Do Short-Chain Fatty Acids have a role in Appetite and the Metabolic Response?

by

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Statement 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 Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes.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.

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Abstract

Humans are living in an increasingly ‘obesogenic’ environment with easily accessed, energy dense foods and sedentary lifestyles, which is resulting in a gradual increase in the population body weight. The need for strategies to prevent the rise in obesity are therefore becoming increasingly urgent. One strategy is to identify and develop foods that enhance satiety. The recently discovered short-chain fatty acid (SCFA) activated G-coupled protein receptors GPR41 and GPR43 are co-localised in L-cells with the anorexigenic gut hormone PYY, and also in adipocytes, with activation stimulating leptin release. Thus SCFA such as acetate and propionate may enhance satiety.

The findings from a series of randomised controlled crossover studies investigating orally (Chapters 3-5) and colonically (Chapter 7) delivered SCFA effects on appetite and metabolic response are reported herein. Appetite was assessed subjectively with a battery of visual analogues scales (VAS), and quantitatively with an ad libitum buffet meal 3-h (Chapters 3-5) or 7-h (Chapter 7) postprandially and using 24-h diet diaries.

The role of propionate was investigated in the first study (Chapter 3). Participants (n=20) consumed breakfast made with propionate-rich sourdough (SOUR, 6.0 mmol propionate), or equally palatable, isocaloric placebo (PL, 0.1 mmol propionate) bread. SOUR did not influence subjective nor quantitative appetite measures and appeared to increase postprandial insulinaemia relative to PL (p=0.061, treatment x time).

By contrast, ingestion of 25 mmol acetate (within vinegar) in an unpalatable (Unpal) or more palatable (Pal) drink alongside a standard breakfast (n=16), significantly increased rated fullness (p<0.0001) and nausea (p=0.001), decreased rated prospective consumption (p=0.036) and hunger (p=0.045) and reduced 24-h EI (p=0.021) and
postprandial glycaemia (p=0.022) relative to PL (no acetate). Appetite measures were significantly correlated to breakfast palatability ratings (p<0.05), and Unpal significantly reduced EI relative to PL (p<0.05), while Pal did not.

In a follow-up study to further examine the role of palatability (Chapter 5), orosensory stimulation with a vinegar-containing drink (VIN) following a standard preload did not significantly alter postprandial metabolites nor alter EI 3 h postprandially. However VAS hunger ratings were significantly lower (p=0.009, treatment x time) relative to PL, suggesting a transient reduction in appetite following orosensory stimulation with VIN.

Colonic SCFA effects were investigated by providing 22.4g inulin, 25.5g L-rhamnose (L-Rha) or no NDC (PL) in split doses at breakfast and lunch alongside standard mixed meals (n=13) following a 6-d run-in (Chapter 7). NDC treatment did not influence quantitative nor subjective appetite measures, except desire to eat a meal (p=0.008, during morning). However, postprandial plasma insulin (p=0.009) and NEFA (p=0.046, following lunch) were significantly influenced (treatment x time effect), with the lowest response for both following L-Rha, previously reported to enhance serum propionate.

Additionally the ability of a novel non-digestible carbohydrate (inulin esterified with propionate) to enhance colonic propionate production was investigated (Chapter 6), although the results were inconclusive.

The findings from these investigations are mixed, but overall do not support a role for SCFA in appetite regulation and are suggestive that previously reported effects may arise, at least in part, from product palatability. L-Rha shows promise as a candidate to reduce postprandial insulin secretion.
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<tr>
<td>Ach</td>
<td>Acetyl Choline</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>ARC</td>
<td>Arcuate Nucleus</td>
</tr>
<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
</tr>
<tr>
<td>BG</td>
<td>Blood glucose</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CIU</td>
<td>Clinical Investigation Unit</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CCNFSDU</td>
<td>Codex Committee on Nutrition and Foods for Special Dietary Uses</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>Conc</td>
<td>Concentration</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>DEBQ</td>
<td>Dutch Eating Behaviour Questionnaire</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>DF</td>
<td>Dietary fibre</td>
</tr>
<tr>
<td>DMH</td>
<td>Dorsomedial nucleus</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
</tr>
<tr>
<td>DPave</td>
<td>Average degree of polymerisation</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure</td>
</tr>
<tr>
<td>EI</td>
<td>Energy intake</td>
</tr>
<tr>
<td>EVAS</td>
<td>Electronic Visual Analogue Scales</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
</tr>
<tr>
<td>FAO</td>
<td>The Food and Agricultural Organisation of the UN</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>FOS</td>
<td>Fructooligosaccharides</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>GER</td>
<td>Gastric Emptying Rate</td>
</tr>
<tr>
<td>GERD</td>
<td>Gastroesophageal reflux disease</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography – mass spectrometry</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal Tract</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GTT</td>
<td>Gut transit time</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HbA1C</td>
<td>Haemoglobin A1C</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis Assessment Model</td>
</tr>
<tr>
<td>IAUC</td>
<td>Integrated area under curve</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IPE</td>
<td>Inulin propionyl ester</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistant / insulin resistance</td>
</tr>
<tr>
<td>IS</td>
<td>Internal standard</td>
</tr>
<tr>
<td>KO</td>
<td>Knockout</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>L-Rha</td>
<td>L-rhamnose</td>
</tr>
<tr>
<td>M</td>
<td>Male</td>
</tr>
<tr>
<td>MD</td>
<td>Maltodextrin / Dextrine maltose</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MSF</td>
<td>Modified sham feeding</td>
</tr>
<tr>
<td>α-MSH</td>
<td>α-Melanocyte stimulating hormone</td>
</tr>
<tr>
<td>3-MV</td>
<td>3-methyl valeric acid</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride (common salt)</td>
</tr>
<tr>
<td>NDC</td>
<td>Non digestible carbohydrate</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>OGGTT</td>
<td>Oral Glucose Tolerance Test</td>
</tr>
<tr>
<td>OF</td>
<td>Oligofructose</td>
</tr>
<tr>
<td>Oxm</td>
<td>Oxyntomodulin</td>
</tr>
<tr>
<td>PE</td>
<td>Propionyl ester</td>
</tr>
<tr>
<td>PL</td>
<td>Placebo</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PP</td>
<td>Pancreatic polypeptide</td>
</tr>
<tr>
<td>ppt</td>
<td>Precipitate</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide YY</td>
</tr>
<tr>
<td>REE</td>
<td>Resting Energy Expenditure</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiratory Quotient</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chain fatty acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SI</td>
<td>Insulin sensitivity</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>SUERC</td>
<td>Scottish Universities Environmental Research Council</td>
</tr>
<tr>
<td>TAG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>tBDMS</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TBS</td>
<td>Tablespoon</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>Tsp</td>
<td>Teaspoon</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scales</td>
</tr>
<tr>
<td>VIN</td>
<td>Vinegar</td>
</tr>
<tr>
<td>WHO</td>
<td>The World Health Organisation</td>
</tr>
</tbody>
</table>
Chapter 1. Literature Review

1.1 Introduction

The prevalence of obesity and consequent co-morbidities such as type 2 diabetes mellitus (T2DM), coronary heart disease and stroke are rising rapidly and projected to continue rising (Foresight, 2007).

Obesity aetiology is governed by a complex and multifaceted combination of biological and behavioural processes influenced by a multitude of cultural, environmental and social determinants. Central to this is the biological system regulating energy homeostasis, which evolved to ensure energy requirements are met, thus promoting a positive energy balance thereby predisposing humans to gain weight. Humans are living in an increasingly ‘obesogenic’ environment with easily accessed, energy dense foods and sedentary lifestyles. These ‘obesogenic’ factors can override homeostatic systems resulting in a gradual increase in the population body weight (BW), a phenomenon that has been coined ‘passive obesity’ (Foresight, 2007).

The need for strategies to prevent the rise in obesity are therefore becoming increasingly urgent. One approach that may be adopted is to identify and develop foods that enhance satiety. The provision of satiety-enhancing foods may result in earlier termination of eating episodes, thereby controlling meal size, thus reducing energy intake (EI) (Blundell and Halford, 1994, Cummings and Overduin, 2007).

The following literature review explores human eating behaviour and appetite regulation. The effects of short-chain fatty acids (SCFA) delivered both orally (for example vinegar) and colonically (via fermentable non digestible carbohydrates (NDC)) on appetite and metabolic response are reviewed, followed by a review of the influence cephalic phase responses and food palatability may have on appetite and the metabolic response.
1.2 Human eating behaviour

1.2.1 General overview

Eating behaviour is a complex phenomenon characterised by discrete eating episodes of meals and snacks separated by intervals of time (de Graaf et al., 2004). Mechanisms to regulate eating patterns and food choices (and therefore nutrient intake) are outcomes that are influenced by a diverse array of interacting biological and environmental inputs (Blundell and Halford, 1994, Blundell et al., 1994). Food intake is regarded not only a biological phenomenon to meet physiological needs, but also a social phenomenon influenced by psychological and environmental stimuli with biological consequences (Blundell and Halford, 1994). These stimuli include cognitive factors such as learned habits (e.g. habitual mealtimes, familiar foods), health beliefs and dietary restraint and also environmental factors such as food availability, time of day, time availability, sensory and nutritional properties of food, social circumstances, cultural influences, stress and boredom (Blundell et al., 1994, Gerstein et al., 2004, Mattes et al., 2005, Blundell and Halford, 1994). Indeed, physiological mechanisms regulating homeostasis may often be overridden by environmental and psychological factors (Figure 1.1).

![Diagram](image)

**Figure 1.1.** Scheme to illustrate the interacting biological and environmental influences on eating behaviour patterns. Adapted from (Blundell and Halford, 1994)
1.2.2 Appetite regulation and the ‘Satiety Cascade’ concept

Appetite has been defined as “the internal driving force for the search, choice and ingestion of food” (de Graaf et al., 2004), while hunger describes the sensations associated with the need for food, thus acting as a biological drive to seek food (Mattes and Friedman, 1993, Mattes et al., 2005, Green et al., 1997).

The influence of food or beverage consumption on appetite is generally considered to occur in two distinct phases, satiation and satiety, also referred to as intra- and inter-meal satiety respectively (Green et al., 1997).

Satiation (intra-meal satiety) is concerned with the processes that take place during ingestion to promote the termination of an eating episode (Cummings and Overduin, 2007, Blundell and Halford, 1994, Green et al., 1997). Once a meal is initiated, hunger sensations diminish as the meal is consumed, while sensations linked with satiation tend to develop (Mattes et al., 2005, Gerstein et al., 2004). Satiation thus controls both meal size and duration.

Satiety (inter-meal satiety) refers to postprandial events following an eating episode that inhibit hunger and further eating (Blundell and Halford, 1994, Cummings and Overduin, 2007). Satiety therefore regulates the interval of time between meals and meal frequency (Cummings and Overduin, 2007), and may also reduce food quantity consumed at the next eating episode (Gerstein et al., 2004).

The Satiety Cascade (Figure 1.2) is a model proposed by Blundell and Halford to conceptualise the processes initiated prior to, during and following an eating episode resulting from interactions between food characteristics (e.g. energy density and nutrient composition, sensory properties, quantity) and psychobiological responses to eating (Blundell and Halford, 1994).
Four overlapping (or cascading) mediating processes have been identified that occur during and following the onset of a meal, divided into: sensory, cognitive, post-ingestive/pre-absorptive and post-absorptive mechanisms. Together these cascading stimuli influence events within and between meals, ultimately to determine satiety and satiation effects, thus influencing eating behaviour. The intensity and time-course of cascade mechanisms, particularly at the post-ingestive and post-absorptive stage will be influenced by food characteristics (Blundell and Halford, 1994, Green et al., 1997). This supports the concept that food characteristics influence the 'satiety power', or capacity of a specific food to suppress hunger and enhance satiety thereby influencing the intermeal interval (Kissileff et al., 1984, Blundell and Halford, 1994).

1.3 Central and peripheral regulation of appetite

The physiological system regulating eating behaviour and energy expenditure (EE), thereby influencing energy homeostasis, comprises a synergy of multiple afferent peripheral signals. These signals include a variety of gut-, pancreas- and adipose-derived hormones, which are centrally integrated in the hypothalamus, primarily within
the arcuate nucleus (ARC) (Arora, 2006, Cummings and Overduin, 2007, Druce and Bloom, 2006, Druce et al., 2004, Stanley et al., 2006) (Figure 1.3).

Physiological regulation of appetite therefore needs to be considered at two levels: peripheral afferent signals such as gut-derived hormones (section 1.3.1) and central processes within the ARC in the hypothalamus and via the brain stem (section 1.3.2).

Figure 1.3. Peripheral and central regulation of energy homeostasis. Continuous lines represent stimulatory effects and dashed lines represent inhibitory effects. Taken from (Murphy and Bloom, 2004).1

1AgRP (agouti-related peptide), GLP-1 (glucagon-like peptide-1), α-MSH (alpha-melanocyte-stimulating hormone), NPY (neuropeptide Y), Oxn (oxyntomodulin), POMC (proopiomelanocortin), PP (pancreatic polypeptide), PYY (peptide YY).

