Please circle if you consider the eating episode to be a meal or a snack. Please write down the time you started eating and the time you stopped eating.

1. Time started eating / drinking:

Brand name.	**Food Items Description**	**Amount / portion.**

Time stopped eating / drinking: **Meal / Snack**

2. Time started eating / drinking:

Brand name.	**Food Items Description**	**Amount / portion.**

Time stopped eating / drinking: **Meal / Snack**

3. Time started eating / drinking:

Brand name.	Food Items Description	Amount / portion.

Time stopped eating / drinking: **Meal / Snack**

PLEASE COMPLETE THIS ACTIVITY RECORD FOR THE EVENING BEFORE YOU ATTEND THE LABORATORY.

Use ONE section per activity. Please write down everything you do from 5.00pm until first thing the morning of the study. Please write down the time you started the activity and the time you stopped.

1. Time started activity:

Type of Activity

Time Activity stopped:

2. Time started activity:

Type of Activity

Time Activity stopped:

3. Time started activity:

Type of Activity

Time Activity stopped:

APPENDIX 10

Chapter 3 systematic review search terms

Key search terms for type of soluble fibre were: -

(fiber) OR (fibre) OR (dietary fibre) OR (non-starch polysaccharide) OR (resistant oligosaccharide) OR (Beta-glucans[mesh] OR beta-glucans) OR (Cellulose[mesh] OR cellulose) OR (hemicellulose) OR (arabinoxylan) OR (arabinogalactan) OR (polyfructose) OR (inulin) OR (oligo fructan) OR (galacto-oligosaccharide OR galactooligosaccharide) OR (Plant gums[mesh] OR plant gum) OR (mucilage) OR (pectin) OR (analogous carbohydrate) OR (indigestible dextrin) OR (resistant maltodextrin) OR (resistant potato dextrins) OR (synthesized carbohydrates compound) OR (polydextrose) OR (resistant starch) OR (lignin) OR (Wax) OR (cutin) OR (tannin) OR (alginate) OR (carrageenan) OR (Chitin[mesh] OR chitin) OR (Fructans[mesh] OR (fructan) OR (Galactans[mesh] OR (galactan) OR (amylose) OR (Dextrins[mesh] OR dextrins) OR (Glycosaminoglycans[mesh] OR glycosaminoglycan) OR (mannan) OR (Trisaccharides[mesh] OR trisaccharide) OR (xylan) OR (fructooligosaccharide OR fructo-oligosaccharide) OR (oligofructose) OR (glucooligosaccharide) OR (gluco-oligosaccharide) OR (cyclodextrin) OR (xylooligosaccharide) OR (xylo-oligosaccharide) OR (guar gum) OR (locust bean gum) OR (psyllium) OR (mannan oligosaccharide OR mannan-oligosaccharide) OR (bran) OR (brans) OR (pulp) OR (pulps) OR (whole grain OR wholegrain) OR (Agar) OR (Barley β-glucan) OR (Barley bran) OR (Barley fibre) OR) Beet fibre OR (Chitosan) OR (corn fiber) OR (Corn fibre) OR (Dextrin) OR (Ethyl hydroxy ethyl cellulose) OR (Fenugreek gum) OR (flaxseed) OR (FOS) OR (glucan) OR (Konjac glucomannan) OR (Lupin kernel fibre) OR (Marine polysaccharide) OR (Methyl cellulose) OR (Oat) OR (Oat bran) OR (Oat fiber) OR (Oat fibre) OR (Oatmeal) OR (pectin) OR (Resistant Starch) OR (Resistant wheat starch) OR (Rye) OR (Rye fibre) OR (Rye fiber) OR (Rye Kernel) OR (Rye-Based) OR (Wheat fibre) OR (Xylan) OR (Xylooligosaccharides) OR (yellow pea fibre) OR (α-cyclodextrin) OR(β-glucan) OR (soluble) OR (fermentable) OR (prebiotic) or (Viscous fibre) OR (Viscous Fiber).

