Abstract

With the rise in obesity, there has been an increased interest in foods which may beneficially affect appetite. Resistant starch (RS) and whole grains (of which RS is a main dietary fibre component) have been proposed to affect satiety and therefore may be beneficial in weight management. There is little direct evidence confirming this in humans. Whilst animal data suggest a positive effect of RS on appetite, the few existing human intervention studies provide inconsistent findings. For whole grains the majority of evidence is from epidemiological work as opposed to intervention studies. Therefore a series of studies was conducted to investigate effects of RS and whole grains on appetite and food intake.

Two studies were conducted using RS. The first investigated the acute (24 hours) effects of 48 g RS in healthy adult males compared with an energy and available carbohydrate matched placebo. Following RS there was a significantly lower energy intake compared with placebo. There was also a significantly lower postprandial insulin response with RS, possibly explained by increased hepatic insulin clearance determined by a higher C-peptide to insulin ratio. In the second study 40 g RS consumed daily for 4 weeks was compared with the placebo, in overweight and obese participants. Effects on food intake were assessed and a frequently sampled intravenous glucose tolerance test (FSIVGTT) was conducted. This study found no effect on either appetite or energy intake, but did find significantly higher glucose, insulin and C-peptide concentrations, measured during the FSIVGTT, with the RS compared with the placebo, possibly explained
by an improved first-phase insulin response. This finding did not translate into
differences in parameters obtained from modelling the FSIVGTT data, but this
and the lack of appetite and food intake differences could be explained by the
small participant numbers.

Two intervention studies were conducted with whole grains incorporated into
bread rolls. The first, a crossover study, involved 3 weeks' daily consumption of
48 g milled whole grain or control, in young healthy adults. Whilst no significant
difference was found between interventions in energy intake or subjective
appetite ratings, a significantly lower systolic blood pressure was observed with
the milled whole grains. The second was an 8 week parallel study (48 g intact or
48 g milled whole grains or control) in overweight and obese adults. No
significant difference was found between groups on energy intake, subjective
appetite ratings, cholesterol or postprandial metabolite concentrations.

RS appears to be a possible satiating ingredient when consumed acutely and,
whilst this was not confirmed in our chronic study, effects may have been
masked by small participant numbers. A novel finding from our RS studies was
an effect on the insulin response. These studies suggest that RS could have a
beneficial role in weight management and favourable metabolic effects. Our
whole grain interventions appear not to agree with epidemiological work that
suggests a beneficial role on appetite, but there maybe effects on blood
pressure regulation. In all instances further investigations are required in other
population groups, with more participants and for longer time periods.
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Finally, I would like to thank my wonderful family, especially my Mum, for all their love, help, patience and encouragement over the last three years; for putting up with numerous conversations about things that really could not be interesting for them and for providing me with the support that I have needed.
Declaration of Originality

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.
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<td>AgRP</td>
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<td>AIR&lt;sub&gt;g&lt;/sub&gt;</td>
<td>First-phase Insulin Response</td>
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<td>ANOVA</td>
<td>Analysis Of Variance</td>
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<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
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<td>cAMP</td>
<td>cyclic Adenosine Monophosphate</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine- and Amphetamine-Regulated Transcript</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CCNFSDU</td>
<td>Codex Committee on Nutrition and Foods for Special Dietary Uses</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>Co</td>
<td>Commercial</td>
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<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<tr>
<td>DEBQ</td>
<td>Dutch Eating Behaviour Questionnaire</td>
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<tr>
<td>DF</td>
<td>Dietary Fibre</td>
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<td>Disposition Index</td>
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<tr>
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<td>Dipeptidyl-peptidase-IV</td>
</tr>
<tr>
<td>E</td>
<td>Experimental</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
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<tr>
<td>EGIR</td>
<td>European Group for the Study of Insulin Resistance</td>
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<td>F</td>
<td>Females</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FSIVGTT</td>
<td>Frequently Sampled Intravenous Glucose Tolerance Test</td>
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<td>GE</td>
<td>Glucose Effectiveness</td>
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<tr>
<td>GI</td>
<td>Glycaemic Index</td>
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<tr>
<td>GIP</td>
<td>Gastric-Inhibitory Polypeptide</td>
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<tr>
<td>Acronym</td>
<td>Term</td>
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<tr>
<td>GL</td>
<td>Glycaemic Load</td>
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<tr>
<td>GLUT</td>
<td>Glucose Transporter</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
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<tr>
<td>GLP-1R</td>
<td>Glucagon-like peptide-1 Receptor</td>
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<td>HDL</td>
<td>High-Density Lipoprotein</td>
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<tr>
<td>HOMA</td>
<td>Homeostasis Model Assessment</td>
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<tr>
<td>iAUC</td>
<td>Incremental Area Under the Curve</td>
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<td>IGD</td>
<td>Institute of Grocery Distribution</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IRS</td>
<td>Insulin Receptor Substrates</td>
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<td>IVGTT</td>
<td>Intravenous Glucose Tolerance Test</td>
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<td>Low-Density Lipoprotein</td>
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<td>Md</td>
<td>Median</td>
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<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<td>MWG</td>
<td>Milled Whole Grain</td>
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<tr>
<td>NEFA</td>
<td>Non-Esterified Fatty Acids</td>
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<td>National Health Service</td>
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<td>NPY</td>
<td>Neuropeptide Y</td>
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<td>NSP</td>
<td>Non-Starch Polysaccharides</td>
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<td>OGTG</td>
<td>Oral Glucose Tolerance Test</td>
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<td>Oxyntomodulin</td>
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<td>PL</td>
<td>Placebo</td>
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<td>POMC</td>
<td>Pro-opiomialcortin</td>
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<td>Pancreatic Polypeptide</td>
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<td>PYY</td>
<td>Peptide YY</td>
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<tr>
<td>RDS</td>
<td>Rapidly Digestible Starch</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>RPM</td>
<td>Revolutions Per Minute</td>
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<td>RS</td>
<td>Resistant Starch</td>
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<tr>
<td>SCFA</td>
<td>Short-Chain Fatty Acids</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<td>SDS</td>
<td>Slowly Digestible Starch</td>
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<tr>
<td>SEM</td>
<td>Standard Error of the Means</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>SFA</td>
<td>Saturated Fatty Acid</td>
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<tr>
<td>Sg</td>
<td>Glucose Effectiveness</td>
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<td>Si</td>
<td>Insulin Sensitivity</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes</td>
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<td>TDF</td>
<td>Total Dietary Fibre</td>
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<tr>
<td>TG</td>
<td>Triglycerides</td>
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<td>TNFα</td>
<td>Tumour Necrosis Factor Alpha</td>
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<tr>
<td>UK</td>
<td>United Kingdom</td>
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<td>USA</td>
<td>United States of America</td>
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<td>VAS</td>
<td>Visual Analogue Scales</td>
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<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
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<tr>
<td>WG</td>
<td>Whole Grain</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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Chapter 1

Introduction
The following chapter describes the background literature relating to the conducted research. The chapter includes introductory information on appetite, physiological regulation of food intake and the role of insulin, as well as a summary of current literature on the effects of consumption of resistant starch and whole grains on appetite, weight regulation and insulin responses.

1.1. Obesity

Obesity has become a global epidemic and rates are increasing in economically less developed countries, as well as in the most developed (World Health Organisation (WHO), 2000a). In a report by the National Audit Office (2001) proportions of the population in England (from data collected in 1998), who are obese (body mass index, BMI ≥30 kg/m²) or overweight (BMI ≥25 kg/m²), were reported to be over half of women and approximately two thirds of men, with one in five adults being obese. It was stated in this report that if levels of obesity increased at the rates observed at the time of the report, then by 2010 over a quarter of adults would be obese (National Audit Office (NAO), 2001).

Obesity has been linked to an increased risk of developing other diseases including cardiovascular disease (CVD), osteoarthritis, respiratory diseases, type 2 diabetes mellitus (T2DM), gastrointestinal diseases, liver diseases, gall bladder disease, reproductive disorders and some cancers (National Audit Office (NAO), 2001, World Health Organisation (WHO), 2000b, World Health Organisation (WHO), 2000a). Indeed it has been stated that obesity is the main
modifiable risk factor for development of T2DM, and the relative risk rises with increasing BMI (World Health Organisation (WHO), 2000a). Central obesity is considered more hazardous to health, due to fat accumulation around organs, and increases co-morbidity risk (Haslam and James, 2005). Central obesity, more than peripheral obesity, is a significant contributor to the development and progression of insulin resistance and the metabolic syndrome (World Health Organisation (WHO), 2000b). Several mechanisms have been proposed as to how the increased risk of insulin resistance due to central obesity arises. These include the presence of the high levels of internal fat itself, but also to the secretion of inflammatory cytokines (for example tumour necrosis factor alpha (TNFα) and interleukins 1 and 6 (IL1 and IL6) from the adipose tissue (Haslam and James, 2005). It has been shown that weight loss, even modest amounts, can be of benefit to many of the associated conditions (World Health Organisation (WHO), 2000b).

1.1.1. **Energy balance**

Energy intake and expenditure are key factors that regulate body weight and a prolonged imbalance between these two factors results in weight gain or loss. Energy balance is closely maintained even within large variations in energy intake and expenditure (Murphy and Bloom, 2004). However, over recent years, changes in lifestyle have resulted in an increase in energy intake (due to more readily available food and higher energy density foods) and decreased energy expenditure (more labour saving devices and less physical activity). Both of
these contribute to an obesogenic environment. Only a small imbalance between intake and expenditure over several years would lead to obesity.

1.2. Appetite

1.2.1. Definitions

Appetite is the desire or physical craving to eat and is typically separated into three parts – hunger, satiation and satiety. In a review by Mattes et al. (2005) hunger is defined as "sensations that promote food consumption", satiation as "sensations that govern meal size and duration", and satiety as "sensations that determine the inter-meal period of fasting". Therefore satiation and satiety are important for determining energy intake. All these aspects of appetite are closely regulated by physiological factors, but are also influenced by external cues (including activity levels, availability of food, the presence or absence of other people and the hedonic properties of the food itself), psychological factors (such as learnt habits and beliefs) and emotional factors (for example response to stress or happiness) (Mattes et al., 2005, Flint et al., 2000).

Appetite is difficult to quantify as it is subjective and varies greatly between individuals, as well as being influenced by many factors (both external and internal) and therefore there is no easy direct assessment; nevertheless indirect measures have been developed (Mattes et al., 2005). The most frequently used indirect methods involve monitoring food intake (for example, through dietary records, food frequency questionnaires or ad libitum test meals), using
biomarkers (for example monitoring changes to concentrations of gut hormones) and the use of questionnaires (for example those that require subjects to rate how they feel in response to a meal) (Mattes et al., 2005).

1.2.2. Physiological regulation

1.2.2.1. Short-term regulation

Appetite is regulated by many physiological factors that interact, including involvement of the hypothalamus and hormones released from the gut (Bloom et al., 2005). The latter act on areas of the brain including the hypothalamus (the main site of action), the brainstem and the vagus nerve (Bloom et al., 2005, Druce et al., 2004) and subsequently modulate appetite regulation.

The hypothalamus is vital for appetite regulation and the subsequent control of food intake (Dhillo, 2007); it receives peripheral signals from the digestive tract (gut peptides) and adipose tissue (through signalling molecules, including leptin and insulin) (Figure 1.1) (Murphy and Bloom, 2004, Wynne et al., 2005). There are several different areas (nuclei) in the hypothalamus, of which the arcuate nucleus (ARC) is thought to be the most important in appetite regulation (Dhillo, 2007, Heijboer et al., 2006), due to the area having an incomplete blood brain barrier. The ARC is essential for interpreting peripheral signals, which act on receptors and consequently cause the release of neuropeptides (Bloom et al., 2005, Murphy and Bloom, 2004, Wynne et al., 2005). There are two classes of neurones in the ARC, those that stimulate food intake (neuropeptide Y (NPY)
and agouti-related peptide (AgRP)) and those that inhibit food intake (pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART)) (Heijboer et al., 2006, Wynne et al., 2005).

Gut hormones that regulate food intake are both anorexigenic (increase satiety) and orexigenic (promote hunger). The hormone ghrelin is unique in being orexigenic and is often termed the "hormone of hunger" (Druce et al., 2004, Wynne et al., 2005, Murphy and Bloom, 2004). Anorexigenic hormones are released postprandially; they act by both direct and indirect mechanisms and promote feelings of satiety. These hormones include cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), oxyntomodulin (Oxm), peptide YY (PYY) and pancreatic polypeptide (PP) (Bloom et al., 2005).

Ghrelin is primarily secreted from endocrine cells in the stomach (De Vriese and Delporte, 2007). The form in which ghrelin is secreted is distinctive as it is made
up of 28 amino acids with an octanoyl group added, which allows ghrelin to exert its actions (De Vriese and Delporte, 2007). In addition to increasing food intake, ghrelin has other functions in the body including stimulation of growth hormone release and increasing gastric emptying, and it has been shown to decrease the secretion of gastric acid in rodent models (Druce et al., 2004, De Vriese and Delporte, 2007). Plasma concentrations of ghrelin are highest when an individual is fasting and fall during a meal in response to changes to glycaemia (De Vriese and Delporte, 2007); lower fasting concentrations of ghrelin are typically found in obesity (De Vriese and Delporte, 2007) and, as individuals loose weight, ghrelin concentrations rise, which partially accounts for problems associated with sustaining weight loss (Druce et al., 2004).

CCK is released rapidly postprandially, primarily from enteroendocrine cells of the duodenum and jejunum, due to the presence of amino acids and fatty acids (Coll et al., 2007, Holt et al., 1992). CCK concentrations stay high for up to five hours (Bloom et al., 2005, Wynne et al., 2005). The release of CCK causes, in addition to effects on appetite, gall bladder contraction, inhibition of gastric emptying, stimulation of gastric motility, and exocrine pancreatic secretions (Bloom et al., 2005, Holt et al., 1992, Wynne et al., 2005). Administration of CCK in humans has been shown to reduce both meal size and duration (Holt et al., 1992). It has been reported that CCK may influence body weight due to interaction with leptin and subsequent enhancement of the effects of leptin (as discussed below, section 1.2.2.2).
GLP-1 and Oxm are synthesised from the processing of the proglucagon gene in the gut (Druce et al., 2004, Small and Bloom, 2004); glucagon is also synthesised from the proglucagon gene in the pancreas and is the principle product of proglucagon processing (Bloom et al., 2005). GLP-1 and Oxm are released from endocrine L cells in the small intestine (Coll et al., 2007), 50% of GLP-1 is released from the small intestine and 50% from the large intestine (Robertson et al., 1999). Oxm promotes satiety and causes decreased secretion of gastric acid, delayed gastric emptying and has a role in the control of weight (Bloom et al., 2005, Coll et al., 2007). Oxm concentrations increase following food intake (Druce et al., 2004) and the associated decrease in food intake observed may be partially explained due to inhibition of ghrelin (Bloom et al., 2005). GLP-1 is secreted in response to absorbed nutrients (glucose, fat and protein) and causes a reduction in food intake and enhanced satiety (Adam and Westerterp-Plantenga, 2005, Bloom et al., 2005). Postprandial release of GLP-1 also delays gastric emptying, reduces secretion of gastric acid and inhibits glucagon release (Druce et al., 2004, Adam and Westerterp-Plantenga, 2005, Murphy and Bloom, 2004). In humans the greatest concentration of GLP-1 (and the biologically active form) is GLP-1_{7-36} (Girard, 2008). Studies have shown that GLP-1 concentrations are lower in obesity and therefore it has been suggested that administration of GLP-1 may enhance satiety and cause weight loss (Bloom et al., 2005, Wynne et al., 2005). GLP-1 is also an incretin (Dhillo, 2007). Incretins are gut hormones that increase the insulin response by stimulating insulin secretion and therefore play a role in blood glucose regulation (Girard,
The insulin produced due to the action of incretins may account for at least 50% of the insulin released in response to oral glucose intake (Kim and Egan, 2008). Once GLP-1 has been released it travels in the circulation and binds with a specific receptor (GLP-1R) on the α and β cells in the Islets of Langerhans (Girard, 2008), which increases intracellular cAMP (cyclic adenosine monophosphate) and calcium concentrations, in turn activating other signalling pathways (Kim and Egan, 2008). Receptor binding and signalling pathway activation results in increased insulin secretion, inhibition of glucagon release and increase in β-cell proliferation and survival (Girard, 2008, Kim and Egan, 2008). GLP-17-36 is rapidly broken down to an inactive form due to the action of protease enzymes (such as dipeptidyl-peptidase-IV (DPP-IV)) (Dhillo, 2007, Wynne et al., 2005). Therefore due to the incretin actions of GLP-1, strategies using analogues of GLP-17-36, that are resistant to DPP, are being trialled for use in treatment of T2DM (Coll et al., 2007, Dhillo, 2007, Wynne et al., 2005).

PYY is secreted from L cells in the distal intestine (predominately the ileum and colon). PYY is secreted in 2 forms, PYY1-36 and PYY3-36, the most active of which is PYY3-36. It has been shown to reduce food intake and promote satiety (Bloom et al., 2005). Concentrations of PYY rise rapidly following a meal (Druce et al., 2004, Coll et al., 2007) in proportion to energy consumed and concentrations stay high for about six hours (Bloom et al., 2005, McGowan and Bloom, 2004). PYY concentrations also rise following high fat intake and in the
presence of gastric acid and CCK (McGowan and Bloom, 2004). PYY is released quickly following ingestion but before nutrients arrive at the distal portion of the intestine, suggesting neuronal and upper gastrointestinal tract involvement (Wynne et al., 2005, McGowan and Bloom, 2004). A further PYY release occurs once the nutrients reach the colon, approximately 4 – 5 hours after the meal (Druce et al., 2004). PYY has been proposed to act as an “ileal brake” which may contribute to satiety and feelings of fullness (Druce et al., 2004). PYY has other documented effects apart from inhibition of food intake, which include slowing gastric emptying and delaying exocrine secretions from the pancreas and gall bladder (Bloom et al., 2005, Druce et al., 2004).

PP is released from endocrine F-cells that are located near to the pancreatic islets (Coll et al., 2007). PP concentrations rise proportionally to the amount of food consumed and concentrations stay high for approximately six hours (Bloom et al., 2005, Murphy and Bloom, 2004, Wynne et al., 2005). PP is not only released relative to calorie intake, but also by gastric distension, in response to blood glucose concentrations and due to the presence of other gut hormones (Murphy and Bloom, 2004). Its actions in addition to those on food intake include inhibition of pancreatic exocrine secretion, gastrointestinal motility and gall bladder contraction. When PP has been administered to normal weight individuals, subsequent food intake was reduced until the following morning (Druce et al., 2004).
1.2.2.2. **Long-term regulation**

Other factors are also important in appetite regulation and reflect longer-term energy stores rather than short-term effects to ingested nutrients. Leptin is a hormone that is released from adipocytes and circulates in the blood at concentrations that are proportional to body fat mass (Druce et al., 2004, Murphy and Bloom, 2004, Wynne et al., 2005). Leptin has been found to reduce food intake (Druce et al., 2004) and its concentrations are seen to fall during starvation (Small and Bloom, 2004). Once released it stimulates anorexigenic neurons and causes negative feedback inhibiting the orexigenic actions (Druce et al., 2004, Dhillo, 2007); this causes food consumption to be inhibited and prevents further body fat mass increase. When leptin is deficient it results in obesity in both humans and rats (Murphy and Bloom, 2004). However, leptin concentrations in obesity do not appear to be deficient, and indeed plasma concentrations are high (due to the high levels of adipose tissue), suggesting that in obesity individuals may be resistant to the actions of leptin (Murphy and Bloom, 2004) and therefore the potential of leptin as a treatment for obesity is limited (Dhillo, 2007). Insulin also acts as a long-term energy signal and is discussed in more detail below (section 1.3).

1.3. **Insulin**

1.3.1. **Mechanism of action**

Insulin is a small peptide secreted from the β-cells of the Islets of Langerhans in the pancreas and is co-secreted (in identical amounts) with connecting peptide
(C-peptide), both of which are part of the same original molecule, proinsulin. These two peptides are removed from the body by different routes, insulin predominantly by the liver and C-peptide by the kidneys (Rossell et al., 1983). As such, insulin and C-peptide have different half lives (5 minutes and 30 minutes respectively) and therefore C-peptide concentrations are normally 5 times higher than insulin in the periphery. Of the insulin that arrives at the liver via the portal system, approximately 50% is removed during 1st pass transit (Polonsky and Rubenstein, 1984), and thus C-peptide is often used as a surrogate marker of insulin secretion (Hills and Brunskill, 2009, Toffolo et al., 1995). Measurements of insulin and C-peptide concentrations taken concomitantly can provide information on both insulin secretion and hepatic clearance (Cobelli et al., 2007, Faber et al., 1981). Hepatic insulin clearance calculated as the ratio of C-peptide to insulin, should still be interpreted with caution due to many other factors that may influence or confound the findings (Polonsky and Rubenstein, 1984).

Whilst it has been considered that C-peptide does not have an active role in metabolism and is inert, only acting to stabilise and ensure the correct formation of insulin molecules, emerging evidence now contradicts this assumption (Hills and Brunskill, 2009, Wahren, 2004). C-peptide may act at a cellular level, by binding to cell membrane receptors and activating signalling pathways. It may also exert an independent beneficial effect on the nephropathy and neuropathy found in diabetes (Hills and Brunskill, 2009, Wahren, 2004).
Insulin secretion is pulsatile and biphasic. In the first phase, insulin is released rapidly due to increases in blood glucose concentrations and in the second phase insulin has a slower sustained release (Gerich, 2002). Insulin has many actions, both those that promote and those that inhibit metabolism. Inhibiting actions include gluconeogenesis (production of glucose from other substances), glycogenolysis (glycogen breakdown), lipolysis (fat breakdown), ketogenesis (production of ketones) and proteolysis (protein breakdown). Enhancements include glucose uptake into muscle and adipose tissue, glycogen synthesis and protein synthesis. Of insulin's actions perhaps the most fundamental is glucose uptake into cells (Trout et al., 2007).

Blood glucose concentrations are tightly regulated and maintained at approximately 5 mmol/l. During fasting, in normal conditions, insulin concentrations are low and blood glucose concentrations are maintained through the release of glucagon (from the α-cells of the Islets of Langerhans), which activates enzymes (such as glucose 6-phosphatase) that produce glucose from glycogenolysis and gluconeogenesis. After ingestion of macronutrients, insulin concentrations rise and the release of glucose from the liver is down-regulated, resulting in conservation of body stores and the use of exogenous carbohydrate for metabolism (Figure 1.2). In the postprandial phase glucose uptake can be both insulin-dependent and independent. Different isoforms of the glucose transporters are expressed in different areas of the body, for example GLUT2 is expressed mainly in the liver, GLUT4 in muscles and fat, GLUT1 in
the brain, kidney and colon, GLUT5 in the jejunum and GLUT3 in many areas including the brain and kidney (Bell et al., 1990). The insulin-dependent uptake in muscle and fat involves insulin binding to cellular membrane receptors (insulin receptor substrates (for example IRS-1)) and activating intracellular signalling pathways, which ultimately results in the translocation of GLUT4 to the membrane surface (Bell et al., 1990, Trout et al., 2007). GLUT2, unlike GLUT4, is continually expressed on the membrane surface to allow bi-directional movement of glucose in the liver (Bell et al., 1990). The main sites of action of insulin are the liver, muscle and adipose tissues.

![Diagram of glucose homeostasis](image)

*Figure 1.2: Glucose homeostasis. Figure adapted from Gaw et al (1999).*

1.3.2. **insulin sensitivity**

Insulin sensitivity is defined as the ability of insulin to control blood glucose concentrations, through glucose uptake into tissues and the inhibition of hepatic glucose output (Trout et al., 2007).
Insulin resistance arises due to the inability of insulin to execute its actions at a given insulin concentration (Wallace and Matthews, 2002), and higher insulin concentrations are therefore required to maintain normal glucose concentrations (Trout et al., 2007). Over long periods of time the insulin produced may become inadequate to maintain the normal glucose concentrations (due to β-cells no longer maintaining sufficient secretion), which would result in high glucose concentrations, and, if this is prolonged, could progress to T2DM. The progression of increased insulin resistance and the effects on insulin production and blood glucose concentrations are shown in Figure 1.3.

![Figure 1.3: Progression of insulin resistance over time and the effects on insulin production and glucose concentrations. Greater production of insulin is required over time to maintain blood glucose concentrations, due to increasing insulin resistance. This eventually leads to reduced insulin secretion and subsequently higher glucose concentrations observed in Type 2 diabetes.](image)

Insulin resistance is associated with other pre-disposing factors such as an increased waist circumference (a marker of visceral obesity), hypertension and
dyslipidaemia. When several of these factors appear concomitantly it is defined as the Metabolic or Insulin Resistance Syndrome. There are different definitions of the Metabolic Syndrome; however, one commonly cited was put forward by the European Group for the Study of Insulin Resistance (EGIR). The EGIR definition of the metabolic syndrome is designed for non-diabetic individuals and is described by the presence of insulin resistance or high fasting insulin concentration (concentrations in the highest 25% of the population), which would equate to being a concentration greater than 60 pmol/l. This needs to be present in combination with two other factors from: fasting hyperglycaemia (greater than 6.1 mmol/l in an individual without diabetes), hypertension (≥140/90 mmHg or treated hypertension), dyslipidaemia (triglycerides (TG) >2.0 mmol/l or HDL-cholesterol <1.0 mmol/l or treated dyslipidaemia) or central obesity (waist circumference ≥94 cm for men and ≥80 cm for women) (Balkau and Charles, 1999). Another frequently used definition is the US National Cholesterol Education Program Adult Treatment Panel III definition (National Institutes of Health, 2001). This definition is predominately lipid based and requires three or more of the following factors to be present: abdominal obesity (assessed by waist circumference ≥102 cm in men or ≥88 cm in women), high TG concentrations (≥1.695 mmol/l (≥150 mg/dl)), low high-density lipoprotein (HDL) (<1.036 mmol/l (<40 mg/dl) in men and <1.295 mmol/l (<50 mg/dl) in women), hypertension (≥130/85 mmHg) or hyperglycaemia (≥6.1 mmol/l (≥110 mg/dl)) (National Institutes of Health, 2001).
Several techniques are used to measure insulin sensitivity, some of which are discussed in more detail below. The "gold standard" technique is the euglycaemic-hyperinsulinaemic clamp, described by DeFronzo et al (DeFronzo et al., 1979). This method investigates glucose uptake directly in the steady state and as such the data do not require modelling. Limitations of the technique include risk of hypoglycaemia, lack of metabolic flexibility (as humans live in a non-steady state) and the relative invasiveness, intensity and cost of the technique (Trout et al., 2007, Wallace and Matthews, 2002). It is also not possible from this technique to measure β-cell function (Cobelli et al., 2007). As the clamp is considered to be the gold standard, other methods have been validated against it with varying precision and these are discussed below.

The most common alternative to the clamp is the intravenous glucose tolerance test (IVGTT). The frequently sampled IVGTT (FSIVGTT) involves a short (few minutes) intravenous infusion of glucose at a steady rate for a set time with collection of frequent blood samples to measure changes to glucose and insulin concentrations. This has also been modified to include a short (normally 5 minutes) infusion of insulin to enhance the individual's own insulin response (Trout et al., 2007, Finegood et al., 1990) and this modification has been shown to improve the estimation of insulin sensitivity (Yang et al., 1987). Many different protocols are used for the IVGTT and there is little standardisation between groups, making comparisons between studies difficult (Wallace and Matthews, 2002). In a review by Bingley et al (1992) a standard protocol, not involving
insulin infusion, was proposed. The IVGTT has the advantage that it provides information not only on insulin sensitivity, but also on β-cell function (Bergman, 2005). Data from an IVGTT require modelling to provide indices of insulin sensitivity. The most validated model is the “Minmod”, a minimal model technique developed by Bergman et al (1981) and this software is extensively used; it has been stated to have become “in effect, the “industry standard” for analysing frequently sampled intravenous glucose tolerance test (FSIVGTT) data” (Boston et al., 2003). This model calculates β-cell function and insulin sensitivity from an IVGTT (both the insulin modified and non-modified versions) and involves two mathematical models, the first of which is a model of insulin kinetics (looking at first and second phase of the β-cell response) and the second, a model of glucose kinetics (measuring insulin sensitivity) (Bergman et al., 1981).

The acute insulin response (first phase) observed in the first 8 – 10 minutes is widely reported from IVGTT (Mari et al., 2008, Cobelli et al., 2007). In the first 10 minutes after the glucose has been administered, there is a peak of blood glucose concentrations, as the infused glucose mixes in the circulation, and an initial peak in insulin concentrations due to activation of the β-cells (Trout et al., 2007). Up to 20 minutes after the glucose administration, the glucose disposal is proposed to be glucose-mediated and after 20 minutes is said to be insulin-mediated (Trout et al., 2007). During the insulin modified IVGTT there are two insulin peaks, one is the endogenously produced insulin and the second is due
to the infused insulin. The sensitivity value for the IVGTT is derived from the glucose concentrations in relation to concentrations of insulin. This technique has been reported to be reproducible; it is less invasive, requires less investigator skill (Trout et al., 2007) and correlates well with the clamp (Beard et al., 1986). However, the IVGTT is time consuming and the results are dependent on the minimal modelling software used and on interpretation (Wallace and Matthews, 2002).

The oral glucose tolerance test (OGTT) has also been used to measure insulin sensitivity. The test involves an oral dose of 75 g of glucose and then collection of blood samples for 2 hours. This test is used clinically to determine an individual's glycaemic status and WHO criteria have been developed for diagnostic purposes. Clinically samples are taken at time zero and 120 minutes, but increased information can be obtained by frequent sampling. This test is limited by poor reproducibility and can be affected by gastric emptying rate (Trout et al., 2007); however it can give an indication of whole body insulin sensitivity (Matsuda and DeFronzo, 1999).

Measures of postprandial oral insulin sensitivity have also been developed to calculate the insulin sensitivity to a meal (Caumo et al., 2000). A value of insulin sensitivity is given based on area under the curve (AUC) calculations for insulin and glucose concentrations and involves minimal modelling of glucose kinetics and a mathematical equation of how quickly glucose is absorbed and appears in
the blood following oral consumption (Caumo et al., 2000). Caumo et al (2000) evaluated the postprandial oral insulin sensitivity value against the sensitivity value obtained in the same subjects by an insulin-modified FSIVGTT. The study found that the two insulin sensitivity values obtained were well correlated ($r_s = 0.89$, $p= <0.01$), although the actual values obtained were significantly different ($p= <0.001$) with the oral test value being approximately twice as high as the value from the IVGTT. Another study has shown that whilst the insulin sensitivity values obtained from the oral insulin sensitivity test are correlated with the insulin sensitivity from an IVGTT, the results were also twice as high and results regarding β-cell function were less comparable (Steil et al., 2004). However, the studies concluded that the oral insulin sensitivity test could still be used to assess changes to insulin sensitivity (Steil et al., 2004, Caumo et al., 2000), especially when other types of test may not be possible due to cost or practical reasons (Caumo et al., 2000). Postprandial tests are also useful when gut function or the incretin response is likely to be of importance.

Measurements of insulin sensitivity have also been calculated from fasting blood samples. The most established and validated is the homeostasis model assessment (HOMA). HOMA provides an estimate of insulin sensitivity (HOMA %S) and β-cell function (HOMA %B) compared with a young healthy reference population (Wallace and Matthews, 2002, Matthews et al., 1985). It uses a computer mathematical model, derived from measures of fasting insulin and glucose concentrations (taken at the same time) in individuals with different
degrees of insulin resistance and β-cell function; from this a subject’s %S and
%B can be estimated (Matthews et al., 1985). Values obtained from HOMA have
been shown to correlate well with the clamp (Bonora et al., 2000, Matthews et
al., 1985). Fasting insulin concentrations are also used in isolation as a marker
of insulin sensitivity and it is assumed that any fasting insulin concentration
greater than 60 pmol/l (representing the top tertile of the general population)
would indicate insulin resistance; this value has been shown to correlate with
other factors of the metabolic syndrome. However, a limitation of the method is
the assay methodology. Fasting insulin concentrations form the basis of the
EGIR Criteria for insulin resistance. Both of these fasting measures rely on the
accuracy of a single sample which may be affected by stress or the pulsate
nature of insulin release and it has been proposed that more than one sample
should be taken to account for these effects (Matthews et al., 1985, Wallace and
Matthews, 2002).