1.3.1 Peripheral hormonal signals to regulate appetite

Peripheral signals involved in regulation of food intake and EE include both short term gut-derived hormones produced acutely in response to ingested nutrients (section 1.3.1.1), and markers of long term energy status released in levels proportional to body adipose stores from the pancreas (insulin) and adipose tissue (leptin and adiponectin)
(section 1.3.1.2). While the former are released phasically during eating episodes, the latter are tonically active. The production, mechanisms and actions of these peripheral signals have been described in a number of excellent and detailed reviews, for example (Cummings and Overduin, 2007, Druce and Bloom, 2006, Druce et al., 2004, Huda et al., 2006, Karra and Batterham, 2010, Murphy and Bloom, 2004, Neary et al., 2004, Stanley et al., 2006, van der Lely et al., 2004, Woods and D'Alessio, 2008, Wren and Bloom, 2007, Wynne et al., 2005, Gardiner et al., 2008).

1.3.1.1 Short term peripheral signals of nutrient intake

Gut-derived hormones are released acutely into the bloodstream from various parts of the gastrointestinal tract (GIT) following a meal in response to the entry of nutrients into the gut lumen. These hormones act both locally, for example to influence gastric emptying, and centrally (see section 1.3.2).

Table 1.1 summarises the main gut peptides identified to date, their sites of production and their main modes of action. As summarised, ghrelin is only known endogenous peripheral orexigenic hormone (appetite enhancing) and it powerfully increases food intake. All the other known gut-derived peptides, for example peptide YY (PYY), cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), pancreatic polypeptide (PP) and oxyntomodulin (Oxm), are anorexigenic (appetite suppressing) (Cummings and Overduin, 2007, Druce and Bloom, 2006, Druce et al., 2004, Huda et al., 2006, Karra and Batterham, 2010, Murphy and Bloom, 2004, Neary et al., 2004, Stanley et al., 2006, van der Lely et al., 2004, Woods and D'Alessio, 2008, Wren and Bloom, 2007, Wynne et al., 2005, Gardiner et al., 2008).
Table 1.1. Summary of main gut peptides involved in appetite regulation.

<table>
<thead>
<tr>
<th>Gut peptide</th>
<th>Main sites of production</th>
<th>Effect on appetite</th>
<th>Control mechanisms to influence appetite</th>
<th>Physiological effects other than appetite</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK</td>
<td>L-cells in jejunum</td>
<td>Released rapidly from GIT</td>
<td>Released rapidly into circulation.</td>
<td>Serotoninergic neurons in the brainstem. May also act via vagal pathway.</td>
</tr>
<tr>
<td>GLP-1</td>
<td>L-cells in ileum, ileum &amp; hypogastrium</td>
<td>Released rapidly from GIT</td>
<td>Released rapidly into circulation.</td>
<td>Serotoninergic neurons in the brainstem. May also act via vagal pathway.</td>
</tr>
<tr>
<td>Oxyntomodulin</td>
<td>Small intestine &amp; colon</td>
<td>Released rapidly from GIT</td>
<td>Released rapidly into circulation.</td>
<td>Serotoninergic neurons in the brainstem. May also act via vagal pathway.</td>
</tr>
<tr>
<td>GLP-1</td>
<td>L-cells in ileum, ileum &amp; hypogastrium</td>
<td>Released rapidly from GIT</td>
<td>Released rapidly into circulation.</td>
<td>Serotoninergic neurons in the brainstem. May also act via vagal pathway.</td>
</tr>
<tr>
<td>PP</td>
<td>Small intestine, colon</td>
<td>Released rapidly from GIT</td>
<td>Released rapidly into circulation.</td>
<td>Serotoninergic neurons in the brainstem. May also act via vagal pathway.</td>
</tr>
<tr>
<td>PYY</td>
<td>L-cells in ileum, ileum &amp; hypogastrium</td>
<td>Released rapidly from GIT</td>
<td>Released rapidly into circulation.</td>
<td>Serotoninergic neurons in the brainstem. May also act via vagal pathway.</td>
</tr>
</tbody>
</table>

**Effect on appetite**
- **Glutaglin**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **CCK**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **GLP-1**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **Oxyntomodulin**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **PP**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **PYY**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.

**Control mechanisms to influence appetite**
- **Glutaglin**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **CCK**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **GLP-1**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **Oxyntomodulin**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **PP**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **PYY**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.

**Physiological effects other than appetite**
- **Glutaglin**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **CCK**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **GLP-1**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **Oxyntomodulin**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **PP**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
- **PYY**: Released rapidly from GIT, directly activates vagal fibers, may also act via NTS and CNNs. May also act via vagal pathway.
1.3.1.2 Long term peripheral signals of adiposity

In addition to peripheral signals produced acutely in response to food intake, appetite and energy homeostasis is also regulated by longer term signals of adiposity: insulin, leptin and adiponectin. Insulin and leptin are anorexigenic signals that act centrally crossing the BBB to enter the hypothalamus by a saturable, receptor mediated mechanism, in concentrations proportional to circulating levels. Mechanisms by which adiponectin acts are less well understood.

Produced by pancreatic B cells, insulin was the first described adiposity signal (Schwartz et al., 1992). Plasma insulin concentrations are largely determined by degree of insulin sensitivity, which are in turn related to total adipose tissue mass and distribution. Circulating plasma insulin concentrations are therefore proportional to adiposity levels, particularly visceral (Porte Jr et al., 2002).

Insulin is also rapidly released in a biphasic pattern in response to ingestion. Central administration dose-dependently reduces food intake acutely and decreases BW chronically in rodents and primates (Ikeda et al., 1986, Woods et al., 1979, Menendez and Antrens, 1991). Effects of peripheral insulin administration are more difficult to investigate due to inducing hypoglycaemia during administration, which confounds interpretation. When glucose concentrations are controlled, acute peripheral administration decreases food intake in rodents and baboons (Nicolaidis and Rowland, 1976, Woods et al., 1984). In normal and overweight humans, a meta-analysis of appetite studies concluded postprandial insulin but not glucose may be associated with short-term appetite regulation (Flint et al., 2007).

Leptin is derived from the ob gene, expressed primarily in white adipose tissue, with circulating levels directly proportional to total fat mass (Maffei et al., 1995). As well as
being determined by energy stores, leptin concentrations are also suppressed with restricted food intake, and elevated during overeating (Frederich et al., 1995, Maffei et al., 1995), although unlike insulin, leptin is not acutely influenced by food intake. Central and peripheral leptin administration to rodents acutely decreases food intake (Ahima et al., 1996), and chronically decreases BW and fat mass (Halaas et al., 1995). Leptin also appears to modulate EE in rodents (Halaas et al., 1995, Pelleymounter et al., 1995), but not in humans. The classical ob/ob mouse, which has a mutation in the ob gene, is leptin deficient, hyperphagic, hyperinsulinaemic, has a low EE and is very obese, all of which may be normalised with leptin administration (Campfield et al., 1995, Halaas et al., 1995, Pelleymounter et al., 1995).

Also secreted from adipose tissue, circulating plasma adiponectin concentrations are approximately 1000 times greater than circulating insulin and leptin concentrations (Tsao et al., 2002). Although exact functions and mechanisms are unknown, adiponectin appears to have a role in energy homeostasis. Circulating concentrations are inversely proportional to adipose tissue mass in rodents, primates and humans (Arita et al., 1999, Hotta et al., 2001, Hu et al., 1996), and significantly increased following food restriction in rodents (Berg et al., 2001) and humans (Hotta et al., 2000). Peripheral administration attenuates BW gain by increasing EE, with no effect on actual intake (Fruebis et al., 2001, Yamauchi et al., 2001).

1.3.2 Central processes in appetite regulation

The hypothalamus is pivotal in appetite regulation and ensuring long-term food intake adjusts over time thus stabilising energy levels stored as fat (i.e. promoting energy homeostasis), by integrating incoming hormonal and neuronal signals (Morton et al., 2006, Wynne et al., 2004). The majority of receptors involved in integrating energy
homeostasis signals are located within the ARC of the hypothalamus. The ARC is located at the base of the hypothalamus near the median eminence, where there is an incomplete blood brain barrier (BBB) allowing easy access by various circulating peptides and hormones (Broadwell and Brightman, 1976). Gut peptides including PYY and GLP-1, are able to cross the BBB via a nonsaturable mechanism, while others such as leptin and insulin are transported via a saturable mechanism (Stanley et al., 2006, Wynne et al., 2005).

The ARC includes two distinct neuronal populations that integrate signals to either inhibit or stimulate food intake, secreting POMC (proopiomelanocortin) or NPY/AgRP (neuropeptide Y and Agouti-related protein) respectively (Cone et al., 2001). While originating in the ARC, these neuronal populations both project into other areas within the hypothalamus and other central areas, often in parallel (Woods and D'Alessio, 2008).

The group of neurones that secrete POMC activate catabolic mechanisms, inhibiting food intake, increasing EE and promoting body fat loss. When secreted, POMC is cleaved to form an anorectic peptide α-melanocyte stimulating hormone (α-MSH). α-MSH exerts its actions primarily via activation of melanocortin 3 and melanocortin 4 receptors (MC3R and MC4R) located on neurons in the hypothalamus and elsewhere in the brain. Central administration of α-MSH inhibits food intake and reduces BW. Leptin is known to stimulate the neurones directly to release POMC and PYY 3-36 is also thought to stimulate POMC release, with peripheral administration increasing POMC expression (Neary et al., 2004, Woods and D'Alessio, 2008).

The group of neurones secreting AgRP and NPY activate anabolic mechanisms, stimulating food intake and body fat synthesis. As an antagonist of MC3R and MC4R, AgRP blockades via actions of α-MSH, thus opposing the inhibition of food intake.
Central AgRP administration acutely increases food intake with relatively long-lasting effects (up to a week), with chronic administration leading to sustained hyperphagia and consequently obesity. NPY is one of the most potent orexigenic ligands and stimulates food intake via activation of Y receptors. Central administration of NPY acutely stimulates feeding although these effects are far shorter-lived than those of AgRP. Chronic central administration leads to sustained hyperphagia and weight gain in rodents. Prior to eating episodes NPY concentrations increase rapidly and remain elevated until eating commences, suggestive of a role for NPY in meal initiation, which is interlinked to the finding that ghrelin activates NPY/AgRP neurons. It is also known that both leptin and insulin inhibit NPY/AgRP neurons. PYY 3-36 is thought to additionally inhibit NPY/AgRP release, with central administration directly into the ARC reducing food intake, with this effect being abolished in Y2 receptor knockout mice (Neary et al., 2004, Woods and D'Alessio, 2008).

In addition to direct action in the ARC, satiety signals also act indirectly via afferent fibres of the vagal nerve to the brain stem. Within the brain stem, NTS neurones such as GLP-1 neurones project into the PVN and lateral hypothalamus, and serotonergic neurones project into the ARC, thus connecting the brain stem to the hypothalamus. In addition to GLP-1, CCK and PP are also known to exert actions via vagal activation. Vagal activation appears to regulate short-term feeding, while the hypothalamus is involved in longer-term energy homeostasis mechanisms (Woods and D'Alessio, 2008).

1.4 Experimental approaches to assess appetite

Due to the complex and diverse factors that can influence ingestive behaviour, as discussed in section 1.3, experimentally it is not straightforward to assess appetite and there is no single ideal method to follow. Factors regulating appetite include both
objective (i.e. unconditioned or physiological) and subjective (i.e. conditioned or learned) components. Debates about the most valid, reproducible and robust methods are ongoing, controversial and subject to various reviews (Kissileff, 1985, Stubbs et al., 1998, Stubbs et al., 2000, Booth, 2009b, Booth, 2009a).

1.4.1 What is the most appropriate setting in which to assess appetite?

The assessment of appetite in a laboratory setting allows the investigator to control the intervention, experimental environment (such as excluding eating cues) and outcome measures, and ensures participants follow the specified protocol (Stubbs et al., 1998). However this control creates an artificial environment resulting in a lack of external validity in relation to normal eating behaviour (Meiselman, 1996, Stubbs et al., 1998), although the internal validity of this type of study is generally good (Stubbs et al., 1998) and could be advantageous in assessing the internal drive to eat (de Graaf et al., 2004).

An alternative is to conduct intervention studies in free-living conditions, for example by monitoring intake with the use of food diaries and/or food frequency questionnaires (FFQs) and asking participants to complete subjective appetite questionnaires. Free living studies have more external validity than controlled laboratory conditions, however it is not possible to unequivocally ascertain desired protocol has been adhered to, food diaries are subject to reporting bias (for example under-reporting and under-eating (Poslusna et al., 2009)) and other errors such as misinterpretation of portion sizes during food diary analysis. It is also not possible to control for external, potentially confounding factors that could affect subjective results (for example stress, availability of food, going out for meals or drinks, etc).

It has been suggested that the best approach is to carry out studies in both laboratory controlled and free-living conditions (Stubbs et al., 1998).
1.4.2 Experimental design of laboratory based appetite studies

The most common experimental approach to investigate the influence of a treatment (e.g. NDC supplement) on appetite is the “preload-test meal” paradigm in which participants are presented with a standard “preload” that includes the treatment under investigation or placebo (PL). Following this, the motivation to eat is usually subjectively assessed (section 1.4.3), then participants are presented with a “test meal” in which EI is measured (section 1.4.4) (Stubbs et al., 1998). This method allows assessment of the effects of the treatment on subsequent eating behaviour (Kissileff, 1985), and is best conducted as a within-subjects repeated measures design (Stubbs et al., 1998). An important consideration is that preload-delivered treatment effects tend to decrease as the time interval between preload and test meal increases (Rolls et al., 1991). However this depends upon the treatment being investigated. For example the influence of an NDC supplement may occur later due to the time taken to reach the colon for fermentation.