Key outcome search terms for outcome measures appetite and food intake were: -

AND (satiation) OR (satiety) OR (Hunger[mesh]) OR (appetite) OR (satisfaction) OR (hunger) OR (desire to eat) OR (prospective food consumption) OR (fullness) OR (subjective feeling) OR (energy intake[mesh]) OR (energy intake) OR (calorie intake) OR (caloric intake) OR (ingestion) OR (food intake) OR (food intakes) OR (food

consumption) OR (food consuming) OR (eating behaviour) OR (ad libitum intake) OR (ad libitum AND intake) OR (intake) OR (consumption)

Key exclusion terms: -

NOT (rat) OR (Rats) OR (Mouse) OR (Mice) OR (pig) OR (pigs) OR (cow) OR (cows) OR (sheep) OR (chicken) OR (dog) OR (dogs) OR (children) OR (child) OR (infants) OR (babies) OR (teenagers) OR (girls) OR (boys) OR (diabetic) OR (patients) OR (diabetes) OR (type 2) OR (hypercholesterolemia) OR (dyslipidaemia) OR (metabolic syndrome) OR (type II) OR (hypercholesterolemic)

Filters: Publication date 01/01/1996 to 2016/12/31; Humans; English

Appendix 11

Task

For the next section, we will present you with 10 images of different drinks. We'd like you to take a close look at each image and answer the questions below each image. The questions relate to how you ***think*** the drinks would score on the characteristics, prior experience of the products is not required

---

1. Which of these two options would you buy right now if they were identical in price? Please select **ONE** item.

Click on your chosen item once to select. Click twice more to deselect. Your chosen item will highlight green.

Participants were presented with 10 different pairs of drinks and asked to select which drink they would buy. After making their selection they were then asked to rate eat of the 2 drinks presented on 5 different dimensions.


COLD-PRESSED
SAVSE
RAW SMOOTHIES
SUPER ORANGE
FEEL FULLER for LONGER
COLD-PRESSED
SAVSE
RAW SMOOTHIES
SUPER ORANGE
NEW FLAVOUR
Ocean Spray
Cranberry Raspberry
FEEL FULLER for LONGER
Ocean Spray
Cranberry Raspberry
NEW FLAVOUR
BOOSTED
blue machine
Naked
100% JUICE SMOOTHIE
NEW FLAVOUR
BOOSTED
blue machine
Naked
100% JUICE SMOOTHIE
FEEL FULLER for LONGER
JOHNSONS JUICE CO.
SMOOTHIE
APPLE BANANA MANGO PINEAPPLE PASSION FRUIT
NEW FLAVOUR
JOHNSONS JUICE CO.
SMOOTHIE
APPLE BANANA MANGO PINEAPPLE PASSION FRUIT
FEEL FULLER for LONGER

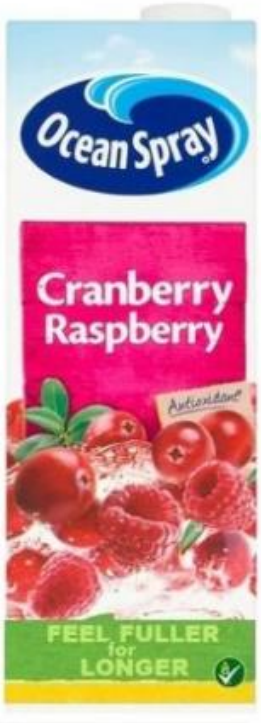

innocent
SUPER SMOOTHIE
NEW FLAVOUR
innocent
SUPER SMOOTHIE
FEEL FULLER for LONGER
A HOLIDAY
Wake me up
Tropicana
PURE PREMIUM
FEEL FULLER for LONGER
ORANGE WITH NO BITS
A HOLIDAY
Wake me up
Tropicana
PURE PREMIUM
NEW FLAVOUR
ORANGE WITH NO BITS


Tropicana
MORNING BOOST
FRUIT AND CEREAL SMOOTHIE
FOREST FRUITS
FEEL FULLER for LONGER
Tropicana
MORNING BOOST
FRUIT AND CEREAL SMOOTHIE
FOREST FRUITS
NEW FLAVOUR
ORANGE & CARROT
JUICE BURST
FEEL FULLER for LONGER
ORANGE & CARROT
JUICE BURST
NEW FLAVOUR


Tropicana
MIXED BERRIES
FEEL FULLER for LONGER
Tropicana
MIXED BERRIES
NEW FLAVOUR


innocent
CITRUS
NEW FLAVOUR
innocent
CITRUS
FEEL FULLER for LONGER

Please take a close look at the drink and answer the questions below.



Now please rate the following characteristics: -

Not at all — Extremely

0 10 20 30 40 50 60 70 80 90 100

How likely are you to buy?

Taste

Filling

Health

Aids Weight Loss

After participants had chosen which drink, they would choose to buy they then rated the 2 drinks on 5 dimensions. The same procedure was repeated for each beverage presented on the choice task.