1.3.3. Role of insulin in weight and appetite

Fasting insulin concentrations are correlated with the degree of obesity (Meistas
et al., 1983). Postprandial insulin concentrations have also been shown to be
higher in individuals with obesity (Meistas et al., 1983). It has been proposed
that the high insulin concentrations observed in obesity could be due to
increased insulin secretion, decreased clearance or a combination (Meistas et
al., 1983, Rossell et al., 1983, Faber et al., 1981) and that insulin resistance
could play a key role.
Insulin has a role comparable to leptin in appetite regulation (long-term signals) as both increase with body adipose tissue mass (Murphy and Bloom, 2004, Wynne et al., 2005). However, the primary difference between leptin and insulin is the postprandial variation in insulin concentrations (Wynne et al., 2005). There are insulin receptors in the hypothalamus and insulin has been shown to stimulate POMC gene expression and inhibit NPY mRNA expression directly (Heijboer et al., 2006) causing a reduction in food intake (Murphy and Bloom, 2004, Wynne et al., 2005). The lack of association between insulin concentrations and appetite in the obese could therefore potentially be explained by central insulin resistance.

There are conflicting data as to whether glucose and/or insulin are involved in short-term appetite regulation. A recent meta-analysis looking at the association between insulin and appetite in single meal interventions in both normal and overweight subjects (Flint et al., 2007) found that insulin was indeed associated with appetite regulation in normal weight subjects, but this association was absent in overweight subjects and a similar effect was not observed with glucose. Flint et al (2007) proposed mechanisms by which insulin may affect appetite, including direct insulin action on the hypothalamus or indirectly via gastrointestinal hormones.
1.3.4. Glycaemic index

The glycaemic index (GI) was first defined by Jenkins et al (1981). GI refers to a food rather than to an individual and so differs from the glycaemic response, which is the change in blood glucose concentrations of an individual in response to a food. GI is therefore defined as a measure of how much 50 g of the available carbohydrate portion of a food raises the blood glucose concentration of an individual and is calculated using the incremental AUC (iAUC). This is then expressed as a percentage of the blood glucose response to 50 g of glucose in the same individual, again calculated using iAUC. Therefore GI allows a quantitative comparison of blood glucose responses following ingestion of equivalent amounts of digestible carbohydrate from different foods. Foods are then classified based on their glycaemic response, with low GI foods being classified as foods that are digested and absorbed slowly, whilst high GI foods are those that are rapidly digested and absorbed (Brouns et al., 2005). GI values are classified as: low GI < 55, intermediate GI between 55 and 70 and high GI > 70 (Bornet et al., 2007).

High GI foods not only cause a higher glycaemic response, but also elicit higher insulinaemic responses compared with low GI (Ludwig, 2000). The higher insulin responses observed following high GI foods have been reported to potentially promote weight gain (Ludwig, 2000). GI is often used as a surrogate marker for the insulin response, as it is easier to measure than insulin concentration, even
though it is actually the insulin response which is of more metabolic importance due to the associated health problems associated with hyperinsulinaemia.

Whilst the available carbohydrate portion of a food primarily determines the GI, other factors also exert an influence. These include the amount of fructose, lactose, soluble fibre, protein and fat that are present within the food (Bornet et al., 2007, Ludwig, 2000). The degree of food processing and particle size of a food can also affect GI, where the more processed a grain, the higher the GI (Englyst et al., 2003). When foods are combined, as typically consumed within a meal, the GI of each food is altered due to the presence of factors other than the food itself in the meal (Bornet et al., 2007). However, whilst there is lack of agreement between studies as to whether the GI of a meal can be predicted from the GI of each individual food, this is commonly used (Bornet et al., 2007). Wolever et al (2006) concluded from their study that the GI of individual foods is a "significant determinant of the glycaemic effect of mixed meals in normal subjects" when properly applied. In the review by Brouns et al (2005) it is recommended that the GI of a meal is based on the GI of the individual food (that has been measured, not taken from tables) and its contribution to the meal to provide an overall GI for the meal.

Another term used is the glycaemic load (GL). GL is defined as the GI of a food multiplied by the amount (in grams) of carbohydrate present and then divided by 100, and is used as a measure of the glycaemic effect of a meal. It is thought to
be a more sensitive measure of the effect of the available carbohydrate on the blood glucose response as it considers the amount of food being consumed (Lunn and Buttriss, 2007).

It has been proposed that low GI foods and low GL meals, may have a beneficial role on risk factors for chronic diseases, especially T2DM, due to their influence on the glycaemic response (Bornet et al., 2007, Brouns et al., 2005) and therefore there is much debate about the GI concept. Another mechanism by which low GI foods could be beneficial is the effect of the glycaemic response on an attenuated postprandial insulin response (Liljeberg et al., 1999).

Brouns et al (2005) reviewed the methodology used for GI to provide a standard protocol. The review provides a list of recommendations for the methodology, some of which are summarised below. Ten people (healthy males and females) should be used to provide reasonable precision, with tests taking place after an overnight fast (between 10 and 14 hours) and each individual consuming an evening meal, of their choice, but the same prior to each test. The reference food that is recommended is glucose, although the review states that other foods (in particular white bread) can be used, but must be calibrated against glucose. When glucose is used it is recommended that the 50 g is dissolved in 250 ml of water and consumed within 10 minutes. As the reference food is used to determine the GI of the test food it is desirable that this is repeated for each individual to reduce variation and therefore the paper recommends the reference
food is repeated at least once to obtain an average value. Glucose measurement is recommended on fingertip capillary blood (either whole blood or plasma). Insulin concentrations are not needed for routine GI measurements, but are recommended for completeness. Blood samples should be collected on fasting and then 15, 30, 45, 60, 90, 120 minutes from time zero, which is considered as after the first bite/sip of the food/drink being tested. The iAUC (ignoring areas below baseline) is the recommended AUC calculation.

Bornet et al (2007) conducted a systematic review on the effect of GI on satiety and weight regulation. This review found that in short-term studies satiety was higher following low GI foods/meals compared with high GI foods/meals. However, results from long-term studies are less conclusive. The role of GI as a predictor of appetite and satiety has since been reviewed by Niwano et al (2009). They also found that evidence from short-term studies indicated that both the glycaemic and insulinaemic responses were related to appetite and satiety, and reactive hypoglycaemia (blood glucose concentrations falling below baseline in response to food) appeared to indicate an earlier return of hunger. This review also found that the relationship between the glycaemic and insulinaemic responses were less established from long-term studies and further well-designed studies were required. A crossover study conducted for 12 weeks, where high or low GI foods were incorporated into normal diets, found no effect of the lower GI on energy intake, satiety or body weight in obese/overweight women (Aston et al., 2008). In this study effects on satiety were assessed at the
end of the 12 week intervention periods, using *ad libitum* meals and visual analogue scales for subjective measures, and, as is acknowledged by the authors, the lack of findings on the test days may have been due to effects of chronic consumption. However, an eight day crossover study, where high or low GI foods were consumed at all meals in a laboratory setting to investigate the effects of GI and GL, also found no significant differences in ratings of appetite or food intake between the diets and no significant difference in glycaemic or insulinaemic responses (Alfenas and Mattes, 2005). Therefore this suggests that overall the data relating to GI and appetite are inconclusive.

1.4. **Fibre**

1.4.1. **Types of fibre**

In 2008 a definition for dietary fibre (DF) was agreed by the Codex Alimentarius Commission (Codex Committee on Nutrition and Foods for Special Dietary Uses – CCNFSDU), where carbohydrate polymers of ten or more monomers, which are not hydrolysed in the small intestine by enzymes, are considered to be DF. They must be edible carbohydrate polymers that occur naturally in foods, or are carbohydrate polymers taken from raw foods (enzymatically, physically or chemically), or are synthetic carbohydrate polymers. The latter two need to have been shown to have a physiological benefit and need to be verified by scientific evidence (Cummings et al., 2009). Until this definition there were several conflicting definitions that considered different components as DF, although the
majority of definitions considered components that are indigestible in the small intestine as being part of DF (Buttriss and Stokes, 2008).

DF has historically been divided into two classes depending on solubility in water, thus classified as either soluble or insoluble. Insoluble DF includes resistant starch, cellulose, hemicellulose and lignins, whilst soluble DF includes glucans, pentose and oligosaccharides (Lunn and Buttriss, 2007). Soluble DF is predominately found in vegetables, fruits, oats and barley, and insoluble DF, in whole grains (especially wheat and rice) and fibrous parts of plants, although most DF-containing foods are comprised of a mixture of both types. The two types have different properties in the body. Soluble DF absorbs water during transit in the gastrointestinal tract and forms viscous gels, whereas insoluble DF increases bulk and softens stools (Lunn and Buttriss, 2007). However, some newly classified DF, such as resistant starch and inulin, do not fit easily in this classification and can exhibit a mixture of effects. As the terms soluble and insoluble do not completely reflect the physiological properties of all DF, they now tend to be classified as viscous or non-viscous DF, although many published studies still refer to the solubility. DF can also be classified as fermentable or non-fermentable, where the distinction is whether or not the DF reaches the colon, where the colonic microflora ferment the DF to produce products such as gases and short-chain fatty acids (SCFA).
Soluble DF has been linked to lowering of postprandial glucose concentrations and positive effects on blood lipid concentrations, which may be mediated through delayed gastric emptying and subsequent slowing of nutrient absorption in the small intestine. However, epidemiological evidence suggests that it may in fact be the insoluble DF, such as those found in whole grains, that are linked to positive health effects (Weickert and Pfeiffer, 2008, Jenkins et al., 2000), for example a reduced risk for many chronic diseases including CVD, cancers (especially of the gastrointestinal tract), T2DM and obesity.

In a review of carbohydrates and DF by Lunn and Buttriss (2007) it is stated that high DF intakes are linked to five primary physiological benefits: improved gastrointestinal health, improved glucose and insulin responses, reduced CVD risk factors, reduced risk of development of some cancers and increased satiety. However, different DF may result in different physiological effects and an individual DF source may not exhibit all of the benefits.

In the United Kingdom (UK) the current recommendation for DF intake, 18 g per day, was set in 1991 (Department of Health, 1991). The definition was based on measurement of non-starch polysaccharides (NSP) by the Englyst method, as NSP was thought to contribute the most quantitatively to DF, could be measured with good precision and was chemically identifiable (Lunn and Buttriss, 2007). According to the method used by the Association of Official Analytical Chemists (AOAC) total DF would be approximately 24 g, which is more in line with the
recommendations of many other countries (Buttriss and Stokes, 2008). The AOAC method incorporates a greater range of components, including resistant starch, sugar alcohols and the short-chain oligosaccharides, which are not accounted for in the Englyst method. The AOAC method is now commonly used by the food industry for labelling purposes (Lunn and Buttriss, 2007).

Whether 18 or 24 g as a recommendation is taken, the average intake of DF in the UK is well below either of these, at approximately 13 g per day (Buttriss and Stokes, 2008).

1.4.2. Effects of fibre on weight and appetite

DF may be beneficial in weight regulation. This diverse group of carbohydrates has been proposed to increase satiety (Slavin and Green, 2007) and this may therefore be one mechanism by which DF impacts on energy balance.

Studies have investigated the interaction between DF and weight, some of which were reviewed by Slavin (2005). This review found that from observational studies there was an inverse relationship between DF intake and body weight. However, this review also found that evidence from intervention studies was less consistent when DF foods or supplements were investigated. Experimental studies using different types of DF have found variable results, with some DF exhibiting satiating qualities and others not. Delargy et al (1995) performed two studies, one investigating the effects of high versus low DF and the other,
varying ratios of insoluble (wheat bran) and soluble (psyllium gum) DF, on food intake and satiety. These studies found that higher DF intakes were associated with reduced energy intake at the next meal, although daily energy intake was not significantly different between the amount or ratios of DF; but there were slight effects on desire to eat after the high insoluble DF meal.

Pereira and Ludwig (2001) reviewed mechanisms by which DF may affect weight. Several mechanisms were discussed and divided into intrinsic, hormonal and colonic effects. Some of the mechanisms proposed included both the lower energy density (intrinsic effect) of DF and the fermentation of DF (colonic effect), which produces SCFA that may stimulate the satiety hormone GLP-1. This review also discussed other mechanisms including the role of other hormones (such as insulin, which is lowered due to slowed carbohydrate absorption) and effects on gut hormones involved in appetite (Pereira and Ludwig, 2001). Other mechanisms have also been proposed, including delayed gastric emptying, that may then prolong the feeling of fullness or slowed nutrient absorption, particularly glucose, which in turn may prolong satiety (Slavin, 2005, Mattes et al., 2005, Pereira and Ludwig, 2001).

1.4.3. Effects of fibre on insulin

Soluble DF forms gels, which have a beneficial effect on glucose and insulin responses due to reduced absorption rates, although results from long-term intervention studies are mixed (Venn and Mann, 2004). Other studies have
shown that in fact it may be the insoluble DF that has beneficial effects on insulin responses and therefore may have a positive role in T2DM control (Wolever, 2000, Weickert and Pfeiffer, 2008).

In a study by Weickert et al (2005) the short-term effects of insoluble DF on glucose were assessed. The study was a randomised crossover study where subjects consumed three portions of macronutrient matched breads, either control white bread, or bread supplemented with purified insoluble DF (10.5 g wheat fibre, 10.6 g oat fibre or 10.4 g resistant starch) for one day and on the following day the control bread was taken. Effects on blood metabolites were assessed on both days. Overall on the day it was consumed the insoluble DF enhanced the insulin response and, on the following day, after the control meal the postprandial glucose measurements were significantly lower. A further study by Weickert et al (2006) investigated the effects of insoluble DF intake on insulin sensitivity, assessed using an euglycaemic-hyperinsulinaemic clamp. This was a three day randomised crossover study, in which subjects consumed three portions of either control white bread or bread enriched by an insoluble oat fibre. The study found an improvement in insulin sensitivity from the insoluble DF intake.

The beneficial effects of insoluble DF on glucose and insulin responses may be mediated through the production of SCFA from fermentation of DF (Weickert and Pfeiffer, 2008). Several mechanisms have been proposed as to how
increased SCFA may affect glucose and insulin responses. The increased SCFA may decrease hepatic production of glucose and therefore alter insulin requirements (Weickert and Pfeiffer, 2008). Increased SCFA (particularly acetate) have been shown to reduce free fatty acid concentrations (Crouse et al., 1968), which are known to inhibit insulin mediated glucose uptake (Boden et al., 1994) and increase ectopic TG storage, therefore improving insulin sensitivity. Increased SCFA from fermentation of DF may also increase production of GLP-1 (as shown in animal models) which, due to the incretin actions of GLP-1, could affect insulin secretion (Pereira and Ludwig, 2001).

There is also evidence to suggest that DF may influence insulin sensitivity (Slavin and Green, 2007, Weickert and Pfeiffer, 2008) and this may be independent from any effects on body weight or appetite. Weickert and Pfeiffer (2008) reported that, based on the studies they reviewed, diets high in insoluble DF were associated with a reduced risk of T2DM and this could be mediated by improved insulin sensitivity, although the exact mechanism is still unclear.

1.5. **Resistant starch**

1.5.1. **Definition**

Resistant starch (RS) is a fermentable carbohydrate, which is not digested or absorbed in the small intestine, but passes to the colon where it is fermented by colonic bacteria to produce SCFA (including butyrate, acetate and propionate), organic acids (lactic acid) and some gasses (hydrogen, methane and carbon...
dioxide) (Champ, 2004). RS has been defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992). RS, a non-viscous insoluble DF, is a high-amylose starch which is more resistant to digestion than amylopectin (rapidly digestible) (Nugent, 2005) and has been proposed to have effects similar to other DF, such as improved bowel function and reduced risk of some cancers, particularly of the bowel and lower gastrointestinal tract (Goldring, 2004).

RS is subdivided into 4 categories, RS1, RS2, RS3 and RS4. RS1 refers to RS where the granules of starch are surrounded by intact cell walls, making the starch physically inaccessible, and is found in grains, seeds and tubers (Brown, 2004, Tapsell, 2004). RS2 refers to starches which are resistant to the action of amylase due to the structure of the granule and are found in foods such as unripe green bananas and raw potatoes (Brown, 2004, Tapsell, 2004). A form of RS2, high-amylose maize starch, is distinctive from other types of RS as it retains its resistant properties during food processing (Brown, 2004). RS3 is formed upon retrogradation of starches (as in cooked and cooled potatoes or pasta, and stale bread) and RS4 starch granules have been chemically or heat modified by manufacturers so that their digestibility is decreased and they resist digestion by enzymes; this enables them to be added to processed foods (Brown, 2004, Tapsell, 2004). The resistance of the starches can be affected by factors such as processing, cooking and chewing (Nugent, 2005).
RS may be a useful way to increase DF intake at a population level, as it is easy to incorporate into food without adverse effects to either taste or texture (Brown, 2004), which from a food industry perspective is extremely important. The safety of high consumption of RS was reviewed by Goldring (2004), who stated that whilst allergic reactions have been observed with other DF (such as inulin), there are no similar reported reactions with RS. However, some studies have reported increased flatulence, bloating and mild laxative effects, whereas other studies have not found effects of intakes up to 60 g per day.

Most of the studies that have involved supplementation with RS to assess its physiological effects have used RS2 and RS3 in their investigations.

1.5.2. Intake levels of resistant starch

The RS intake in the UK is low and was reported to be approximately 2.76 g per day (Tomlin and Read, 1990) although this estimate is now two decades old. There are differences between countries in RS intake, which can be accounted for by differences in habitual diets. Currently there are no dietary recommendations for the amount of RS specifically that should be consumed within the diet, although it is included in the 24 g DF recommendation by AOAC method.
1.5.3. **Health benefits of resistant starch**

Many potential physiological effects of RS have been suggested, including improvements in bowel health, in glucose and insulin responses and blood lipid concentrations, as well as increases in satiety (Nugent, 2005, Higgins, 2004). Changes and improvements in these and other physiological effects may all therefore have a beneficial role in several chronic diseases, including obesity, CVD, T2DM, metabolic syndrome and some cancers (Nugent, 2005, Higgins, 2004).

1.5.4. **Effects of resistant starch on weight and appetite**

Many studies investigating the effects of RS have measured body weight as an outcome measure; some of these (Muir et al., 2004, de Roos et al., 1995, Heijnen et al., 1996, Phillips et al., 1995) have reported that subjects' body weight remained stable throughout the study. However, in many of the studies, subjects were asked to keep their diets constant or follow their normal dietary pattern, in order to assess other primary outcome measures (Phillips et al., 1995, Muir et al., 2004, Heijnen et al., 1996), which may account for the lack of change in body weight. In the study by de Roos et al (1995) the subjects were able to eat freely and yet body weight still remained constant. Similarly, in a study in overweight and obese subjects that investigated the effects of RS supplementation on blood lipids, glucose and insulin, no change in body weight was observed (Park et al., 2004). Another study found that after supplementation with 30 g RS for 4 weeks there was no change in body weight.
or BMI, but that there was a small significant increase in lean body mass (Robertson et al., 2005).

Although the evidence for an effect of RS consumption on body weight is limited, different mechanisms have been proposed as to how RS may result in weight loss. These include lower energy intakes due to RS having a lower energy value per gram compared with rapidly digestible starch (RDS) (Raben et al., 1994, Champ, 2004, Nugent, 2005). It has also been stated that RS has similar properties to other DF (Phillips et al., 1995) and therefore may effect satiety and weight regulation, but further well controlled long-term interventions are required.

A review by Tapsell (2004) examined the effects of RS on the metabolic syndrome. It was stated that it is possibly the effects on slower absorption of glucose and the subsequent effects on lowering insulin concentrations that may affect hunger, fat storage and weight regulation.

RS may also affect weight regulation due to direct effects on appetite. Several mechanisms have been proposed, but the actual mechanism, if any, is not known. The production of SCFA from colonic fermentation of RS may cause an increase in the production of GLP-1, or a decrease in the release of glucose from the liver, which in turn may alter the requirement for insulin and therefore
indirectly affect satiety (Pereira, 2002). RS may also affect appetite through mechanisms similar to those of other DF, as discussed previously.

Animal studies consistently show positive effects of RS on appetite, and rodent studies have also demonstrated effects of RS on gut hormone release (Keenan et al., 2006, Zhou et al., 2006, Zhou et al., 2008). These results have yet to be fully demonstrated in humans.

A human study by de Roos et al (1995) investigated the effect of one week supplementation with 30 g RS2, or 30 g RS3, or glucose, incorporated into habitual diets. Overall the study found that supplementation with either type of RS had little effect on appetite or food intake, (although subjects reported being slightly more satiated with RS2) and caused no change to energy or macronutrient intake.

A study by Raben et al (1994) investigated the effects of acute ingestion of RS compared with digestible starch, both mixed into an artificially sweetened fruit syrup. The study found postprandial glucose, insulin, gastric-inhibitory polypeptide (GIP) and GLP-1 concentrations were significantly lower after RS compared with the digestible starch, and that feelings of fullness and satisfaction were greater with digestible starch than RS. However, the interpretation was confounded as the texture of the drinks was different (the digestible starch was thick whilst the RS was liquid) which may have resulted in differences in gastric-
emptying, and the fact that postprandial measures were only taken for 5 hours and the RS may need longer to have an effect.

In a study by van Amelsvoort and Weststrate (1992) the effects of different ratios of amylose to amylopectin in meals were assessed. It was found that immediately after consumption the high amylose was more satiating and that the effect lasted for up to 6 hours; although results may have been adversely affected by palatability. In a further study by Weststrate and van Amelsvoort (1993) there were no significant differences between different ratios of amylose and amylopectin in subjective appetite ratings.

A study by Robertson et al (2005) investigated the effects of four week supplementation with RS on insulin sensitivity (assessed using an euglycaemic-hyperinsulinaemic clamp and by postprandial oral insulin sensitivity using the minimal model method described by Caumo et al (2000)). As part of the outcome measures from the study, effects on ghrelin, GLP-1 and leptin were measured. The study found no significant difference on GLP-1 or leptin between the RS and placebo, but did find a significant increase in fasting concentrations of ghrelin.

Combining data there appears to be no effect or only a weak link between RS intake and satiety in the short term although further research is required (Higgins, 2004, Nugent, 2005).
1.5.5. Effects of resistant starch on insulin

As discussed above, viscous DF is thought to lower insulin and glucose concentrations when consumed acutely. As RS is a non-viscous DF it would not be expected to affect these postprandial responses. Some studies have shown that RS consumption causes reduced glycaemic and insulinaemic responses when it has been used as a replacement for normal flour or other carbohydrates. However, in some of these studies (Raben et al., 1994, Park et al., 2004) the substitution with RS has resulted in differences in the amount of available (glycaemic) carbohydrate, GL and energy between the supplements, which could explain the effects observed on lower insulin and glucose responses.

A study by Robertson et al (2003) matched the amount of available carbohydrate between the RS supplement and the placebo. This study found that, after one day’s consumption of 60 g RS compared with an available carbohydrate matched placebo, postprandial glucose and insulin concentrations were lower, in response to a standard fibre-free meal. There was also a higher insulin sensitivity (assessed by postprandial oral insulin sensitivity using the minimal model method described by Caumo et al (2000)) following the RS than the placebo.

A study by Behall and Hallfrisch (2002) looked at ratios of amylose in breads and the amount required to affect glucose and insulin concentrations. This study found that 50% (equivalent of approximately 8 g RS) or more amylose was
required to affect the glucose concentrations and 60% (equivalent of approximately 11.5 g RS) or more to affect insulin concentrations. In a study by Behall and Howe (1995) the effects of 14 week consumption of high versus low amylose on insulin, glucose and TG concentrations were assessed. It was found that the insulin response was lower following high amylose than low amylose, but the glucose responses were similar. The study also found that the TG concentrations were lower with the high amylose starch.

In a study by van Amelsvoort and Weststrate (1992) the effects of amylose to amylopectin ratios in meals were assessed; they found lower glucose and insulin concentrations following the high amylose meals in the first hour after consumption, but the glucose concentrations between 2 and 6 hours post meal were higher with the high amylose starch. Another study by Weststrate and van Amelsvoort (1993) also investigated the effects of the different ratios of amylose and amylopectin, on postprandial insulin and glucose concentrations. They found that in response to the test breakfasts there were no significant differences between the supplements for glucose or insulin concentrations, although these were slightly lower with the high amylose. After the test lunches the glucose concentrations were significantly lower with the high amylose than the low amylose, but there were no significant differences between the starches for the insulin concentrations. A further study was conducted by Heijnen et al (1995) where the effects of different ratios of amylose and amylopectin, in different food matrices, on glucose and insulin concentrations were assessed.
They found that in drinks the glucose and insulin responses were lower with the high amylose ratio, the glucose responses were lower in puddings with high amylose and there were similar effects on glucose and insulin when the different ratios were mixed into breads. Overall this study found that different physio-chemical properties of foods, into which amylose is mixed, impact on the subsequent effects observed on postprandial glucose and insulin concentrations.

Studies have investigated the effects of RDS and slowly digestible starch (SDS) on glucose and insulin responses. SDS, similarly to RDS, is digested completely in the small intestine (unlike RS), but the digestion of SDS is slower than that of RDS (Cummings and Englyst, 1995, Englyst et al., 1996). One study gave participants (healthy and those with T2DM) 50 g of each of the starches, on 2 different occasions, and collected blood samples over a 6 hour postprandial period (Seal et al., 2003). The study found higher glucose and insulin concentrations with the RDS compared with the SDS in both participant groups. Those with T2DM also attended for an additional study visit where the SDS dose was increased to 89.7 g in order to try and match the glycaemic response observed with the RDS. Whist the glycaemic response with the greater dose of SDS was higher than the lower dose of SDS, it was still lower than RDS, although these differences were not significant (Seal et al., 2003). In a study by Ells et al. (2005) the effects of RDS and SDS on glucose and insulin were also compared. In this study participants consumed 75 g of each starch and attended
for a 6 hour postprandial study day, at the start (to assess acute changes) and end of the 14 days (to assess adaptation to 75 g/day), where the starch was consumed at a breakfast test meal. This study found significantly greater changes in glucose and insulin concentrations after the RDS compared with the SDS, although the 14 day adaptation period did not alter the findings.

The study by de Roos et al (1995), investigated the effects of one week supplementation with 30 g RS2, or 30 g RS3, or glucose, incorporated into habitual diets. Urine was also collected to assess C-peptide concentrations as a marker of insulin secretion. The study found that C-peptide concentrations were significantly lower following RS3 than either the RS2 or glucose, but were highest following the week's supplementation with glucose, which the authors suggest shows that RS decreases insulin secretion.

The study by Robertson et al (2005) with four weeks' supplementation of RS compared with an energy and carbohydrate matched placebo found an improvement in insulin sensitivity (measured by postprandial oral insulin sensitivity using the minimal model method described by Caumo et al (2000) and an euglycaemic-hyperinsulinaemic clamp) with RS compared with placebo.

Nugent (2005) reviewed studies that had investigated the effect of RS on insulin and glucose responses and found mixed results, with some showing decreased glucose and insulin responses and some no effects, although it was noted that
there was no evidence for a detrimental effect of RS consumption on either insulin or glucose. The review also stated that it is difficult to compare the different studies as they have used different protocols and have used test meals that have varied in macronutrient content and amounts of available carbohydrate and DF, all of which may impact on insulin and glucose responses.

Higgins (2004) also reviewed effects of RS on insulin and glucose responses and looked at both acute and chronic effects. The acute studies reviewed showed mixed findings, for which proposed reasons included different types of RS being used in the studies, the macronutrient and DF content of tests meals being different and the effects of processing of the starch in the test meal. However, overall Higgins concluded that RS causes a small decrease in postprandial glucose responses and a greater effect on postprandial insulin concentrations. From the review of the chronic studies it was indicated that whilst it appears that RS improves insulin sensitivity in rodent studies, there were less available data for an effect in humans.

1.6. **Whole grains**

1.6.1. **Definition**

Whole grains are comprised of three layers; the bran, germ and endosperm. Each of these layers has a different nutritional composition with the presence of both nutrients and non-nutrients. The bran contains DF, B vitamins, phytonutrients (for example flavonoids and indoles) and some proteins; the
endosperm is mostly made up of starch, with some proteins and a small amount of vitamins and minerals; the germ contains minerals (including iron and zinc), vitamin E, B vitamins and phytochemicals (Lang and Jebb, 2003). Commonly, when whole grains are milled the bran and germ layers (most nutrient dense parts) are removed leaving just the endosperm that is predominately digestible starch and thus of less nutritional value (Slavin, 2004, Smith et al., 2003). Although many refined flours are fortified to replace some of the lost nutrients, it is unclear whether this would provide the same effects as when the nutrients are present together in whole grains in natural amounts (Smith et al., 2003). The structure of a whole grain is shown in Figure 1.4.

![Figure 1.4: Structure of a whole grain. Taken from Slavin (2004).](image)

A grain can be termed a ‘whole grain’ even after processing, if all three layers are present in the proportions they are normally found in the native whole grain. The most often used and accepted definition of a whole grain, as stated by the
American Association of Cereal Chemists (AACC) (1999) is: "Whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis". Similarly, in the UK, the Institute of Grocery Distribution (IGD) (2008) whole grain Working Group recently defined whole grains as including those which have been processed in some manner, as long as the three main components are present in their normal proportions. The IGD (2008) definition states that a whole grain is "the edible entire grain after removal of inedible parts such as the hull and glume. It must include the entire germ, endosperm and bran".

The definition for a wholegrain food that is most commonly used is the one by the USA Food and Drug Administration (FDA) (1999), where in order for a food product to be considered a wholegrain food, the whole grains must make up 51% or more, by weight of the portion that is typically consumed. The IGD (2008) recommend that, in order to claim that a product contains whole grains on the food label, it must contain at least 8 g whole grain per serving of the food.

1.6.2. Intake levels of whole grains

The consumption of whole grains in the UK is low. In a review by Lang et al (2003) intakes of whole grains in the UK were assessed, from two National surveys, the Dietary and Nutritional Survey of British Adults (aged 16-64 years) in 1986-87 and the National Diet and Nutrition Survey (in adults aged 65 and
more) in 1994-95. From both surveys it appeared that a third of the UK adults surveyed did not consume whole grains daily and that 90% of those questioned consumed less than 3 servings per day. They also found that whole grain-intake increased with age and was higher in non-smokers and those with non-manual jobs. Whole grain-intake in British Adults in 1986-87 has also been compared with more recent intake data, from the National Diet and Nutrition Survey 2000-2001, by Thane et al (2007). This study found that whole grain-intake actually decreased from a median intake of 16 g per day (in 1986-87) to 14 g per day (in 2000-01). Interestingly, the proportion of adults consuming no whole grains has increased over time (from 31% to 39%). In both surveys whole grain-intake was lower in smokers than non-smokers, manual workers versus non-manual workers and older age groups consumed more whole grains than those in younger age groups.

There are many varieties of whole grains including, but not limited to, wheat, oats, rye, maize, rice and barley, although in the UK the most regularly consumed whole grains are wheat, rice and maize (Seal et al., 2006). Whilst all types of whole grain are comprised of the bran, germ and endosperm the actual nutrient content differs between grains, for example wheat grains are high in insoluble DF, whilst oats are high in soluble DF (Smith et al., 2003). In the work by Lang et al (2003), described above, it was found that wholegrain food choice also differs. In the younger group (adults aged 16 -64 years), the majority of whole grain-intake came from breads (48%) and then from breakfast cereals.
(29%); in older adults (aged 65 and over) most was from breakfast cereals (46%) and then wholemeal breads (42%).

Currently there is no dietary recommendation for whole grain consumption in the UK. However, higher amounts of DF rich foods are encouraged (Lang and Jebb, 2003), as well as whole grain varieties of starchy foods.

1.6.3. Health benefits of whole grains

Whole grains have been proposed to be beneficial in many chronic conditions including obesity, CVD, some cancers and T2DM (Seal et al., 2006, Slavin, 2004, Smith et al., 2003). Although there are some intervention data, the majority of the evidence comes indirectly from epidemiological studies.

The health benefits of whole grains may come either from the individual components or from the different components working synergistically. Some whole grains contain high amounts of DF and in particular fermentable carbohydrates, RS (predominately RS1) and oligosaccharides (Smith et al., 2003, Slavin, 2004). These fermentable carbohydrates have individually been shown to be beneficial, mostly likely due to fermentation and production of SCFA. Indeed it was stated in a review by Brown (2004) that "RS1 is likely to provide an important health contribution in whole grain foods". Whole grains are also a good source of phytosterols (plant sterols and stanols), that lower blood cholesterol concentrations due to their similar structure to cholesterol (Slavin,
2004), and are a good source of antioxidants (water and fat soluble), that are known to be important as a protective mechanism in many chronic diseases (Slavin, 2004).