1.4.3 Use of subjective rating scales

Visual Analogue Scales (VAS) are the most commonly used systems to subjectively assess appetite. VAS comprise a straight line, often 100mm long, with each end describing the most extreme positive and negative sensations of the attribute under investigation. For example when asked “How hungry do you feel?”, the two extremes are usually “not at all hungry” and “I have never been more hungry” (Stubbs et al., 2000). Participants are asked to mark the point on the line that best represents their sensation of the attribute at that time and a temporal profile of the attribute is generated by repeating the VAS measurement at various timepoints.

VAS were initially designed to assess pain and were then developed for use in appetite regulation research. A variety of questions are usually asked to encompass the
multidimensional aspects of appetite (Mattes et al., 2005), with the original battery of six questions used summarised in Table 1.2 (Hill and Blundell, 1982). Further questions have since been added including assessing appetite for specific food groups, for example “Would you like to eat something sweet?” (Stubbs et al., 1998).

Table 1.2. VAS questions designed for use in appetite research. With the phases used to describe each extreme. Adapted from (Hill and Blundell, 1982)

<table>
<thead>
<tr>
<th>Question</th>
<th>Negative Extreme</th>
<th>Positive extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>How hungry do you feel?</td>
<td>Not at all hungry</td>
<td>As hungry as I have ever felt</td>
</tr>
<tr>
<td>How full do you feel?</td>
<td>Not at all full</td>
<td>As full as I have ever felt</td>
</tr>
<tr>
<td>How strong is your desire to eat?</td>
<td>Very weak</td>
<td>Very strong</td>
</tr>
<tr>
<td>How much do you think you could eat right now?</td>
<td>Nothing at all</td>
<td>A large amount</td>
</tr>
<tr>
<td>Urge to eat</td>
<td>No urge to eat</td>
<td>Strong, want to eat now, waiting is very uncomfortable</td>
</tr>
</tbody>
</table>

VAS offer a quick, easy to use and easily interpreted method to assess appetite in a standardised way allowing comparisons between different studies (Stubbs et al., 2000). However the reliability of VAS in appetite research is debated, not least due to difficulties in objectively validating their use. Stubbs et al. (2000) suggested VAS reliability can be assessed by considering their:

(a) Ability to predict eating behaviour (e.g. food intake): Studies have shown variable results in the ability of VAS to predict food intake. In free-living conditions, significant but small correlations between subjective hunger ratings and subsequent self-recorded EI over a 7 d period have been reported (de Castro and Elmore, 1988, Mattes, 1990). Mattes found this was true only on week days, not weekend days, and within-subjects analysis found no association between hunger ratings and subsequent EI
in the following hour (Mattes, 1990). However free-living conditions may not be relevant to preload-test meal studies, and food diaries are vulnerable to mis-reporting. When carried out under controlled conditions, *ad libitum* food intake was significantly and positively correlated to pre-meal hunger, desire to eat and prospective consumption ratings and negatively correlated to fullness ratings in older subjects (65-85 years). However in younger subjects (18-35 years) this correlation was only significant for fullness and prospective consumption (Parker et al., 2004). Drapeau and colleagues found the 1 h AUC for fullness ratings following a standard breakfast were also a good predictor of *ad libitum* EI following the same standard breakfast provided on a different day (Drapeau et al., 2005). Furthermore, mean appetite ratings collected for 4.5 h prior to an *ad libitum* lunch were more strongly correlated with subsequent EI than were pre-meal ratings, and were significant for hunger, fullness, prospective food consumption and satiety ratings (Flint et al., 2000).

Results have therefore been variable, with stronger correlations seeming to occur in controlled rather than free-living conditions. Therefore overall hunger ratings do not necessarily appear to predict food intake, while fullness ratings may have a stronger relationship with subsequent EI.

(b) *Sensitivity to experimental manipulations:* There is some evidence VAS are sensitive to experimental manipulations such as changes in diet composition and administration of appetite stimulating and inhibiting drugs (Stubbs et al., 2000). However Stubbs et al. (2000) observed VAS are not necessarily sensitive to relatively modest manipulations.

(c) *Reproducibility:* Variable results have been found for within-subject test-retest-reliability of VAS ratings. Similar postprandial temporal profiles have been found
following the same standard meal on different days (Flint et al., 2000, Nair et al., 2009). However considerable variation is observed, probably from combination of true biological day-to-day variation and methodological variation (Flint et al., 2000, Raben et al., 1995), and possibly from conditioned effects of having previously experienced the same meal (Raben et al., 1995). Flint et al. reported relatively low reproducibility for fasting and peak ratings, and better reproducibility for mean 4.5 h ratings, particularly prospective consumption ($r^2 = 0.76$ and 0.72 with and without previous standard diet) and fullness ($r^2 = 0.66$ and 0.58 with and without previous standard diet) (Flint et al., 2000).

Between-subject reliability is more difficult to assess as differences in scores may arise from inter-individual differences in interpretation of the VAS (Stubbs et al., 2000). It was found that in order to detect a 5 mm difference in mean 4.5 h fullness ratings, 20 participants should be recruited for a crossover study, while 120 participants are required per group in a parallel design study (Flint et al., 2000), highlighting the weakness of parallel studies in appetite research.

Thus overall a number of limitations and weaknesses in the use of VAS to assess appetite have been highlighted by the validation studies, reflecting the difficulties in conducting research into appetite regulation. This is largely because data generated by VAS is a measure of the participant's interpretation of their sensations and motivations regarding the attribute being investigated rather than an absolute outcome of an underlying physiological response (Stubbs et al., 2000, Stubbs et al., 1998).

These limitations do not mean VAS are not useful and valid for use in appetite research, but rather they should be carefully interpreted in combination with other measures of appetite such as food intake data, within a crossover study design.
1.4.4 Quantitative assessment of appetite

Within the “preload-test meal” paradigm, quantitative assessment of preload influence on subsequent appetite is made by measuring EI at an *ad libitum* test meal. Participants are presented a pre-weighed meal in a quantity far exceeding the usual portion size and are asked to eat until they feel comfortably full after which the meal is re-weighed to determine EI and macronutrient intake (Hill et al., 1995).

*Ad libitum* meals are served either as a homogenous single meal or a mixed item buffet. The former can only assess effects on EI while a mixed buffet also assesses effects on macronutrient selection. However the variable intake of macronutrients during a mixed item buffet may confound results due to differing satiating properties, as protein appears to be more satiating than fat and CHO (Gregersen et al., 2008).

Despite being widely used in appetite studies, the reproducibility of EI in *ad libitum* meals has not been extensively investigated. Good correlation was found in EI and macronutrient intake on two separate occasions when participants were presented a mixed 31 item cold buffet meal 4 h following a standard preload. The inter- and intra-subject coefficient of variations (CV) for EI were 35.1% and 8.2% respectively, demonstrating a high degree of variability between subjects, again highlighting the strength of a crossover design. The authors concluded the mixed buffet test meal is a reproducible method to assess macronutrient preferences (Arvaniti et al., 2000), however the sample size was not large (n=14) and participants only attended two sessions. Nair and colleagues reported EI of a mixed 18 item cold buffet provided on three study occasions was highly reproducible within subjects, although macronutrient reproducibility was more variable, being good for protein, moderate for fat and low for CHO. The CV was not reported (Nair et al., 2009).
Similarly EI of a homogenous \textit{ad libitum} buffet test meal (mix of pasta, minced meat, vegetable and cream) provided on two occasions 4.5 h following a standard preload was significantly correlated between each test day (Gregersen et al., 2008). A similar intra-individual CV to the Arvanti study of 8.9\% was found, although this study was better powered (n=55). The authors concluded 17 participants are required to detect a 500 kJ difference in EI with prior diet standardisation in a crossover study with a power of 0.8 (Arvaniti et al., 2000).

Overall, evidence to date indicates an \textit{ad libitum} test meal following a preload is a reproducible method to assess EI, with a homogenous meal being easier and less laborious to prepare, less expensive and wasteful, and removing the potentially variable satiating effects of a mixed buffet. Mixed buffet may also lack external validity, as presenting participants with a wide food choice is not representative of everyday life and may result in over-eating (Hill et al., 1995), as found by Arvanti and colleagues.

Whether EI of an \textit{ad libitum} test meal reflects appetite is controversial. Many factors (e.g. hedonic food properties, cognitive factors, environment) influence the relationship between appetite and food intake (de Graaf et al., 2004). It is advisable to standardise test meal conditions by using the same room, with constant light, and free from distractions and to separate subjects to avoid social interaction (Kissileff, 1985). De Graaf and colleagues conclude that when directly measured under standardised conditions, actual food intake may serve as a post-hoc indicator of appetite (de Graaf et al., 2004).
1.5 Fibre and non-digestible carbohydrates (NDC)

1.5.1 Defining dietary fibre

Dietary fibre (DF) was originally defined to describe the non-digestible components of plant cell walls (Hipsley, 1953) and was further expanded to “the skeletal remains of plant cells that are resistant to digestion (hydrolysis) by enzymes of man” (Trowell, 1972). This was amended to “dietary fibre consists of the plant polysaccharides and lignin which are resistant to hydrolysis by digestive enzymes of man” (Trowell et al., 1976) to account for plant cell components other than the cell wall being hydrolysis resistant.

Since then, the definition of DF has been under debate by various committees and organisations, with contrasting definitions in use worldwide, some based on analytical methods to isolate DF, and others based on chemical or physiological properties (Panel on the Definition of Dietary Fiber et al., 2001).

Efforts are being made to reach consensus on a universal definition to avoid confusion. A joint expert consultation of WHO and the FAO (FAO of the UN, 1998) recommended carbohydrates (CHO), including DF, should be classified according to their chemical divisions, namely sugars, oligosaccharides, polysaccharides and appropriate subgroups. It was considered ‘dietary fibre’ should only describe components with well established health benefits, in particular vegetable, fruit and wholegrain cell wall polysaccharides, and should not include synthetic, isolated or purified oligosaccharides and polysaccharides that do not have well established physiological effects. The Scientific Update therefore proposed DF should be defined as “intrinsic plant cell wall polysaccharides” (Mann et al., 2007), which could include the non-starch polysaccharides (NSPs) cellulose, hemicellulose and pectin, and resistant starch (FAO of the UN, 1998). However it was acknowledged that while this definition facilitates...
easy measurement and therefore clear food labelling, it does not easily translate into nutritional effects (Mann et al., 2007).

For this reason, the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU), which sets global food standards, revisited the issue and agreed a new definition for DF in 2008 to include isolated and synthetic polysaccharides:

"Dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by endogenous enzymes in small intestine of human beings and belong to the following categories:

- Edible CHO polymers naturally occurring in food as consumed
- CHO polymers, which have been obtained from raw material in food by physical, enzymatic, or chemical means and which have been shown to have physiological effect of benefit to health by generally accepted scientific evidence to competent authorities
- Synthetic CHO polymers, which have been shown to have physiological effect of benefit to health by generally accepted scientific evidence to competent authorities” (Cummings et al., 2009, Codex Alimentarius Commission, 2008)

The definition included a footnote that the inclusion of hydrolysis resistant CHO with 3 to 9 monomeric units (i.e. oligosaccharides) should be at the discretion of national authorities (Codex Alimentarius Commission, 2008, Harris and Pijls, 2009).

In view of the complex definition for DF, reference will often be made to Non-Digestible Carbohydrates (NDC) in this thesis, which will be used to include any hydrolysis resistant CHO, regardless of molecular size.
1.5.2 Defining prebiotics

It is also important to define the term 'prebiotics' when considering DF. Prebiotics are non-digestible compounds that upon reaching the colon are selectively fermented to stimulate activity and/or growth of health-enhancing indigenous colonic microflora, such as *Bifidobacterium* and other lactic acid-producing bacteria (Kelly, 2008, Roberfroid, 2007). Inulin-type fructans are the most widely studied prebiotics, with galactooligosaccharides (GOS) also fulfilling the inclusion criteria (Roberfroid, 2007). Various other products have also been suggested as prebiotics, but further research is required to ascertain if they meet the prebiotic inclusion criteria (Kelly, 2008).

1.5.3 Inulin-type fructans

Inulin-type fructans are linear oligo- and polysaccharides with prebiotic properties that include mainly β-(2→1) fructosyl-fructose glycosidic linkages (Roberfroid, 2005a). As digestive enzymes are specific for α-glycosidic bonds, inulin-type fructans resist enzymatic hydrolysis in the small intestine, and therefore proceed undigested to the colon where they are fermented (Kelly, 2008).

Inulin-type fructans are considered long chain with a degree of polymerisation (DP) ≥10, medium chain with a DP of 5-9 and short chain with DP of 2-4. They are often termed inulin, oligofructose (OF) or fructooligosaccharides (FOS) respectively, although these terms are not consistently used (Kelly, 2008).

Natural food sources include onions, leeks and garlic, with Jerusalem artichokes and chicory being rich sources (contain >15 %) (Franck, 2002). The dietary intake of inulin-type fructans is estimated to be 2 - 12 g per day in the UK (van Loo et al., 1995).

Commercially, inulin-type fructans are isolated by extraction from chicory root or synthesis from smaller molecules (e.g. sucrose) (Kelly, 2008). Commercial inulin-
type fructan preparations are a non-homogenous mixture with varying DP, and are described by the average DP (DP\text{ave}) and DP range. This can be further processed to change product composition and therefore properties, as summarised in Table 1.3 (Franck, 2002, Kelly, 2008).

<table>
<thead>
<tr>
<th></th>
<th>Standard inulin</th>
<th>Inulin HP</th>
<th>Oligofructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP range</td>
<td>2 to 60</td>
<td>10 to 60</td>
<td>2 to 7</td>
</tr>
<tr>
<td>DP\text{ave}</td>
<td>12</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>Appearance</td>
<td>White powder</td>
<td>White powder</td>
<td>White powder</td>
</tr>
<tr>
<td>Taste</td>
<td>Neutral</td>
<td>Neutral</td>
<td>Moderately sweet</td>
</tr>
<tr>
<td>Water solubility (g/l)</td>
<td>120</td>
<td>25</td>
<td>&gt;750</td>
</tr>
<tr>
<td>Use in foods</td>
<td>Fat replacer</td>
<td>Fat replacer</td>
<td>Sugar replacer</td>
</tr>
</tbody>
</table>

The DP appears to influence the site of fermentation in the colon and side-effects. Fermentation of shorter chain fructans occur more proximally while longer chain fructans appear to resist proximal fermentation and are fermented more distally. Furthermore FOS and OF tend to be associated with more gastrointestinal side effects than standard inulin at the same dose (Kelly, 2008).

### 1.6 Short-chain fatty acids

#### 1.6.1 Definition of short-chain fatty acids

Short-chain fatty acids (SCFA) are organic fatty acids formed in the colon when NDC resist digestion and absorption in the small intestine, instead proceeding to the colon to undergo bacterial fermentation. SCFA comprise 1 to 7 carbon (C) units, with acetate (2 C units), propionate (3 C units) and butyrate (4 C units) being the predominant anions in the colon (Cummings et al., 1987, Topping and Clifton, 2001), accounting for approximately 90-95% of total colonic SCFA (Dass et al., 2007). The abundance of the
major colonic SCFA is normally acetate > propionate > butyrate (Cummings et al., 1987), with daily colonic production estimated to be approximately 100 to 200 mmoles (Cook and Sellin, 1998). Figure 1.4 shows the chemical structure of the main SCFA.

![Chemical structures of SCFA](image)

Figure 1.4. Chemical structures of the SCFA acetate, propionate and butyrate.

1.6.2 The fermentation process in the large intestine

The main routes by which NDC are fermented are summarised in Figure 1.5.

![Fermentation process diagram](image)

Figure 1.5. Main routes of CHO fermentation by colonic microflora in the colon. Adapted from (Macfarlane and Macfarlane, 2003).
Once they arrive in the colon, NDC-derived polysaccharides, oligosaccharides and disaccharides are hydrolysed to monosaccharides. The monosaccharides are metabolised within colonic bacteria cells to pyruvate and acetyl CoA, predominantly via the Krebs cycle, and to a lesser extent via the pentose phosphate pathway (FAO/WHO, 1997, Topping and Clifton, 2001). Pyruvate and acetyl CoA are then further metabolised via a range of intermediate fermentation products to SCFA (particularly acetate, butyrate and propionate), and gaseous by-products including hydrogen (H₂) and carbon dioxide (CO₂) and in some individuals methane (CH₄). Heat is also released in this exothermic process (Macfarlane and Macfarlane, 2003, Topping and Clifton, 2001).

1.6.3 Fate of SCFA

Figure 1.6 provides an overview of the fate of the SCFA once generated in the GIT.

![Figure 1.6. Summary of fates of colonic acetate, propionate and butyrate.](image-url)
SCFA are efficiently absorbed, with only 5-10% of generated SCFA excreted within faeces (Cummings et al., 1987, Roberfroid, 2005b). Human intubation studies have demonstrated butyrate, propionate and acetate are rapidly absorbed at similar rates along various regions of the large bowel in a concentration-dependent manner (Ruppin et al., 1980). Large quantities are metabolised by the large intestinal epithelium (Bergman, 1990), with their oxidation supplying around 60-70% of colonocyte energy needs (Topping and Clifton, 2001, Roediger, 1995). Of the three main SCFA, butyrate is the preferred colonocyte fuel (Bergman, 1990, Topping and Clifton, 2001, Roediger, 1995).

The majority of acetate and propionate produced are absorbed across the colonic epithelium, with only relatively small amounts oxidised within colonocytes. Acetate and propionate, along with any residual butyrate not oxidised by colonocytes, are transported via the portal vein to the liver (Topping and Clifton, 2001), with approximately 90% of propionate and 50-70% of acetate extracted by the liver during a single pass (Wong et al., 2006, Roberfroid, 2005b). The fraction of acetate, propionate and butyrate not absorbed or utilised by the liver is distributed to the other body organs and tissues for metabolism via the general circulation, although very little propionate and butyrate is remaining at this point.

Data from sudden death victims demonstrates this progressive reduction in SCFA concentrations as they progress from portal to peripheral circulations (Table 1.4).

Table 1.4. Mean SCFA concentrations in human blood taken from portal, hepatic and peripheral sources. Samples from sudden death victims (n=6) within 4 h of death (Cummings et al., 1987)

<table>
<thead>
<tr>
<th>Blood Source</th>
<th>Total SCFA*</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/l</td>
<td>µmol/l</td>
<td>% total</td>
<td>µmol/l</td>
</tr>
<tr>
<td>Portal</td>
<td>375</td>
<td>258</td>
<td>69</td>
<td>88</td>
</tr>
<tr>
<td>Hepatic</td>
<td>148</td>
<td>115</td>
<td>78</td>
<td>21</td>
</tr>
<tr>
<td>Peripheral</td>
<td>79</td>
<td>70</td>
<td>89</td>
<td>5</td>
</tr>
</tbody>
</table>

* Total concentration of acetate + propionate + butyrate
1.6.4 Factors influencing SCFA production

The exact molar ratios and quantities of SCFA are dependent on a number of factors including the fermentable NDC substrate (Cook and Sellin, 1998, Wong et al., 2006), the colonic microflora composition and quantity (Roberfroid, 2005b, Wong et al., 2006) and the gut transit time (Cook and Sellin, 1998, Wong et al., 2006, Miller, 2004).

(a) Fermentable substrate: Studies have demonstrated that the fermentation of different NDC sources result in distinct SCFA profiles, in terms of both overall quantity and molar ratios of SCFA produced (Macfarlane and Macfarlane, 2003). This variability may arise from particular substrates favouring the growth of particular colonic bacteria (Nyman, 2002) and the substrate solubility, with more soluble NDC being more easily accessed by hydrolytic enzymes (FAO/WHO, 1997). However some soluble fibres, for example alginates and carragheenan, are poorly fermented (FAO/WHO, 1997).

(b) Colonic Microflora: The human colon is resided by a vast bacterial population, estimated to be at least $10^{10}$ to $10^{11}$ cfu/g wet weight (Hill, 1995) with over 50 genera and greater than 400 species of bacteria having been identified in human faeces (Gibson and Roberfroid, 1995, Hill, 1995, Savage, 1986, Topping and Clifton, 2001), although microbial diversity within the human gut is thought to be grossly underestimated at present (Blaut et al., 2002). Anaerobes, which include the Bacteroides, Bifidobacterium, Eubacterium, Streptococcus and Lactobacillus species, are the predominant microflora. Of these, Bacteroides are generally present in the greatest numbers, often comprising over 30% of the colonic microfloral population (Gibson and Roberfroid, 1995, Topping and Clifton, 2001). There is marked inter-individual variation in microbial composition (Hopkins et al., 2002), which will impact on SCFA production profiles.
Fermentation end-products vary with different microbes (Macfarlane and Macfarlane, 2003). Furthermore even within the same microbe, end-products can vary with the amount of CHO substrate available and the microbe growth rate (Macfarlane and Macfarlane, 2003).

(c) Gut Transit Time (GTT): The GTT (time taken for food eaten to travel along the GIT from the mouth to the anus) greatly influences bacterial physiology and metabolism, and thus the fermentation process. When GTT is slower (by ingesting senna or cisapride) significantly increased fecal SCFA concentrations are observed, while when the GTT is faster (by ingesting operamide) significantly lower fecal SCFA levels are observed. This may be due to the slower transit time allowing greater opportunity for substrate fermentation (El Oufir et al., 1996, Lewis and Heaton, 1997).

1.7 SCFA G-protein coupled receptors (GPR)

In 2003 two G-protein coupled receptors, GPR41 and GPR43 (also known as FFA3 and FFA2 respectively), were identified as being activated by SCFA (Brown et al., 2003, Le Poul et al., 2003). Interest in GPR41 and GPR43 is increasing and a number of papers regarding these receptors have now been published (Xiong et al., 2004, Tazoe et al., 2009, Tazoe et al., 2008, Samuel et al., 2008, Le Poul et al., 2003, Karaki et al., 2008, Karaki et al., 2006, Hong et al., 2005, Dass et al., 2007, Covington et al., 2006, Brown et al., 2003), although their physiological relevance is not yet resolved.

GPR43 is equally sensitive to activation by propionate, butyrate and acetate, while sensitivity for GPR41 is in the order propionate ≥ butyrate > acetate (Tazoe et al., 2008, Brown et al., 2003, Le Poul et al., 2003, Xiong et al., 2004). Acetate is more selective for GPR43 than GPR41 (Tazoe et al., 2008).
1.7.1 GPR patterns of expression

GPR41 and GPR43 are expressed in a variety of tissues including adipose and the GIT. Initial studies indicate GPR41 is primarily expressed in adipose (Brown et al., 2003, Le Poul et al., 2003, Xiong et al., 2004) while GPR43 is more widely distributed throughout many tissues, including immune cells, adipose tissue and the GIT (Brown et al., 2003, Le Poul et al., 2003, Covington et al., 2006).

Both GPR41 and GPR43 are expressed in the human colon (Karaki et al., 2008, Tazoe et al., 2009). GPR43 is more abundant and widely distributed than GPR41, with cell counts of 0.33±0.01 and 0.01±0.01 per crypt respectively. Western blot analysis suggests both receptors are located only in the mucosa, with neither detected in either submucosa or muscle (Karaki et al., 2008, Tazoe et al., 2009). Evidence to date indicates both receptors are expressed in enteroendocrine L-cells containing the anorexigenic gut peptides PYY and GLP-1. All PYY-immunoreactive cells were immunoreactive for GPR43, however only low levels of GPR41 immunoreactivity were found in PYY-immunoreactive cells (Karaki et al., 2008, Tazoe et al., 2009), indicating all PYY-expressing cells also express GPR43 but do not necessarily express GPR41. Furthermore, double-immunostaining indicates GPR41 and GPR43 are not colocalised in the same enteroendocrine cells (Tazoe et al., 2009).

1.7.2 Role of GPR41 and GPR43 in adipose tissue

In vitro and in vivo evidence from murine models indicates activation of GPR41 and GPR43 by SCFA ligands may mediate leptin expression (Covington et al., 2006, Xiong et al., 2004) and inhibit lipolysis (Ge et al., 2008, Hong et al., 2005) respectively.

In vitro, leptin expression was upregulated following treatment of murine adipocytes with SCFA solutions, while oral provision of propionate to mice in vivo led to elevated circulatory leptin concentrations. Propionate was the most potent ligand in
vitro, and the stimulatory effects of propionate treatment were abolished in cells infected with a virus that targeted GPR41 mRNA, suggesting GPR41 is the relevant receptor (Xiong et al., 2004). However these findings are not corroborated by Hong and colleagues who did not detect GPR41 in adipose tissue (Hong et al., 2005).

A role for GPR43 in adipocyte functionality is indicated by an observed up-regulation of GPR43 expression in mice fed a high fat diet (41 % fat) relative to a normal fat diet (8.5 % fat) (Hong et al., 2005). In vitro treatment of isolated adipocytes with propionate significantly elevated GPR43 expression (Hong et al., 2005), with acetate and propionate treatment inhibiting lipolysis (Hong et al., 2005, Ge et al., 2008). This inhibition was abolished when treating adipocytes isolated from GPR43 KO mice (Ge et al., 2008), suggesting this effect is mediated via GPR43 activation. Oral provision of acetate in vivo to live mice significantly suppressed plasma free fatty acids (FFA), while no suppression was observed following similar treatment of GPR43 KO mice (Ge et al., 2008).

Thus SCFA may have a role in energy homeostasis via GPR41 activation to stimulate leptin expression, and may suppress NEFA release via GPR43 activation to inhibit lipolysis.

1.7.3 GPR41 and GPR43 and gut peptides

The discovery that GPR41 and GPR43 receptors are co-localised with the anorexigenic ‘ileal brake’ gut peptide PYY in enteroendocrine L-cells in the rodent and human colon (Karaki et al., 2006, Karaki et al., 2008, Tazoe et al., 2009) indicates luminal colonic SCFA may have a role in appetite regulation. In addition to expressing and releasing PYY, L-cells also produce the anorexigenic gut peptides GLP-1 and Oxm, although
studies have not determined if these gut peptides are co-localised with GPR41 and GPR43 receptors.

When administered in vitro to a vascularly infused rat colon lumen, solutions of propionate, butyrate, but not acetate stimulate PYY secretion (Plaisancié et al., 1996), with no significant release of GLP-1 (Plaisancié et al., 1995). In vivo, luminal administration of SCFA solutions in live rats increases subsequent blood PYY concentrations and reduces upper GI motility, an effect reproduced with a PYY infusion (Cherbut et al., 1998). Similarly, in live large white pigs, ileal infusion of an SCFA solution results in a significantly higher postprandial PYY release than infusing with saline, with no significant effect on GLP-1 (Cuche and Malbert, 2000) (Figure 1.7).