Although evidence suggests high intakes of whole grains are beneficial, disentangling the effects of whole grains compared with DF alone is not always possible. It has been consistently found that individuals who consume higher amounts of whole grains also are likely to have other healthier lifestyle habits, such as not smoking, high activity levels, high consumption of fruits and vegetables and lower fat intakes (McKeown et al., 2002, Lang and Jebb, 2003). These factors serve as confounders to the interpretation of the whole grain data, although many of the studies do claim to adjust for these factors within their statistical analysis.

1.6.4. **Effects of whole grains on weight and appetite**

Whole grains have been proposed to be protective against the development of obesity (Koh-Banerjee and Rimm, 2003). However, there are limited studies, especially interventional, which have looked directly at the relationship between whole grains and body weight, with most of the existing evidence coming indirectly from epidemiology. Cohort studies do suggest an inverse association between body weight and higher levels of whole grain-intake (Koh-Banerjee and Rimm, 2003). However, some care needs to be taken when interpreting the data due to confounding factors, such as the often self-reported anthropometric
measurements and whole grain-intake data obtained from questionnaires. Some of the evidence proposing a link between whole grains and weight comes from studies in which weight was not a primary outcome measure, but measured simply from routine.

The Nurses' Health Study carried out over a period of 10 years found that women who had higher whole grain-intakes at baseline tended to weigh less than those with lower intakes, whereas for refined grain-intakes, high intakes were associated with higher body weights (Liu et al., 2003). They also found that, although BMI (calculated from self-reported weights) in the whole cohort increased over time, the increase was smaller in those who consumed whole grains rather than refined grains. Liu et al also reported that over a 10 year period whole grain-intake did increase slightly, by 0.13 servings per 1000 kcal. An inverse association has also reported between weight gain over 8 years and high levels of whole grain-intake from data from another prospective cohort study, the Health Professionals' Follow-up Study (Koh-Banerjee et al., 2004).

In a study by McKeown et al (2002) it was found that after adjustment for confounding factors (such as age and gender) those who consumed high whole grain-intakes had lower BMI than those with low whole grain-intakes. The study also found that there was a significant inverse relationship between whole grain intake and waist to hip ratios, concentrations of fasting insulin, total cholesterol
and LDL cholesterol. However, the relationship with insulin concentrations was lessened when corrections were made for DF and magnesium intakes.

Data from the Physicians’ Health Study examined the link between whole and refined grain breakfast cereal intake and weight change in men (Bazzano et al., 2005). The study found that those who consumed breakfast cereals gained less weight than those who did not, although they did not find a significant difference between whole grain and refined grain cereals. The study also found that those who ate breakfast cereal also exhibited other healthier lifestyle choices (such as not smoking and having high physical activity levels). The authors attributed a lack of association between whole and refined grains to only assessing intake of breakfast cereals and not total daily grain intake.

A review by Seal (2006) discussed proposed mechanisms by which whole grains could lower CVD risk, including the beneficial effect of high whole grain intake on body weight. This review summarised several large studies (some of which were discussed above) that had investigated the effects of whole grains where weight and/or anthropometrics had been outcome measures. It was concluded that there appeared to be a small inverse relationship between whole grain intake and body weight, but that further longer-term intervention studies were required.
The exact mechanism, if any, by which whole grains may affect weight is not known, although various hypotheses have been proposed. These include effects of whole grains on the promotion of satiety, delayed digestion and absorption of nutrients and lower postprandial glucose and insulin concentrations (Slavin, 2004). The effects of whole grains on body weight regulation have been specifically postulated by Pereira (2002) and included the lower energy density of whole grain foods, the promotion of satiation (due to low palatability and effects of chewing) and prolonged satiety due to effects on gastrointestinal and pancreatic hormones. The presence of high amounts of DF and RS and their associated effects may also be a mechanism, as discussed above (Pereira, 2002), although as yet it has not been fully demonstrated that the effects of whole grains are fully independent from those of DF.

Although whole grains have been proposed to affect satiety (Slavin, 2004, Pereira, 2002), there is no direct evidence confirming this effect, although some indirect evidence exists.

In a study investigating the effects of whole grain intake on insulin sensitivity assessed using an euglycaemic-hyperinsulinaemic clamp (Pereira et al., 2002), all meals provided to the subjects for 6 weeks, were isoenergetic for energy requirements and weight maintenance. The subjects completed a daily questionnaire regarding ratings of hunger, which indicated a trend for less hunger between meals with whole grains compared with refined grains.
Another study investigated the effect of particle size (a measure of grain processing) on satiety and glycaemic response (Holt and Miller, 1994). Four grades of wheat, whole grains, cracked grains, coarse wholemeal flour and fine wholemeal flour, were used. It was found that the satiety scores were higher in the less processed grains than with the refined grains. The glucose and insulin findings were inversed with the fine wholemeal flour producing higher responses than the less processed grains.

1.6.5. Effects of whole grains on insulin

In a review by Koh-Banerjee and Rimm (2003) improvement in insulin sensitivity was discussed as an indirect mechanism by which whole grains may be beneficial in weight regulation, due to the effects of high insulin concentrations on weight (as discussed above, section 1.3.3).

The relationship between whole grain intake and insulin sensitivity was discussed in a study by Steffen et al (2003). This study found that higher whole grain intake was associated with lower BMI and improved insulin sensitivity (measured by euglycaemic insulin clamp) in adolescents, and this association was strongest in adolescents with higher BMIs.

Data from a prospective cohort study showed an inverse association between whole grain-intake and several of the outcomes investigated, including BMI,
inflammation, fasting insulin and insulin resistance (assessed by HOMA) (Lutsey et al., 2007).

The relationship between whole grain-intake and insulin sensitivity was also assessed in a study by Liese et al (2003). Data collected from the Insulin Resistance Atherosclerosis Study, combined insulin sensitivity, assessed by an insulin modified FSIVGTT, and whole grain-intake, assessed from food frequency questionnaires. The study found that on average the subjects consumed less than 1 serving of whole grain per day, but that high whole grain-intake was positively associated with insulin sensitivity and lower fasting insulin concentrations, when adjustments were made for lifestyle factors (such as smoking, energy intake and energy expenditure). When the authors adjusted for BMI and waist circumference the relationship remained, although at a lower significance level.

A study by Pereira et al (2002) investigated the effects of a 6 week whole grain intervention on insulin sensitivity in overweight adults. This was of a crossover design with all food on both legs (refined grain diet or whole grain diet) provided to the subjects and insulin sensitivity assessed by an euglycaemic-hyperinsulinaemic clamp. It was found that insulin sensitivity was increased following the whole grain compared with the refined grain intervention and fasting insulin concentrations were 10% lower with the whole grains compared with the refined grains.
The effect of whole grains on insulin sensitivity, as measured by a euglycaemic-hyperinsulinaemic clamp, was also assessed in a recent study by Andersson et al (2007). This study found no improvement in insulin sensitivity in overweight subjects, from 6 week supplementation with either whole grain foods (112 g per day of whole grain ingredient) compared with refined grain foods, both supplied to be incorporated into habitual diets.

The combined effects of whole grains on insulin sensitivity were reviewed by McKeown (2004), in which potential mechanisms by which whole grains improve insulin sensitivity were proposed. These include (i) the high level of DF that would increase SCFA production and therefore improve insulin sensitivity, (ii) DF may reduce absorption of nutrients, leading to a reduced postprandial response of glucose and insulin, (iii) the high level of magnesium in whole grains could improve insulin signalling and regulate the action of insulin in peripheral tissues. Alternatively it is proposed that it may be the lower GI of whole grain foods that could positively affect blood glucose and insulin concentrations, or the mechanism may be the effects of whole grains on body weight, as discussed above.

Whilst grains that have been processed are still considered to be whole grains, as per the definitions described above (section 1.6.1), the structure/form that the whole grain is in can have different effects on the metabolic response. Several studies have shown that when the whole grain is in the intact form there is a
lower postprandial glycaemic and insulinaemic response than when the whole grain has been processed (Bjorck et al., 1994). For example, a study by Holm and Björck (1992) investigated the effects of different breads (3 types of white wheat bread, 2 types of coarse breads and 3 high soluble DF rich breads) on glucose and insulin responses. The authors found that the coarse bread containing intact wheat kernels and the soluble DF rich bread (oat bran) produced the lowest metabolic response. An older study by Heaton et al. (1988) investigated the effect of particle size (including whole grains, cracked grains and milled flours) of different whole grains (wheat, maize and oat) on glucose and insulin responses. This study found that for the wheat whole grains the glucose AUCs tended to be higher with the flours than with the less processed grains, although not significantly, whilst the insulin AUCs increased as the grains were more processed. The glucose AUCs were also not significantly different between the 3 types of maize or between the 3 types of oats. For the maize, insulin responses were higher with the flour than the other 2 types, whereas for the oats there was not a difference. Overall this suggests that particle sizes of wheat and maize may have different metabolic effects. However, whilst these studies show particle size to affect the metabolic response, a study by Behall et al. (1999) comparing the effects of white bread, whole grain breads (one made with traditional whole grain wheat flour and the other with fine ground whole grain wheat flour) found no effect of the different particle sizes on the insulin or glucose AUC.
The higher glucose and insulin responses observed with greater processed grains, compared with less processed grains, are most likely to be due to the change in structure which would increase accessibility of the carbohydrates to be digested and absorbed (Bjorck et al., 1994, Venn and Mann, 2004).

1.7. Aims of research

RS, a major DF component of whole grains, has been proposed to affect satiety, but there is little direct evidence in humans to confirm this. Whole grains have also been proposed to affect satiety and therefore may have a beneficial role in weight management; there is however, little direct evidence, with most coming from epidemiological studies.

This research was designed to test the hypothesis that the inclusion of RS into habitual diets, both acutely and chronically, would reduce appetite and food intake. It was also hypothesised that short and long-term consumption of whole grains, incorporated into bread rolls, would reduce appetite and food intake.

Therefore this research aimed to determine the effects of both RS and whole grains on appetite and food intake. In order to achieve these aims, a series of studies were conducted to investigate the role of both short and long term consumption of RS and whole grains on appetite and food intake. Some mechanisms by which these products exert their effects were also considered.
Chapter 2

General Methods
This chapter describes the common methods used within the clinical studies and associated laboratory analysis; any deviations from these methods are described in each individual chapter in the study protocol section.

2.1. **Participant Recruitment**
All participants for the following clinical studies were recruited from the University staff and student population, by email and poster advertisements, and from the general population, using poster, email and, in some instances, newspaper advertisements. Individuals who had participated in previous studies and expressed interest in future studies were also contacted.

2.2. **Screening**
Prior to each study all participants were screened to ensure that they met the specific study inclusion criteria. The screening session always involved discussion of the study with the participants and completion of a health and lifestyle questionnaire. For the appetite studies, the Dutch Eating Behaviour Questionnaire (DEBQ) was completed. For the studies where blood was to be taken, haemoglobin and blood glucose concentrations were checked prior to inclusion. In all studies participants gave informed written consent. A favourable ethical opinion for each study was obtained from the University of Surrey’s ethics committee and, for the study described in Chapter 4, from Surrey Research Ethics Committee, as part of the study was conducted on NHS premises.
2.2.1. Health and Lifestyle Questionnaire

These self-certificate medical questionnaires were tailored to each study, but in general included questions regarding the participants' past and current medical history, any medications they were taking and questions specific to their lifestyle, including smoking, exercise and alcohol intake (see Appendix 1).

Specific exclusion or inclusion criteria for each study are detailed in the individual chapters; however general exclusion criteria for the studies included individuals with current or previous medical conditions, including cardiovascular disease (for example stroke and angina), diabetes, gastrointestinal diseases (for example Crohn's disease, Coeliac disease, Irritable Bowel Syndrome), liver disease, endocrine diseases and clinical depression or other psychological disorders. Other exclusion criteria included individuals with anaemia, those following weight reducing diets and those not weight stable for at least the preceding three months. Participants were also excluded if they had participated in another clinical trial in the preceding three months, if they had a history of drug or alcohol abuse in the last two years, were pregnant or lactating females, or were taking certain prescription medications and supplements. There were no exclusions based on ethnicity for any of the studies.

2.2.2. The Dutch Eating Behaviour Questionnaire

Factors that can influence food intake need to be considered when changes to appetite are a primary outcome measure. Thus at screening participants for the
appetite studies completed the DEBQ (see Appendix 2) to ensure they were not highly restrained, emotional or external eaters.

The DEBQ was developed in the 1980s and the 33 questions are based around the psychosomatic (emotional eating) theory, externality theory and the theory of restrained eating (van Strien et al., 1986). The psychosomatic theory concentrates on emotional eating, which is described as excessive eating in response to states of arousal (such as stress or fear) when normally these emotions would result in loss of appetite (van Strien et al., 1986). The externality theory is portrayed by excessive eating in response to food cues irrespective of feelings of hunger/fullness (van Strien et al., 1986). The theory of restrained eating suggests that an individual's "natural weight" is set higher than others' and therefore there is constant pressure to reduce weight, even in the presence of hunger, resulting in continuous control by dieting; once this control is lost, excessive eating results (van Strien et al., 1986).

The DEBQ questions require a response of never, seldom, sometimes, often, very often and not relevant. These are accorded a score of 1 – 5 with not relevant scoring 0. Each of the questions are separated into those relating to emotional, restrained and external eating (as indicated by the colours on the questionnaire, green for external, blue for emotional and red for restrained). The values for each category are averaged to provide a score. If a participant responds "not relevant" to a question the division for that category is reduced by
one for each not relevant response. A high cut-off of 4.5 for all studies was used in order to exclude only those who were highly responsive in any one category.

2.2.3. Blood glucose and haemoglobin concentrations

Haemoglobin concentrations were checked on capillary blood and analysed on an haemoglobin HemoCue® 201+ (HemoCue, Sweden). Fasting blood glucose was checked on screening; this was also assessed on capillary blood and analysed on a HemoCue® glucose 201+ analyser (HemoCue, Sweden). Those with concentrations outside of standard normal ranges, >6 mmol/l for fasted blood glucose and <13 g/dl for men and <12 g/dl for women for haemoglobin concentrations (World Health Organisation (WHO), 1968), were excluded from study participation. In Chapter 6 screening blood samples were collected by venepuncture.

2.3. Anthropometric measurements

For each study anthropometric measurements were taken using the standardised procedures indicated below; all measurements were taken by the same investigator within a study in order to reduce inter-operator variation.

2.3.1. Height

Height was measured using a standard stadiometer. The participants removed their shoes and stood straight with their heels together and their back against the stadiometer. Height was recorded in centimetres to the nearest 0.1cm.
2.3.2. **Weight, Body Mass Index and % Body Fat**

Weight, BMI and % body fat were taken on Tanita scales, which measure body fat by bioimpedance (Tanita TBF-300, Tanita, United Kingdom). Prior to the measurement all participants removed shoes and socks, they were asked not to wear moisturiser and to go to the toilet just before the measurement was taken in order to standardise water content on each visit. All measurements were taken to one decimal place.

2.3.3. **Waist and Hip Circumferences**

Waist and hip circumferences were taken with the participants in a relaxed position. In order to standardise the measurements, waist circumference was taken around the navel and hip circumference at the participants' widest point around the hip bone. All measures were taken to the nearest 0.1 cm.

2.3.4. **Blood Pressure**

Blood pressure measurements were taken on each study visit using an automatic blood pressure cuff (Omron MX3 Plus, Omron Healthcare Europe, United Kingdom). Participants sat and relaxed for five minutes prior to the measurement. Three readings were taken on the relaxed non-dominant arm, whilst the participant remained silent. In Chapters 3 and 6 the three readings were averaged and in Chapters 4 and 5 the third reading was recorded.
2.4. **Subjective Appetite Ratings**

Feelings of hunger and fullness vary between individuals and thus monitoring subjective feelings are important aspects of appetite research. Subjective appetite measures are most frequently taken using questionnaires, in particular visual analogue scales (VAS). Initially VAS were used to assess pain and have evolved for use in other areas, such as appetite (Wewers and Lowe, 1990). The VAS used in the following studies have previously been validated by Flint, *et al* (2000). VAS are quick and easy to use and allow for simple measurement of subjective appetite; they also allow for differences in feelings to be calculated over time and between individuals (Flint *et al*., 2000, Stubbs *et al*., 2000). The reliability of VAS in appetite research has been evaluated; in some studies it has been suggested that the reproducibility is low (Raben *et al*., 1995), although the authors suggest this may be due to routine daily changes in biological processes. More recent studies and reviews have reported VAS to have good reproducibility for appetite research use (Flint *et al*., 2000, Stubbs *et al*., 2000).

VAS are a 100 mm horizontal line with extremes of feeling at each end (*Figure 2.1*). Participants place a mark on the line according to how they feel at the time, and are told to regard the statements at the ends of the line as the extremes of feeling that have ever been felt. The questions were given to participants in booklets (a new booklet each time) with each question on a separate page, to ensure that the marks could not be copied or compared with previous responses.
I am not hungry at all I have never been more hungry

Figure 2.1. An example of one of the questions used in the visual analogue scales.

The measurement was taken from the left end of the line to the participants' mark to obtain the appetite rating. The questions used in this research are shown in Appendix 3 and are standardised questions in appetite research.

In the studies two fasting VAS were completed (at least 15 minutes apart) and then further VAS were completed every 30 minutes during the postprandial study period.

2.5. **Actual Food intake**

Effects on food intake were assessed using two methods. The first investigated the effects on food intake at an *ad libitum* test meal and the second investigated differences in food intake over longer periods of time using diet diaries.

2.5.1. **Ad libitum Test Meal**

At the end of each postprandial study day participants were presented with a large pre-weighed *ad libitum* test meal. This was a homogenous meal designed to exceed normal portion sizes, and was comprised of 400 g dry weight fusilli pasta (Tesco, UK), 500 g Ragu® original tomato sauce (Unilever Foods, UK), 100 g mild cheddar cheese (Tesco, UK) and 30 g vegetable oil (Tesco, UK). The nutritional composition of the meal is shown in Table 2.1. The meal provided
27.2% of energy from fat, 14.1% of energy from protein and 58.7% of energy from carbohydrate.

Table 2.1: Nutritional composition of the *ad libitum* test meal.

<table>
<thead>
<tr>
<th>Energy</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Sugar</th>
<th>Fat</th>
<th>Saturates</th>
<th>Fibre</th>
<th>Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>KJ</td>
<td>Kcal</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(mg)</td>
</tr>
<tr>
<td>9765</td>
<td>2315</td>
<td>81.5</td>
<td>339.1</td>
<td>44.7</td>
<td>70.0</td>
<td>25.3</td>
<td>15.9</td>
</tr>
</tbody>
</table>

The reproducibility of homogenous test meals to assess *ad libitum* intake has been reported (Gregersen et al., 2008); it was found that the measurement of energy intake, from the *ad libitum* test meal used, was reproducible. Other test meals, such as a buffet meal, have been used in previous studies (Martins et al., 2007b), which have been shown to be useful in not only obtaining energy intake at an *ad libitum* meal, but also at identifying macronutrient choice. The use of buffet meals for assessment of *ad libitum* intake has been evaluated and has been found to be highly reproducible for assessment of energy and macronutrient intake (Arvaniti et al., 2000). In the studies described in the following chapters the primary outcome from the *ad libitum* test meal was overall energy intake and therefore a homogenous meal was considered to be the most appropriate.

The *ad libitum* meal was weighed before and after consumption in order to calculate weight consumed. Participants consumed the meal in an isolated room with no distractions and were told to consume as much as they wanted until
comfortably full; the participants were also informed that they could take home any of the meal that was not consumed to prevent over-consumption.

The total energy and macronutrient composition of the meal was identical on each visit; however as the pasta absorbed a slightly different amount of water each time during cooking the energy density per gram of food varied. An adjustment for this was made on each visit to allow calculation of the actual amount of energy consumed.

2.5.2. Diet Diaries

Diet diaries (Appendix 4) were used to assess energy and macronutrient intake either on one day or over a seven day period. Participants recorded all they consumed during this period in as much detail as possible. Standard portion sizes of some foods and photographs were included in the back of the diary to assist participants in providing more accurate details about the food they consumed.

The completed diaries were discussed with the participants immediately on return to ensure that they were legible and any further details were recorded. The diaries were analysed for average daily intakes of energy, protein, carbohydrate, sugar, total fat, saturated fat, total DF (TDF), alcohol and sodium. Analysis was performed using WinDiets Professional Version (Robert Gordon University, Aberdeen, UK) nutrition analysis programme. When the diaries were
analysed, generic foods in the nutritional analysis programme were used and were standardised across all diaries, unless specific food brands had been provided by the participants. If specific food details were given the nutritional information for these products was obtained and added into the programme.

As dietary information was taken from the specific food packets wherever possible, the DF values obtained would have been measured by the AOAC measurement method. However, where generic foods within the program were used the AOAC cannot be guaranteed as the DF measurement method.

2.6. **Resistant Starch and Placebo Supplements**

The supplements used in the studies investigating the effects of RS were manufactured and supplied by the National Starch Company, LLC (NJ, USA). The RS supplement (Hi-Maize® 260), a high-amylose maize starch, is comprised of 60% RS2 and 40% RDS; the RS portion of the supplement was measured by the AOAC TDF method 991.43. The placebo (PL) supplement (Amioca®) is comprised of 100% RDS.

In the RS studies the amount of each supplement given was balanced to provide the same amount of RDS and therefore provided an identical amount of available carbohydrate between the two supplements, resulting in products which provided a similar glycaemic load. In these studies, no energy value for fermentation was assigned to the RS component of the supplement.
2.7. **Blood Collection and Analysis**

Participants were requested to consume a similar evening meal on the day before each study visit and to fast for 10-12 hours. They were also asked to avoid alcohol, caffeine and strenuous exercise on the day before each study visit.

2.7.1. **Blood collection**

In the studies blood was collected from an intravenous cannula inserted into the antecubital vein. Blood was collected into sodium oxalate tubes for glucose analysis, potassium EDTA for insulin and lipid analysis, and potassium EDTA with 200 KIU aprotinin per ml of whole blood for C-peptide analysis to prevent enzymatic degradation. The samples were kept chilled until centrifuged at 3000 RPM for 10 minutes using a Heraeus® centrifuge (Thermo Electron Corporation, Waltham, MA). Aliquots were taken and stored at -20°C until batch analysis at the end of each study, to minimise inter-assay variation.

2.7.2. **Glucose**

Glucose concentrations were analysed by one of two methods, although the method within each individual study was standardised.

(1) Plasma glucose concentrations were measured using an IL Test™ Glucose (oxidase) kit (Instrumentation Laboratory, UK) for the ILab650 (Instrumentation Laboratory, UK). This method measures glucose by the enzymatic colorimetric
method, where the measured glucose concentration is proportional to the increase in absorbance generated by the red dye:

\[ \beta-D-Glucose + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2 \]

\[ 2H_2O_2 + \text{phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{red quinoneimine} + 4H_2O \]

The sensitivity of the method was 0.1 mmol/l. Two quality controls (2.4 mmol/l and 5.1 mmol/l) were measured at the beginning and end of each run. The inter assay CVs were <2%.

(2) Plasma or whole blood glucose concentrations were measured on a YSI 2300 STAT Plus™ (YSI Life Sciences, UK). This method involves an immobilised enzyme biosensor. The glucose oxidase is immobilised between two membranes, as the glucose passes through the membrane it becomes oxidised and produces hydrogen peroxide which is in turn oxidised at the platinum electrode. The current that is then produced is proportional to the glucose concentration.

\[ \text{D-Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-glucono-5-lactone} + H_2O_2 \]

A standard glucose solution of 10 mmol/l was measured at the beginning and end of each run. The inter assay CVs were <1%.

2.7.3. Insulin

Plasma insulin concentrations were measured by radioimmunoassay (RIA) using a commercially available Human Specific Insulin kit (Millipore, UK). All
samples were measured in duplicate. The assay uses a single labelled antigen that competes with the insulin in the blood sample for binding to the antibody. The assay had low cross-reactivity with Human Proinsulin, <0.2%.

The limit of detection of the assay was 2 μU/ml. Two quality controls (with an expected range of 5.9 -12.3 μU/ml and 20.4 – 42.4 μU/ml) were measured at the beginning and end of each assay run. The inter-assay variation across all the studies was <15% and the intra-assay variation was <10%.

2.7.4. C-peptide

Plasma C-peptide concentrations were measured by radioimmunoassay using a commercially available Human C-peptide RIA kit (Millipore, UK). All samples were measured in duplicate. The assay uses a single labelled antigen that competes with the C-peptide in the blood sample for binding to the antibody. The assay had a low cross-reactivity to Human Proinsulin, <4%.

The limit of detection of the assay was 0.1 ng/ml. Two quality controls (with an expected range of 0.28 – 0.58 ng/ml and 1.4 – 2.8 ng/ml) were measured at the start and end of each assay run. The inter-assay variation was <20% and the intra-assay variation was <10%.
2.7.5. **Triglyceride (TG)**

Plasma TG concentrations were measured using an IL Test™ Triglyceride kit (Instrumentation Laboratory, UK) for the ILab650. This method measures TG by the enzymatic method, where the concentration of quinoneimine produced is proportional to the TG concentration:

\[
\text{Triglycerides} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol + fatty acids}
\]
\[
\text{Glycerol + ATP} \xrightarrow{\text{glycerol kinase}} \text{glycerol-3-phosphate + ADP}
\]
\[
\text{Glycerol-3-phosphate + O}_2 \xrightarrow{\text{glycerolphosphate oxidase}} \text{dihydroxyacetone phosphate + H}_2\text{O}_2
\]
\[
\text{H}_2\text{O}_2 + 4\text{-chlorophenol + 4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye + H}_2\text{O}_2
\]

The sensitivity of this method was 0.02 mmol/l. Two quality controls (1.5 mmol/l and 2.5 mmol/l) were measured, with inter-assay CVs of <2.5%.

2.7.6. **Non-esterified Fatty Acids (NEFA)**

NEFA concentrations were measured using a RANDOX NEFA kit (RANDOX Laboratories Ltd, UK) on the ILab650. This method measures NEFA by the colorimetric method:

\[
\text{NEFA + ATP + CoA} \xrightarrow{\text{Acyl CoA Synthetase}} \text{Acyl CoA + AMP + PPi}
\]
\[
\text{Acyl CoA + O}_2 \xrightarrow{\text{Acyl CoA Oxidase}} 2,3\text{-trans-Enoyl-CoA + H}_2\text{O}_2
\]
\[
2\text{H}_2\text{O}_2 + \text{TOOS + 4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{purple adduct + 4H}_2\text{O}
\]
\[
\text{TOOS = N-ethyl-N-(2hydroxy-3-sulphopropyl)m-toluidine}
\]
Two quality controls (0.11 mmol/l and 1.09 mmol/l) were measured. The inter-assay variation was <4%.

2.7.7. Cholesterol

Total cholesterol concentrations were measured using an IL Test™ Cholesterol kit (Instrumentation Laboratory, UK) for the ILab650. This method measures total cholesterol by bichromatic analysis, where the concentration of quinoneimine produced is proportional to the total cholesterol concentration of the sample:

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest-4-en-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4\text{H}_2\text{O}
\]

The sensitivity of the method was 0.1 mmol/l. Two quality controls (2.4 mmol/l and 5.1 mmol/l) were measured. The inter-assay CV was <2.5%.

High-density lipoprotein (HDL) cholesterol concentrations were measured using Randox Direct HDL-Cholesterol kit (Randox Laboratories Ltd, UK) on the ILab650. This method measures HDL-cholesterol directly by the enzymatic clearance method. The measurement has two reaction steps; the first enzymatically removes chylomicrons, very-low-density lipoprotein (VLDL) cholesterol and low-density lipoproteins (LDL) cholesterol, and the second measures the HDL-cholesterol concentrations after it has been released by
detergents. The concentration of the dye produced is then proportional to the HDL-cholesterol concentration:

**Step 1:** Cholesterol ester $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + $\text{O}_2$ $\xrightarrow{\text{cholesterol oxidase}}$ Cholestenone + $\text{H}_2\text{O}_2$

$2\text{H}_2\text{O}_2$ $\xrightarrow{\text{Catalase}}$ $2\text{H}_2\text{O} + \text{O}_2$

**Step 2:** Cholesterol ester $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + $\text{O}_2$ $\xrightarrow{\text{cholesterol oxidase}}$ Cholestenone + $\text{H}_2\text{O}_2$

$2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{HDAOS} \xrightarrow{\text{Peroxidase}}$ Quinone pigment + $4\text{H}_2\text{O}$

HDAOS = N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

Three quality controls (0.82, 1.32 and 1.88 mmol/l) were measured. Inter-assay CV was <6% for each quality control.

LDL-cholesterol concentrations were then calculated using the equation described by Friedewald *et al* (1972):

$$ \text{LDL-cholesterol} = \text{total cholesterol} - \text{HDL-cholesterol} - \left( \frac{\text{TG}}{5} \right) $$

2.8. **Insulin Sensitivity Calculations**

On the study mornings when blood was collected, fasting insulin sensitivity and $\beta$-cell function (%S and %B respectively) were assessed using the homeostatic model assessment (HOMA) (Matthews *et al.*, 1985) by the HOMA2 Calculator.
version 2.2 (University of Oxford, UK). The following equations are used by HOMA:

\[ \text{\( \beta \)-cell function (\%) = } 20 \times \frac{\text{insulin}}{(\text{glucose} - 3.5)} \]

\[ \text{Insulin sensitivity (\%) = } 100 \div \left( \frac{\text{insulin} \times \text{glucose}}{22.5} \right) \]

Oral postprandial insulin sensitivity to the test meals was also calculated on the study mornings when blood was collected using the minimal model method described by Caumo et al. (2000). The model provides an estimate of an individual's insulin sensitivity \( S_{(\text{oral})} \) following carbohydrate consumption in a single meal. The method uses cumulative integrated AUC for glucose and insulin concentrations, and assumes that total glucose disposal from the system, at the end of the study period investigated or once basal values have been reached, equals the glucose entering the peripheral circulation and allows for first pass extraction by the liver. Insulin-independent mechanisms also contribute to glucose disposal and a constant rate of glucose effectiveness (GE) has been assumed for the whole time period. The equation below is used by the model (Caumo et al., 2000); \( f \) denotes the fraction of ingested carbohydrate reaching the peripheral circulation as glucose, a nominal value of 1 was used for all studies. \( D_{\text{oral}} \) is the amount of ingested carbohydrate per kg body weight (mg/kg), AUC is the area from baseline until the end of the test period investigated, GE was fixed at 0.024dl/kg-min (the value given and used by Caumo et al. (2000)).
2.9. Statistical Analysis

AUC were always calculated using the trapezoid method. All statistical analyses were carried out using SPSS 15.0 and 16.0 for Windows (Chicago, USA). The data were checked for normality of distribution, using the Kolmogorov-Smirnov test and, where normally distributed, parametric tests were conducted; the corresponding non-parametric tests were used where data were not normally distributed or in some instances when the study participant numbers were small (≤10). Statistical significance was taken as \( p<0.05 \). Specific statistical tests utilised in each study are detailed in the study chapter. Values given in the text are mean and standard error of mean (SEM), unless the test was non-parametric, in which case the median (Md) is stated.
Chapter 3

Effects of Acute Consumption of Resistant Starch on Appetite
3.1. **Introduction**

RS, a fermentable carbohydrate, has been proposed to have properties similar to other types of DF (Champ, 2004) and therefore may affect satiety. As RS is non-viscous and is not thought to affect the absorption of nutrients or gastric emptying (Robertson et al., 2005), effects on satiety, if indeed there are any, are unclear. Few studies have investigated the effects of including RS in the diet and the subsequent effects on appetite. Whilst animal studies show consistent positive effects on appetite regulation, data in human studies are mixed (Higgins, 2004). In particular, there are no studies in humans that have explored the effects on appetite when RS is included as part of a meal, when compared with an energy and available carbohydrate matched placebo.

The postprandial effects of RS are also not known; however, as a non-viscous DF, it would not be predicted to affect glucose absorption. Nevertheless a recent study has shown that short-term consumption of RS2 improves postprandial glucose metabolism in healthy individuals (Robertson et al., 2005).

3.2. **Aims and objectives**

The study aimed to investigate the acute effects of 48 g RS2, on energy intake, subjective appetite measures and changes to postprandial metabolites, compared with a placebo. This was a randomised, single-blind balanced crossover study, in which the 48 g RS was consumed as part of mixed meals and was divided equally between breakfast and lunch (providing 24 g at each meal).
3.3. **Hypothesis**

The inclusion of 48 g RS, over two meals, will prolong satiety and decrease intake at both the *ad libitum* test meal and over the 24 hour period.

3.4. **Study Design**

3.4.1. **Participants**

Twenty young healthy adult males participated in the study (Table 3.1). The participants included in the study had a mean score of 2.1 (SEM 0.22) on the restraint scale, 1.98 (SEM 0.13) on the emotional scale and 3.1 (SEM 0.11) on the external eating scale from the results of the DEBQ. At screening participants completed a food preferences questionnaire (Appendix 5).

**Table 3.1: Participant measurements taken on the morning of the first study visit, n=20.**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.8 ± 0.82</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.2 ± 1.62</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.2 ± 2.48</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 0.65</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>85.5 ± 2.07</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>97.4 ± 1.42</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>15.0 ± 1.17</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>120.8 ± 1.69</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>74.4 ± 2.16</td>
</tr>
</tbody>
</table>

3.4.2. **Protocol**

Participants attended the investigation unit on two occasions, at least one week apart. During the visit participants consumed either the RS or PL within standard
breakfast and lunch meals. The timeline for the study days is shown in Figure 3.1. The study received a favourable ethical opinion from the University of Surrey’s Ethics Committee (EC/2006/118/SBMS) and all participants gave informed written consent.

On each study day anthropometric measurements and blood pressure were taken. Participants remained in the unit for the entire postprandial period (seven hours), whilst minimising activity, and were able to drink water *ad libitum*.

Participants were cannulated and two fasting blood samples and VAS were taken, at -15 minutes and just before time zero. The test breakfast containing the RS or PL was given at time zero. The test lunch (containing a second dose of the test carbohydrate) was served at 180 minutes. VAS and blood samples were taken every 30 minutes during the seven hour postprandial study. All blood samples were analysed for glucose (using the ILab method described in Chapter 2), insulin and C-peptide concentrations.
At the end of the seven hours and following the two meals containing the test starch, participants were provided with the *ad libitum* test meal (as described in Chapter 2). The participants were then free to leave the unit completing a diet diary for the remainder of the day to assess overall 24 hour intake. Bowel habit diaries (Appendix 6) were completed on the day of the study and the following day to assess gastrointestinal tolerance of the starches.

3.4.3. **Starches**

In this study 80 g of Hi-Maize\(^\circledR\) 260 (providing 48 g RS and 32 g RDS) and 32 g of Amioca\(^\circledR\) were used (starches described in detail in Chapter 2).

The starches were tested for incorporation prior to the start of the study to deliver two products with a similar taste and texture. When incorporated into milkshakes, the starches dissolved well but the RS tasted powdery and left a greater aftertaste, compared with the corresponding PL drink. In jelly, as used in Robertson *et al* (2003), the starches worked well at low amounts but higher amounts resulted in unpleasant tasting products that resembled the texture of mousse rather than jelly. Various amounts of Hi-Maize\(^\circledR\) 260 were mixed with mousse (Angel Delight\(^\circledR\), Premier Foods, UK). The best amount, in an individual portion, was 40 g of the Hi-Maize\(^\circledR\) 260, which resulted in a good consistency, less aftertaste, was an acceptable portion size and differed very little in taste and texture from the corresponding PL mousse. Mousse was therefore chosen as the delivery vehicle for this study.
Two portions of the mousse were used to provide a total of 48 g RS (24 g at each meal), a level similar to that which has been used in previous studies without accompanying adverse gastrointestinal effects and at a level high enough to potentially elicit an effect on appetite. The participants were offered a choice of three flavours of mousse (chocolate, butterscotch and raspberry), but consumed the same flavour for both meals and on each visit. The nutritional composition of one portion of each flavour of mousse and starch are shown in Table 3.2. The mousses provided an identical glycaemic carbohydrate load and differed, between starch type, only in weight and DF content.

Table 3.2: Nutritional composition of one portion of mousse with each starch.

<table>
<thead>
<tr>
<th></th>
<th>Chocolate</th>
<th>Butterscotch</th>
<th>Raspberry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RS</td>
<td>PL</td>
<td>RS</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>162.0</td>
<td>138.0</td>
<td>162.0</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>907</td>
<td>907</td>
<td>911</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>216</td>
<td>216</td>
<td>217</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>4.3</td>
<td>4.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>36.9</td>
<td>36.9</td>
<td>36.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>5.7</td>
<td>5.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>24.5</td>
<td>0.5</td>
<td>24.1</td>
</tr>
</tbody>
</table>

3.4.4. Meals

The breakfast and lunch meals were standardised between visits with each participant consuming identical food (except for the starch) on both visits. The food given to the participants was of typical portion sizes for an adult male and
overall the two meals combined were low in DF (mean DF intake 5.5 g ± 0.21), excluding the RS portion.

All food was weighed before each meal. The test breakfast consisted of 30 g Rice Krispies® (Kellogg's, UK) with 100 g semi-skimmed milk (Tesco, UK) and one portion of the mousse with the starch. The test lunch was sandwiches (Kingsmill® white bread (Allied Bakeries, UK) with Flora Light®, (Unilever Foods, UK)) containing either Tesco Healthy Living Honey Cured Roast Ham (Tesco, UK) or mild cheddar cheese (Tesco, UK), with the same filling being consumed on each study day by a participant. Participants were also given ready salted crisps (Walkers®, Walkers Snack Foods Ltd, UK), a sugar-free orange drink (Tesco No Added Sugar Orange Squash, Tesco, UK) and one portion of the mousse with the starch.

At lunch on the first visit participants were able to regulate their intake from the offered food (except for the mousse which they were required to fully consume on all visits). Whatever was not consumed was weighed and the participants were then required to consume an identical amount on the subsequent visit to ensure the energy and macronutrient intake were matched with only the presence of the RS differing. A similar study design has been used successfully in previous studies (Weststrate and van Amelsvoort, 1993). The mean values for the amounts consumed at the meals are shown in Table 3.3.
Table 3.3: Mean intake at breakfast and lunch on both study days, n=20.

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Lunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>1584 ± 2.5</td>
<td>3865 ± 152.8</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>379 ± 0.6</td>
<td>924 ± 36.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9.6 ± 0.03</td>
<td>35.6 ± 1.54</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>67.2 ± 0.20</td>
<td>111.9 ± 3.36</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>7.9 ± 0.03</td>
<td>38.8 ± 2.87</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>0.5 (24*) ± 0.05</td>
<td>5.0 (24*) ± 0.20</td>
</tr>
</tbody>
</table>

* = resistant starch meals only

3.4.5 Calculations and Statistical Analysis

The ratio of C-peptide to insulin was calculated using the AUC for both measures up to two hours after each meal (0 – 120 minutes and 180 – 300 minutes) and overall until two hours after the test lunch (0 – 300 minutes) and this was used as a surrogate marker of hepatic insulin clearance.

The data were normally distributed and therefore paired samples t tests were used to compare between the groups. All time course data were analysed by repeated measures ANOVA, with starch and time as independent variables and the measurements themselves as the continuous dependent variable.

3.5. Results

Both starches were well tolerated by the participants with no adverse gastrointestinal effects reported on either the study day or the following day.
Chapter 3

The fasting insulin sensitivity (95.9 ± 7.5 % vs 97.0 ± 8.9 %, RS and PL legs respectively) and β-cell function (105.3 ± 5.9 % vs 111.4 ± 9.8 %, RS and PL legs respectively) as assessed by HOMA (described in Chapter 2), were not significantly different at the start of each study day, which confirms that the participants were in a similar metabolic state. Values for each variable were close to 100 % as the participants used for the study were young and healthy.

3.5.1. Test meal

Intake of 48 g RS split over breakfast and lunch caused a reduced energy intake at the *ad libitum* test meal at the end of the 7 hour postprandial period (Figure 3.2) compared with the PL (5241 ± 313 kJ vs 5606 ± 345 kJ, *p*=0.033). The weight consumed was not significantly different between the starches, although there was a trend that participants consumed less weight of food on the RS leg than the PL leg (775.4 ± 46.2 g vs 821.9 ± 48 g, *p*=0.061).

![Figure 3.2: Energy intake at the *ad libitum* test meal for each intervention. Mean ± SEM, n=20. Paired samples t-test showed significant difference between interventions (* p=0.033).](image-url)
3.5.2. Subjective appetite ratings

Despite the lower intake at the *ad libitum* test meal there were no significant differences between the two starches for the subjective appetite ratings, measured by VAS. This was true for ratings of hunger (Figure 3.3), fullness and prospective food consumption. There also were no significant differences between the starches for ratings of thirst, or desire for different foods (sweet, salty, savoury or fatty foods).

![Figure 3.3](image)

*Figure 3.3:* Subjective appetite ratings in response to the question "how hungry do you feel?" Mean ± SEM, n=20. Supplements were consumed at the test meals represented by the dashed lines. Repeated measures ANOVA showed no significant difference between the supplements for any of the subjective appetite ratings.

3.5.3. 24 hour intake

Over the entire 24 hour period there was a significantly lower energy intake following the 48 g RS compared with the PL; from 12603 ± 519 kJ compared with 13949 ± 755 kJ (p=0.044) respectively (Table 3.4). Following RS, the mean energy intake was 104% of calculated habitual energy requirements (calculated
with the Schofield Equation (Schofield et al., 1985) and applying a moderate activity level of 1.6 for all participants) compared with 116% for the PL leg.

Table 3.4. 24 hour intake following supplementation with 48 g RS or PL, measured from 24 hour diet diaries, n=20. Comparisons were made with paired samples t test.

<table>
<thead>
<tr>
<th></th>
<th>RS Mean</th>
<th>SEM</th>
<th>PL Mean</th>
<th>SEM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>12603</td>
<td>519</td>
<td>13949</td>
<td>755</td>
<td>0.044</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2994</td>
<td>123</td>
<td>3312</td>
<td>180</td>
<td>0.048</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>104.9</td>
<td>5.63</td>
<td>115.3</td>
<td>6.87</td>
<td>0.060</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>424.4</td>
<td>18.40</td>
<td>452.6</td>
<td>23.00</td>
<td>NS</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>97.2</td>
<td>6.1</td>
<td>104.7</td>
<td>8.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>96.7</td>
<td>4.56</td>
<td>110.0</td>
<td>4.79</td>
<td>0.017</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>39.2</td>
<td>2.18</td>
<td>45.2</td>
<td>2.37</td>
<td>0.014</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>65.1</td>
<td>0.95</td>
<td>16.7</td>
<td>0.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>4375</td>
<td>195</td>
<td>4757</td>
<td>243</td>
<td>0.048</td>
</tr>
</tbody>
</table>

The lower energy intake can be primarily attributed to a significantly lower fat intake, following the RS (13.3 g lower) compared with the PL (Table 3.4). There was also a trend towards a lower protein intake with RS compared with PL, whilst the carbohydrate intake was not significantly changed. There was also a significantly lower sodium intake with RS than with PL.

As expected, there was a significant difference in DF during this 24 hour period (difference of 48.4 g) attributed directly to the supplementation with 48 g RS.
3.5.4. Metabolite analysis

The postprandial plasma glucose concentrations were not significantly different between the RS and the PL (Figure 3.4). The total AUC (for the seven hour postprandial period) was not significantly different between starches; however, when the response to each meal was compared, 0 – 120 minutes and 180 – 300 minutes, (as used in an OGTT), no significant difference was found for the test lunch, but the AUC for the RS leg was significantly lower than for the PL leg at the test breakfast (677.7 ± 22 mmol/l.min vs 710.5 ± 21 mmol/l.min, p=0.025).

There was a significantly lower postprandial insulin response following the RS compared with the PL over the whole acute study period (p=0.029) (Figure

![Plasma Glucose Concentrations](image.png)

*Figure 3.4: Postprandial plasma glucose concentrations after consumption of 48 g RS compared with PL. Mean ± SEM, n=20. Supplements were consumed at test meals represented by the dashed lines. Repeated measures ANOVA showed no significant difference between the starches.*
3.5A). However, the corresponding C-peptide concentrations were not significantly different between the two starches (figure 3.5B).

![Figure 3.5: Postprandial plasma insulin (pmol/l) (A.) and C-peptide (nmol/l) (B.) concentrations after consumption of 48 g RS compared with PL. Mean ± SEM, n= 20. Starches were consumed at test meals represented by the dashed lines. Repeated measures ANOVA showed the plasma insulin response was significantly lower (p=0.029) following RS and no significant differences between the starches for the C-peptide concentrations.](image-url)
The AUC for two hours after each meal were also compared for insulin and C-peptide. For insulin they were significantly lower with the RS than the PL for both meals (breakfast = 30456.5 ± 3933.3 pmol/l.min vs 39655.8 ± 6655.2 pmol/l.min, \( p = 0.02 \), lunch = 34605.5 ± 4176.2 pmol/l.min vs 41607.6 ± 6752.5 pmol/l.min, \( p = 0.039 \)). There were no significant differences at either meal for C-peptide AUC.

Consequently there was a trend towards significance for a higher molar ratio of C-peptide:insulin for the RS leg compared with the PL leg for 0 – 120 minutes and for 0 to 300 minutes and no significant difference for 180 – 300 minutes, which is a surrogate marker to indicate an increase in hepatic insulin clearance (Table 3.5). There was, however, no significant difference in postprandial oral insulin sensitivity between the two starches at either test meal (Table 3.5).

Table 3.5. C-peptide to insulin AUC ratio and postprandial oral insulin sensitivity following consumption of 48 g RS or PL, n=20. Comparisons made with a paired samples \( t \) test.

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th></th>
<th>PL</th>
<th></th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>C-peptide:insulin AUC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-120 minutes</td>
<td>6.52</td>
<td>0.39</td>
<td>5.74</td>
<td>0.43</td>
<td>0.065</td>
</tr>
<tr>
<td>C-peptide:insulin AUC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180-300 minutes</td>
<td>6.45</td>
<td>0.49</td>
<td>6.08</td>
<td>0.46</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide:insulin AUC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-300 minutes</td>
<td>6.69</td>
<td>0.37</td>
<td>6.13</td>
<td>0.42</td>
<td>0.059</td>
</tr>
<tr>
<td>Oral ( S_1 ) Breakfast (dl/kg/min/μU/ml)</td>
<td>3.36 ( \times 10^{-3} )</td>
<td>0.44 ( \times 10^{-3} )</td>
<td>8.50 ( \times 10^{-3} )</td>
<td>5.58 ( \times 10^{-3} )</td>
<td>NS</td>
</tr>
<tr>
<td>Oral ( S_1 ) Lunch (dl/kg/min/μU/ml)</td>
<td>5.65 ( \times 10^{-3} )</td>
<td>1.11 ( \times 10^{-3} )</td>
<td>4.43 ( \times 10^{-3} )</td>
<td>0.63 ( \times 10^{-3} )</td>
<td>NS</td>
</tr>
</tbody>
</table>

\( S_1 \) = oral insulin sensitivity for each meal, calculated by minimal model (described in Chapter 2)
3.6. **Discussion**

This study found that after consumption of 48 g RS (split equally over the test breakfast and lunch meals) there was a lower energy intake at both the *ad libitum* test meal and over the whole 24 hour period, without an associated effect on subjective appetite ratings. The study also found a significant novel effect of the RS on lowering the postprandial insulin response. To our knowledge this is the first study where RS has been provided to participants as part of a mixed meal and compared with a placebo, where available carbohydrate and energy load have been matched, and so provides novel information on the role of DF as a bioactive compound in appetite regulation.

Previous studies investigating the effects of RS on appetite have replaced proportions of digestible carbohydrate with RS and therefore the amount of glycaemic carbohydrate provided has varied between the supplements which would confound the interpretation of the results. Indeed in one study consumption of RS appeared to cause a reduction in subjective feelings of satiety (Raben et al., 1994). However, a limitation of the study was that there were differences in texture between the supplements, one liquid and the other semi-solid, with liquids being known to be less satiating than solid foods (Mourao et al., 2007). The study by Raben *et al* (1994) also matched the supplements by weight of starch and therefore the supplements differed in energy and available carbohydrate content. A recent study found greater satiety (measured by VAS) following consumption of muffins containing 8 g RS
compared with other fibres (Willis et al., 2009); however this study provided no information on actual food intake. In studies where the effects of different ratios of amylose to amylopectin ratio on appetite were investigated, it was found that the high amylose meals (least digestible) were the most satisfying but also the least palatable (van Amelsvoort and Weststrate, 1992). Another study, that also varied amylose and amylopectin ratios, found no significant effect between their treatments on VAS ratings (Weststrate and van Amelsvoort, 1993).

In our study, none of the participants would have been classified as under-reporters based on the Goldberg cut-off values for under-reporting (Goldberg et al., 1991); indeed, on both study days, the participants consumed more than their estimated requirements (104% for RS leg and 116% for the PL leg) and therefore there was likely to be an element of over-consumption. This over-consumption may have arisen because the participants were given food and did not need to prepare/cook it themselves; nevertheless the over-consumption was less on the RS leg than PL leg. The over-consumption may explain why no effects were found on any of the subjective appetite ratings.

The breakfast and lunch meals provided to the participants were based on standard portion sizes rather than set to basal metabolic rate (BMR), as only part of the daily intake was provided to the participants and an outcome of the study was to investigate any effects on the metabolic response, which may have been masked if intakes were set to BMR. The only difference between the two
legs, apart from the 48 g of RS, could have been a slight difference in the energy density of the meals due to the presence of the RS; however, the difference in energy density was minor (for breakfast and lunch combined the density per g for meals was 7.2 kJ/g for the RS leg and 7.7 kJ/g for the PL leg) and the GL of the two legs was identical.

The lower energy intake over 24 hours appeared to be primarily explained by a significantly lower fat intake following the RS. However the reason for this lower fat intake is unclear, as the participants did not report a difference in desire for fatty foods on the subjective appetite ratings during the postprandial study. This difference in macronutrient choice following RS consumption would require further investigation to determine whether it was an incidental finding or a true effect. There also did not appear to be an influence of the high RS intake on DF intake later in the day; however there may have been an effect on the following day’s DF intake that was not monitored in this study.

Although in the present study a reduced energy intake was found following the RS, it is not possible from the design of this study to determine the mechanisms for this effect. However, as the main effect on food intake appeared to occur at the ad libitum test meal at the end of the postprandial study and later in the evening, a possible mechanism could be the fermentation of the RS in the colon, by colonic microflora and the subsequent effects of the production and action of
SCFA, which has been hypothesized as a mechanism for the effects of DF on appetite (Pereira and Ludwig, 2001).

Whilst the metabolism of SCFA may result in potential additional energy being provided to the body, this was not accounted for in this study and no value was assigned for the fermentation of the RS. There was no way of assessing SCFA absorption in the participants and an amount cannot be assumed as this is highly variable. This variability depends on factors including, but not limited to, extent of fermentation and metabolism within the colon (Wolever et al., 2002), variations in colonic gut transit time (Macfarlane and Macfarlane, 2003), the colonic microbiota and the SCFA produced but lost to the faeces (although it has been estimated that less than 5% of produced SCFA are lost to the faeces (Topping and Clifton, 2001)). Published works have attributed an energy value of 8 kJ/g for Hi-Maize® 260 (Behall and Howe, 1996, Livesey, 1994) assuming 100% fermentation and 100% absorption of resulting SCFA, compared with the 16 kJ/g for the PL. In this study we matched the starches by GL as this was the largest confounder to the glycaemic response. When taking into consideration the amounts of each starch given it would have resulted in energy doses of 640 kJ for the RS and 512 kJ for the PL, with a maximal difference of only 128 kJ. This therefore does not account for the full difference in 24 hour energy intake observed.
It is possible that the increase in production of SCFA may consequently increase production of satiety hormones from the colon, such as PYY (Cuche et al., 2000, Cherbut, 2003). However, so far the only evidence of an effect of RS on these hormones, particularly PYY and GLP-1, has been shown in rodent studies (Keenan et al., 2006, Zhou et al., 2006, Zhou et al., 2008). Rodents are, in terms of the gastrointestinal tract, anatomically different; indeed the large bowel of a human has been said to comprise 17% of the gastrointestinal tract, whereas in a rodent it makes up 61% (Topping and Clifton, 2001) and thus rodents are a poor model for the human colon. In addition, some studies in humans that have measured changes to GLP-1 after consumption of RS have not found the concentrations to be elevated (Raben et al., 1994, Robertson et al., 2005), either acutely or following chronic intake.

As insulin and C-peptide are co-secreted, the lower postprandial insulin concentration detected was likely to be due to increased hepatic insulin clearance, as there was a trend towards significance between the two starches for the molar ratio of C-peptide to insulin. An increase in hepatic insulin clearance has previously been reported following RS intake over 24 hours (Robertson et al., 2003). It has been proposed that the increase in production of SCFA and their exposure to the liver may ultimately be responsible for the increase in insulin clearance, although this remains to be fully clarified. However, it is difficult from this study to determine exactly what the mechanism is for an effect of RS on lower postprandial insulin responses and further
investigations, specifically tailored to determine the mechanisms would be required.

The lower energy intake observed in this study following RS consumption as part of a mixed meal could have beneficial implications in weight management and, potentially, weight loss; however further studies are required to confirm whether a similar finding is shown in other population groups such as the overweight or obese, and to determine the actual mechanisms for the effect. Although the dose in this study was well tolerated for a single day, and a lower energy intake was observed, further investigations are needed to establish whether lower doses would also produce a clinically relevant finding. A lower postprandial insulin response was also observed, was entirely novel, and could change our understanding of the acute effects of non-viscous DF. Increased intakes of RS in the diet may therefore have beneficial implications in weight management and metabolic control.

This work has recently been accepted for publication in the British Journal of Nutrition. Please see published work and abstracts on page 226.
Chapter 4

Effects of Chronic Consumption of Resistant Starch on Appetite and Insulin Clearance
4.1. **Introduction**

As the findings from the acute RS and appetite study indicated a reduced energy intake over 24 hours (Chapter 3), and there are few chronic studies investigating the effects of RS consumption on appetite, this follow-on study was designed to determine the effects of longer-term RS ingestion on appetite and food intake. In contrast to Chapter 3, obese or overweight individuals were the target group, as weight loss, rather than maintenance, is often a primary goal. A novel finding from Chapter 3 was a significantly lower postprandial insulin response with RS. As such, this study was also designed to determine whether the lower postprandial insulin response would be observed following chronic consumption and elucidate possible mechanisms.

4.2. **Aims and objectives**

The aim was to investigate the effects on appetite, food intake and the insulin response following consumption of 40 g RS daily for four weeks, compared with a placebo, in a randomised, single-blind crossover study, combining both a postprandial feeding study and an insulin-modified FSIVGTT.

The main objectives were to investigate the effects of RS on appetite, including effects on actual food intake (using *ad libitum* test meals and a seven day diet diary) and subjective measures of appetite (using VAS). The effects of RS on the insulin response and insulin sensitivity were also assessed using an insulin-modified FSIVGTT.
4.3. **Hypothesis**

The inclusion of 40 g RS per day for four weeks will cause a decrease in participants' intake both in their habitual diets and at a pre-weighed *ad libitum* meal at the end of the intervention. The RS will cause an increase in insulin clearance and an improvement in insulin sensitivity.

4.4. **Study Design**

4.4.1. Participants

In addition to the general inclusion criteria (described in Chapter 2) participants required for the study were adult males (aged between 20 and 60 years) with a waist circumference greater than 94 cm (37 inches) and adult females (aged between 20 and 60 years) with a waist circumference greater than 80 cm (31.5 inches) and, for both males and females, a BMI of 26 – 35 kg/m².

Due to time constraints for recruitment, seven healthy adult males and females (5 M, 2 F) completed this pilot study (**Table 4.1**). The participants included had mean scores of 2.07 (± 0.22), 3.54 (± 0.10) and 2.81 (± 0.31) on the emotional, external and restrained scales of the DEBQ, respectively.

Eight participants started the study, with one drop-out who did not tolerate taking the placebo in their diet (due to gastrointestinal upset). The other seven participants completed the study.
### Table 4.1: Baseline anthropometric measurements and fasting blood values, n=7.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.6 ± 5.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.1 ± 4.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91.2 ± 4.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 0.7</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>98.9 ± 2.6</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>108.1 ± 1.5</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>30.1 ± 3.6</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>120.6 ± 1.5</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>72.6 ± 4.4</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.75 ± 0.2</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>75.98 ± 10.4</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>0.88 ± 0.2</td>
</tr>
<tr>
<td>Fasting Non-esterified fatty acids (mmol/l)</td>
<td>0.50 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.35 ± 0.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.13 ± 0.1</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.04 ± 0.2</td>
</tr>
<tr>
<td>HOMA % S²</td>
<td>72.2 ± 12.0</td>
</tr>
<tr>
<td>HOMA % B³</td>
<td>147.4 ± 17.9</td>
</tr>
</tbody>
</table>

1 Value for five participants
2 HOMA %S = fasted oral insulin sensitivity, assessed by homeostatic model assessment
3 HOMA %B = β-cell function, assessed by homeostatic model assessment

#### 4.4.2. Starches

In this study 67 g of Hi-Maize® 260 (providing 40 g RS and 27 g RDS) and 27 g of Amioca® were used (starches described in Chapter 2). The starches were provided in pre-weighed sachets and participants consumed two sachets daily for four weeks. Participants were free to include the starches into their habitual
diets in a method and time of day of their own choice, although mixing with a
cold liquid (especially milk) was advised as the best method of incorporation.

4.4.3. Protocol

A favourable ethical opinion was given for this study by the University of
Surrey's Ethics Committee (EC/2008/80/FHMS – fast track) and from Surrey
Research Ethics Committee (08/H1109/112) as the FSIVGTT was conducted on
NHS premises, and all participants gave informed, written consent.

Following recruitment, a fasting baseline blood sample was collected to
ccharactrishe the participants. Pariicipants atended for a "practice" morning,
which was identical to study morning 1, to allow them to become familiarised
with the procedures and environment. At the end of each four weeks
intervention, participants attended for two study mornings. The two intervention
periods were separated by a four week washout period (Figure 4.1).

Figure 4.1: Timeline for the study
Study morning 1 – Appetite Assessment. Anthropometric measurements and blood pressure were taken on arrival. Participants were cannulated and two fasting blood samples and VAS were taken. At time zero a standard commercially available fibre-free milkshake (Nurishment®, ENCO Products Ltd, UK) breakfast (Table 4.2) was consumed, from a choice of three flavours (each participant had the same flavour on each visit). VAS and blood samples were taken every 30 minutes for three hours, with two additional blood samples collected at 15 and 45 minutes postprandially for insulin and glucose measurement. An *ad libitum* test meal was provided at the end of the three hours to quantify food intake.

Table 4.2: Nutritional composition of the fibre-free breakfast (Nurishment® Drinks), per 420 g.

<table>
<thead>
<tr>
<th>Flavour</th>
<th>Energy (KJ)</th>
<th>Protein (g)</th>
<th>Carbohydrate (g)</th>
<th>Sugar (g)</th>
<th>Fat (g)</th>
<th>Saturates (g)</th>
<th>Fibre (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry</td>
<td>1940</td>
<td>462</td>
<td>21.0</td>
<td>67.6</td>
<td>67.6</td>
<td>11.8</td>
<td>7.1</td>
</tr>
<tr>
<td>Chocolate</td>
<td>1726</td>
<td>412</td>
<td>21.0</td>
<td>55.9</td>
<td>55.9</td>
<td>11.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Vanilla</td>
<td>1804</td>
<td>428</td>
<td>21.0</td>
<td>60.0</td>
<td>60.0</td>
<td>12.6</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Study morning 2 – Insulin sensitivity, secretion and metabolic clearance were assessed by an insulin-modified FSIVGTT. Participants arrived fasted and a cannula was placed into each arm in the anticubital vein, one for sampling and the other for infusions. At time zero glucose (50% dextrose, Baxter Healthcare Ltd, UK) (0.3 g/kg body weight, with a maximum dose of 25 g) was infused for 5 minutes using an infusion pump (IVAC 560 volumetric pump). After 20 minutes
the insulin (Actrapid®, Novo Nordisk, UK) (0.03 U/kg body weight) infusion commenced and was infused (Graseby 3100 syringe pump) for 5 minutes. Blood samples for glucose, C-peptide and insulin concentrations were taken at 1, 3, 5, 7, 10, 15, 19, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 60, 90, 120, 150 and 180 minutes (protocol modified from those previously described by Bingley et al (1992), Vicini et al (1999) and Trout et al (2007)). Blood glucose concentrations were measured immediately by the YSI method (described in Chapter 2). Insulin and C-peptide concentrations were measured by RIA on all the plasma samples by batch analysis at the end of the study.

Seven day diet diaries (to assess dietary intake) and bowel diaries (to assess gastrointestinal tolerance), were completed for the last week of each intervention.

4.4.4. Calculations and Statistical Analysis

All postprandial data from the appetite study morning were analysed using repeated measures ANOVA with starch and time as independent variables and the measurements themselves as the continuous dependent variable. The AUCs for the time course data were also calculated and then compared using paired samples t-tests. All other data were compared using paired samples t-tests. Non-parametric tests were also run due to small participant numbers and the consequent difficulty in assessing normality accurately. However, as statistical significance did not differ between tests, parametric tests are reported as the more powerful statistical test.
The raw glucose, insulin and C-peptide data from the FSIVGTT data were analysed using repeated measures ANOVA with starch and time as independent variables and the measurements themselves as the continuous dependent variable.

The data from the FSIVGTT were also modelled using Bergman's minimal model (Bergman et al., 1981). The program used was the MINMOD Millennium version, which has been described by Boston et al. (2003). This version of the minimal model uses two equations, one of which is the glucose minimal model, which attempts to determine the glucose dynamics and the other the insulin minimal model, which attempts to determine the insulin dynamics (Bergman, 2005). Four key indices are reported from the modelling of IVGTT data. These are: (i) insulin sensitivity ($S_I$) which measures the ability of insulin to promote glucose clearance; (ii) the first-phase insulin response ($AIR_g$) that assesses the initial insulin secretion; (iii) glucose effectiveness ($S_G$) which measures the ability of glucose to cause its own disposal separately to the effect of increased insulin (an insulin-independent glucose disposal); (iv) the disposition index (DI) (which is equal to the $AIR_g*S_I$) which provides a measure of β-cell function as it proposes that there is an increase in β-cell secretion due to increased insulin resistance to avoid impaired glucose responses (Bergman, 2005).
4.5. **Results**

The starches were equally well tolerated by participants, with a weekly bowel movement frequency of $7.3 \pm 0.8$ on the RS leg and $8.9 \pm 1.3$ on the PL leg, which were not significantly different. A similar number of gastrointestinal complaints (flatulence and looser stools) were recorded for both starches.

4.5.1. **Anthropometrics**

There were no significant differences between the RS and PL for any of the anthropometric measurements taken or for resting blood pressure (Table 4.3). There was also no significant difference between the RS and PL when percentage changes from baseline were calculated and compared.

**Table 4.3**: Anthropometric measurements taken at baseline and end of each intervention, n=7.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline Mean ± SEM</th>
<th>End of RS Leg Mean ± SEM</th>
<th>End of PL Leg Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>91.2 ± 4.2</td>
<td>90.9 ± 4.4</td>
<td>90.6 ± 4.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 0.7</td>
<td>29.2 ± 1.1</td>
<td>29.2 ± 0.9</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>98.9 ± 2.6</td>
<td>99.6 ± 2.5</td>
<td>99.6 ± 2.5</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>108.1 ± 1.5</td>
<td>107.5 ± 1.4</td>
<td>108.7 ± 1.4</td>
</tr>
<tr>
<td>Body Fat (%)¹</td>
<td>30.1 ± 3.6</td>
<td>27.7 ± 2.9</td>
<td>28.0 ± 3.0</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>120.6 ± 1.5</td>
<td>121.4 ± 2.9</td>
<td>120.7 ± 2.2</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>72.6 ± 4.4</td>
<td>74.9 ± 2.1</td>
<td>72.3 ± 3.0</td>
</tr>
</tbody>
</table>

¹Value for five participants for baseline measurement
4.5.2. **Subjective appetite ratings**

There was no significant difference between the starches for subjective ratings of prospective food consumption following the fibre-free breakfast; however the responses differed significantly over time for each starch \(p=0.003\) (Figure 4.2). There were no significant differences between the starches for subjective ratings of hunger or fullness following consumption of the fibre-free breakfast. There was a significantly greater desire for fatty foods on the RS leg compared with the PL leg \(p=0.008\) (Figure 4.3), but there were no significant differences between the starches in desire for salty, sweet or savoury foods; however, the postprandial responses for each starch differed over time for sweet \(p=0.044\) and savoury \(p=0.014\). There were also no significant differences between the starches for AUC for any of the questions, except in relation to the question regarding desire for fatty foods \(p=0.011\).