Figure 1.7. Postprandial plasma a) PYY and b) GLP-1 concentrations during ileal infusion of SCFA (—○—) or saline (—△—). PYY AUC was significantly higher with SCFA treatment (p<0.05). GLP-1 was not significantly influenced. Results shown as mean with error bars representing the SEM (n=5). From (Cuche and Malbert, 2000)

A limited number of infusion investigations have been carried out in human volunteers, although these studies were less conclusive.

Caecal infusion of a mixed SCFA solution (54 and 90 mmol/180ml, 70% acetic, 20% propionic, 10% butyric acid) delivered over 1 h to human volunteers did not significantly influence subsequent GLP-1 or PYY concentrations relative to saline, although proximal gastric tone was significantly decreased, suggesting an ileal brake effect (Ropert et al., 1996). However the study design was flawed with infusions delivered in a non-random order, and at different times of the day, thus confounding
interpretation of these data. Saline was infused on the morning of day 1, 90 mmol SCFA solution infused on the afternoon of day 1, and 54 mmol SCFA solution infused on the morning of day 2.

In a separate 4-way crossover study, acetate solution was administered per rectum (60 mmol / 300ml) and intravenously (i.v.) (20 mmol Na acetate / 100ml) and compared to per rectum water (300ml) and i.v. saline (100ml). Repeated measures ANOVA analysis found acetate treatment and rectal delivery significantly increased plasma GLP-1 (p=0.01 and p<0.01 respectively) and PYY (p<0.0001 for both). A significant treatment x time interaction for postprandial PYY (p<0.001) but not GLP-1 levels was found by two-way repeated measures ANOVA. The authors concluded these results indicate colonic fermentation may stimulate gut hormones via distension of the colon and increasing SCFA concentrations (acetate vs. saline) (Freeland and Wolever, 2010). However per rectum infusions are not replicating events in the colon, therefore this data should be interpreted with caution when considering fermentable NDC effects.

Overall from these studies, luminal SCFA appear to activate PYY (Cherbut et al., 1998, Cuche and Malbert, 2000, Freeland and Wolever, 2010, Plaisancié et al., 1995, Plaisancié et al., 1996) and possibly GLP-1 (Freeland and Wolever, 2010) release, implicating SCFA may have a role in regulating appetite. As sensitivity to stimulation in vitro is in the order propionate ≥ butyrate >> acetate for PYY secretion (Plaisancié et al., 1996) GPR41 is implicated as the responsible receptor for modulating these effects.

1.7.4 SCFA in the GIT and intestinal motility

Both in vitro and in vivo evidence indicates luminal SCFA influence intestinal motility via interactions with the lumen mucosa, suggesting a possible role for SCFA in eliciting the ileal brake effect.
In animal models propionate and butyrate dose-dependently elicit phasic and tonic contractions when applied *in vitro* to the lumen colon muscle strips isolated from rats, while no contractions are observed with acetate (Mitsui et al., 2005, Yajima, 1988). These contractions are accompanied by an increase in electrical current (indicating colonic electrical activity) when the submucosal nerve is intact (Yajima, 1988).

Propionate-induced effects are attenuated by Ach and 5-HT$_4$ receptor antagonists and cyclooxygenase (COX) inhibitor, implicating cholinergic motor neurones, 5-HT$_4$ receptors and COX products (e.g prostglandins) as eliciting these effects (Mitsui et al., 2005). Tazoe and colleagues hypothesise GPR41 rather than GPR43 are responsible for mediating these effects as the sensitivity of responses appear to occur in the order propionate ≥ butyrate >> acetate (Tazoe et al., 2009).

Luminal administration of SCFA solutions *in vivo* to the proximal colon in conscious rats significantly accelerated colonic transit. This effect disappeared with prior vagotomy or pre-treatment with a 5-HT$_3$ antagonist, indicating 5-HT$_3$ receptors and sensory neurons in the colonic mucosa mediated these effects (Fukumoto et al., 2003). Ileal infusion of an SCFA solution into conscious large white pigs decreased the amplitude but increased the frequency of distal and terminal contractions compared to a infusion of saline (Cuche and Malbert, 2000).

SCFA solutions have also been reported to alter GIT motility in human volunteers. Ileal infusion of an SCFA solution (66% acetate, 24% propionate, 10% butyrate) over 1 h significantly increased the number of motor events following infusion than when air or saline were administered (Kamath et al., 1988). In a separate study, proximal gastric tone was significantly decreased by ileal SCFA administration (Ropert et al., 1996).

However, infusion of an SCFA solution (67.5% acetate, 17.2% propionate, 9.2% butyrate, 1.9% isobutyrate, 3% isovalerate, 1.2% valerate) into the duodenum of
intubated volunteers (n=8) over 12 h, did not differ significantly alter the motility index as compared to saline. The authors concluded SCFA do not alter jejunal motility in contrast to previous work (Masliah et al., 1992).

Overall from these studies, it appears that luminal SCFA influence gastrointestinal motility in the ileum and colon, and may therefore have a role in the ileal brake effect. This may be via interaction with either GPR41 and/or GPR43 and secretion of PYY. Although GPR43 is present in higher levels in the GIT than GPR41, GPR41 is implicated as being the responsible receptor for modulating these effects as sensitivity to stimulation in vitro is in the order propionate ≥ butyrate >> acetate (Mitsui et al., 2005, Tazoe et al., 2009, Yajima, 1985, Yajima, 1988).

1.8 SCFA and appetite

As outlined in section 1.7.3, the SCFA-activated receptor GPR43 is co-localised luminally in enteroendocrine L-cells alongside the anorexigenic gut hormone PYY, which is suggestive of a role for SCFA in appetite regulation. The effect of SCFA on appetite have been investigated both via oral provision of SCFA (see section 1.8.1) and by colonic delivery of SCFA via fermentable NDC such as inulin-type fructans (see section 1.8.2) as discussed below.

1.8.1 Oral provision of SCFA and appetite

A limited number of studies investigating the effect of orally ingested SCFA, in particular acetate (delivered as vinegar) (Hlebowicz et al., 2008, Mettler et al., 2009, Ostman et al., 2005) and propionate (delivered as sodium (Na) propionate) (Frost et al., 2003, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995) on subsequent satiety have been published, as summarised in Table 1.5.
Table 1.5. Summary of human clinical studies investigating the acute effects of oral supplementation with acetate or propionate on appetite.  

<table>
<thead>
<tr>
<th>Study</th>
<th>SCFA</th>
<th>Study design &amp; participants</th>
<th>Test meals</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Liljeberg et al., 1995)</td>
<td>Prop</td>
<td>6-way crossover study Healthy (n=11) 6 F, 5 M</td>
<td>Bread (supplying 50 g CHO) as part of breakfast: - wholemeal bread (PL) or - wholemeal bread with added sourdough, lactic acid, Ca-lactate or Na-propionate (low &amp; high conc, ~15 mmol &amp; 45 mmol acetate)</td>
<td>• Postprandial satiety: Prop bread sig ↑ AUC (0-180 min) (high dose) and sig ↑ score at 45 min (low &amp; high conc), 95 min (high conc), 120 min (low conc) relative to PL • Acceptability: Sig ↓ score for prop (high conc), lactic acid, sourdough</td>
</tr>
<tr>
<td>(Liljeberg and Bjorck, 1996)</td>
<td>Prop</td>
<td>3-way crossover study Healthy (n=11) 8 F, 4 M</td>
<td>Bread (supplying 50 g CHO) given as part of breakfast (1585 kJ total): - wholemeal bread (PL) or - wholemeal bread with added lactic acid or Na-propionate (high dose only ~ 45 mmol)</td>
<td>• Postprandial satiety: Prop bread sig ↑ satiety AUC (0-180 min) and sig ↑ score at 15 &amp; 45 min relative to PL &amp; lactic acid bread (using single bipolar scale)</td>
</tr>
<tr>
<td>(Frost et al., 2003)</td>
<td>Prop</td>
<td>2 x 2 crossover study Healthy (n=10) 6 F, 4 M</td>
<td>Tomato pasta meal: - with or without fat (30 g sunflower oil &amp; 3 g (~31 mmol) Na propionate) - with or without psyllium viscous fibre</td>
<td>• Postprandial appetite: No sig effect on VAS ratings • GLP-1: Psyllium + fat sig ↑ IAUC relative to PL &amp; psyllium alone • Ad libitum intake 4 h postprandially: No sig effects • Nausea ratings: Fat containing meals sig ↑ ratings</td>
</tr>
<tr>
<td>(Ostman et al., 2005)</td>
<td>Acetate (vinegar)</td>
<td>Dose response crossover study Healthy (n=12) 10 F, 2 M</td>
<td>Bread (supplying 50 g CHO) for breakfast: - white bread (PL) or - white bread soaked in 18, 23 or 28 g vinegar (= 18, 23, 28 mmol acetate)</td>
<td>• Postprandial satiety: Dose dependent ↑ in satiety. 28 mmol sig ↑ score at 30, 90, 120 min &amp; sig ↑ AUC (0-120) (using single bipolar scale)</td>
</tr>
<tr>
<td>(Hlebowicz et al., 2008)</td>
<td>Acetate (vinegar)</td>
<td>4 way crossover study Healthy (n=13) 7 F, 6 M</td>
<td>Bread (supplying 50 g CHO) for breakfast: - white wheat bread (PL) or - white or wholemeal or wholegrain wheat bread in 28 g white wine vinegar (= 28 mmol acetate)</td>
<td>• Postprandial satiety: Vinegar + wholegrain sig ↑ AUC compared to other treatments &amp; sig ↑ score at 15, 30, 45, 60, 90 min compared to PL &amp; vinegar + white bread (using single bipolar scale)</td>
</tr>
<tr>
<td>(Metzler et al., 2009)</td>
<td>Acetate</td>
<td>2 x 2 crossover study Healthy (n=27) 18 F, 9 M</td>
<td>Milk rice pudding and glucose drink (supplying 75 g CHO) for breakfast: - with or without added 28 mm mol acetic acid - with or without added cinnamon</td>
<td>• Postprandial satiety: IAUC did not differ between treatments. Main effect of acetic acid approaching sig (p=0.064), sig ↑ score at 15 &amp; 30 min for cinnamon + acetic acid (using single fixed point scale)</td>
</tr>
</tbody>
</table>

1 All studies were carried out in healthy volunteers the morning after an overnight fast, 2 any values quoted are the mean values, 3 Prop = propionate
First considering propionate and effects on appetite, two studies from the same group examined the acute effects of a bread baked with added Na propionate when consumed as part of a mixed meal for breakfast. These studies report Na propionate significantly increases rated satiety, particularly when a high dose of propionate is used (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995). The actual dose was not reported by the authors, but is calculated as 15 and 45 mmol for low and high dose breads respectively.

However, conclusions regarding effects on satiety were solely based on subjective data generated from a single bipolar rating scale, and no quantitative assessment of appetite was made. The rating scale used fairly confusing language, with one extreme representing extreme hunger (score -10) and the other extreme representing extreme satiety (score +10). Although satiety and hunger could be considered to lie at polar opposite ends of appetitive sensation scales, satiety is not simply the absence of hunger (Mattes et al., 2005) and it is therefore confusing and inappropriate to have them on the same rating scale.

Furthermore in both studies, the statistical tests used were inappropriate for the data. Wilcoxon signed ranks test was used for comparisons at each timepoint (rather than repeated measures ANOVA for comparisons over time) and to compare the AUC (rather than repeated measures ANOVA or Friedman’s ANOVA to compare >2 groups). It is therefore difficult to comment on the validity of these conclusions.

By contrast, when mixed into a pasta meal consumed at breakfast, Na propionate (3g, ~ 31 mmol) combined with polyunsaturated fat (PUFA, provided as 30 g sunflower oil) did not significantly alter subjective appetite ratings, or EI of an ad libitum test meal served 4 h postprandially (Frost et al., 2003). There was however a significantly increased GLP-1 IAUC and significantly delayed gastric emptying with Na propionate treatment. A significant increase in nausea was also observed. However as PUFA was
delivered alongside the propionate, it is not possible to determine if the observed effects are attributed to the added PUFA, propionate, or both and if the higher energy content of the PUFA/propionate test meals had an effect.

Looking now at effects of acetic acid, a dose-response increase in satiety ratings were reported following the provision of bread soaked with 0 g (PL), 18 g, 23 g and 28 g vinegar (equivalent to 0, 18, 23 and 28 mmol acetic acid) for breakfast (Table 1.6), with a significantly higher AUC following the 28 g dose relative to PL (Ostman et al., 2005).

Table 1.6. Satiety score AUC (0-120 minutes) following test breakfast of white bread (containing 50 g CHO) soaked in vinegar. Taken from (Ostman et al., 2005).

<table>
<thead>
<tr>
<th>Amount of vinegar (g)</th>
<th>N</th>
<th>Satiety Score AUC Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>210$^{a}$</td>
<td>45</td>
</tr>
<tr>
<td>18</td>
<td>10</td>
<td>363$^{ab}$</td>
<td>38</td>
</tr>
<tr>
<td>23</td>
<td>11</td>
<td>435$^{ab}$</td>
<td>75</td>
</tr>
<tr>
<td>28</td>
<td>11</td>
<td>512$^{b}$</td>
<td>105</td>
</tr>
</tbody>
</table>

$^{ab}$ Mean values marked with different letters significantly different (Wilcoxon signed-ranks test).