![Subjective appetite ratings](image)

**Figure 4.2:** Subjective appetite ratings in response to the question "how much do you think you can eat?" Following the fibre-free breakfast at the end of the RS and PL interventions. Mean ± SEM, \(n=7\). Repeated measures ANOVA showed no significant difference between starches, but a significant starch*time interaction \(p=0.003\).
4.5.3. Test meal

There was no significant difference in either weight or energy intake between the two starches at the *ad libitum* test meal (Figure 4.4). However, there appeared to be a slightly lower energy intake with the RS than with the PL, which was not significant most likely due to the small sample size.

**Figure 4.3**: Subjective appetite ratings in response to the question "Would you like to eat something fatty?" Following the fibre-free breakfast at the end of the RS and PL interventions. Mean ± SEM, n=7. Repeated measures ANOVA showed a significant difference between starches (*p*=0.008).

**Figure 4.4**: Energy intake (kJ) at the *ad libitum* test meal consumed at the end of the postprandial period following the fibre-free breakfast at the end of each intervention. Mean ± SEM, n=7. Paired samples *t*-test showed no significant difference between the starches.
4.5.4. **Cholesterol**

There were no significant differences between the RS and PL legs for total plasma cholesterol, HDL-cholesterol or LDL-cholesterol (Table 4.4).

**Table 4.4:** Cholesterol concentrations prior to and following consumption of 40 g RS or the PL per day for 4 weeks, n=7.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline Mean ± SEM</th>
<th>End of RS Leg Mean ± SEM</th>
<th>End of PL Leg Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmo/l)</td>
<td>3.35 ± 0.2</td>
<td>3.44 ± 0.2</td>
<td>3.36 ± 0.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mmo/l)</td>
<td>1.13 ± 0.1</td>
<td>1.13 ± 0.1</td>
<td>1.23 ± 0.1</td>
</tr>
<tr>
<td>LDL-cholesterol (mmo/l)</td>
<td>2.04 ± 0.2</td>
<td>2.05 ± 0.2</td>
<td>1.91 ± 0.2</td>
</tr>
</tbody>
</table>

4.5.5. **Postprandial metabolite analysis**

There were no significant differences between the starches for fasting glucose (plasma samples measured by YSI method described in Chapter 2), insulin or NEFA concentrations (Table 4.5), or for the postprandial glucose, insulin or NEFA concentrations (Figure 4.5) following the standard fibre-free breakfast.

**Table 4.5:** Fasting metabolite concentrations prior to and following consumption of 40 g RS or the PL per day for 4 weeks, n=7.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline Mean ± SEM</th>
<th>End of RS Leg Mean ± SEM</th>
<th>End of PL Leg Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.75 ± 0.2</td>
<td>4.93 ± 0.1</td>
<td>5.02 ± 0.1</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>75.98 ± 10.4</td>
<td>77.91 ± 12.6</td>
<td>78.07 ± 7.4</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>0.88 ± 0.2</td>
<td>1.27 ± 0.4</td>
<td>1.10 ± 0.3</td>
</tr>
<tr>
<td>Fasting NEFA (mmol/l)</td>
<td>0.50 ± 0.1</td>
<td>0.56 ± 0.2</td>
<td>0.62 ± 0.1</td>
</tr>
</tbody>
</table>
Figure 4.5: Postprandial glucose (A), insulin (B) and NEFA (C) concentrations for the two supplements following the fibre-free breakfast at the end of each intervention. Mean ± SEM, n=7. Repeated measures ANOVA revealed no significant differences between supplements for any of the measurements.
There were also no significant differences between the starches for fasting (Table 4.5) or postprandial TG concentrations (Figure 4.6).

Figure 4.6: Postprandial TG concentrations for RS and PL, following the fibre-free breakfasts at the end of the interventions. Mean ± SEM, n=7. Repeated measures ANOVA revealed no significant differences between the starches.

There were no significant differences between the two starches in fasting insulin sensitivity (HOMA %S) or β-cell function (HOMA %B) as calculated by the HOMA model (described in Chapter 2) (Table 4.6).

There was also no significant difference between the starches in postprandial oral insulin sensitivity (method described in Chapter 2) (Table 4.6) following the fibre-free breakfasts at the end of the interventions.
Table 4.6. Indices of insulin sensitivity following consumption of 40 g RS or the PL per day for 4 weeks, n=7. Comparisons were made with a paired samples t test.

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th>SEM</th>
<th>PL</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA % S&lt;sup&gt;1&lt;/sup&gt;</td>
<td>73.3</td>
<td>12.1</td>
<td>65.6</td>
<td>6.8</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA % B&lt;sup&gt;2&lt;/sup&gt;</td>
<td>136.4</td>
<td>14.4</td>
<td>133.9</td>
<td>10.1</td>
<td>NS</td>
</tr>
<tr>
<td>Oral Si&lt;sup&gt;3&lt;/sup&gt;Breast (dl/kg/min/µU/ml)</td>
<td>1.10 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.23 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>1.97 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.76 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup>HOMA %S = fasted oral insulin sensitivity, assessed by homeostatic model assessment.
<sup>2</sup>HOMA %B = β-cell function, assessed by homeostatic model assessment.
<sup>3</sup>Si = oral insulin sensitivity to breakfast meal, calculated by minimal model.

4.5.6. Diet diaries

There were no significant differences between the RS and PL for mean daily energy or macronutrient intake as measured from the seven day diet diaries completed during the final week of each intervention (Table 4.7). However, total DF intake was significantly higher following the RS compared with the PL (56.5 ± 2.3 g vs 18.2 ± 2.7 g, p=<0.001), attributable to the RS supplement.

Table 4.7: Average daily energy and macronutrient intake following supplementation with 40 g RS or the PL, measured from seven day diet diaries, n=7. Comparisons were made with a paired samples t test.

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th>SEM</th>
<th>PL</th>
<th>SEM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>8913</td>
<td>792</td>
<td>8941</td>
<td>399</td>
<td>NS</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2122</td>
<td>189</td>
<td>2129</td>
<td>95</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>89.6</td>
<td>8.0</td>
<td>89.8</td>
<td>8.8</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>254.8</td>
<td>27.3</td>
<td>244.1</td>
<td>15.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>79.0</td>
<td>9.7</td>
<td>78.2</td>
<td>5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>56.5</td>
<td>2.3</td>
<td>18.2</td>
<td>2.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
4.5.7. FSIVGTT

The data presented for the FSIVGTT are for six participants, due to cannulation problems for one subject. Wilcoxon Signed-Rank tests were used, to compare AUC and values from the model, due to the smaller participant numbers.

The FSIVGTT glucose concentrations following consumption of 40 g RS per day for 4 weeks were significantly higher ($p=0.045$) than the concentrations following the PL (Figure 4.7A). There was also a significantly higher insulin response following consumption of the RS for 4 weeks compared with the PL ($p=0.003$) which was accompanied by a significant treatment*time interaction ($p=0.012$) (Figure 4.7B). The C-peptide concentrations were also significantly higher following 4 weeks consumption of RS compared with the PL ($p=0.039$), which also had a significant treatment*time interaction ($p=<0.001$) (Figure 4.7C).

The AUC were significantly higher with the RS than with the PL for the glucose ($Md\ 949.7\ \text{mmol/l.min vs Md\ 922.0}\ \text{mmol/l.min, p=0.028}$) and insulin responses ($Md\ 47485.0\ \text{pmol/l.min vs Md\ 33709.5}\ \text{pmol/l.min, p=0.028}$), whilst there was a trend for a higher C-peptide response with the RS compared with the PL ($Md\ 233.5\ \text{nmol/l.min vs Md\ 196.7}\ \text{nmol/l.min, p=0.075}$).

Examples of the output graphs from the MINMOD Millennium program are shown in Figure 4.8. These graphs are for the mean glucose and insulin concentrations from the FSIVGTT for both the RS (Figure 4.8A) and PL legs (Figure 4.8B).
Figure 4.7: FSIVGTT glucose (A), insulin (B) and C-peptide (C) concentrations for the RS and PL legs at the end of 4 weeks supplementation. Glucose infused at time zero and insulin at time 20 minutes. Mean ± SEM, n=6. Repeated measures ANOVA showed significant difference between starches for glucose (p=0.045), insulin (p=0.003) and C-peptide (p=0.039).
Figure 4.8: Output graphs from the MINMOD Millennium program for mean glucose (mg/dl) and insulin (mU/l) responses from the FSIVGTT for the RS (A) and PL (B) legs. Blue lines/circles indicate glucose observations, white circles indicate unweighted glucose observations, green circles/lines indicate insulin observations, red lines indicate the fit of the model to the glucose observations and the black lines indicate the IAGD, which is the insulin attributable glucose disposal (instantaneous % of glucose disposal due to the action of insulin).
Results from the modelling of the glucose and insulin data indicated no significant difference between the two supplements for insulin sensitivity ($S_i$), first-phase insulin responses ($\text{AIR}_9$) glucose effectiveness ($S_g$) or the disposition index (DI) Table 4.8.

Table 4.8. Indices from the MINMOD Millennium program for modelling of the FSIVGTT data following consumption of 40 g RS or the PL, n=6. Comparisons were made with Wilcoxon Signed-Rank tests.

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th>PL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_i$</td>
<td>Median</td>
<td>Inter-quartile range</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>2.53</td>
<td>1.52 - 4.35</td>
<td>3.32</td>
</tr>
<tr>
<td>\text{AIR}_9</td>
<td>543.5</td>
<td>369.1 - 1441.4</td>
<td>392.5</td>
</tr>
<tr>
<td>$S_g$</td>
<td>0.024</td>
<td>0.019 - 0.029</td>
<td>0.026</td>
</tr>
<tr>
<td>DI</td>
<td>1304.9</td>
<td>913.9 - 3848.0</td>
<td>1343.6</td>
</tr>
</tbody>
</table>

Inter-quartile range 25th and 75th percentiles.

4.6. Discussion

This study was the first to our knowledge that investigated the effects of longer-term (4 week) consumption of RS on appetite and food intake when compared with an energy and carbohydrate matched placebo. However, unlike in the acute RS and appetite study (Chapter 3), no significant difference was found in food intake following consumption of 40 g RS for four weeks, compared with the PL. The data from the FSIVGTT indicated a significant difference between the RS and PL for concentrations of glucose, insulin and C-peptide; however, there was
no significant difference between the supplements when the data were modelled.

A major limitation of the study and an important reason as to why no significant differences were found, and why no conclusion can be completely drawn from the study, is the very small participant numbers (n=7) due to time constraints. However, ethics were obtained for 20 participants based on findings from previous studies, where changes to appetite and insulin responses were observed, and it is hoped more individuals will be recruited in the future to confirm or refute the current findings. The original power calculations for the study were based on changes to appetite ratings, where there was a calculated 80% probability of detecting a 20% increase in subjective hunger scores with a SD (standard deviation) of 17% based on the work by Martins et al. (2007a), and changes to postprandial insulin responses, where there was a calculated 89% probability of detecting a 5035 µU/ml.min difference in postprandial insulin AUC between the two treatments based on a SD of 6693 µU/ml.min taken from the work described in Chapter 3. Based on the data obtained so far, power calculations (on the ad libitum test meal data and the parameters obtained from the modelling of the FSIVGTT data) were performed to determine the number of participants required to elicit a significant difference. These showed that, to determine significant differences between the treatments at 5%, with an 80% probability, 10 individuals would be needed based on the ad libitum test meal data and 14 individuals based on the FSIVGTT data.
In this study, effects on food intake were assessed by diet diaries recorded during the final week of each intervention and from food intake at the *ad libitum* test meal after consumption of a standard fibre-free breakfast. No significant effect was observed on food intake from either measurement. From the seven day diaries there was only an average 28 kJ per day difference in mean intake between the two supplements. At the *ad libitum* test meal there was a lower energy intake (394 kJ) following the RS (4521 ± 541 kJ) compared with the PL (4915 ± 533 kJ). A possible hypothesis as to why there was such a small difference in intake from the diet diaries compared with that measured with the *ad libitum* test meal, could be the nature of how the intakes were assessed. At the *ad libitum* meal (single-meal) the participants were isolated, with any distractions removed, and were asked to consume as much as they wanted until comfortably full; therefore enabling them to focus more clearly on the meal and being more aware of when they were actually full. When the diet diaries were completed, participants were free to consume according to their normal dietary patterns and would have been subjected to influences of everyday life, including external cues and habits. External influences (such as the availability and olfactory properties of foods and the presence/absence of other individuals), which are removed in the *ad libitum* setting, could have influenced the amount of food consumed. Alternatively, satiety signals may have been ignored in the free-living situation and therefore food may have been consumed according to normal patterns, rather than in relation to hunger, which is important when translating single-meal findings to weight management. This hypothesis is
corroborated in a review of biomarkers of satiation and satiety by de Graaf et al (2004) where it was reported that many factors (cognitive and external) can affect food intake, but when food intake is measured under controlled conditions this can act as a measure of appetite. This suggests that the *ad libitum* intake in our study could have provided a more accurate marker of intake than the reported diet diaries, although not perhaps an accurate marker of free-living conditions.

Our study also found no significant difference between the two starches on subjective appetite ratings (hunger, fullness or prospective food consumption), but again this may have been because of habitual overriding of internal cues, and therefore the participants may have been unable to distinguish between the two supplements. A study by Barkeling et al (2007) investigated the relationship between eating patterns and feelings of fullness and hunger in obese subjects (a control group of lean individuals was also studied). Subjective appetite ratings were taken before and after fixed energy breakfast and lunch meals in a laboratory setting. The obese subjects were divided into two groups at screening, one group who experienced appetite sensations related to eating and the other whose eating was not related to appetite sensations (allocated in response to screening questions). The authors reported that all groups showed changes to appetite ratings in response to the meals, which they suggest shows that those in the group where eating was not related to appetite sensations could in fact note changes under certain conditions; and that the laboratory
setting used is different to real-life settings where other effects can influence appetite. This could support the hypothesis from our study discussed above, that the lower energy intake observed with the ad libitum test meal (compared with the dietary records) could have been due to the participants being more aware of their appetite sensations.

A significant difference was found between the RS and PL for ratings of desire for fatty foods, with participants appearing to have a greater desire for fatty foods after the RS compared with the PL. The reason for this greater desire for fatty foods is unclear, especially as there was no significant difference between the supplements in consumption of fatty foods from the seven day diet diaries, and the result contradicts our own earlier findings (Chapter 3), where participants consumed less fat over the 24 hour period with the RS compared with the PL. This greater desire for fatty foods may therefore be a coincidental finding especially as the subject numbers were small.

There are few long-term human interventions investigating the effects of RS on appetite and food intake. In a study by de Roos et al (1995), where two types of 30 g RS (Type 2 and Type 3) or glucose were consumed, each for one week, it was found that there was little effect on appetite (although subjects were more satiated following RS2) or energy and macronutrient intakes from either type of RS. However, whilst the supplements were matched for total carbohydrate content, the proportion of available carbohydrate was lower in both of the RS
supplements. One possible reason for the lack of effect on appetite and food intake could also be the short duration of the intervention. One week, when supplements are consumed in participants’ habitual diets with possible influences from external factors (as discussed above), may be insufficient time for effects on appetite to become evident, especially if the primary mechanism is due to the products of fermentation, which may increase over time. Similarly the dose of RS used in de Roos’ study may have been too low to elicit an effect on appetite. In a study where the effects on appetite and ad libitum energy intake of consumption of fermentable (pectin and β-glucan) and non-fermentable (methylcellulose) DF for 3 weeks were assessed, it was found that satiety was higher following the non-fermentable DF, whilst there were no effects on reported energy intake (Howarth et al., 2003). However, again the 3 week intervention may have been insufficient time for the body to adapt to the fermentable DF and therefore the intervention did not affect appetite and food intake.

In our study, when using the Goldberg cut-off values to estimate under-reporting, two participants on the RS leg and three on the PL leg (of which two were the same as the ones on the RS leg) would have been classified as under-reporters. These participants were included in the analysis for the 7 day diet diaries due to the small participant numbers. However, when the analysis was re-run with the three under-reporters excluded from both legs of the study, there were no changes to the results for energy or macronutrient intake.
As has been demonstrated in previous studies with RS (discussed in Chapter 1), our study found no effect of RS intake on body weight, when the starches were consumed within habitual diets. This has also been found in two other recent studies conducted by our group (Johnston et al. and Robertson et al., unpublished). The lack of effect of RS on body weight has been demonstrated in both normal weight (de Roos et al., 1995, Heijnen et al., 1996) and overweight individuals (Park et al., 2004). In contrast to the lack of effect of RS on body weight in humans, rodent studies have reported effects of high RS consumption on lower epididymal fat pad weight compared with low RS diets (de Deckere et al., 1993) and rats fed a high amylose-resistant cornstarch diet had decreased abdominal fat compared with those on a control diet (Keenan et al., 2006).

Our study found no significant differences between the RS and PL legs for any of the blood lipids measured (total, HDL and LDL cholesterol or postprandial NEFA and TG concentrations). Whilst there is evidence in rodent studies for a lipid lowering effect of RS (de Deckere et al., 1993) the evidence in humans is less clear; indeed in a review by Nugent (2005) it was stated that overall RS consumption may be lipid neutral. A study by Robertson et al. (2005) found no effect of 4 week supplementation with RS compared with a placebo on fasting TG or NEFA concentrations, although postprandial NEFA concentrations were significantly lower with RS. A study by Heijnen et al. (1996) where 30 g RS2, 30 g RS3 or glucose were consumed for 3 weeks each within habitual diets of normolipidaemic individuals, also found no effect on total, HDL or LDL
cholesterol or TG. Higgins et al (2004) also found no significant difference with 4 different amounts of RS on fasting and postprandial TG concentrations. However, in contrast to this evidence, a parallel study conducted in overweight and obese subjects, where subjects consumed either 24 g per day RS or regular corn starch for 3 weeks, the RS resulted in a decrease in total and LDL cholesterol whilst no effect was demonstrated with the regular corn starch (Park et al., 2004). A study by Behall and Howe (1995) found that after 14 week consumption of a high amylose cornstarch diet (70% amylose and 30% amylopectin) the TG concentrations were significantly lower during the study than after a high amylopectin diet (standard cornstarch 30% amylose and 70% amylopectin). In the light of the mixed findings from the published work further investigation would be required to determine what effect, if any, consumption of RS has on lipid concentrations in humans.

The postprandial glucose and insulin concentrations in response to the standard fibre-free breakfast given in our study did not differ between the RS and PL. However, in a study by Behall and Howe (1995) following 14 week consumption of high or low amylose cornstarch, glucose responses were similar between the two diets, but the insulin responses were significantly lower with the high amylose diet compared with the low amylose diet. This suggests that the participant numbers may indeed have been too small in our study to detect significant differences; alternatively 4 weeks may have been insufficient time for
effects on metabolites to occur, as demonstrated by an effect being observed after 14 weeks in the Behall and Howe (1995) study.

In our study, when the glycaemic and insulinaemic responses were investigated using the FSIVGTT, there was a significant difference between the RS and PL for glucose, insulin and C-peptide concentrations. In all instances the metabolite responses were significantly higher with the RS than with the PL. However, when the data were modelled there were no significant differences between the starches for the parameters obtained. The raw data indicates that there is an improved first-phase insulin response with the RS compared with the PL whilst the AIR₉ values obtained from the model were not significantly different; these apparent contradictory findings are likely to be due to a lack of study power for the modelling of the data to determine differences between the treatments. Improvements in the first-phase insulin response due to consumption of RS, could have beneficial implications in diabetes, as the reduction of first-phase insulin is a primary defect of β-cell functioning in the development of diabetes (Gerich, 2002). This finding would need further investigation with more participants to determine whether it is a true physiological effect.

Both the oral insulin sensitivity (in response to the standard fibre-free meal) and the insulin sensitivity values obtained by the FSIVGTT were not significantly different between the RS and the PL, but both measures produced insulin sensitivity values that were higher with the PL than with the RS. These findings
contradict those of previous studies where consumption of RS has been seen to improve insulin sensitivity compared with a placebo, measured by oral insulin sensitivity in one study and by an euglycaemic-hyperinsulinaemic clamp in another study (Robertson et al., 2003, Robertson et al., 2005). It is therefore likely that the lack of finding of an effect of consumption of RS on insulin sensitivity in our study compared with the PL is due to the very small participant numbers and thus more participants would need to be added to this body of work before a final conclusion can be drawn.

Overall it would appear from this study that longer-term (4 week) consumption of RS by overweight individuals does not impact on appetite or food intake. The study also found that there appears to be a treatment effect of the RS on the insulin response, although further investigation is required to confirm this finding. All of the results from the study are probably affected by the small participant numbers and therefore it is difficult to draw any concrete conclusions from the study. Further investigation with more participants (as is planned) would be required to confirm or disprove the preliminary findings.
Chapter 5

Whole Grain Products
Chapter 5

This chapter describes three studies, using specially developed whole grain bread rolls, to determine glycaemic index, acceptability to participants and short-term effects on appetite and food intake. This was important preliminary data, necessary before commencing a longer-term intervention using these rolls.

5.1. Introduction

There is epidemiological evidence suggesting whole grains have a beneficial role in several chronic diseases (Seal et al., 2006) and that they may regulate body weight and thus could be beneficial in obesity prevention. However, there are few intervention studies that directly examine this (Koh-Banerjee and Rimm, 2003).

As the majority of evidence for the effect of whole grains on weight comes from cohort studies, it is unclear if the benefits reported are due to the whole grains themselves or because those who consume high intakes of whole grains also tend to have other healthier lifestyle habits. These studies alone are therefore too weak to provide evidence of a causal relationship.

For use in a controlled intervention study bread rolls were specially manufactured by Premier Foods. These rolls delivered a high level of whole grains to individuals in order to determine if this dietary factor does possess health benefitting properties. The studies in this chapter provide necessary information in terms of taste and GI of the bread roll products, which could
confound the interpretation of the data in the intervention study, with reference to the beneficial effects of the whole grains themselves. An initial small crossover study using the rolls is also outlined in this chapter, which was conducted to provide initial data on the effect of whole grains on appetite, as there is no current evidence for this effect in the literature.

5.2. **Aim**

The overall aim of the studies described in this chapter was to acquire information regarding the glycaemic index, taste and short-term appetite effects of the developed whole grain roll products to complement data obtained from a chronic study.

5.3. **The Products**

The bread rolls were developed, manufactured and supplied by Premier Foods.

5.3.1. **Description**

When the products were developed there were no UK recommendations for the level of whole grains to include in the diet; however, it was recommended that levels of intake should be increased. The 2005 Dietary Guidelines for Americans recommend that individuals consume three or more servings of whole grains per day. A serving is considered to equate to 16 g of the whole grain ingredient (Whole Grains Council, USA) and therefore 48 g of whole grains would be the recommended intake per day.
Based on these recommendations the experimental products developed for the following studies provided 48 g whole grain (wheat grains), in a two roll serving. Premier Foods developed the bread rolls as a controlled and reproducible vehicle to provide the specified whole grain. Two types of whole grain rolls were developed, one where the grains were in the intact form and the other where the product contained exactly the same ingredients and level of whole grain, but where the grains had been milled. This would allow for the influence of whole grain structure to also be investigated, as both the intact and milled whole grain products fulfil the definition of whole grains. A refined grain roll, baked from identical dough, containing no whole grains was also produced and used as a control. The bread rolls used in the study are shown in Figure 5.1.

**Figure 5.1:** Bread rolls used within the studies.
5.3.2. **Nutritional information**

The nutritional information for each roll (per 100 g of product and per average roll) is shown in Table 5.1. The basic bread dough was identical for all three rolls, the only differences arising from the addition of whole grains in different structures.

**Table 5.1**: Nutritional composition of the developed bread rolls, per 100 g and per average roll.

<table>
<thead>
<tr>
<th></th>
<th>Intact whole grain rolls</th>
<th>Milled whole grain rolls</th>
<th>Refined grain rolls – Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per 100g</td>
<td>Per 80.0g</td>
<td>Per 100g</td>
</tr>
<tr>
<td>Energy (KJ)</td>
<td>1243</td>
<td>994</td>
<td>1200</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>293</td>
<td>234</td>
<td>283</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>10.0</td>
<td>8.00</td>
<td>9.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>59.5</td>
<td>47.60</td>
<td>57.4</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>1.5</td>
<td>1.20</td>
<td>1.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.7</td>
<td>1.36</td>
<td>1.6</td>
</tr>
<tr>
<td>Saturates (g)</td>
<td>0.22</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>Fibre (g)¹</td>
<td>5.6</td>
<td>4.48</td>
<td>5.4</td>
</tr>
<tr>
<td>Sodium (g)</td>
<td>0.29</td>
<td>0.23</td>
<td>0.28</td>
</tr>
<tr>
<td>Moisture (g)</td>
<td>21.8</td>
<td>17.44</td>
<td>24.5</td>
</tr>
</tbody>
</table>

¹ Fibre measured by the AOAC method

5.4. **Study 1 – Taste test**

It is important when developing products for use in intervention studies that they are acceptable to the consumer, which ultimately would enhance study compliance. Differences in taste and acceptability could result in differing postprandial responses, irrespective of nutritional properties. Therefore a taste test on the developed experimental rolls was carried out to gather data on the acceptability and organoleptic properties of the products. Commercially available

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Study 1 contains the products of joint research between Laura Tripkovic and myself. Whilst the planning and data collection were conducted in collaboration, all analysis was conducted by myself.
products were chosen to which the three developed bread rolls could be compared. These were matched as closely to the developed breads as possible, but as there are no commercially available products that are the same as the developed products these were predominately visually matched.

5.4.1. Aim
To investigate the similarities and differences between the developed bread rolls (intact and milled whole grain rolls and control rolls) and compare them to visually similar commercially available bread products.

5.4.2. Hypothesis
The three developed products will not differ significantly from each other, which might impact on appetite and food intake in the intervention study.

5.4.3. Study Design
5.4.3.1. Participants
Thirty adult males and females (15 M, 15 F) participated in the study. Individuals suitable for inclusion for the taste test met similar criteria to those who would be participating in the intervention. The main inclusion criteria for the taste test were individuals without food allergies or intolerances, those without bowel complaints, such as irritable bowel syndrome, those who did not have other problems associated with foods high in DF (such as poor dentition) and those who did not regularly consume high intakes of whole grains.
5.4.3.2. Protocol

The study was a blinded, comparison sensory evaluation, using a Difference Test, Hedonic Scale and Visual Analysis. Participants attended for a maximum of 45 minutes to complete questionnaires regarding the breads. The University of Surrey’s Ethics Committee gave a favourable ethical opinion for this study (EC/2007/02/SBMS) and all participants gave informed, written consent.

There were six samples, three experimental rolls and three commercial products (Figure 5.2). The six breads were randomly assigned a number for the test.

![Figure 5.2: Commercially available bread products for taste test comparison. A = milled whole grain roll equivalent, B = refined grain roll equivalent, C = intact whole grain roll equivalent.](image)

The taste test was guided by the methodology in the British Standards in Sensory Analysis (BS ISO 6658:2005, Standards Policy and Strategy Committee). As per these Standards, the test was performed mid-afternoon, one
to two hours after the participants had consumed lunch, to eliminate possible
influences of appetite. The test was conducted in controlled conditions. All
participants were placed in individual areas and all surroundings and equipment
were kept as neutral as possible to reduce external influences. The participants
remained silent throughout the test and were unable to communicate either with
each other or the investigators. All six samples were provided to the participants
as 1 inch squares and were on a covered plate. Each sample was identified by a
randomly assigned number. A glass of water was provided.

Three tests were used to determine information on the breads, a difference test,
a visual analysis and a hedonic scale. The difference tests involved participants
rating, on a scale of 1 to 9 (one being the most negative and nine the most
positive), properties of each of the breads (Figure 5.3). The properties rated
were aftertaste, saltiness, moistness, roughness, chewiness and hardness.

<table>
<thead>
<tr>
<th>1) Moistness</th>
<th>Just right</th>
<th>Very moist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very dry</td>
<td>1 2 3</td>
<td>4 5 6 7 8 9</td>
</tr>
</tbody>
</table>

Figure 5.3: An example of one of the questions asked in the difference tests. Participants
were asked to circle the answer they felt most appropriate for each of the breads and
questions.

The visual analysis gauged the visual acceptability of each of the samples from
photographs and participants were asked to rank them from 1st choice to 6th.
The hedonic scale involved the participants rating how much they liked or
disliked each bread (Figure 5.4).
5.4.3.3. Statistical Analysis

The data were analysed using non-parametric statistics. Each grain type was compared with their corresponding commercial product using Wilcoxon Signed-Rank. The three test breads were compared using the Friedman test and when significant differences were found, Post Hoc Wilcoxon Signed-Rank tests, with a Bonferonni adjustment. Data are presented in the text as the median and on graphs as the median and inter-quartile range. On all graphs and tables intact whole grain rolls are referred to as "WG", milled whole grain rolls as "MWG" and control rolls as "C".

5.4.4. Results

5.4.4.1. Difference Test

There were no significant differences in aftertaste between the experimental and commercial rolls of the same grain, or between the three experimental rolls. The experimental and commercial intact whole grain rolls and control rolls did not differ significantly in ratings of saltiness. The experimental milled whole grain...
rolls were perceived as significantly less salty than the matching commercial rolls ($Md_6$ vs $5M_d$, $p=0.044$). The ratings of saltiness were not significantly different between the experimental rolls (Figure 5.5).

![Figure 5.5](image)

**Figure 5.5:** Results from difference test for saltiness, $n=30$ for all groups. $E =$ experimental, Co = commercial. Numbers on graph represent outliers at each point. Wilcoxon Signed Rank test showed no significant difference between $E$ and Co WG and C rolls and a significant difference between MWG $E$ and Co rolls (* $p=0.044$). Friedman test showed no significant difference between the $E$ rolls.

All three experimental rolls (intact, milled and control) were significantly drier and more bitty than their commercial counterparts ($Md_3$ vs $Md_3$, $p=0.009$, $Md_1$ vs $Md_5$, $p=<0.001$ and $Md_2$ vs $Md_3$, $p=0.014$, respectively for moistness; $Md_3$ vs $Md_6$, $p=<0.001$, $Md_5$ vs $Md_7$, $p=<0.001$ and $Md_6$ vs $Md_7$, $p=0.024$, respectively for roughness). There was a significant difference between the three experimental breads when compared for moistness ($p=0.007$). Post Hoc tests revealed that the intact whole grain rolls were significantly less dry than the milled whole grain rolls ($p=0.008$) (Figure 5.6). The three experimental rolls were also significantly different ($p<0.001$) for roughness, with Post Hoc tests...
revealing the difference lay between all three products, intact vs milled ($p=0.001$), intact vs control ($p<0.001$) and milled vs control ($p<0.001$) (Figure 5.6).

All three experimental rolls (intact, milled and control) were significantly more chewy and hard than their commercial pair ($Md_2 \text{ vs } Md_4$, $p<0.001$, $Md_3 \text{ vs }$}
Md 5, $p<0.001$ and Md 3 vs Md 5.5, $p<0.001$, respectively for chewiness; and Md 4 vs Md 5 $p<0.001$, Md 3 vs Md 7 $p<0.001$ and Md 4 vs Md 6.5 $p<0.001$, respectively for hardness). There was no significant difference between the three experimental rolls for chewiness or hardness (Figure 5.7).

Figure 5.7: Results from difference tests for chewiness (A) and hardness (B), n=30 for all groups, except WG E for hardness n=29. E = experimental, Co = commercial. Numbers on graph represent outliers at each point. All three E rolls significantly more chewy and harder than their Co pair. There were no significant differences between the three E rolls for chewiness or hardness.
5.4.4.2. Visual Analysis

The visual ranking of the breads from the photographs, revealed the order of acceptability to be:

1\textsuperscript{st} Intact whole grain commercial
2\textsuperscript{nd} Milled whole grain commercial
3\textsuperscript{rd} Milled whole grain experimental
4\textsuperscript{th} Intact whole grain experimental
5\textsuperscript{th} Control experimental
6\textsuperscript{th} Control commercial

5.4.4.3. Hedonic Scale

Figure 5.8 shows the frequency of response for each of the breads on the hedonic scale. The density of the colour on the charts signifies the degree of liking/disliking, with the orange shades indicating liking, blue/green shades disliking and white indicates neither like nor dislike.

From all of the breads it appears that the control rolls were the most disliked with the commercial roll being the least acceptable of the two rolls. From the two types of whole grain rolls the commercial intact and milled rolls appeared to be more acceptable than the corresponding experimental rolls.
Figure 5.8: Frequency of response on the hedonic scale for each bread, n=30. A = intact whole grain experimental, B = intact whole grain commercial, C = milled whole grain experimental, D = milled whole grain commercial, E = control experimental, F = control commercial. Orange colours represent liking, blue/green colours represent disliking and white represents neither like nor dislike.
Comparison of the experimental breads against commercial breads showed the experimental milled whole grain rolls were significantly more disliked than the corresponding commercial roll \((Md = 6 \text{ vs } Md = 3, p=0.001)\), there was a trend towards the experimental intact whole grain roll being more disliked than the corresponding commercial product \((Md = 4 \text{ vs } Md =3, p=0.097)\) and there was no significant difference between the two types of control roll (Figure 5.9). When the three experimental rolls were compared there was a significant difference \((p=0.042)\) and Post Hoc analysis revealed the difference lay between the intact whole grain rolls and control rolls \((Md = 4 \text{ vs } Md = 7, p=0.013)\) (Figure 5.9), with the intact whole grain rolls being more preferred than the control rolls.

![Figure 5.9: Hedonic rating for each bread, n=30. E = experimental, Co = commercial. Wilcoxon Signed-Rank tests showed a significant difference between MWG E and MWG Co (* p=0.001). The Friedman Test showed a significant difference between the three E groups († p=0.042).](image-url)
5.5. **Study 2 – Glycaemic Index**

This section of the chapter will outline the study undertaken to determine the GI of the three developed bread rolls. The methodology for this study followed the guidelines outlined in the paper by Brouns et al. “Glycaemic index methodology” (2005), which is the accepted protocol for GI measurement.

5.5.1. **Aim**

To determine the GI and insulin response of the developed whole grain bread rolls (intact and milled) and the control roll (refined grain).

5.5.2. **Hypothesis**

The refined grain bread roll will have a GI similar to that of published GIs for commercially available white bread. The whole grain rolls will have lower GIs than the refined grain roll, with the roll containing grains in the intact form having a lower GI than the milled grain roll.

5.5.3. **Study Design**

5.5.3.1. **Participants**

Twelve young, healthy participants were recruited and nine (5 M, 4 F), completed the study. The participant characteristics are shown in **Table 5.2**. Of the participants who dropped out of the study, one stopped due to sleep deprivation, which could have impacted on blood glucose responses and the other two due to problems with time commitments due to personal work load.
Table 5.2: Baseline characteristics of the participants, n=9.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.9 ± 1.86</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>74.4 ± 3.90</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.2 ± 4.47</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 1.00</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>87.1 ± 2.60</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>102.6 ± 2.37</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>23.5 ± 1.91</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>118.7 ± 4.98</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>71.0 ± 3.26</td>
</tr>
</tbody>
</table>

5.5.3.2. Protocol

The study was a randomised crossover study investigating the glycaemic index of the three manufactured test bread rolls. Participants attended for six study mornings, when they consumed one of the three bread rolls (intact, milled or control rolls) and, on three occasions, the reference food (glucose). Each study morning was separated by at least one day for washout. The University of Surrey's Ethics Committee gave a favourable ethical opinion for this study (EC/2008/66/FHMS) and all participants gave informed, written consent.

Participants were given a weighed amount of bread (weighed to 0.1 g) that provided 50 g of available carbohydrate (Table 5.3). The reference food used was 50 g of glucose powder (Thornton and Ross Ltd, UK) in 250 ml water. All
participants started with a glucose drink as their first test product. The bread rolls and two other glucose drinks were randomised for the subsequent visits for each participant using a web-based randomisation program.

Table 5.3: Amounts of product given to provide 50 g available carbohydrate

<table>
<thead>
<tr>
<th>Available Carbohydrate (g)</th>
<th>Amount of product (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain &quot;intact&quot; roll</td>
<td>50</td>
</tr>
<tr>
<td>Whole grain &quot;milled&quot; roll</td>
<td>50</td>
</tr>
<tr>
<td>Refined grain &quot;control&quot; roll</td>
<td>50</td>
</tr>
</tbody>
</table>

On the day before each study day participants were requested to refrain from strenuous exercise and alcohol, and were asked to consume the same evening meal the evening before each study morning and then fast for 12 hours. On the first study morning anthropometric measurements and blood pressure were taken.

On each study morning a fasting capillary blood sample was taken by finger prick. After this the participants consumed either the test roll with 250 ml of water or the glucose drink. Participants consumed the products within 10 minutes. Further capillary blood samples were taken at 15, 30, 45, 60, 90 and 120 minutes after the first mouthful of the test product. Participants returned for the next study morning after at least one day to allow for washout.
After consumption of the breads rolls, the participants completed a questionnaire regarding the taste, texture, smell, palatability and aftertaste of each of the tested products. These questionnaires required the participant to rate the products on a scale of 1 to 5, with 1 being unpleasant and 5 very acceptable.

On each occasion approximately 300 µl of blood was collected into heparinized tubes. All samples were centrifuged and the plasma was immediately analysed for plasma glucose concentrations, using the YSI method (described in Chapter 2). All plasma samples were then frozen at -20°C prior to analysis of insulin concentrations by RIA (as described in Chapter 2).

5.5.3.3. Calculations and Statistical Analysis

The iAUC for each participant’s glucose response to each product was calculated using the trapezoid method, ignoring all points that went below baseline, according to Brouns et al (2005). A mean glucose response was then used to compare the bread samples. The glycaemic index of each product for each participant was then calculated by dividing the iAUC of the test food by the iAUC of the mean glucose scores (reference food), times 100. The overall GI of each product was then calculated as the mean value from the 9 participants.

\[
\frac{\text{iAUC of the test food}}{\text{iAUC of the reference food}} \times 100
\]
The glucose and insulin data were also analysed using repeated measures ANOVA with bread type and time as independent variables and the measurements themselves as the continuous dependent variable. AUC were also calculated for the insulin and glucose responses to each bread, using the trapezoid method, and compared using one-way repeated measures ANOVA. The questionnaire data regarding the breads' properties were analysed using the Friedman test and when significant differences were found, Post Hoc Wilcoxon Signed-Rank tests, with a Bonferonni adjustment. On all graphs and tables intact whole grain rolls are referred to as "WG", milled whole grain rolls as "MWG" and control rolls as "C".

5.5.4. Results

5.5.4.1. Glycaemic Index

The GI of the bread rolls are shown in Table 5.4. The results suggest that the milled grain roll and the control roll have similar a GI whilst the intact grain roll has a lower GI. However, the difference between the GI of the breads was not significantly different.

Table 5.4: Glycaemic Index of the three test bread rolls.

<table>
<thead>
<tr>
<th></th>
<th>GI</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain &quot;intact&quot; roll</td>
<td>60.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Whole grain &quot;milled&quot; roll</td>
<td>81.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Refined grain &quot;control&quot; roll</td>
<td>78.3</td>
<td>10.6</td>
</tr>
</tbody>
</table>
These values would be classified as high GI for the milled whole grain and control rolls (high GI >70) and medium GI for the intact grain rolls (medium GI 55-70). The control roll GI is similar to that of commercially available white wheat bread, which has previously been assigned an average GI of 75 ± 2 (Atkinson et al., 2008).

5.5.4.2. Glucose and Insulin Responses

The fasting insulin sensitivity and β-cell function (assessed by the homeostatic model assessment, described in Chapter 2) were not significantly different at the start of the study days, which confirms that the participants were in a similar metabolic state at the start of each study day, Table 5.5.

Table 5.5: Fasted insulin sensitivity and β-cell function on each study day, n=9. Comparisons were made with one-way repeated measures ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>Average Glucose</th>
<th>WG</th>
<th>MWG</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>HOMA % S¹</td>
<td>147.3</td>
<td>13.1</td>
<td>141.6</td>
<td>17.3</td>
<td>146.8</td>
</tr>
<tr>
<td>HOMA % B²</td>
<td>85.3</td>
<td>8.1</td>
<td>92.7</td>
<td>8.8</td>
<td>88.4</td>
</tr>
</tbody>
</table>

¹HOMA %S = fasted oral insulin sensitivity, assessed by homeostatic model assessment.
²HOMA %B = β-cell function, assessed by homeostatic model assessment.

Figure 5.10 shows the mean plasma glucose response to all four products. There was a greater response to the glucose drink and a similar response for the three test rolls. There was no significant difference between the three rolls when the glucose responses were compared using repeated measures ANOVA.
However, there was a treatment*time interaction, indicating that the responses differed over time between the breads. Post Hoc analysis indicated that the significant difference arose between the intact grain rolls and the control rolls. This difference over time could be explained by the lower drop in plasma glucose concentrations noted with the intact grain roll, whilst the control roll plasma glucose concentrations remained fairly constant.

![Graph showing postprandial plasma glucose response to four products](image)

**Figure 5.10:** Postprandial plasma glucose response to the four products. Mean ± SEM; n=9. Repeated measures ANOVA showed no significant differences between the plasma glucose responses for three bread rolls.

The mean insulin responses to the four products are shown in **Figure 5.11**. As with the plasma glucose concentration there is a greater insulin response to the glucose drink than the three test rolls. The insulin response of the three rolls is comparable, with a slightly lower response for the milled grain rolls than for the other two, although these differences were not significant.
There was also no significant difference between the three test breads for the glucose or insulin responses measured by AUC (Figure 5.12).

Figure 5.12: AUC for the three bread rolls for:
A = Plasma glucose response.
B = Plasma insulin response
Mean ± SEM; n=9. One-way repeated measures ANOVA showed no significant differences between breads for either the plasma glucose or insulin responses.
5.5.4.3. **Product Evaluation**

**Figure 5.13** shows the total score for each bread. Each question was marked on a scale of 1 to 5, where 5 was considered very acceptable, so a maximum score of 25 could have been achieved. As can be seen, overall the milled whole grain roll appeared to be most acceptable to the participants with a median score of 17, whilst the control rolls were least acceptable with a median score of 13. There was a significant difference between the breads ($p=0.014$), with the Post Hoc test indicating the significant difference was between the milled whole grain and control rolls, $p=0.017$.

![Figure 5.13: Total score from the evaluation questionnaire for each of the three bread rolls. Median and inter-quartile range; n=9. Friedman test showed a significant difference between the three breads ($p=0.014$).](image)

**Figure 5.14** shows the results for each bread in response to each of the evaluation questions. There was no significant difference between the breads for smell or taste.

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There were significant differences between the breads for palatability \( (p=0.039) \), with the milled whole grain rolls being more preferable than the control rolls \( (p=0.014) \); and for ratings of texture \( (p=0.004) \), where the intact whole grain rolls were much less acceptable than the milled whole grain rolls \( (p=0.009) \).

A significant difference was also found between the breads for ratings of aftertaste \( (p=0.011) \), where the aftertaste of the control rolls was least acceptable to the participants and these rolls were significantly different to the intact whole grain rolls \( (p=0.011) \). There was also a trend towards significance with the milled whole grain and control rolls \( (p=0.034) \).
5.6. **Study 3 – Short-term effects of whole grains on appetite**

This section of the chapter describes a short-term appetite study using the developed bread rolls. Results from studies 1 and 2 indicated the milled whole grain and control rolls had comparable GIs and the milled whole grain rolls appeared to be the more acceptable of the two whole grain rolls. Therefore this pilot appetite study used the milled whole grain rolls compared with the control rolls to determine the effect of whole grains on appetite and food intake.

5.6.1. **Aim**

The aim was to investigate the effects on appetite and satiety of three weeks' consumption of milled whole grain bread rolls (providing 48 g whole grain per day) compared with the refined grain bread rolls. This was measured subjectively using VAS and quantitatively using actual food intake (at a test meal and over one week using diet diaries). The effects of the milled rolls on anthropometric measurements were also assessed.

5.6.2. **Hypothesis**

The inclusion of 48 g of milled whole grains per day for three weeks will promote satiety and decrease participants' intake compared with refined grain at an *ad libitum* test meal and their habitual food intake measured by a seven day diet diary.
5.6.3. Study Design

5.6.3.1. Participants

Fourteen young healthy adults (5 M, 9 F) participated in the study (Table 5.6). The participants included in the study had mean scores of 2.27 (± 0.23), 2.88 (± 0.10) and 1.91 (± 0.16) on the emotional, external and restrained scales of the DEBQ, respectively.

Table 5.6: Baseline anthropometric measurements taken on the first study morning, n=14.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 ± 1.43</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.9 ± 2.36</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.0 ± 2.89</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 ± 0.76</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>77.8 ± 2.45</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>92.7 ± 2.78</td>
</tr>
<tr>
<td>Body Fat (%)¹</td>
<td>22.3 ± 2.83</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>114.2 ± 2.75</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>70.4 ± 1.61</td>
</tr>
</tbody>
</table>

¹= Value for 13 participants

5.6.3.2. Protocol

The study was a randomised balanced crossover study investigating the effects of 48 g milled whole grain on measures of appetite and satiety compared with refined grain. Participants consumed two of the milled whole grain bread rolls or two of the refined grain bread rolls (control) daily for three weeks, separated by a three week wash-out period (Figure 5.15). A favourable ethical opinion was
given for this study by the University of Surrey's Ethics Committee (EC/2007/65/FHMS) and all participants gave informed, written consent. Participants could consume the rolls at any time of day and with any fillings of their choice.

A diet diary was completed for a week before the start of the study to obtain baseline dietary intake. Participants attended for a study morning at the beginning and end of each intervention. On each study morning anthropometric measurements and blood pressure were taken. The participants completed two fasting VAS and were given a standard commercially available milkshake breakfast (Nurishment®, ENCO Products Ltd, UK), as described in Chapter 4, section 4.4.3 and Table 4.2. Each participant consumed the same flavour on all visits. Following breakfast, VAS were completed every 30 minutes for three hours, after which participants were given the *ad libitum* test meal.
Participants began consuming the bread rolls on the day after the study day and completed a seven day diet diary during the last week of each intervention.

At the end of each intervention an evaluation questionnaire regarding the taste, texture, smell, aftertaste, palatability and ease of incorporation of the bread rolls into their normal diets was completed. The participants were asked to rate these factors from 1 to 5 where 1 was considered unpleasant and 5 pleasant.

5.6.3.3. Calculations and Statistical Analysis
The VAS data were analysed using repeated measures ANOVA with bread type and time as independent variables and the measurements themselves as the continuous dependent variable. The AUC for each VAS question was also calculated and then compared using paired samples t-tests. Comparisons were made between treatments (using percentage change from the beginning to end of each treatment) and between the ends of each treatment after the three week intervention, using paired samples t-tests. Responses to the evaluation questionnaires were analysed using the Wilcoxon Signed-Rank tests. On all graphs and tables milled whole grain rolls are referred to as “MWG” and control rolls as “C”.

5.6.4. Results
5.6.4.1. Anthropometrics
Anthropometric measurements taken at baseline and end of each intervention are shown in Table 5.7.
There was no effect of whole grain intake on % body fat, waist or hip circumference or diastolic blood pressure. However, there was a significant difference between breads for systolic blood pressure \( (p=0.016) \) which decreased during the milled whole grain intervention and increased during the control intervention, as shown in Figure 5.16. There was also a significant difference between the ends of the milled whole grain and control interventions for systolic blood pressure, \( 112.4 \pm 2.56 \text{ mmHg} \) versus \( 117.1 \pm 1.71 \text{ mmHg} \) \( (p=0.038) \).
There was also a trend towards significance for body weight and therefore BMI. The percentage change for body weight on the milled whole grain intervention reduced by an average of $0.38 \pm 0.27\%$, whilst increased by an average of $0.49 \pm 0.44\%$ on the control intervention ($p=0.07$). This translated as an average decrease in BMI of $0.09$ kg/m$^2$ on the milled whole grain intervention and an average increase of $0.09$ kg/m$^2$ on the control intervention.

5.6.4.2. Subjective appetite ratings

There were no significant differences in VAS ratings for hunger (Figure 5.17), prospective food consumption, fullness or desire for different foods (sweet, salty, savoury or fatty) either within each intervention or between interventions when the responses were compared. It should be noted that the subjective appetite ratings were in response to the fibre-free meal on the test mornings and not to the products themselves.
Figure 5.17: Subjective appetite ratings in response to the question “how hungry do you feel?”
A) Pre and post MWG intervention.
B) Pre and post C intervention.
C) Post each intervention.
Mean ± SEM, n=14. No significant differences observed when comparisons were made with repeated measures ANOVA.

There was also no significant difference between the AUC, for the VAS ratings relating to hunger, fullness, prospective food consumption, or desire for different foods, when the ends of both interventions were compared and when the difference between beginning and end of each intervention were compared.

5.6.4.3. Test Meal
There was no significant difference in either weight consumed or in energy intake at the ad libitum test meal within interventions or between interventions (absolute difference between beginning and end of each intervention was calculated) (Figure 5.18). However, following the control intervention there was
an increase in intake (mean increase of 367 kJ ± 270 kJ) at the *ad libitum* test meal which was not observed with the milled whole grain intervention (mean decrease of 0.7 kJ ± 300 kJ).

![Figure 5.18: Energy intake at the *ad libitum* test meal, pre and post each intervention. Mean ± SEM, n=14. Comparisons made with a paired samples *t* test showed no significant differences within or between interventions.](image)

5.6.4.4. Diet Diaries

There was no significant difference in average daily energy intake between the seven day diaries completed at the end of milled whole grain intervention compared with the control intervention (*Figure 5.19*). However, participants consumed on average 495 ± 370 kJ more during consumption of the control rolls compared with the period during which the milled whole grain rolls were consumed.
There was no significant difference in protein or fat intake between the interventions (Figure 5.20). Carbohydrate intake tended to be higher during the control compared with the milled whole grain intervention (369.3 ± 21.2 g versus 344.5 ± 19.8 g, \( p=0.054 \)) (Figure 5.20), sugar intake also tended to be higher following the control compared with the milled whole grain (126.3 ± 15.5 g versus 115.3 ± 15.4 g, \( p=0.072 \)), although neither difference was significant. When the diaries were analysed the bread rolls were included within the analysis and this resulted in a significantly higher total DF intake with the milled whole grain rolls compared with the control rolls (30.1 ± 1.7 g versus 25.5 ± 1.8 g, \( p=<0.001 \)) (Figure 5.20). Sodium intake was significantly lower with the milled whole grain compared with the control (3192 ± 204 mg versus 3674 ± 199 mg, \( p=0.027 \)).
Percentage change for each intervention was calculated from the pre study diary and the diary completed during the final week of each intervention. There were no significant differences between the two interventions for percentage change for energy or macronutrients. There was a significant difference between the percentage changes for total DF intake, which increased by 40.1 ± 6.9% for the milled whole grain intervention compared with 17.3 ± 6.8% for the control intervention (p=0.001).

5.6.4.5. Evaluation Questionnaires

The scores from each of the questions, where the breads were rated 1 to 5 (with 5 being the highest score in each category), showed the two breads to be closely matched in ease of incorporation into diets, palatability, taste, texture,
smell and aftertaste (Figure 5.21). There were no significant differences between the breads for any of the questions.

![Figure 5.21: Mean response to each question on the evaluation questionnaire for the MWG rolls and C rolls. Median and inter-quartile range, n=14. No significant differences between the breads for any of the questions. Comparisons made with Wilcoxon Signed-Rank Tests.](image)

5.7. **Discussion**

These three studies were conducted to obtain preliminary information on the properties of the developed bread rolls prior to a long-term intervention. Information gathered included data on the taste and organoleptic properties, GI and effects on appetite of short-term consumption of the experimental whole grain (intact and milled) and control rolls.

Assessing the palatability of test products is important as differences between products could affect both the postprandial response and appetite, and foods
that are found to be highly unpalatable could negatively impact compliance in a
study. The cephalic phase (which occurs before and during the start of a meal
and plays a role in nutrient absorption) is affected by the organoleptic properties
of food and is stimulated by the smell, sight, taste and thought of foods
(Robertson, 2006). The sensory properties of foods have important implications
on food intake, in particular the amount which is consumed. The effects of
sensory perception on food intake and subjective appetite have been reviewed
by Sorensen _et al._ (2003). It was stated that studies have shown highly palatable
foods positively influence food intake, but that effects on subjective appetite are
more mixed, with some palatable foods increasing hunger, some decreasing
hunger and some having no effect at all (Sorensen _et al._, 2003). The palatability
of the three experimental grain rolls was assessed in the taste test and as a
secondary outcome of the other studies in this chapter.

Results from the difference test in Study 1 revealed that, for the majority of the
factors explored, the experimental rolls were rated lower than the corresponding
commercially matched rolls. This finding was not too surprising as the rolls
provided a much higher amount of whole grain per serving than the commercial
products, which would have influenced the sensory properties of the rolls.
However, the experimental and commercial rolls were not significantly different
between the same types of grain for ratings of aftertaste, which is one factor that
could affect appetite ratings to a greater extent. If the experimental rolls were to
be provided on a commercial basis, then further investigation into the
organoleptic properties would be necessary to improve the products. For all but two of the factors explored there were no significant differences between the three experimental rolls. The two factors where the ratings were significantly different were for roughness of the rolls, where the experimental intact whole grain rolls were perceived as more bitty, and for ratings of moistness, with the milled grain rolls being significantly drier than the intact grain rolls. These findings were not surprising due to the presence of the whole grains in the rolls. Overall, the three experimental rolls appeared to be comparably rated and therefore effects on appetite due to the organoleptic properties are likely to be similar for all three rolls.

The hedonic properties of the breads were also assessed in Study 1 and again the commercial rolls appeared to be preferred over their experimental counterparts. The experimental control rolls were disliked more than either of the two whole grain rolls. Similarly the visual analysis revealed that the control rolls were the least visually acceptable. These differences may impact on the findings from a chronic appetite study. The data from the evaluation questionnaires completed in Study 2 confirmed that the control rolls were the least acceptable of the experimental rolls. The control rolls were rated as least acceptable for palatability and aftertaste, whilst the intact whole grain rolls were rated as having the worst texture, which again agrees with the findings of Study 1. The results from the evaluation questionnaire completed in Study 3, following 3 week consumption of either the milled whole grain or control rolls showed no
significant differences between the two breads for the factors assessed. This finding indicates that when the breads are consumed over a longer time period there is less perceived difference in organoleptic properties as the participants were able to incorporate the rolls into their habitual diets, with fillings of their choice rather than consuming them in isolation as in studies 1 and 2.

Bakke and Vickers (2007) recently conducted a taste test to determine consumer liking of whole wheat and refined breads. They compared "laboratory-produced" refined and whole wheat breads and commercially available whole wheat and refined breads. In contrast to our study, their study reported that, when the results were investigated as a whole, the refined grain products were preferred compared with the whole wheat products. However, the commercial refined and whole wheat samples were equally well liked, which is similar to our findings regarding our experimental control and milled whole grain rolls in Study 3. Overall it would suggest that liking of the products based on their organoleptic properties is very subject specific and may depend on the habits of the participants, which was not investigated in our study. The evaluation data obtained from Study 3 (short-term crossover study) may provide more valuable information on the liking of the products and their effect on food intake, compared with the taste test (Study 1), as the amount ingested was greater compared with the small square given at the taste test. Indeed in the review by Sorensen et al (2003) it is stated that pleasantness ratings from taste tests are not a good indicator of the actual amounts that different individuals would
consume, but can provide information on differences between intakes of different foods for an individual.

For the GI study it was hypothesised that the control rolls would have the highest GI, followed by the milled whole grain rolls and then the intact whole grain rolls. In fact the results indicated that despite the presence of the whole grains in the milled rolls, the GI of these rolls was not significantly different to that of the control rolls (both in the high GI category), whilst the intact whole grain rolls had a GI in the "medium" category. This finding of a similar GI between the milled whole grain rolls and control rolls in our study was unexpected, especially in view of studies that have demonstrated that the more processed and the smaller the particle size of cereal foods the higher the GI (Englyst et al., 2003). Our data suggest that, although both of the whole grain roll types used in the study met the definition of a whole grain (all three components were present in amounts found naturally) the processing of the grain resulted in contrasting GIs. This may suggest that in order to beneficially affect the glycaemic response, consumption of intact whole grains would be preferable to milled whole grains. In a review by Venn and Mann (2004) it was found that, from the studies examined, grains that had an intact cellular structure resulted in lower postprandial responses than those where the grains had been milled, which would agree with the lower GI finding of the intact whole grains in our study, even though there was no significant difference in the glycaemic or insulinaemic responses.
Our GI values are slightly higher than for the published GI of similar breads. For example Atkinson et al (2008) published values of 75 ± 2 for “white wheat breads”, 74 ± 2 for “whole wheat/whole meal bread” and 53 ± 2 for “speciality grain bread”. This difference may be due to our smaller sample size (one participant less than the recommend number of participants in the GI methodology paper (Brouns et al., 2005)) and the fact that the published values in this table are an amalgamation of various studies. The number of participants included was one less than in the recommend paper due to difficulties with retention of the participants. However, the inclusion of 9 participants rather than 10 is unlikely to have notably changed the results of the study.

The glycaemic and insulinaemic responses were not significantly different between the three rolls, although the insulin AUC increased in a stepwise order of grain processing. This increase in insulin AUC is similar to the finding observed in the study by Heaton et al (1988) where the more processed the wheat whole grain, the higher the insulin response (whole<cracked<coarsely milled flour <finely milled flour); they also found the glucose AUCs to be higher with the flours than with the whole and cracked grains, but not significantly. Holm and Björck (1992) also found that a whole grain wheat bread which contained intact wheat kernels resulted in a greater reduction in the glucose and insulin responses compared with the other breads investigated.
A recent study by Najjar et al. (2009) investigated the effects of four different breads (white, whole wheat (made with ground whole wheat and whole grain flours), sourdough and whole wheat barley (made with ground whole wheat and barley flours)) on postprandial glucose and insulin responses. They found that the whole wheat barley bread glucose response was significantly lower than the whole wheat bread, and the whole wheat bread had the highest glucose response; whilst overall the sourdough bread had the lowest response. As in our study, there was no significant difference between the breads for the postprandial insulin responses. Contrary to our findings and the findings in the study by Najjar et al., an older study by Holt and Miller (1994) found that the level of processing of wheat grains was a determinant in glycaemic and insulinaemic responses, with the greater processing resulting in higher postprandial glucose and insulin responses. The difference between the findings of these studies could be explained by a difference in the amounts of test products given, with our study and the study by Najjar et al. giving portions of bread to provide 50 g available carbohydrate, whilst Holt and Miller gave equal portions of carbohydrate for each test bread but based this on body weight (0.75 g / kg body weight) therefore delivering a mean of 62.7 g. A study by Behall et al. (1999) which investigated the effects of different particle sizes of whole-grain (white bread, whole wheat bread or ultra-fine whole wheat bread) on glucose and insulin responses, also gave equal carbohydrate based on body weight (1 g / kg body weight). They found that the particle size did not significantly affect the glycaemic or insulinaemic responses. These studies contribute conflicting
evidence as to the glycaemic or insulinaemic effects of different grain types and, as the protocols varied between the studies, comparison is difficult.

The insulin responses in our study may have also been affected by measuring the insulin concentrations on capillary blood samples. The capillary samples were small due to problems with some participants being unable to collect a full sample at all time points. Consequently, all samples were analysed at 50 µl, as opposed to 100 µl samples, (values obtained were therefore doubled as per the instructions given with the assay kit) and some were only analysed as singletons rather than duplicates, which therefore could mean the values were less accurate.

Despite some data in acute studies showing that GI may affect appetite and food intake, the data from long-term studies shows less conclusive evidence (as discussed in Chapter 1). If a difference in glucose and insulin responses was observed in a follow-on chronic intervention study using these rolls, the difference in GI could be considered as a possible explanation; although this is unlikely as no significant difference was found between the rolls for their GI values or in either their glucose or insulin responses and thus differences in an intervention study may indeed reflect direct effects of chronic whole grain consumption.

The short-term intervention study indicated no effect on appetite or food intake following 3 week consumption of the milled whole grain rolls compared with the
control rolls. However, there was a significant difference between the interventions for systolic blood pressure, which increased during the control intervention and decreased following consumption of the milled whole grain.

To our knowledge this is the first study that has directly compared whole grain intake to refined grain intake and measured effects of short-term consumption on actual food intake. None of the 14 participants in this study would have been classified as under-reporters for either the milled whole grain or control intervention diaries according to the Goldberg cut-off values (Goldberg et al., 1991) and therefore all were included in the dietary analysis. Whist there was no significant difference in energy intake between the control and milled whole grain interventions at the ad libitum test meal or from the 7 day dietary records, participants consumed less with the milled grain than with the control. At the ad libitum test meal there was an average increase in energy intake of 367 kJ on the control intervention compared with an average decrease of 0.7 kJ (effectively no change) on the milled whole grain intervention. This could potentially demonstrate a protective role of whole grains in food intake regulation. The energy intake assessed from the 7 day diet diaries was also 495 kJ per day lower with the milled whole grain intervention than with the control. The energy intakes were calculated as a percentage of estimated energy requirements (using the Schofield Equation (Schofield et al., 1985) and an activity factor of 1.6) and were lower with the milled whole grain (99.4%) than with the control (104.8%) intervention. This again could suggest a potential
protective role of high whole grain intake in food intake regulation compared with high refined grain intake, although further investigation would be required as these findings were not significantly different and therefore do not provide actual evidence for this hypothesis. The lack of findings could be due to the participants being healthy, normal weight adults and therefore able to regulate their intake closely. The participants habitual DF intakes (22.3 ± 1.9 g), were higher than the UK average intake of 13 g per day (Buttriss and Stokes, 2008) and this may have been a contributing factor as to why there was little effect on energy intake in this study. Further investigation with other population groups, such as the overweight or obese, or those with lower habitual DF intakes, would therefore be beneficial.

A recently published intervention study by Isaksson et al (2008) investigated the effects of whole grains compared with refined grains on food intake over 24 hours. Their study involved three test days where participants consumed whole grain rye porridge (2 occasions) or refined wheat bread (1 occasion) for breakfast and then, at lunch consumed, whole grain wheat pasta (after one rye porridge breakfast) or refined wheat pasta (after the other rye porridge breakfast and the bread breakfast), energy intake was then assessed at an ad libitum test meal and for the whole day from self-reported intake later in the evening and the following mornings breakfast. There was no effect of treatment on energy intake at the ad libitum test meal, during the evening or on breakfast the following morning. The participants in the study by Isaksson et al were older (mean age
40.7 years) than those included in our study (mean age 26 years), but also had a mean BMI in the healthy range (23.3 kg/m\(^2\)) which was similar to ours (21.8 kg/m\(^2\)), which again could explain the lack of an effect on food intake, or could reflect that longer-term consumption of these products is required to allow for affects on appetite to be observed.