By contrast, a crossover study by the same group investigated the effects of vinegar (28g = 28 mmol acetic acid) and form of grain (i.e. refined, milled or wholegrain). The postprandial satiety score AUC was significantly higher following wholegrain + vinegar relative to the other 3 treatments, with none of the other treatments altering satiety (Hlebowicz et al., 2008). As wholegrain bread without added vinegar was not investigated it is not possible to conclude if the increased satiety arose from the wholegrain structure of the bread alone or it was an additive effect of the grain structure with the vinegar. As satiety scores did not differ between white bread with and without vinegar, vinegar did not appear to influence appetite in this study.
Both these previous studies (Hlebowicz et al., 2008, Ostman et al., 2005) again used the single rating bipolar scale to assess appetite used by the same group to investigate Na propionate effects (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995).

A 2 x 2 crossover study by a different group investigating the additive effects of acetic acid (28 mmol) and cinnamon mixed into a glucose drink and milk rice pudding found the satiety IAUC did not significantly differ between treatments, although a main effect of acetic acid approaching significance (p=0.064) was found (Mettler et al., 2009). Satiety was assessed with a single fixed point scale, rather than a variety of VAS, although the exact wording used on the questionnaire was not reported.

The effects of long-term supplementation with vinegar on anthropometric measures was reported in a recently published parallel study (Kondo et al., 2009) (Table 1.7).

| Table 1.7. Mean [SD] anthropometric and metabolic variables following daily consumption of a drink containing 0, 15 or 30ml apple cider vinegar (PL, Low, High respectively) for 12 wk. Taken from (Kondo et al., 2009). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | PL              | Week 0 Low      | High            | PL              | Week 12 Low     | High            |
| BW (kg)         | 74.2 [11.0]     | 74.9 [10.1]     | 73.1 [8.6]      | 74.6 [11.3]     | 73.7 [10.3]     | 71.2 [8.3]      |
| Waist circ (cm) | 90.2 [6.8]      | 90.8 [6.4]      | 90.5 [6.5]      | 90.4 [6.9]      | 89.4 [6.5]      | 88.6 [6.3]      |
| TAGs (mmol/l)   | 1.71 [0.50]     | 1.70 [0.60]     | 1.78 [0.55]     | 1.68 [0.67]     | 1.39 [0.58]     | 1.31 [0.54]     |
| Total chol (mmol/l) | 5.53 [0.82]    | 5.31 [0.82]    | 5.70 [0.83]    | 5.45 [0.87]    | 4.99 [0.82]    | 5.37 [0.83]    |
| LDL-C (mmol/l)  | 3.39 [0.83]     | 3.12 [0.73]     | 3.48 [0.74]     | 3.44 [0.73]     | 3.00 [0.76]     | 3.37 [0.77]     |
| HDL-C (mmol/l)  | 1.36 [0.24]     | 1.35 [0.32]     | 1.41 [0.34]     | 1.38 [0.26]     | 1.38 [0.34]     | 1.41 [0.33]     |

1 Values marked with different letters significantly different (by ANCOVA with post-hoc Bonferroni).
2 Mean values at week 12 marked # are significantly different from the value at baseline (week 0).
Healthy obese volunteers were randomised to PL (n=58, 50 completed), low dose (n=59, 54 completed), or high dose (n=58, 51 completed) treatment, consuming a beverage containing 0, 15 or 30 ml apple cider vinegar respectively (equivalent to 0, 12.5 and 25 mmol acetic acid) daily for 12 wk. At the end of the intervention, those in both the low and high dose treatment groups had a significantly lower BW, BMI, % body fat, waist and hip circumference, waist to hip ratio, visceral fat area (VFA), subcutaneous fat area (SFA) and TAG concentrations relative to PL (Table 1.7 shows selected data). Additionally total fat area and systolic blood pressure were significantly lower in the high dose group relative to PL.

From the above studies, there is some evidence that SCFA may acutely effect appetite. However there were a number of methodological weaknesses, particularly in terms of limitations in the methods to investigate appetite, and the use of inappropriate statistical analyses. Studies to date have not measured quantitative effects, effects on gut peptides or subjective effects using validated VAS, except in one study (Frost et al., 2003), however PUFA was delivered alongside the propionate in that study. The potentially confounding effects of palatability on appetite have also been over-looked. It is therefore difficult to draw firm conclusions regarding the effect of oral SCFA on subsequent appetite and satiety, although these findings warrant further investigation.

1.8.2 Colonic SCFA via NDC and appetite

There is growing evidence to indicate DF and other NDC may have a role in BW regulation, with a number of studies suggesting intake of DF and/or wholegrains are inversely associated with BW and/or BMI (Burton-Freeman, 2000, Delzenne and Cani, 2005, Howarth et al., 2001, Koh-Banerjee et al., 2004, Koh-Banerjee and Rimm, 2003, Melanson et al., 2006, Pereira and Ludwig, 2001, Slavin, 2005, Harland and Garton,
DF and other NDC may therefore potentially have a role in the prevention and treatment of obesity. Furthermore various studies have demonstrated DF may enhance satiety and reduce appetite, thus helping to control subsequent food intake (Berti et al., 2005, Burton-Freeman, 2000, Delzenne and Cani, 2005, Howarth et al., 2001, Pereira and Ludwig, 2001, Slavin, 2005). However the mechanisms by which this may occur are not fully understood. It is possible that SCFA generated during colonic fermentation may mediate effects on appetite via interaction with the SCFA activated receptors GPR41 and GPR43 in colonic enteroendocrine L-cells, and GPR41 in adipocytes to modulate PYY and leptin production respectively (see sections 1.7.2 and 1.7.3).

Focusing on the fermentable NDC inulin-type fructans, which as well as being classified as a DF are also prebiotics (see section 1.5.2), initial rodent model studies gave evidence that inulin-type fructans may enhance satiety (Cani et al., 2006b, Cani et al., 2004, Cani et al., 2007, Daubioul et al., 2002, Kok et al., 1998, Cani et al., 2005). Chronic OF supplementation in rodents significantly decreases EI (Cani et al., 2004, Cani et al., 2005, Cani et al., 2007), suppresses BW gain (Cani et al., 2005, Cani et al., 2006b, Cani et al., 2007) (although not in all cases (Kok et al., 1998, Cani et al., 2004)) and suppresses fat mass gain (Cani et al., 2004, Cani et al., 2005, Cani et al., 2006b, Cani et al., 2007), increases proximal colon GLP-1 (7-36) amide (Kok et al., 1998, Cani et al., 2004, Cani et al., 2005, Cani et al., 2007), increases portal plasma GLP-1 (7-36) (Cani et al., 2004, Cani et al., 2005, Cani et al., 2007) and GIP (Kok et al., 1998) concentrations, reduces plasma ghrelin concentrations (Cani et al., 2004) and increases the caecum mass (Kok et al., 1998, Cani et al., 2004, Cani et al., 2007). Most studies examined OF (low DP inulin-type fructan), with few investigating...
higher DP inulin-type fructans (e.g. inulin HP). Supplementing rats diets with Synergy 1 (mixture of low and high DP inulin-type fructans) resulted in significantly suppressed BW gain (Daubioul et al., 2002) and fat mass gain (Cani et al., 2004), reduced EI (Daubioul et al., 2002, Cani et al., 2004), reduced proximal colon GLP-1 (7-36) concentrations (Cani et al., 2004) and reduced caecum mass (Cani et al., 2004). Furthermore, supplementation with inulin HP (high DP inulin-type fructan) significantly suppressed BW gain and increased caecum mass, but did not influence proximal colon GLP-1 (7-36) concentrations or fat mass gain (Cani et al., 2004).

Following on from this promising data from animal models, a few human studies have investigated acute (summarised in Table 1.8) and more have investigated chronic (summarised in Table 1.9) effects of supplementing with inulin or OF on appetite and/or anthropometries. The results from these are variable and difficult to draw firm conclusions from, partly due to study limitations.

As summarised in Table 1.8, acute supplementation with Raftilose P95 (a low DP OF) found no significant influence of OF on satiety ratings nor ad libitum food intake (Peters et al., 2009). The satiety ratings were completed in a free-living setting rather than under controlled conditions. It is therefore possible that participants may not have complied with the protocol, for example eating extra foods, and external conditions could have varied in between treatments potentially confounding the results.

Furthermore acute supplementation with inulin HP (high DP inulin-type fructan) significantly reduced 24 h EI relative to PL, although satiety ratings were not significantly altered (Archer et al., 2004), and the investigators did not assess effects on ad libitum intake in a controlled setting.
Table 1.8. Summary of human clinical studies investigating the acute effects of supplementation with inulin-type fructans on appetite.\(^1,2\)

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design &amp; participants</th>
<th>Inulin-type fructan used</th>
<th>Test meals</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Archer et al., 2004)</td>
<td>3-way crossover</td>
<td>Orafti inulin HP</td>
<td>Sausage patties in English Muffin matched for mass, protein &amp; avail CHO but not E, either: Full fat patty (PL) - Inulin patty (24 g inulin) - Lupin kernel fibre patty (24 g fibre)</td>
<td>• Satiety ratings: single 150 mm continuous scale measured at 0-225 min • 24h food intake: 24 h free-living intake weighed &amp; recorded</td>
<td>• Satiety ratings: Lupin fibre IAUC sig higher than PL, IAUC for inulin &amp; PL not sig different • 24 h food intake: 24 h EI sig lower following inulin compared to PL NB: measurements after breakfast were free-living</td>
</tr>
<tr>
<td></td>
<td>n=33 (33M)</td>
<td>DP range 10-60 DP(_{ave}) ~ 25 24 g used as a fat replacer in study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI: 20.5-38.7 (mean 27.4) kg/m(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Peters et al., 2009)</td>
<td>4-way crossover</td>
<td>Orafti Rafitolose P95</td>
<td>Meal replacement bar matched for E (~190kcal) &amp; protein eaten on 2 consecutive days (at 08.00 on both days &amp; 16.30 on day 1 only): Commercially available oat bar (PL) - Barley bar: 8 g barley replacing oats - FOS bar: 8 g OF replacing oats - Barley + OF bar: 8 g barley &amp; 8 g FOS replacing oats</td>
<td>• Satiety ratings: Various 60 mm EVAS completed every 0.5 h at 0-660 min &amp; 0-540 min on days 1 &amp; 2 • Food intake: Ad libitum buffet meal served at 240 min (12.00) on both days</td>
<td>• Satiety ratings: No sig influence of treatment on VAS AUC • Food intake: Did not differ between treatments</td>
</tr>
<tr>
<td></td>
<td>n=21 (5M, 16F)</td>
<td>DP range 2-8 DP(_{ave}) ~ 4 8 g added to meal replacement bar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI: 21.7-30.3 (mean 25.9) kg/m(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Perrigue et al., 2009)</td>
<td>2 x 2 way crossover</td>
<td>Inulin (exact product not specified) DP not specified</td>
<td>Breakfast bar eaten at 08.00 then at 10.00 (T=0 min) consumed preload: Low E yogurt (180 kcal) - High E yogurt (440 kcal) - Low E yogurt + 6 g inulin (180 kcal) - High E yogurt + 6 g inulin (440 kcal) - Equal vol orange juice (180 kcal) - No beverage / yogurt (PL)</td>
<td>• Satiety ratings: Various 100 mm EVAS completed every 20 min at 0-120 min • Food intake: Ad libitum buffet meal served at 120 min (12.00)</td>
<td>• Satiety ratings: Fullness sig ↑ by both high E &amp; low E + inulin yogurts compared to low E no inulin yogurt. • Food intake: Both high E &amp; low E + inulin yogurts sig ↓ EI compared to PL, but total intake including preload sig ↑ with high E yogurt</td>
</tr>
<tr>
<td></td>
<td>n=38 (18M, 20F)</td>
<td>6 g added to yogurt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BMI: 18-30 (mean 23.4 (M), 21.4 (F)) kg/m(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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\(^1\) All studies were carried out in the morning after an overnight fast.

\(^2\) EVAS = electronic VAS
Similarly consumption of a yogurt containing inulin (inulin used not specified) significantly decreased subsequent *ad libitum* EI in a controlled setting relative to “no preload” (PL), although the overall EI including the preload and the *ad libitum* intake was more than the “no preload” condition (Perrigue et al., 2009). Fullness ratings were significantly increased by yogurt containing inulin relative to yogurt with no added inulin (Perrigue et al., 2009), although it was unclear from the paper if hunger and the desire to eat had been significantly altered by inulin.

A greater number of chronic supplementation studies using OF (e.g. Raftilose P95) or mixed DP inulin-type fructans (e.g. Synergy-1) have been published investigating effects on appetite (summarised in Table 1.9), again with variable results and study limitations. There are no published studies investigating high DP inulin (e.g. inulin HP).

Looking first at chronic effects of OF on subjective and quantitative appetite measures. In a 2-way crossover study, daily supplementation with 16 g Raftilose P95 for 2 wk was found to significantly increase satiety ratings after breakfast and dinner, and reduce hunger and prospective consumption ratings following dinner compared to PL. *Ad libitum* EI was significantly reduced at breakfast and lunch but not dinner, and mean daily EI was significantly lower during the OF supplementation period (Cani et al., 2006a). However as the preload test meals were provided *ad libitum* rather than being a fixed, standardised preload, data generated postprandially is meaningless. It further appears from the paper that conditions were not controlled as participants were free to leave and eat (provided they noted it down) in between each *ad libitum* meal (at breakfast, lunch and dinner).
Table 1.9. Summary of human clinical studies investigating the chronic effects of supplementation with inulin-type fructans on appetite.¹⁻³

<table>
<thead>
<tr>
<th>Study</th>
<th>Inulin-type fructan</th>
<th>Participants, study design &amp; protocol</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Fiche et al., 2003)</td>
<td>Orafti Raftilose P95</td>
<td>Single-blind 2-way crossover Patients with GERD (n=9, 5M/4F) given low residue diet daily for 7 d with either: - 3 x 6.6g sucrose (PL) - 3 x 6.6g OF (washout ≥ 3 wk)</td>
<td>MTT at end of each intervention (324 kcal including 6.6 g PL or OF): • Postprandial plasma: GLP-1, PYY &amp; CCK, analysed from 0-240 min, every 15 min for 1st hour then every 30 min thereafter • Breath H₂: Collected at same times as VAS</td>
<td>• Postprandial plasma: OF sig ↑ GLP-1 AUC relative to PL. No sig changes for PYY &amp; CCK • Breath H₂: Sig ↑ at baseline &amp; postprandially with OF compared to PL</td>
</tr>
<tr>
<td>(Cani et al., 2006a)</td>
<td>Orafti Raftilose P95</td>
<td>Single-blind 2-way crossover Volunteers (n=10, 5M/5F, BMI 18.5-27.4 kg/m²) supplemented daily for 2 wk with either: - 2 x 8g DM (PL) - 2 x 8g OF</td>
<td>MTT at end of each intervention: • Satiety ratings: Various 100 mm VAS completed every 0.5 h after breakfast (0-240 min), lunch (240-540 min) &amp; dinner (540-840 min) • Food intake: Ad libitum buffet meal served at breakfast, lunch &amp; dinner (0, 240, 540 min respectively) • Mean daily intake: Food diary completed during supplementation period</td>
<td>• Satiety ratings: OF effects relative to DM: - After breakfast: sig ↑ satiety. No effect on hunger, fullness, prospective food consumption - After lunch: No sig differences - Dinner: sig ↑ satiety, sig ↓ hunger &amp; prospective food consumption • Food intake: Sig lower EI with OF compared to DM at breakfast &amp; lunch &amp; for overall 24 h period. Intake at dinner not sig different • Mean daily intake: Sig ↓ EI with OF than DM</td>
</tr>
<tr>
<td>(Whelan et al., 2006)</td>
<td>Mixture of OF and inulin, no further details given</td>
<td>Double-blind 2-way crossover Volunteers (n=11, 5M/6F, BMI 22.0-25.0 kg/m²) consumed daily for 2 wk as sole nutrition source to meet E requirements: - Standard enteral formula, Nestle Nutren 1.0 (PL) - Formula with added pea fibre, inulin &amp; OF, Nestle Nutren Fiber</td>
<td>• Satiety ratings: Various 100 mm VAS completed hourly on days 1, 4, 8, 11 during supplementation period from 08.00-21.00 h • Body weight: Measured at start &amp; end of each supplementation period • Intake of formula: Actual intake in comparison to prescribed intake monitored using compliance diary</td>
<td>• Satiety ratings: Fibre formula sig ↑ mean &amp; minimum fullness, minimum satiety &amp; mean hourly ratings for fullness • Body weight: Weight loss occurred during both supplementation periods but did not differ between standard &amp; fibre formulas • Food intake: Mean intake of standard &amp; fibre formula was 1988 &amp; 1937 ml/d, compared to mean prescribed intake 2272 ml/d. Mean intake of FOS &amp; pea fibre was 9.8 &amp; 18.3 g/d with fibre formula (not significant).</td>
</tr>
</tbody>
</table>

¹ MTT carried out in the morning after an overnight fast, ² any values quoted are the mean values.
³ DM = Maltodextrin, GERD = Gastroesophageal reflux disease, MTT = meal tolerance test, OF = Oligofructose
### Table 1.9 Cont. Chronic effects of inulin-type fructans on appetite.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inulin-type fructan</th>
<th>Participants, study design &amp; protocol</th>
<th>Outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cani et al., 2009)</td>
<td>Orafti Synergy 1 Mixure of low &amp; high DP (OF &amp; inulin)</td>
<td>Double-blind parallel study Volunteers (n=10, 5M/5F, mean BMI 21.6 [SD 0.99] kg/m²) consumed daily for 12 wk either: - 2 x 8g DM (PL) - 2 x 8g OF</td>
<td>Study day at start &amp; end of 2 wk: - Satiety ratings: Various 100 mm VAS completed at 0, 30, 60, 120 &amp; 180 min - Food intake: Ad libitum buffet breakfast - Postprandial capillary plasma: Glucose, GLP-1, PYY, GIP, PP, insulin analysed at 0, 10, 30, 60, 120 min - Breath H2: Collected at same times as VAS - 24 h intake: Recorded in food diary on each study day</td>
<td>- Satiety ratings: No sig differences between OF &amp; DM over time relative to baseline - Food intake: No sig influence of treatment - Postprandial capillary plasma: OF sig ↑ PYY &amp; GLP-1 at T=10 min &amp; sig ↑ GIP over time relative to baseline &amp; sig ↓ AUC glucose - Breath H2: Baseline &amp; postprandial AUC sig ↑ with OF compared to DM - 24 h intake: No sig influence of treatment</td>
</tr>
<tr>
<td>(Parnell and Reimer, 2009)</td>
<td>Orafti Rafilose P95 DP range 2-8 DPave ~ 4</td>
<td>Double-blind parallel study Volunteers (n=48, 9M/39F, mean BMI &gt;25 kg/m², n=39 completed) consumed daily for 2 wk either (matched for E levels): - 7.89g DM (PL) - 21g OF</td>
<td>MTT at start &amp; end of study: - Satiety ratings: Various 100mm VAS completed when blood collected - Postprandial plasma: Active ghrelin &amp; GLP-1, PYY, GIP, insulin, leptin, glucose analysed at 0, 15, 30, 60, 120, 240, 360 min - Food intake: 3 d food diary recorded at baseline &amp; every 3 wk thereafter - Anthropometrics: Measured BW &amp; assessed lean &amp; fat mass &amp; central mass every 3 wk (with DEXA)</td>
<td>- Satiety ratings: No sig influence of treatment - Postprandial capillary plasma: OF sig ↑ PYY, ↓ ghrelin &amp; ↓ leptin AUC relative to baseline. No sig effect on GLP-1, GIP, glucose, insulin AUC. Sig month x diet interaction found for PYY, insulin, ghrelin AUC (2 factor ANOVA) - 24 h intake: OF sig ↓ EL, CHO &amp; protein relative to DM at 6 wk, and sig ↓ EL relative to baseline (but not DM) at 12 wk - Anthropometrics: At 12 wk, OF sig ↓ BW, fat mass &amp; central fat</td>
</tr>
<tr>
<td>(Antal et al., 2008)</td>
<td>Jerusalem artichoke concentrate containing OF (in Hungarian)</td>
<td>Parallel study Volunteers (n=51, 16M/35F, obese) consumed daily for 12 wk either (isocaloric): - low E diet (PL) - low E diet + 14g OF</td>
<td>At start &amp; end of study (difficult to determine exact methods as paper in Hungarian): - Satiety ratings: Fullness &amp; hunger sensations - Anthropometrics: BMI &amp; % body fat</td>
<td>- Satiety ratings: OF ↓ hunger sensation (uncertain if significant) - Anthropometrics: OF sig ↓ BMI &amp; % body fat</td>
</tr>
</tbody>
</table>

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1. MTT carried out in the morning after an overnight fast, 2. any values quoted are the mean values.
3. DM = Maltodextrin, GERD = Gastroesophageal reflux disease, MTT = meal tolerance test, OF = Oligofructose. 4. Paper was in Hungarian, only abstract translated into English.
By contrast, Parnell and Reimer found satiety ratings and EI were not significantly altered by daily supplementation for 12 wk with 21 g OF (Raftilose P95) relative to PL (Parnell and Reimer, 2009), contradicting the results from Cani and colleagues (2006). However this parallel study, while having a fairly high sample size (n=48), was not sufficiently powered to assess subjective appetite effects being far lower than the 120 participants per group suggested by Flint and colleagues (2000).

Finally, a recent parallel study conducted by a Hungarian group reported supplementation with 14 g Jerusalem Artichoke concentrate (containing OF) daily for 12 wk reduced rated hunger and significantly reduced BMI and % body fat levels (Antal et al., 2008). However the paper was difficult to critically review as it was in Hungarian, it was unclear if effects on hunger were significant.

Looking now at effects of mixed inulin-type fructans on subjective and quantitative appetite measures, Whelan and colleagues found supplementation for 2 wk significantly increased daily mean and minimum fullness and minimum satiety ratings in a crossover study (Whelan et al., 2006). However it was not clear from the paper if appetite VAS were completed in controlled or free-living conditions and as pea fibre was also included in the active treatment, observed results may not have been solely due to the inulin-type fructans.

In a separate parallel study Cani found supplementation with Synergy-1 did not influence postprandial subjective appetite ratings following a meal tolerance test preload (Cani et al., 2009). However this study was flawed in design. It was vastly underpowered (only 5 participants per treatment) and the meal tolerance test preload was *ad libitum* rather than fixed which confounds interpretation of postprandial data.
Studies have also found contradictory evidence regarding effects on gut peptides. Raftilose P95 supplementation for 7 d significantly increased postprandial plasma GLP-1 AUC with no effect on PYY or CCK in GERD patients (Piche et al., 2003), while in healthy volunteers supplementation for 2 wk significantly increased the PYY AUC with no influence on GLP-1 (Parnell and Reimer, 2009).

Supplementation with Orafti Synergy 1 was also found to increase the postprandial GLP-1 and PYY response (Cani et al., 2009) however as noted above this study had a number of design flaws. The study was underpowered, and postprandial measures were made following an *ad libitum* rather than fixed meal tolerance test preload. Furthermore significant results for plasma data were only found when reported relative to baseline, and capillary rather than venous plasma was collected, the use of which has not been validated for gut peptide analysis (although authors did note this and values were reported to be comparable to venous values).

Less compelling is anthropometric and food intake data from chronic intervention studies, although these were not the main outcomes of interest for all studies except two (Antal et al., 2008, Parnell and Reimer, 2009), so these studies were not designed specifically to explore these outcomes. None of the studies except two (Antal et al., 2008, Parnell and Reimer, 2009) found a significant influence of inulin-type fructan supplementation on BW post intervention (Alles et al., 1999, Antal et al., 2008, Davidson et al., 1998, Forcheron and Beylot, 2007, Giacco et al., 2004, Jackson et al., 1999, Luo et al., 1996, Luo et al., 2000) or on food intake during the intervention (Alles et al., 1999, Giacco et al., 2004, Pedersen et al., 1997, Forcheron and Beylot, 2007). The study by Antal and colleagues reported daily supplementation with 14 g Jerusalem Artichoke concentrate (containing OF) for 12 wk significantly reduced BMI and % body fat levels (Antal et al., 2008).
Therefore overall, while a number of studies have investigated the influence of supplementation with inulin-type fructans on markers of appetite in humans, the results to date are contradictory making it difficult to draw firm conclusions. This is in part due to variable dosages and differing choices of inulin-type fructan supplements and various confounding factors introduced by flawed study designs. Furthermore few studies have investigated the high DP inulin-type fructans specifically.

1.9 SCFA and metabolic response

The idea SCFA may have a role in influencing metabolic mechanisms is strengthened by the discovery of the SCFA activated receptors GPR41 and GPR43, possibly influencing lipolysis and leptin release and ileal brake mechanisms (summarised in section 1.7). Effects of SCFA on the metabolic response and associated mechanisms have been investigated both via oral provision of SCFA (see section 1.9.1) and colonic delivery of SCFA via fermentable NDC such as inulin-type fructans (see section 1.9.2) as discussed below.

1.9.1 Oral SCFA and metabolic response

There is increasing evidence to indicate that in addition to possibly influencing appetite, oral SCFA ingestion may blunt glycaemic and insulinaemic responses.

Early evidence for this was the finding that intragastric co-administration of a starch and acetic acid solution to rats (n=6) significantly blunted the glycaemic response relative to no acetic acid (Ebihara and Nakajima, 1988). Furthermore, consumption of a sucrose drink containing 60 ml strawberry vinegar, by human volunteers (n=7) lowered the postprandial insulin AUC by 20 % relative to PL (no added vinegar), although no differences in the glycaemic response were found (Ebihara and Nakajima, 1988).
Since then, a number of studies investigating the acute effects of oral SCFA supplementation have been conducted, primarily with acetate (supplied as vinegar) and propionate and also lactate. Most studies investigated effects on postprandial glycaemia and insulinaemia, but some considered other metabolic parameters.

Table 1.10 summarises findings from these studies. Evidence to date appears to support the idea that oral supplementation with acetate (given as vinegar) and propionate acutely influences postprandial insulin responses in healthy volunteers (Torsdottir et al., 1992, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Darwiche et al., 2001, Frost et al., 2003, Johnston et al., 2004, Ostman et al., 2005, Johnston and Buller, 2005, Ebihara and Nakajima, 1988).