Our study also found no effect of either the control or milled whole grain interventions on subjective appetite ratings. There are few other intervention studies that have investigated the effects of whole grain intake on appetite and satiety using subjective appetite ratings. The study by Isaksson et al. (2008) found that consumption of whole grain rye porridge at breakfast resulted in reports of greater satiety. Other studies where appetite ratings were measured have found consumption of whole grains (80% wheat and the remainder from other whole grain sources) indicated a trend towards less hunger between meals compared with refined grain consumption (Pereira et al., 2002). In a study by Holt and Miller (1994) satiety scores were found to be higher following the least processed grains compared with the refined grains. These studies, contrary to ours, suggest that whole grains may have a role in appetite regulation and therefore this would be an interesting area to further explore in a population where positive effects on appetite would be most desirable, such as in the overweight.
Our investigation found a trend towards a significant difference in weight change (and therefore BMI) between the two interventions. This finding is in line with the epidemiological evidence (as discussed in Chapter 1) that suggests a beneficial role of whole grains on weight regulation. In our study the participants were of normal weight and so only a minor effect on body weight would have been expected. Our finding does not agree with very recent epidemiological evidence from a study based in the UK (Thane et al., 2009). This study investigated the relationship between whole grain intake and adiposity or BMI, from data collected from two dietary surveys, the Dietary and Nutritional Survey of British Adults (aged 16-64 years) in 1986-87 and the National Diet and Nutrition Survey (in adults aged 19-64) in 2000-01. They found little association between whole grain-intake and body weight or BMI in either survey or with waist circumference in the later survey (Thane et al., 2009). However, our data do agree with that obtained from another recent study, conducted in an American female population, which found that individuals with higher whole grain intakes had a lower BMI (Good et al., 2008). The finding in the study by Good et al (2008) is similar to that seen in a recent cross-sectional investigation, conducted in the Netherlands, where it was found that whole grain-intake was lower in those with a higher BMI (van de Vijver et al., 2009). The relationship between whole grain intake and adiposity from observational studies was recently systematically reviewed by Harland and Garton (2008). This review found that overall a lower BMI and central adiposity were associated with a higher whole grain-intake. Therefore our intervention data showing a trend towards a decrease in weight
following milled whole grain consumption for 3 weeks adds valuable information to the effects of whole grains on weight regulation, although further investigation, over longer time periods and in other population groups (particularly the overweight), is required to confirm or refute the finding.

An interesting incidental finding from the study was an effect on systolic blood pressure, which decreased during the milled whole grain intervention and increased during the control intervention, with a significant difference of 4.7 mmHg between the ends of each intervention. This lower systolic blood pressure occurred in individuals who were normotensive and therefore this difference would be considered clinically significant. Other studies have demonstrated an effect of whole grain-intake on hypertension. In a prospective study high whole grain-intake at baseline resulted in less hypertension at 10 years follow-up (Wang et al., 2007). An inverse relationship between whole grain intake and hypertension in males was also reported in a recent prospective cohort study (Flint et al., 2009). In a crossover intervention study different types of whole grains (barley, whole wheat/brown rice and a half and half mix of the two) consumed for 5 weeks each, resulted in significantly lower systolic and diastolic blood pressures (Behall et al., 2006).

The lower blood pressure observed in our study following the milled whole grains could be explained by the lower sodium intake (482 mg/day lower) compared with the control intervention. However, this was assessed from self-
reported diet diaries and therefore the accuracy of these needs to be considered before concluding that this was a contributing factor.

Overall, the three studies in this chapter have shown that the three breads were equally as acceptable to the participants, the GI values were not significantly different (although the two whole grain rolls had a GI in different categories) and there could be a moderate effect of the milled whole grains on food intake. These findings suggest whole grains may affect appetite and that the rolls could be a useful means of delivering a controlled amount of whole grains for use in an intervention study and findings from an intervention study may not be affected by differences in taste or GI.
Chapter 6

Effects of Chronic Consumption of Whole Grains on Appetite
6.1. **Introduction**

As there is little previous interventional evidence for a role of whole grains on appetite, satiety or food intake, and having determined that RS, a main DF constituent of whole grains, has some effects on food intake (Chapter 3), a long-term intervention was conducted, using the developed bread rolls. Due to the lack of findings on food intake in normal-weight individuals (Chapter 5) the participants in this study were overweight/obese individuals, who would be the target group for weight loss. The effects of the whole grain rolls, in both forms, were investigated and compared with each other and the control roll.

6.2. **Aims**

The aim of the study was to investigate the acute (single meal) and chronic (eight weeks) effects of whole grain (both intact and milled) consumption on appetite, food intake and postprandial metabolites, compared with refined grains. Effects on appetite and food intake were assessed subjectively using VAS and quantitatively using ad libitum test meals and diet diaries. Effects of the different breads on anthropometrics and blood pressure were also assessed.

6.3. **Hypothesis**

The inclusion in the diet of whole grains (intact or milled) will cause a reduction in food intake and beneficially affect the metabolic response, after eight weeks consumption, compared with refined grain. Whole grains included in a single meal will also have beneficial effects on food intake and metabolic responses.

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This Chapter contains the products of joint research between Laura Tripkovic, Nicola Muirhead and myself. Some data were shared, but each of us had our own particular outcome measures. Whilst the planning and data collection were conducted in collaboration, glucose and lipid concentrations measured by Nicola Muirhead and insulin concentrations by Laura Tripkovic and myself, all statistical analysis and discussion included in the Chapter are my own work.
6.4. **Study Design**

6.4.1. **Design**

The study was a randomised, parallel dietary intervention. Participants were randomly assigned to consume two bread rolls, containing 48 g of intact whole grains, 48 g milled whole grains or control rolls (containing refined grain only), daily for eight weeks. A favourable ethical opinion was given for this study by the University of Surrey’s Ethics Committee (EC/2006/89/SBMS) and all participants gave informed, written consent.

6.4.2. **Participants**

Twenty-one individuals participated in this pilot study. In addition to the general criteria (described in Chapter 2) participants required for this study were adult males (aged 30 to 55 years) with a waist circumference greater than 94 cm (37 inches) and postmenopausal females with a waist circumference greater than 80 cm (31.5 inches). Other criteria included, a BMI of 25 – 35 kg/m², fasting insulin greater than 60 pmol/l (EGIR criteria for Insulin Resistance) and exclusion of those who regularly consumed high whole grain-intakes (three or more servings per day) (assessed by a questionnaire, Appendix 7).

Twenty-two participants started the study, with one drop-out due to gastrointestinal intolerance of the whole grains. All other 21 participants completed the study.
Chapter 6

The 21 participants had mean scores of 2.12 (± 0.18), 3.07 (± 0.14) and 2.39 (± 0.12) on the emotional, external and restrained scales of the DEBQ, respectively. The participants’ baseline anthropometrics are shown in Table 6.1.

Table 6.1: Baseline anthropometric measurements, n=21.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.6 ± 2.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.5 ± 1.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>89.8 ± 3.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9 ± 0.7</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>102.5 ± 2.2</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>108.7 ± 1.5</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>31.2 ± 1.4</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>127.2 ± 2.3</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>77.1 ± 1.7</td>
</tr>
</tbody>
</table>

The 21 participants were randomly (by a web-based randomisation program) assigned to one of the three groups with seven participants (5 M, 2 F) in each group.

6.4.3. Protocol

Before starting the study participants completed a seven day diet diary to obtain baseline dietary intakes. Participants were requested to consume a low-fibre evening meal prior to each study morning, before fasting for 10-12 hours.
Participants attended for three study mornings during the eight week intervention, shown in the study timeline (Figure 6.1). The study investigated both acute (single meal) and chronic (eight week) effects of whole grain intake.

![8 week intervention with intact whole grain rolls, milled whole grain rolls or control rolls](image)

Figure 6.1: Timeline for the 8 week intervention study.

Study mornings 1 and 3, at the beginning and end of the intervention, were identical. On each occasion anthropometrics and blood pressure were taken. Participants were cannulated and two fasting samples and VAS were taken. At time zero participants consumed a nutritionally balanced low-fibre preload (hot chocolate) (Table 6.2).

Table 6.2: Nutritional composition of the preload (hot chocolate), per 250 ml.

<table>
<thead>
<tr>
<th></th>
<th>Energy (KJ)</th>
<th>Protein (g)</th>
<th>Carbohydrate (g)</th>
<th>Sugar (g)</th>
<th>Fat (g)</th>
<th>Saturates (g)</th>
<th>Fibre (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2130</td>
<td>510</td>
<td>19.5</td>
<td>59.4</td>
<td>58.8</td>
<td>21.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

The hot chocolate was made to a standard recipe, comprised of 18 g Cadbury’s drinking chocolate (Cadbury® UK Ltd, UK), 20 g glucose powder (Thornton and
Ross Ltd, UK), 31 g skimmed milk powder (Marvel®, Premier Foods, UK), 200 g skimmed milk (Tesco, UK) and 20 g mild olive oil (Tesco, UK).

VAS and blood samples were taken every 30 minutes for three hours. At the end of the three hour period participants were given the *ad libitum* test meal.

Study morning 2 occurred 24 hours after study morning 1. On this occasion breakfast was two of the test bread rolls (to which the participants had been randomly assigned), with a standard amount of margarine (20 g Flora®, Unilever Foods, UK), to determine any acute effects of the whole grains. VAS and blood samples were collected as in study mornings 1 and 3 and participants were provided with the *ad libitum* test meal to quantify food intake.

A seven day diet diary was completed for the final week of the eight week intervention period to determine changes to habitual intake.

6.4.4. **Calculations and Statistical analysis**

Normal distribution was tested with the Kolmogorov-Smirnov test. Data were analysed both parametrically and non-parametrically due to the small participant numbers in each group and the inability therefore to assess normality accurately. Results from the parametric tests (as the more powerful statistic) are shown, as there were few differences between the parametric and non-parametric tests in terms of significance and the majority of the data were normally distributed.
Time course data from the acute postprandial morning (study morning 2) were analysed using mixed between-within subjects ANOVA, with roll type as the independent between-subjects variable, time as the independent within-subjects variable and the measurements as the continuous dependent variables. AUC for postprandial data and data from the ad libitum test meal were compared using a one-way between groups ANOVA with Post Hoc tests (Tukey).

Data collected pre and post intervention (study mornings 1 and 3) were compared within and between groups, to determine whether an individual treatment elicited a response and whether there were treatment effects. Within group analysis involved repeated measures ANOVA for postprandial data and paired samples t-test comparisons of AUC and other pre/post data.

Differences between fasting blood concentrations were compared using one-way between groups ANOVA. Differences between treatments for postprandial data were compared using mixed between-within subjects ANOVA, with roll type and visit as independent between-subjects variables, time as the independent within-subjects variable and the measurement as the continuous dependent variable. The difference (either percentage change or absolute difference) between pre and post data (and postprandial AUC) were compared using one-way between groups ANOVA with Post Hoc tests (Tukey).

On all graphs and tables intact whole grain rolls are referred to as “WG”, milled whole grain rolls as “MWG” and control rolls as “C”.

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6.5. **Results**

There were no significant differences between the three groups (Table 6.3) for any of the baseline anthropometric measurements or for systolic blood pressure. However, there was a significant difference between the groups for diastolic blood pressure, $p=0.029$. Post Hoc tests revealed the difference lay between the milled grain group and the intact whole grain (82.4 ± 3.6 mmHg vs 71.9 ± 2.1 mmHg, $p=0.023$).

**Table 6.3**: Baseline measurements, $n=7$ (5 M and 2 F) in each group.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WG Group Mean ± SEM</th>
<th>MWG Group Mean ± SEM</th>
<th>C Group Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.9 ± 4.6</td>
<td>51.4 ± 1.8</td>
<td>45.4 ± 0.76</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.6 ± 2.8</td>
<td>176.1 ± 3.8</td>
<td>175.9 ± 3.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.5 ± 5.2</td>
<td>90.9 ± 8.4</td>
<td>92.1 ± 6.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 1.2</td>
<td>28.9 ± 1.5</td>
<td>29.5 ± 1.0</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>101.7 ± 3.2</td>
<td>103.6 ± 5.0</td>
<td>102.1 ± 3.8</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>108.5 ± 2.9</td>
<td>108.0 ± 2.8</td>
<td>108.7 ± 2.6</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>29.6 ± 3.1</td>
<td>32.4 ± 2.1</td>
<td>31.6 ± 2.4</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>120.9 ± 4.0</td>
<td>132.1 ± 3.0</td>
<td>128.7 ± 3.9</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>71.9 ± 2.1</td>
<td>82.4 ± 3.6</td>
<td>77.0 ± 1.4</td>
</tr>
</tbody>
</table>
6.5.1. **Acute postprandial results**

This section of the results shows the acute single meal response (study morning 2) to each of the three test breads. Due to cannulation problems for some participants the metabolite data are not complete, but sample size for each variable is indicated in figures and tables.

6.5.1.1. **Subjective appetite ratings**

There were no significant differences between the three test rolls for the subjective ratings of hunger, fullness or prospective food consumption ([Figure 6.2](#)), when analysed either by repeated measures ANOVA or comparison of AUC. There also were no significant differences between the rolls for ratings of thirst, or desire for different foods (sweet, salty, savoury or fatty foods).
6.5.1.2. Test meal

There were no significant differences in either weight or energy intake (Figure 6.3) between the three groups at the *ad libitum* test meal. Despite
those in the intact whole grain group indicating they felt they could eat the least
on the subjective appetite ratings, they consumed the most at the test meal.

![Figure 6.3: Energy intake (kJ) at the ad libitum test meal at study morning 2. Mean ± SEM, n=7 in each group. One-way between groups ANOVA showed no significant differences between the three rolls.](image)

6.5.1.3. **Metabolite analysis**

There was no significant difference between the fasting concentrations of the three groups for glucose (measured by the ILab method described in Chapter 2), NEFA or TG (Table 6.4).

**Table 6.4:** Fasting concentrations of metabolites measured on the acute day, n=7 (5 M and 2 F) in WG and C groups, n=6 (4 M and 2 F) in MWG group.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WG Group Mean ± SEM</th>
<th>MWG Group Mean ± SEM</th>
<th>C Group Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.11 ± 0.2</td>
<td>5.00 ± 0.1</td>
<td>5.22 ± 0.2</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>1.22 ± 0.2</td>
<td>1.26 ± 0.2</td>
<td>1.72 ± 0.3</td>
</tr>
<tr>
<td>Fasting NEFA (mmol/l)</td>
<td>0.47 ± 0.05</td>
<td>0.48 ± 0.10</td>
<td>0.44 ± 0.03</td>
</tr>
</tbody>
</table>

Metabolites measured by Nicola Muirhead
There were no significant differences between the three groups for postprandial plasma glucose or NEFA concentrations. The postprandial TG concentrations (Figure 6.4) were not significantly different between the groups; however, the TG concentrations for each bread type exhibited different patterns over time ($p=0.078$).

![Figure 6.4: Acute postprandial TG concentrations for each of the breads. Mean ± SEM, n=7 in WG and C groups and n=6 in MWG group. Mixed between-within subjects ANOVA showed no significant differences between the three breads, but a trend towards different responses over time ($p = 0.078$).]

6.5.2. Chronic whole grain intervention results

This section of the results shows the pre and post (study mornings 1 and 3) eight week intervention comparisons for the three rolls, where the postprandial data is in response to a nutritionally balanced low-fibre preload (hot chocolate). Again due to cannulation problems, some of the data are incomplete (data for six participants only in the intact whole grain group).
6.5.2.1. Anthropometrics

Anthropometric measurements taken at baseline and end of each intervention are shown in Table 6.5.

Table 6.5: Anthropometric measurements taken at baseline and post each intervention, n=7 (5 M and 2 F) in each group. Mean±SEM.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WG Group Baseline</th>
<th>Post</th>
<th>MWG Group Baseline</th>
<th>Post</th>
<th>C Group Baseline</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>86.5±5.2</td>
<td>86.8±5.1</td>
<td>90.9±8.4</td>
<td>90.8±8.4</td>
<td>92.1±6.4</td>
<td>93.7±6.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3±1.2</td>
<td>28.5±1.2</td>
<td>28.9±1.5</td>
<td>28.9±1.5</td>
<td>29.5±1.0</td>
<td>30.0±1.0</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>101.7±3.2</td>
<td>101.4±2.9</td>
<td>103.6±5.0</td>
<td>101.8±4.8</td>
<td>102.1±3.6</td>
<td>102.8±3.4</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>108.5±2.9</td>
<td>108.5±2.9</td>
<td>108.0±2.8</td>
<td>107.7±4.1</td>
<td>109.7±2.6</td>
<td>111.5±2.9</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>29.6±3.1</td>
<td>29.6±3.1</td>
<td>32.4±2.1</td>
<td>32.7±2.3</td>
<td>31.6±2.4</td>
<td>31.0±2.7</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>120.9±4.0</td>
<td>123.6±5.0</td>
<td>132.1±3.0</td>
<td>125.4±3.9</td>
<td>128.7±3.9</td>
<td>129.1±4.6</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>71.9±2.1</td>
<td>79.6±2.5</td>
<td>82.4±3.6</td>
<td>78.4±2.4</td>
<td>77.0±1.4</td>
<td>72.0±2.4</td>
</tr>
</tbody>
</table>

There were no significant differences between the start and end of the control intervention for any of the anthropometric or blood pressure measurements. For the milled grain group there was a significant decrease from beginning to end of the intervention only for waist circumference (103.6 ± 5.0 cm vs 101.8 ± 4.8 cm, p=0.045). With the intact whole grain group there was a significant difference only in diastolic blood pressure, where it increased from the start of the intervention to the end (71.9 ± 2.1mmHg vs 79.6 ± 2.4 mmHg, p=0.007).
When the differences between the start and end of interventions were calculated as percentage change and then compared between groups, there were no significant differences for any of the anthropometric measurements. However, there was a significant difference between the breads for diastolic blood pressure ($p=0.008$) (Figure 6.5). The Post Hoc test revealed the difference lay between the intact whole grain and the milled whole grain groups ($10.9 \pm 2.9 \text{ mmHg vs } -4.1 \pm 3.9 \text{ mmHg, } p=0.025$) and the intact whole grain group and the control group ($10.9 \pm 2.9 \text{ mmHg vs } -6.2 \pm 4.1, p=0.011$).

![Figure 6.5: Percentage change for diastolic blood pressure from beginning to the end of each intervention. Mean ± SEM, n=7 in each group. Significant difference between the three breads ($p=0.008$), Post Hoc tests revealed the difference to be between WG and MWG rolls (* $p=0.025$) and WG and C rolls († $p=0.011$).]

6.5.2.2. Subjective appetite ratings

There were no significant differences within each intervention for any of the subjective appetite ratings. However, there was a trend for higher ratings of fullness at the end of the milled whole grain intervention ($p=0.071$) (Figure 6.6).
Figure 6.6: Subjective appetite ratings in response to the question "how full do you feel?" pre and post MWG. Mean ± SEM, n=7. Repeated measures ANOVA showed a trend for participants to feel fuller at the end of the intervention compared with the start.

When the VAS AUC pre and post interventions were compared for the intact whole grain group there were no significant differences. For the milled whole grain intervention AUC, there was just a trend for a higher rating post intervention compared with pre in relation to fullness (7937.1 ± 1013.3 mm.min vs 6079.0 ± 841.5 mm.min, p=0.062). With the control intervention AUC desire for fatty foods was significantly lower pre intervention compared with post (6182.1 ± 2467.3 mm.min vs 7538.6 ± 2711.0 mm.min, p=0.044) (Figure 6.7).

There were no significant differences between the three breads for any of the subjective appetite ratings when the differences in AUC between the start and end of each intervention were compared.
6.5.2.3. Test meal

There were no significant differences at the *ad libitum* test meal, in weight or energy intake, either within each intervention or between interventions when absolute differences in intake were compared (Figure 6.8).

---

**Figure 6.7**: AUC for the C intervention in response to the question "Would you like to eat something fatty?" Mean ± SEM; n=7. Paired samples t-test showed AUC significantly higher post intervention compared with pre (*p=0.044).

**Figure 6.8**: Energy intake at the *ad libitum* test meal, pre and post each intervention. Mean ± SEM, n=7 in each group. No significant differences either pre and post each intervention or between interventions.
6.5.2.4. Diet diaries

Due to two final diet diaries not being returned by the participants, the 7 day dietary data for the milled whole grain group is for 5 participants only. The study rolls were included in the dietary analysis as stated by the participants.

Within treatment effects showed that in the intact whole grain group there was a significant increase in energy intake during the intervention ($p=0.019$), and a significant increase in protein ($p=0.011$), carbohydrate ($p=0.002$) and DF intake ($p=0.006$) (Table 6.6). With the milled grain group there was a trend towards a decrease in fat ($p=0.076$) and saturated fat ($p=0.058$) intake during the intervention (Table 6.7). During the control intervention there was a trend towards an increase in energy ($p=0.055$) and fat intake ($p=0.081$) and a significant increase in saturated fat ($p=0.019$) intake (Table 6.8).

Table 6.6. Average daily intakes calculated pre and post the WG intervention, n=7. Comparisons made with a paired samples $t$ test.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>SEM</th>
<th>Post</th>
<th>SEM</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>8082</td>
<td>587</td>
<td>9352</td>
<td>616</td>
<td>0.017</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1927</td>
<td>142</td>
<td>2226</td>
<td>147</td>
<td>0.019</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>75.3</td>
<td>5.1</td>
<td>88.9</td>
<td>3.2</td>
<td>0.011</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>233.3</td>
<td>14.9</td>
<td>299.7</td>
<td>19.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>95.4</td>
<td>9.9</td>
<td>85.7</td>
<td>10.3</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>71.9</td>
<td>9.7</td>
<td>67.5</td>
<td>6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>23.8</td>
<td>3</td>
<td>22.9</td>
<td>2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>19.5</td>
<td>1.9</td>
<td>27.2</td>
<td>2.6</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Table 6.7. Average daily intakes calculated pre and post the MWG intervention, n=5. Comparisons made with a paired samples t test.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>9613</td>
<td>893</td>
<td>9922</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2291</td>
<td>213</td>
<td>2371</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>88.5</td>
<td>11.4</td>
<td>86.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>268.6</td>
<td>23.3</td>
<td>304.9</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>120.2</td>
<td>12.8</td>
<td>107.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>89.4</td>
<td>8.9</td>
<td>77.2</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>34.3</td>
<td>4.2</td>
<td>27.6</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>21.4</td>
<td>3.4</td>
<td>23.4</td>
</tr>
</tbody>
</table>

Table 6.8. Average daily intakes calculated pre and post the C intervention, n=7. Comparisons made with a paired samples t test.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>8605</td>
<td>624</td>
<td>9707</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2049</td>
<td>149</td>
<td>2308</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>82.3</td>
<td>11.9</td>
<td>85.9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>246.0</td>
<td>15.1</td>
<td>278.5</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>103.9</td>
<td>22.7</td>
<td>98.4</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>63.5</td>
<td>6.8</td>
<td>75.1</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>21.2</td>
<td>2.5</td>
<td>29.3</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>15.3</td>
<td>2.0</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Actual differences in intake between pre and post interventions were calculated and then compared between groups for treatment effects. There were no significant differences between the three breads in differences in average daily energy intake (Figure 6.9). There were also no significant differences between groups for protein, carbohydrate or sugar intake.
However, there was a significant difference between the three groups for fat (p=0.029) and saturated fat intake (p=0.005) (Figure 6.10). Post hoc tests revealed the difference for fat intake lay between the milled whole grain group (decreased during the intervention) and control group (increased during the intervention) (p=0.030) and, for saturated fat intake, lay between the milled whole grain group and control group (p=0.004) and between the intact whole grain group and the control group (p=0.057), where intakes increased in the control group and decreased in the two whole grain groups.

There was also a treatment effect for total DF intake which increased in all groups (Figure 6.11), and was significantly different between the three rolls (p=0.032). Post Hoc analysis revealed the difference lay between the intact whole grain and the control groups (p=0.035).
Figure 6.10: Absolute difference in average daily fat and saturated fat (SFA) intake between the start and end of the interventions calculated from the diet diaries. Mean ± SEM, n=7 in WG and C groups and n=5 in MWG group. Significant difference between the three breads for fat (p=0.029) and SFA (p=0.005) intake. Post Hoc tests showed difference for fat intake was between MWG and C (* p=0.030) and for SFA was between the MWG and C († p=0.004) and WG and C (‡ p=0.057).

Figure 6.11: Difference in average daily total DF intake calculated from the seven day diet diaries completed pre and post the interventions. Mean ± SEM, n=7 in WG and C groups and n=5 in MWG group. Significant difference between the three groups (p=0.032) with Post Hoc analysis revealing the difference was between the WG and C groups (* p=0.035).
6.5.2.5. Cholesterol

There were no significant differences between the three groups for plasma total cholesterol, HDL-cholesterol or LDL-cholesterol concentrations at the start of the study or after the intervention. There were also no significant differences between the beginning and end of the milled whole grain and control interventions for total plasma cholesterol, HDL-cholesterol or LDL-cholesterol. However, for the intact whole grain intervention total plasma cholesterol significantly increased during the intervention (3.9 ± 0.4 mmol/l vs 4.3 ± 0.4 mmol/l, p=0.046) and there were trends towards significant increases for both HDL-cholesterol (1.0 ± 0.1 mmol/l vs 1.1 ± 0.1 mmol/l, p=0.095) and LDL-cholesterol (2.7 ± 0.3 mmol/l vs 2.9 ± 0.3 mmol/l, p=0.071).

When the absolute difference between pre and post was calculated and the three interventions compared for treatment effects, there was no significant difference between the breads for HDL-cholesterol and LDL-cholesterol; however, there was trend towards a significant difference between the breads for total plasma cholesterol (p=0.069), with Post Hoc analysis determining the difference lay between the intact whole grain and the milled whole grain interventions (0.39 ± 0.1 mmol/l vs -0.18 ± 0.2 mmol/l, p=0.058) (Figure 6.12).
6.5.2.6. Metabolite analysis

Fasting insulin sensitivity and β-cell function (HOMA %S and %B, respectively) were calculated by the HOMA model (described in Chapter 2) for each participant for study mornings 1 and 3. There were no significant differences for either HOMA %S or %B between the three groups at the start or end of the study (Table 6.9), within each intervention from study morning 1 to 3 or between groups when the absolute differences were compared.
Table 6.9: Fasting concentrations of metabolites and fasting insulin sensitivity and β-cell function pre and post each intervention, n=7 (5 M and 2 F) MWG and C groups, n=6 (4M and 2F) WG group. Mean± SEM.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>WG Group</th>
<th>MWG Group</th>
<th>C Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.06± 0.2</td>
<td>5.08± 0.2</td>
<td>4.99± 0.2</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>119.2± 22.6</td>
<td>121.5± 26.0</td>
<td>107.7± 19.1</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>1.19± 0.1</td>
<td>1.35± 0.2</td>
<td>1.15± 0.2</td>
</tr>
<tr>
<td>Fasting NEFA (mmol/l)</td>
<td>0.50± 0.05</td>
<td>0.44± 0.06</td>
<td>0.54± 0.06</td>
</tr>
<tr>
<td>HOMA % S1</td>
<td>66.8± 10.9</td>
<td>65.8± 1.7</td>
<td>54.2± 9.9</td>
</tr>
<tr>
<td>HOMA % B2</td>
<td>143.9± 27.5</td>
<td>143.8± 26.6</td>
<td>170.9± 23.3</td>
</tr>
</tbody>
</table>

1 HOMA %S = fasted oral insulin sensitivity, assessed by homeostatic model assessment.
2 HOMA %B = β-cell function, assessed by homeostatic model assessment.

There were no significant differences in fasting glucose concentrations between the three groups at the start or end of the study (Table 6.9). There were also no significant differences either within or between the three groups for measurements of postprandial glucose concentrations (Figure 6.13).
Figure 6.13: Postprandial glucose response (mmol/l) for each of the three grains. A = WG, B = MWG, C = C. Mean ± SEM, n=6 in WG group and n=7 in MWG and C groups. Repeated measures ANOVA showed no significant differences for any of the groups.
There were no significant differences in fasting insulin concentrations between the three groups at the start or end of the study (Table 6.9). When postprandial insulin concentrations were analysed by the mixed between-within subjects ANOVA there was no significant difference between the three breads. However, the test did reveal differences between visit (pre/post) and grain type \((p=0.058)\), visit (pre/post) and time (postprandial period) \((p=0.026)\) and visit (pre/post) grain type and time \((p=0.059)\). Therefore repeated measures ANOVA (comparing the postprandial data for pre to post) were conducted for each bread type to determine where the differences were. There was no significant difference between pre and post for the control intervention and the values were not different over time for each visit. For the intact whole grain intervention the postprandial response was not significantly different between visits, but there was a trend for the responses to differ over time for each visit \((p=0.068)\). For the milled whole grain intervention there was also no significant difference for the response between visits, but again the postprandial responses were different over time for each visit \((p=0.035)\) (Figure 6.14).
Figure 6.14: Postprandial insulin response (pmol/l) for each of the three grains. A = WG, B = MWG, C = C. Mean ± SEM, n=6 in WG group and n=7 in MWG and C groups. Repeated measures ANOVA showed no significant differences for the C group and whilst the WG and MWG groups did not differ between visits, the postprandial responses were different over time (WG p=0.068, MWG p=0.035).
The AUC for the postprandial insulin concentrations were calculated for each bread and each visit, and compared. There were no significant differences within each treatment group. When the absolute differences between pre intervention and post were compared there was a trend towards a significant difference between the three breads ($p=0.057$). The Post Hoc analysis revealed the difference lay between the intact whole grain and control groups ($p=0.046$) (Figure 6.15).

![Figure 6.15: Absolute difference in postprandial insulin AUC between the values obtained pre and post each intervention. Mean ± SEM, n=6 in the WG group and n=7 in the MWG and C groups. One-way between groups ANOVA showed a trend towards a significant difference between the three grain groups ($p=0.057$).](image)

The postprandial oral insulin sensitivity for each study visit was calculated (by the minimal model method, described in Chapter 2). When compared within interventions there was a significant increase in oral insulin sensitivity from the start to the end of the intact whole grain intervention ($p=0.014$), but no difference
between the other interventions (Figure 6.16). When the absolute differences were compared the three interventions were not significantly different.

![Figure 6.16: Postprandial oral insulin sensitivity (Si) pre and post each intervention. Mean ± SEM, n=6 in the WG group and n=7 in the MWG and C groups. Significant difference between pre and post WG intervention (* p=0.014) and no significant differences for the MWG and C groups or between the interventions when absolute differences were compared.](image)

There were no significant differences in fasting NEFA concentrations between the three groups at the start or end of the study (Table 6.9). There were also no significant differences either within or between the three groups for postprandial NEFA concentrations. However, there was a trend towards a lower NEFA AUC post milled whole grain intervention compared with pre (55.5 ± 6.9 mmol/l.min vs 47.8 ± 5.2 mmol/l.min, p=0.06).

There were no significant differences in fasting TG concentrations between the three groups at the start or end of the study (Table 6.9). For all three interventions the postprandial TG concentrations were higher post intervention compared with pre. When the postprandial TG responses were analysed with
the mixed between-within subjects ANOVA there was not a significant difference between the three groups. However, the test did reveal a significant difference between visit (pre/post) ($p=0.022$). Follow-up repeated measures ANOVA for each bread type were therefore conducted to determine where the difference lay. There was no significant difference between pre and post intervention for either the milled whole grain or control groups, whilst for the intact whole grain group there was a visit (pre/post) time interaction ($p=0.008$), but there was not a significant difference between visits. The AUC for the three interventions, despite all being higher post interventions compared with pre (Figure 6.17), were not significantly different either within each intervention or between interventions when the absolute differences were compared.

![Figure 6.17](image.png)

**Figure 6.17**: AUC for postprandial TG concentrations pre and post each intervention. Mean ± SEM, $n=6$ in the WG group and $n=7$ in the MWG and C groups. No significant difference either within interventions or between interventions when absolute differences compared.
6.6. **Discussion**

The results from the acute postprandial study day and the chronic intervention will initially be discussed separately and then overall conclusions will be drawn.

6.6.1 **Acute postprandial results**

It was hypothesised that when whole grains (intact or milled) were consumed at a single meal there would be a reduction in food intake, at an *ad libitum* test meal, compared with refined grains (control). It was also hypothesised that the consumption of the whole grains, of either form, would beneficially affect the metabolic response.

In fact we found no significant difference between the three breads for energy intake at the *ad libitum* test meal. Indeed intakes were the reverse to that hypothesised, with more consumed with the intact whole grain rolls and least with the control rolls. No significant difference was also found between the three breads for any of the subjective appetite ratings, but there were conflicting findings within the same grain type (those who consumed the intact whole grain rolls felt most hungry, but rated that they could eat the least, whilst those who consumed the control rolls were least hungry and full, but felt they could eat the most). There were also no significant differences between the three breads for postprandial glucose, NEFA or TG concentrations following each bread type.
An initial power calculation was conducted based on a metabolically significant fall (20%) in postprandial insulin AUC and gave an estimate of 17 participants per group. As there was no similar previously conducted study, we aimed to recruit 20 participants per group, with an interim power calculation to be conducted on the data obtained from 12 participants per group to confirm the correct number of subjects required. Therefore 36 participants were aimed to be recruited. Due to difficulties with recruitment a total of 7 participants per group were all that could be recruited in the time frame. The parallel study design combined with the eventual small sample size resulted in a major limitation for determining significant differences between groups. This was especially difficult for the subjective appetite ratings, which by their very nature are particular to an individual and feelings of hunger for one person may not be the same feelings experienced by another, therefore making it difficult to compare between groups, which may account for the lack of findings. Indeed in a review of biomarkers of satiation and satiety by de Graaf et al (2004) it was stated that as individuals differ in their responses VAS are "preferably used in within-subject studies". One study that has investigated the reproducibility, power and validity of VAS in single meal studies (Flint et al., 2000), reported that when using unpaired designed studies more participants would be required to determine differences in VAS ratings, with a minimum of 32 to ascertain a 10 mm difference in hunger and fullness ratings. VAS may therefore have provided valuable data in our study had we been able to recruit the original number of participants.
The low participant numbers and parallel nature of the study could also explain the lack of significant findings between the breads at the *ad libitum* test meal and for the metabolic postprandial responses. The use of a single dish *ad libitum* test meal has been evaluated and was found, in a study by Gergersen *et al* (2008), to be reproducible. However, as with the findings regarding VAS, Gergersen *et al* found that *ad libitum* test meals are better in studies using paired designs where smaller participant numbers can be used compared with unpaired designed studies.

The TG concentrations, even though not significantly different at fasting or for the postprandial concentrations, did differ over time, and were highest following the control rolls. The different concentrations could be explained by the different fasting TG concentration or may differ between the groups simply due to there being different participants in each group, or may in fact be due to a blood lipid lowering effect of consumption of whole grains. Further investigation, using a paired designed study, would be necessary, as it is not possible to draw conclusions from the findings of this study.

Three hours post consumption of whole grains may be insufficient time for the whole grains to influence either food intake or postprandial metabolite responses. Indeed, as was observed in our acute RS and appetite study (Chapter 3) effects on food intake occurred after 7 hours suggesting that fermentation, by colonic microflora, of the RS could be a mechanism by which it
exerted its effects on food intake. As RS is a major DF component of whole grains, fermentation may be an important means by which whole grains could affect appetite and therefore 3 hours would not have been sufficient time for this to occur.

6.6.2 Chronic whole grain intervention results

We hypothesised that the inclusion of whole grains of either structure (intact or milled) in habitual diets for 8 weeks would result in a reduction of food intake and beneficial effects on the metabolic response, compared with refined grain.

Contrary to the hypothesis there were no significant differences either between interventions or within each intervention for weight or energy consumed at the ad libitum test meal. There was also no effect of any of the three interventions on the subjective appetite ratings for hunger or prospective food consumption. Similarly, for the intact whole grain and control rolls there was no effect of consumption over 8 weeks on ratings of fullness; however for the milled whole grain rolls there was a trend towards significance for the participants to feel fuller at the end of the intervention than before the start. This could indicate a role of whole grains in the milled form on satiety and may not have reached significance due to the small participant numbers. There were no differences between the three interventions for any of the subjective appetite ratings, but again potential effects may have been masked due to the small participant numbers and the parallel design of the study, as discussed in section 6.6.1.
Data from the diet diaries were compared within interventions and between interventions. The degree of under-reporting was assessed in all diaries using the Goldberg cut-off limits (Goldberg et al., 1991). In each group there was at least one participant classified as an under-reporter. When these were excluded from the analysis it did not alter the findings from the between groups comparison and therefore as exclusion of these individuals would have lead to even smaller participant numbers they were included in the full dietary analysis. When effects within interventions were investigated it was found that in all groups average daily energy intake increased from the start to the end of the interventions. This was significantly so for the intact whole grain rolls (1270 kJ per day increase), explained by a significant increase in both protein and carbohydrate intake. There was a trend towards a significant increase during the control intervention (1102 kJ per day increase), explained mostly by an increase in fat intake, whilst for the milled whole grain intervention the increase was not significant (309 kJ per day increase). These increases in energy intake could be due solely to the addition of the rolls into the habitual diets as opposed to substitution or could be due to the participants adding additional foods (such as spreads or jam) to the products to increase the palatability, as this was found to be low for all the rolls in the taste test (Chapter 5).

When treatment effects between interventions were compared there was no significant difference between the interventions for average daily energy, carbohydrate or protein intake. There was however a significant difference
between the interventions for fat intake, which decreased with both whole grain interventions and increased with the control intervention. This increase in fat intake agrees with the findings from the subjective appetite ratings where those on the control intervention had a greater desire for fatty foods at the end of the intervention compared with the start. The reason for this finding is unclear, but may be due to the control rolls themselves, which were less well liked according to the hedonic ratings in the taste test (Chapter 5), which could have resulted in more addition of other products to the rolls thereby increasing fat intakes.

During all three interventions DF intake increased, but the increase was greatest in the intact whole grain group and smallest in the control group. This was not surprising as the rolls were included in the analysis, when stated by the participants, and the whole grain rolls had a greater DF content than the control rolls. It is not the most ideal measure of compliance (for this study or for the crossover study in Chapter 5), as the participants may just have included them in the diaries as opposed to in their diets. Recent studies have shown that changes to plasma concentrations of alkylresorcinols maybe a useful biomarker of whole grain intake (Landberg et al., 2009, Linko-Parvinen et al., 2007), and this should be considered for future studies to confirm compliance.

The 21 participants were randomly allocated to one of the three breads, with 5 males and 2 females in each group. At baseline the three groups' anthropometrics were well matched with no significant differences between the
groups. The changes to anthropometrics during each intervention were compared, with no differences arising in the intact whole grain and control groups, and only a mean decrease in waist circumference of 1.8 cm from start to end of the intervention in the milled whole grain group. This decrease in waist circumference could just be due to variations in where the measurement was taken, although this was standardised as much as possible between visits.

A recently published parallel study by Katcher et al (2008), compared the effects of a 12 week hypocaloric diet with inclusion of whole grains encouraged in one group and avoided in another group (control group). This study had similar anthropometric outcomes to ours (waist circumference, weight, blood pressure and percentage body fat) and found that weight decreased significantly in both groups (unsurprisingly due to the hypocaloric diet), but that there was a greater decrease in the refined grain group. They also found a significantly greater decrease in percentage abdominal body fat in the whole grain group compared with the control group. The differences in findings between our study and the study by Katcher et al could be explained by the longer duration or greater participant numbers (24 in whole grain group and 23 in control group) of the Katcher study or due to the fact that the participants in their study were encouraged to consume all grain products from whole grain sources and therefore consumed on average 5 servings of whole grain per day, compared with our participants just consuming the whole grain rolls which provided an equivalent of 3 servings. Whilst this intervention study and our study did not find
significant effects of whole grain intake on body weight, there is much epidemiological evidence that does propose an effect of higher whole grain intake on weight regulation, as discussed in Chapter 1. However, the epidemiological evidence does need to be interpreted with caution and cannot prove cause and effect.

At baseline and during the interventions there were no significant differences in systolic blood pressure between the groups. However, at baseline diastolic blood pressure was significantly lower in the intact whole grain group compared with the milled whole grain group. For the milled whole grain and control interventions, whilst diastolic blood pressure did decrease during the study, this was not significant, but there was a significant increase of 7.7 mmHg during the intact whole grain intervention. This increase in diastolic blood pressure was significantly different to the decrease in diastolic blood pressure observed in the other two groups. However, at the start of the study the intact whole grain group’s diastolic blood pressure was significantly lower and therefore the increase observed during the intervention resulted in a blood pressure that was similar to that of the other groups. The blood pressure finding from this study did not confirm the finding that we observed in the short-term crossover study (Chapter 5) where there was a reduction in systolic blood pressure following the milled whole grain intervention. The reason for the difference in findings is unclear, but may be due to differences between the participants included in each study (normal weight individuals in the short-term crossover study compared
with overweight/obese individuals in this study) and their habitual diets, activity levels or the adaptation to their normal lifestyle of including the rolls into their diets.

At the start of the study there were no significant differences between the three groups for any of the fasting blood concentrations measured (total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, insulin, NEFA or TG) or for fasting insulin sensitivity (HOMA %S) or β-cell function (HOMA %B), suggesting that although the participants were not randomised based on these characteristics they were in fact well matched and any differences observed between the groups at the end of the study would be unlikely to be due differences between the groups at the start of the study. There were however, no significant differences between the three groups for any of the fasting parameters at the end of the intervention period.

There was a significant increase in total cholesterol (average increase of 0.39 mmol/l) during the intact whole grain intervention, accompanied by a trend towards a significant increase for HDL-cholesterol (average increase of 0.09 mmol/l) and LDL-cholesterol (average increase of 0.26 mmol/l). A decrease was observed for all three types of cholesterol during the milled whole grain intervention (average decrease of 0.23 mmol/l for total cholesterol, 0.05 mmol/l for HDL-cholesterol and 0.21 mmol/l for LDL-cholesterol) although these did not reach significance and virtually no change was observed with the control
intervention. The difference observed between the two types of whole grain could be due to the small participant numbers in each group and further investigation with larger participant numbers (as the study was originally designed for) or a cross-over design study should be conducted before conclusion relating to the effects of whole grains on cholesterol can be drawn. In the study by Katcher et al (2008) described above, effects of the whole grains compared with the control group on blood lipids were assessed. They found that whilst total, LDL and HDL cholesterol and TG concentrations decreased significantly in each group, this difference was not significant between the groups and that the decreases were due to the changes in weight that were observed.

The postprandial metabolite data indicated mixed results for each metabolite measured, but it needs to be borne in mind that these postprandial concentrations were in response to a nutritionally balanced low-fibre preload (hot chocolate) rather than to the whole grains themselves. These mixed findings again could be explained due to the small participant numbers and the parallel nature of the study. Whilst there appeared to be no effects of treatment on the postprandial glucose concentrations, there were effects of treatment on the postprandial insulin responses. For both types of whole grain there appeared to be a decrease in the postprandial insulin response at the end of the intervention (moderately in the milled whole grain group and more noticeably in the intact whole grain group), whereas in the control group the postprandial
insulin response appeared to increase during the intervention. The lowered
insulin response observed in our study with the intact whole grain rolls would
agree with published data showing that whole grains with an intact structure
produce lower glucose and insulin responses compared with whole grains that
have been processed in some manner resulting in the disruption of the structure
of the grain and therefore making the carbohydrate within the grain more
accessible to digestion and absorption (Bjorck et al., 1994). The lower insulin
response resulted in a significantly higher oral insulin sensitivity for the intact
whole grain group at the end of the intervention. This finding agrees with some
previous data that has shown improvements in insulin sensitivity with high whole
grain intakes, as discussed in Chapter 1, the studies by Steffen et al (2003)
(insulin sensitivity assessed by insulin modified FSIVGTT) and Pereira et al
(2002) (insulin sensitivity assessed by an euglycaemic-hyperinsulinaemic
clamp). The effect of the whole grains on the insulin response in our study could
be due to the differences in GI of the products, especially as more of an effect
was observed with the intact whole grain rolls which had the lower GI (medium
GI of 60.7), although the insulin responses in the GI study, and the GI values
themselves, were not significantly different between the three breads. As
discussed in Chapter 1 there are mixed findings in previous studies of an effect
of whole grains on insulin sensitivity, when measured by clamps. The studies by
with whole grains, whilst Andersson et al (2007) found no improvement in insulin
sensitivity. In light of this and our findings further investigation with well defined protocols (clamp or IVGTT) to specifically investigate the effect of whole grains on insulin responses would be required.

In our study there appeared to be little effect of the 8 week interventions on the postprandial NEFA concentrations. For all three interventions the TG concentrations where higher at the end of the study compared with the beginning, although this was not significant either within or between interventions. Whole grains have been proposed to possibly have beneficial lipid lowering effects as they contain high amounts of phytosterols, which are known to lower blood cholesterol concentrations, and high levels of DF (Slavin, 2004). An inverse association between whole grain intake and total and LDL cholesterol was reported in a study by McKeown et al (2002). However, a recent crossover intervention study where a diet rich in whole grains was compared with a diet a rich in refined grains found no effect of 6 week intervention on blood lipid concentrations (Andersson et al., 2007). Due to the mixed findings of an effect of whole grains on blood lipids, further investigation would be required to confirm or deny any beneficial effects.

6.6.3 **Overall conclusions**

Consumption of whole grains, either in the intact or milled form, did not alter food intake, subjective appetite ratings or postprandial metabolite concentrations, when consumed at a single meal, compared with refined grains.
There was also little effect on energy intake, subjective appetite ratings or postprandial metabolite concentrations between the interventions, in response to the fibre-free meal, after the rolls were consumed for 8 weeks. The findings from this study do not agree with the epidemiological evidence that suggests a beneficial role of whole grains in appetite and weight regulation. This may be because it is acknowledged that those who habitually consume high whole grain intakes also have other healthier lifestyle habits (such as not smoking, high activity levels and high fruit and vegetable intakes) and therefore it may not be the whole grains alone that have beneficial effects, but all these factors working together to confer the beneficial effects observed in the epidemiological studies. Therefore the lack of significant findings on food intake in our study could indicate that whole grains do not exert an effect on food intake regulation. However, our study findings were affected by the small participant numbers and therefore further investigation would be required, either with more participants or with a study with a crossover design, to determine whether whole grains affect appetite and food intake when ingested chronically.
Chapter 7

Review of Findings
7.1. **Summary**

With the global increase in obesity rates and its associated risk of other health issues, such as a higher prevalence of insulin resistance and T2DM, various strategies have been investigated to determine how this problem could be tackled to prevent a further rise and potentially assist with weight loss. These strategies have included investigations into individuals’ diets and specific dietary components that could be altered to subsequently impact on weight, insulin resistance and T2DM. One such component that has provoked considerable interest is DF, which has been proposed to increase satiety and therefore may have a beneficial role in weight management and potentially weight loss. RS, a type of DF, and whole grains (in which RS is a major component of the DF) have been proposed to affect satiety. The aims of this research were therefore to elucidate the effects of RS and whole grains on appetite and food intake, with the view that, if positive effects on food intake could be proven, these could be beneficial for combating the rising obesity problem. Both acute and chronic consumption effects were investigated in normal and overweight individuals.

Despite there being consistent positive evidence of an effect of RS on appetite in rodent studies, the data from direct human studies are mixed. In both of our studies using RS, the RS was compared with an energy and available carbohydrate matched placebo, therefore providing an identical amount of glycaemic carbohydrate between the supplements. Differences in glycaemic carbohydrate have been a confounding factor in some of the previous work investigating the effects of RS on appetite. Our work has shown that when RS
was ingested acutely (24 hours) at a high dose (48 g) there was a reduction in food intake, although no effect on subjective appetite ratings. This effect was not reproduced in the chronic study (40 g RS daily for 4 weeks), although the study was underpowered. A novel finding from the acute study was a lower postprandial insulin response with the RS, possibly explained by increased hepatic insulin clearance. This finding was therefore further explored in the chronic study using a FSIVGTT. The raw data from the FSIVGTT indicated significantly higher glucose, insulin and C-peptide concentrations with the RS compared with the PL, possibly explained by improved first-phase insulin response, although the parameters obtained from modelling the data were not significantly different. However, again, the study was underpowered and therefore potential effects may have been masked, so further investigation would be required. If consumption of RS does indeed have beneficial effects, not only on the postprandial response, but also on the first-phase insulin response, this could be beneficial for the management of weight, insulin resistance and diabetes.

There is epidemiological evidence for an effect of whole grains on weight regulation and therefore a role in satiety has been proposed, but there is little intervention data to confirm this. Bread rolls containing 48 g whole grain (the minimum amount recommended to be consumed in America) were specially developed for use in a chronic intervention study. These rolls provided whole grains in both the intact and milled form to determine whether differences in grain structure affected the outcome measures. Prior to the chronic intervention
three studies were conducted. The taste test revealed that whilst the experimental breads were rated less highly than the commercially available breads, the three developed breads were not perceived as too dissimilar to each other in terms of liking and on the properties of the rolls assessed. Therefore differences in the organoleptic properties of the breads would be unlikely to influence any observed effects on appetite in an intervention study. Although the GI of the rolls resulted in two products having a GI in the high category (milled whole grain and control) and one in the medium category (intact whole grain) the difference was not statistically significant. As the GI, glucose and insulin responses in this study were not significantly different, this is unlikely to be a contributing factor if any differences were observed on the glucose and insulin responses in a chronic intervention study. The short-term crossover (3 week) study comparing the milled whole grain rolls and the control rolls revealed no significant difference between the two rolls on appetite and food intake. A significantly lower systolic blood pressure was observed following the milled whole grain intervention compared with the control. The participants in this study were healthy, of normal weight and had high fibre diets before the study, which may have masked potential effects of increased whole grain intake on food intake and appetite.

Acute ingestion of the rolls at a single meal (study morning 2 of the 8 week intervention study), by overweight and obese participants, did not alter food intake, subjective appetite ratings or postprandial metabolites. The chronic (8 week) whole grain intervention found no difference between the three rolls for
subjective appetite ratings or energy intake (at the *ad libitum* test meal and from intakes calculated from the diet diaries), but there did appear to be moderate effects on energy and macronutrient intakes within each treatment group (energy intake increased in all three groups). Similarly, for the cholesterol concentrations and the postprandial metabolites there appeared to be little difference between the interventions, but some effects within the different treatments. These findings would require further investigation with more participants and/or in a crossover designed study, to confirm whether these are true effects of whole grain consumption or are incidental findings. Both whole grain interventions appeared to improve the postprandial insulin responses, more so with the intact than with the milled whole grains, which could be explained by the lower GI of intact whole grains compared with the GI of the milled whole grain and control rolls, although this is unlikely.

Overall these studies have shown that when ingested acutely RS may have a role as a satiating ingredient. Whilst this finding was not observed in the chronic intervention, the study was underpowered and there appeared to be a lower energy intake at the *ad libitum* test meal which may have reached significance with more participants. Although the role of RS in satiety is still not completely defined, a clear and novel finding from our two studies, was an effect of RS consumption, both acutely and chronically, on insulin responses. More participants would be required to determine exactly what the mechanism for an effect on the insulin response is, but our data appear to indicate an improvement
in first-phase insulin secretion or increased hepatic insulin clearance. Our studies using whole grains appeared not to agree with the epidemiological evidence suggesting a role of whole grains in satiety and therefore weight regulation. When ingested in the short-term (3 weeks) there was a beneficial effect of the milled whole grains on blood pressure regulation; this finding was not confirmed in the chronic intervention (8 weeks) in overweight/obese participants. Whist the whole grain study was underpowered the lack of significant findings on appetite and food intake may indicate that whole grains do not actually affect food intake/weight. It may in fact not be the whole grains alone that confer beneficial effects, as suggested from epidemiological studies, but instead it is the overall healthier lifestyle habits, that those who habitually consume high whole grain diets follow, which provides beneficial protective effects.
7.2. Conclusions

- Acute (24 hour) consumption of 48 g RS reduced energy intake at an *ad libitum* test meal at the end of the 7 hour postprandial period and over the whole 24 hour period in healthy, young adult males, compared with an energy and available carbohydrate matched placebo.

- Acute (24 hour) consumption of 48 g RS reduced the postprandial insulin response in healthy, young adult males compared with an energy and available carbohydrate matched placebo, possibly explained by an increase in hepatic insulin clearance.

- 40 g RS per day for 4 weeks in overweight/obese participants did not affect food intake or subjective appetite ratings compared with an energy and available carbohydrate matched placebo.

- Following 40 g RS per day for 4 weeks in overweight/obese participants there were significantly higher glucose, insulin and C-peptide concentrations, measured during an FSIVGTT, with the RS compared with the placebo. However, parameters obtained from the modelling of the data were not significantly different.

- 3 week daily consumption of 48 g milled whole grain in healthy young adults did not significantly affect appetite or food intake compared with refined grain intake, although food intake increased during the refined grain intervention and remained fairly constant during the milled whole grain intervention.
• 48 g milled whole grain per day for 3 weeks may have a beneficial role in blood pressure regulation compared with refined grain in healthy young adults.

• Acute ingestion of 48 g whole grains (intact or milled) or control rolls by overweight/obese at a single meal resulted in no significant differences between the breads in food intake, subjective appetite ratings or postprandial metabolites.

• 8 week daily consumption of either 48 g whole grains (in both the intact and milled form) or refined grain in overweight/obese participants did not affect subjective appetite and had little effect on energy intake.

• There was little effect of 8 week daily whole grains consumption (in the intact and milled form) on postprandial metabolites in overweight/obese participants.

Overall conclusions:

o RS appears to be a possible candidate as a satiating ingredient when consumed acutely. Although the chronic study did not confirm this effect, the subject numbers were small and therefore could mask a potential beneficial product in weight management.

o Intervention with 48 g whole grains, in either form, has little effect on appetite or food intake compared with refined grain and therefore these studies do not agree with epidemiological evidence.
7.3. **Future work**

The work conducted in this thesis has been interesting and informative. Whilst the RS work has indicated that inclusion of a high dose of RS in the diet may have acute appetite effects, as well as interesting metabolic effects, the work with whole grains has shown less positive effects on appetite and food intake, potentially disproving the epidemiological evidence. In both instances further areas of research have been highlighted and would be interesting to pursue given more time.

- Continue with the long-term RS and appetite and FSIVGTT study with more participants which would provide valuable information on both the metabolic effect, but also on any effects on appetite and food intake from which further research could progress.
- Inclusion of normal weight individuals to the long-term RS and appetite and FSIVGTT study would allow the determination of whether there are effects on appetite that are overridden in the overweight population.
- If the long-term consumption of RS indicates an improvement in the first-phase insulin response then another long-term crossover intervention (of a longer period of time 8-12 weeks) in individuals with clinically diagnosed impaired glucose tolerance and/or diabetes, as the target group for a clinical improvement, would be conducted to determine whether a beneficial effect could be achieved.
- Conduct an appetite and RS study for longer time periods to allow for adaptation for fermentation and monitor effects on fermentation.
(changes to SCFA in blood or urine) to determine if this is the mechanism for an effect on appetite. The effects on blood lipids could be further explored in this study. This study could be conducted as a dose response study, with increasing amounts of RS being given to determine the optimal amount needed to affect fermentation/appetite/blood lipids. Alternatively this could be conducted as a longer-term study (for example 16 weeks), with study days conducted every 4 weeks to determine the minimum length of time needed for an effect on fermentation/appetite/blood lipids to occur, and whether adaptation occurs at a point at which further consumption at that dose does not have a continuing effect.

- Carry out a long term whole grain crossover intervention in overweight/obese individuals, using commercially available whole grain products to determine overall intake effects on appetite from commercially available products and incorporate a biomarker to assess compliance.

- Carry out a whole grain study further investigating the blood pressure effect, utilising 24 hour monitoring and other vascular markers to determine whether whole grains do beneficially affect blood pressure. This could be conducted in two population groups, those with and those without hypertension, to determine where the beneficial effect would lie.
Published Work
Full Papers


Abstracts


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Appendices
Appendix 1

An example of the Health and Lifestyle Questionnaire (self-certificate medical questionnaire) used in the studies. Each questionnaire was tailored from this to suit the particular study.

Self-Certificate Medical Questionnaire

Study:

Name: .................................................... Date of Birth: .........................

Address: ..........................................................................................................

Contact Telephone Number: ........................................................................

GP Name: ......................................................................

GP Address: ......................................................................................................

Please tick all any of the following that apply:

☐ I have no prior or present history of Coronary Heart Disease, Angina or Stroke
☐ I have no prior or present history of Type 1 or Type 2 diabetes
☐ I have no prior or present history of anaemia
☐ I have no prior or current history of gastrointestinal diseases (for example Crohn's disease, Coeliac disease, Irritable Bowel Syndrome)
☐ I have no prior or present history of liver disease
☐ I have no prior or present history of endocrine disorders
☐ I have no prior or present history of, nor am I currently being treated for, clinical depression and/or other psychological disorders
☐ I have no prior or present history of eating disorders, including anorexia or bulimia nervosa
☐ I have no prior or present history of drug or alcohol abuse within the last 2 years
☐ I am not currently taking or have taken any regular medication prescribed by my GP in the last 6 months (if you are please state what you are taking)

☐ I am not currently taking or have taken any supplements in the last 6 months

Have you had any other medical conditions or recent hospital visits?
YES / NO
If you answered yes, could you provide details?

Are you currently on a weight-reducing diet or other dietary restriction?
YES / NO
If yes, please provide details.

Are you allergic to any foods?
YES / NO
If yes, please state what foods you are allergic to.

Do you have any religious dietary requirements?
YES / NO
If yes, please provide details.

Have you been involved in a clinical trial in the last 3 months?
YES / NO

Do you smoke?
YES / NO
If yes, how many per day?

Do you drink alcohol?
YES / NO
If yes, how many units per week? (See below)

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Measure</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary strength lager (4%) e.g. Carling, Fosters</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Strong lager (5.2%) e.g. Stella Artois, Kronenburg</td>
<td>Pint</td>
<td>3</td>
</tr>
<tr>
<td>Strong lager e.g. Stella Artois, Carlsberg Export, Grolsch</td>
<td>440ml can</td>
<td>2.2</td>
</tr>
<tr>
<td>Beer/ordinary strength Ale e.g. John Smith’s, Guinness</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Std 175ml</td>
<td>2</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Lg. 250ml</td>
<td>3</td>
</tr>
<tr>
<td>Spirits</td>
<td>Std 25ml</td>
<td>1</td>
</tr>
<tr>
<td>Spirits</td>
<td>Lg. 35ml</td>
<td>1.4</td>
</tr>
<tr>
<td>Alcopop e.g. Smirnoff Ice, Bacardi Breezer, Reef</td>
<td>275ml</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Signed_________________________________________ Date__/__/__

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Appendix 2

The Dutch Eating Behaviour Questionnaire completed at screening. The coloured asterisks indicate the questions relating to external eating (green), emotional eating (blue) and restrained eating (red).

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

Visual Analogue Scale (VAS) questions used in this research. Each of the questions was presented to the participants on a separate page of a booklet, and a fresh booklet was given to the participants at each time point. Participants marked the line according to how they felt at the time and were told to regard the ends of the lines as the most extreme sensation they have ever felt.

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>How hungry do you feel?</td>
<td>I am not hungry at all</td>
</tr>
<tr>
<td>How much do you think you can eat?</td>
<td>Nothing at all</td>
</tr>
<tr>
<td>How full do you feel?</td>
<td>Not at all full</td>
</tr>
<tr>
<td>How thirsty do you feel?</td>
<td>Not at all thirsty</td>
</tr>
<tr>
<td>Would you like to eat something sweet?</td>
<td>No, not at all</td>
</tr>
<tr>
<td>Would you like to eat something salty?</td>
<td>No, not at all</td>
</tr>
<tr>
<td>Would you like to eat something savoury?</td>
<td>No, not at all</td>
</tr>
<tr>
<td>Would you like to eat something fatty?</td>
<td>No, not at all</td>
</tr>
</tbody>
</table>
Appendix 4

An example page from the 7 day diet diary given to participants, to show how the diaries should be completed.

Example diary pages

What did you eat?

<table>
<thead>
<tr>
<th>Date</th>
<th>23/7/2003</th>
<th>Day of the Week</th>
<th>Tuesday</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Time of day</th>
<th>Food &amp; Drink (Please describe in detail, including brand names where appropriate)</th>
<th>Amount Eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00am</td>
<td>Muesli - Sainsbury - no added sugar</td>
<td>Med bowl - pic 3a</td>
</tr>
<tr>
<td></td>
<td>Semi skimmed milk</td>
<td>200ml</td>
</tr>
<tr>
<td></td>
<td>Brown sugar</td>
<td>1 heaped tsp</td>
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<tr>
<td></td>
<td>Toast - Granary bread - thick sliced</td>
<td>2 slices</td>
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<tr>
<td></td>
<td>margarine (Flora)</td>
<td>Thick spread</td>
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<tr>
<td></td>
<td>honey</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td></td>
<td>tea</td>
<td>1 mug</td>
</tr>
<tr>
<td></td>
<td>semi-skimmed milk (in tea)</td>
<td>Splash - 1 tbsp</td>
</tr>
<tr>
<td>10.30am</td>
<td>Coffee with whole milk (in coffee)</td>
<td>1 mug</td>
</tr>
<tr>
<td></td>
<td>packet of crisps (Walkers)</td>
<td>25g</td>
</tr>
<tr>
<td>1.00pm</td>
<td>Lunch from canteen: sandwich (white bread)</td>
<td>2 medium slices</td>
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<tr>
<td></td>
<td>margarine (brand unknown)</td>
<td>thick</td>
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<tr>
<td></td>
<td>egg mayonnaise</td>
<td>3 tbsp</td>
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<tr>
<td></td>
<td>mustard and cress</td>
<td>handful</td>
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<tr>
<td></td>
<td>cucumber</td>
<td>6 slices</td>
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<tr>
<td></td>
<td>Blackberry and apple pie</td>
<td>Pic 3a - Large</td>
</tr>
<tr>
<td></td>
<td>Custard</td>
<td>Pic 5d - Medium</td>
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<tr>
<td></td>
<td>orange juice</td>
<td>250ml</td>
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<tr>
<td>3.30pm</td>
<td>Tea with whole milk &amp; white sugar</td>
<td>1 mug (as before)</td>
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<td>Twix</td>
<td>2 finger</td>
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<tr>
<td>6.00pm</td>
<td>Tea with whole milk &amp; white sugar</td>
<td>Mug (as before)</td>
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<td></td>
<td>boiled brown rice</td>
<td>Pic 3a - Large</td>
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<tr>
<td></td>
<td>chicken curry</td>
<td>Pic 5b - Large</td>
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<tr>
<td></td>
<td>mineral water (Sainsbury's)</td>
<td>1/4 pint</td>
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<tr>
<td></td>
<td>Strawberry yoghurt (Sainsbury's low fat)</td>
<td>159g</td>
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<tr>
<td>10.30pm</td>
<td>lager (brand unknown)</td>
<td>330ml bottle</td>
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</tbody>
</table>

Notes/comments/recipes:

Chicken curry - 7oz chicken, 1 med onion, 1 tbsp polyunsaturated margarine, 1tbsp curry powder, 3/4 pint chicken stock, 1tbsp mango chutney, 3 tbsps sultanas, 1/4 pint low-fat yoghurt, 1 tbsp plain flour.
Food preference questionnaire completed in the Effects of Acute Consumption of Resistant Starch on Appetite

Food Preferences Questionnaire

Study: The Acute Effects of Resistant Starch on Appetite and Satiety

1. a) Are you a vegetarian?
   □ YES □ NO

   b) If you answered yes to question 1.a), do you eat –
      Cheese □ YES □ NO
      Eggs □ YES □ NO
      Milk □ YES □ NO

2. Do you usually have breakfast?
   □ YES □ NO

3. a) Are you allergic to any foods?
   □ YES □ NO

   b) If you answered yes to question 3.a), please state what foods you are allergic to –

   .............................................................................................................................
   .............................................................................................................................
   ......  

4. a) Do you have any religious dietary requirements?
   □ YES □ NO

   b) If you answered yes to question 4.a), please state –

   .............................................................................................................................
   .............................................................................................................................
Appendix 6

An example page from the 7 day bowel habit diary used in the RS studies. Participants were required to answer the start questions and then complete the second page for each of the 7 days.

**General Information**

The aim of the diary is to obtain a detailed description of your bowel habit. Every day for the next 7 days, please record your bowel movements. Every time you pass a faecal sample please place a tick in the appropriate box in the table provided. Also record the time at which you pass the stool and the length of time spent on the toilet.

At the end of the 7 day period please answer the following, deleting where appropriate:

Would you say your bowel habit over the past 7 days has been as normal? Y/N
If no, please say how it has changed

Would you describe your bowel habit as being
leak / normal / constipated

Have you followed your normal daily routine? Y/N
If no, please state how it has changed

Have you taken any medication over the last 7 days? Y/N
Please provide details of drug and when taken

Have you menstruated in the past 7 days? Y/N
If yes, please specify dates
### Appendix 7

Food list questionnaire used to measure habitual whole grain intake and to exclude those who regularly consumed high whole grain intakes.

<table>
<thead>
<tr>
<th>Food (1 portion)</th>
<th>How many spoons/slices of the food do you have per portion?</th>
<th>Daily 5+</th>
<th>Daily 4</th>
<th>Daily 3</th>
<th>Daily 2</th>
<th>4 x week</th>
<th>2 x week</th>
<th>1 x week</th>
<th>Fortnightly</th>
<th>Once a month</th>
<th>Quarterly</th>
<th>Yearly</th>
<th>Never</th>
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