The picture for the glycaemic response is less clear with some studies suggesting oral SCFA blunt the glycaemic response (Liljeberg and Bjorck, 1998, Torsdottir et al., 1992, Brighenti et al., 1995, Liljeberg and Bjorck, 1996, Sugiyama et al., 2003, Ostman et al., 2005), while others did not find a significant effect in healthy volunteers (Ebihara and Nakajima, 1988, Darwiche et al., 2001, Johnston et al., 2004, Leeman et al., 2005, Hlebowicz et al., 2008) or were inconclusive (Liljeberg et al., 1995, Frost et al., 2003).
Table 1.10. Summary of human clinical studies investigating the acute effects of oral supplementation with SCFA on the metabolic response.\(^1\)\(^-\)\(^3\)

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design &amp; participants</th>
<th>Test meals</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ebihara and Nakajima, 1988)</td>
<td>2-way crossover study Healthy (n=7) 1 F, 6 M</td>
<td>CHO matched sucrose drink comprising either: - 53.6 g sucrose in 300 ml water (PL) or - 50 g sucrose + 60 ml strawberry vinegar (contains 6% acetic acid)</td>
<td>Glucose: Not significantly influenced by vinegar Insulin: AUC 20% lower with vinegar than PL (p value not reported)</td>
</tr>
<tr>
<td>(Todesco et al., 1991)</td>
<td>2-way crossover study Healthy (n=6) 3 F, 3 M</td>
<td>Bread (supplying 50 g CHO) for breakfast, either: - white bread (PL) or - white bread + 3.3 g (= 34.4 mmol) Na propionate</td>
<td>Glucose: AUC sig ↓ by 47.6% with propionate compared to PL</td>
</tr>
<tr>
<td>(Torsdottir et al., 1992)</td>
<td>3-way crossover study Healthy (n=8) 8 M</td>
<td>Isovolumetric &amp; isocaloric meals (280 kcal, 58% E from CHO): - Composite high glycaemic meal including bread and fruit juice (PL) - Composite meal + fresh vegetables - Composite meal + fermented vegetables (=SCFA)</td>
<td>Glucose &amp; insulin: IAUC for 0-120 min sig ↓ with added fresh &amp; fermented vegetables compared to PL (IAUC: PL &gt; fresh veg &gt; fermented) Somatostatin: Did not differ significantly between treatments</td>
</tr>
<tr>
<td>(Brighenti et al., 1995)</td>
<td>3-way crossover study Healthy (n=5) 1 F, 4 M</td>
<td>50 g CHO challenge (white bread) after eating 100 g lettuce dressed with either: - 10 g olive oil + 1.5 g NaCl (PL) - 10 g olive oil + 20 ml white wine vinegar - 10 g olive oil + 20 ml neutralised vinegar</td>
<td>Glucose: AUC sig ↓ with vinegar compared to neutralised vinegar &amp; PL (AUC: PL = neutralised vinegar &gt; vinegar) with CHO challenge Plasma acetate: Rose within 15 min following vinegar (regular &amp; neutralised), returning to baseline by 60 min</td>
</tr>
<tr>
<td>(Liljeberg et al., 1995)</td>
<td>6-way crossover study Healthy (n=11) 6 F, 5 M</td>
<td>Bread (supplying 50 g CHO) given as part of breakfast (1590 kJ total): - wholemeal bread (PL) or - wholemeal bread with added (^2)sourdough, lactic acid, Ca-lactate or Na-propionate (≈15 &amp; 45 mmol)</td>
<td>Glucose &amp; insulin: Glucose AUC for 0-45 min &amp; insulin AUC for 0-45 &amp; 0-95 min sig ↓ with propionate (both concs), sourdough &amp; lactic acid compared to PL. No sig differences 0-120 AUC Acceptability: Sig ↓ score for high conc (≈45 mmol) propionate, lactic acid &amp; sourdough breads</td>
</tr>
</tbody>
</table>

\(^1\)All studies were carried out in the morning after an overnight fast, \(^2\)any values quoted are the mean values

\(^3\)Studies with sourdough used lactate producing microbiota in the starter culture
Table 1.10 Cont. Acute effects of SCFA on metabolic response.1-3

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design &amp; participants</th>
<th>Test meals</th>
<th>Results</th>
</tr>
</thead>
</table>
| (Liljeberg and Bjorck, 1996) | 3-way crossover study Healthy (n=12) 8 F, 4 M | Bread (supplying 50 g avail starch) given as part of breakfast (1585 kJ total): - wholemeal bread (PL) or - wholemeal bread with added lactic acid or Na-propionate (−45 mmol) | **Glucose & Insulin:** AUC sig ↓ with propionate & lactic acid compared to PL for 0-45, 0-95 & 0-120 min. Propionate AUC sig lower than both PL and lactic acid  
**GER:** Sig delayed propionate compared to PL and lactic acid (assessed by paracetamol clearance) |
| (Liljeberg and Bjorck, 1998) | 2-way crossover study Healthy (n=10) 7 F, 3 M | Bread (supplying 50 g avail starch) given as part of breakfast (1554 kJ total): - white wheat bread (PL) or - white wheat bread soaked in 20 g white vinegar (≈ 20 mmol acetate) | **Glucose & Insulin:** ↓ GI & II with added vinegar (GI=64, II=65) compared to PL (GI & II =100). p values not given  
**GER:** Sig delayed with vinegar compared to PL (assessed by paracetamol clearance) |
| (Darwiche et al., 2001) | 2-way crossover study Healthy (n=9) 4 F, 5 M | Bread (supplying 50 g CHO) given as part of breakfast (energy not given): - white wheat bread (PL) or - white wheat bread with added Na-propionate | **Glucose:** No effect on AUC except 0-20 min sig ↓ with propionate  
**Insulin:** AUC sig ↓ with propionate at T = 0-20, 0-35 & 0-50 min  
**GER:** Sig delayed with propionate compared to PL (assessed by realtime ultrasonography) |
| (Frost et al., 2003) | 2 x 2 crossover study Healthy (n=10) 6 F, 4 M | Tomato pasta meal: - with or without fat (30 g sunflower oil [PUFA] & 3 g (~ 31 mmol) Na propionate) - with or without psyllium viscous fibre | **Glucose & insulin:** Glucose IAUC for 0-240 min sig ↓ with psyllium+fat compared to PL. Insulin IAUC not significantly different  
**GLP-1:** IAUC sig ↑ for psyllium+fat compared to PL & psyllium alone  
**GER:** Sig delayed for psyllium+fat compared to PL (assessed by paracetamol)  
**Nausea ratings:** sig ↓ with fat-containing meals |
| (Sugiyama et al., 2003) | GI study Healthy (n=9-11) for each food tested from volunteer pool (n=58) | Reference or test food (supplying 50 g CHO): - reference food: Satou rice – tested 2-3 times by each volunteer - test foods: SCFA-containing products – vinegarred rice, rice & pickled food, rice & fermented soybean | **Glucose:** GI sig ↓ for vinegared rice (GI=67) & rice + fermented soybean (GI=68) than reference food (GI=100). No sig diff from reference rice with pickled food (GI=75) |

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1 All studies were carried out in the morning after an overnight fast; any values quoted are the mean values  
2 IR = insulin resistant, T2DM = type 2 diabetes mellitus  
3
### Table 1.10 Cont. Acute effects of SCFA on metabolic response.\(^1\)\(^3\)

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design &amp; participants</th>
<th>Test meals</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Johnston et al., 2004)</td>
<td><strong>Crossover &amp; Parallel study</strong>&lt;br&gt;Healthy (n=8)&lt;br&gt;IR (n=11)&lt;br&gt;T2DM (n=10)</td>
<td>Breakfast of bagel, butter &amp; orange juice (87 g CHO) consumed after drinking either:&lt;br&gt;- 60 g water + 1 tsp saccharine (PL) or&lt;br&gt;- 20 g apple cider vinegar + 40 g water + 1 tsp saccharine</td>
<td><strong>Glucose:</strong> Sig ↓ with vinegar in IR but not healthy or T2DM volunteers&lt;br&gt;&lt;br&gt;<strong>Insulin:</strong> Sig ↓ with vinegar in IR &amp; healthy but not T2DM&lt;br&gt;&lt;br&gt;NB – blood samples only collected at 0, 30 &amp; 60 min</td>
</tr>
<tr>
<td>(Ostman et al., 2005)</td>
<td><strong>Dose-response crossover study</strong>&lt;br&gt;Healthy (n=12)&lt;br&gt;10 F, 2 M</td>
<td>Bread (supplying 50 g available CHO) for breakfast:&lt;br&gt;- white bread (PL) or&lt;br&gt;- white bread soaked in 18 g, 23 g or 28 g white vinegar (equiv. 18, 23, 28 mmol acetate)</td>
<td><strong>Glucose:</strong> Sig ↓ GI &amp; II for 0-90 min with 28 g added vinegar (GI=77, II=78) compared to PL (GI &amp; II=100). GI &amp; II at 0-120 min not sig different &amp; lower vinegar doses did not sig influence GI or II&lt;br&gt;&lt;br&gt;NB – blood samples only collected at 0, 30 &amp; 60 min</td>
</tr>
<tr>
<td>(Leeman et al., 2005)</td>
<td><strong>4-way crossover study</strong>&lt;br&gt;Healthy (n=13)&lt;br&gt;10 F, 3 M</td>
<td>Bread or potatoes (supplying 50 g available CHO):&lt;br&gt;- white wheat bread (reference) or&lt;br&gt;- freshly boiled potatoes or&lt;br&gt;- boiled then stored (for 24 h) potatoes or&lt;br&gt;- boiled then stored (for 24 h) potatoes soaked in 28 g white vinegar (equiv. 28 mmol acetate)</td>
<td><strong>Glucose &amp; insulin:</strong> Vinegar did not significantly alter GI or II compared to boiled then stored potatoes without vinegar</td>
</tr>
<tr>
<td>(Johnston and Buller, 2005)</td>
<td><strong>4-way crossover study</strong>&lt;br&gt;Healthy (n=11)&lt;br&gt;10 F, 1 M</td>
<td>Bagel, butter &amp; orange juice (87 g CHO) or teriyaki chicken with rice (52 g CHO) consumed after either:&lt;br&gt;- 60 g water + 1 tsp saccharine (PL) or&lt;br&gt;- 20 g apple cider vinegar + 40 g water + 1 tsp saccharine</td>
<td><strong>Glucose:</strong> Vinegar sig ↓ IAUC with bagel meal but not with chicken &amp; rice&lt;br&gt;&lt;br&gt;<strong>Insulin:</strong> Vinegar sig ↓ IAUC with bagel and chicken &amp; rice meals&lt;br&gt;&lt;br&gt;NB – blood samples only collected at 0, 30 &amp; 60 min</td>
</tr>
<tr>
<td>(Hlebowicz et al., 2008)</td>
<td><strong>4-way crossover study</strong>&lt;br&gt;Healthy (n=13)&lt;br&gt;7 F, 6 M</td>
<td>Bread (supplying 50 g available CHO) for breakfast:&lt;br&gt;- white wheat bread (reference) or&lt;br&gt;- white or wholemeal or wholegrain wheat bread soaked in 28 g white wine vinegar (equiv. 28 mmol acetate)</td>
<td><strong>Glucose:</strong> No sig difference between AUC for 0-120 min&lt;br&gt;&lt;br&gt;<strong>GER:</strong> Did not differ between treatments (assessed by real time ultrasonography)</td>
</tr>
<tr>
<td>(Mettler et al., 2009)</td>
<td><strong>2 x 2 crossover study</strong>&lt;br&gt;Healthy (n=13)&lt;br&gt;7 F, 6 M</td>
<td>Milk rice pudding and glucose drink (75 g CHO):&lt;br&gt;- with or without added 28 mmol acetic acid&lt;br&gt;- with or without added 4 g cinnamon</td>
<td><strong>Glucose:</strong> No sig difference between IAUC for 0-120 min. Blood glucose sig ↓ at T=15 min by acetic acid + cinnamon</td>
</tr>
</tbody>
</table>

\(^1\)All studies were carried out in the morning after an overnight fast. *\(^3\)any values quoted are the mean values,<br><br>**IR** = insulin resistant. **T2DM** = type 2 diabetes mellitus
Looking in more detail at a study investigating propionate, Liljeberg and Bjorck found that providing volunteers (n=12) with a mixed breakfast made using bread with added Na propionate significantly reduced the postprandial glucose and insulin AUC relative to bread matched for CHO levels (50g) with added lactic acid or with no additive (PL) (Liljeberg and Bjorck, 1996) (Figure 1.8). However, the AUC were compared by Wilcoxon signed ranks analysis, which is an inappropriate test for >2 treatments.

![Figure 1.8. Incremental A) blood glucose and B) serum insulin response following breakfast made with PL wholemeal bread (V WMB) or WMB with added Na propionate (O WMB-p) or lactic acid (□ WMB-la). Points with different letters differ significantly as assessed by Wilcoxon signed ranks test. Values shown as mean with error bars representing the SEM (n=12). Taken from (Liljeberg and Bjorck, 1996).](image)

When investigating the acute effects of vinegar supplementation Ostman and colleagues found a dose-dependent drop in postprandial glycaemia and insulinaemia following provision of bread soaked in 0 g (PL), 18 g, 23 g and 28 g vinegar (equivalent to 0, 18, 23 and 28 mmol acetic acid) for breakfast (n=12). The highest dose (28 mmol acetic acid) resulted in the lowest responses (Ostman et al., 2005).

SCFA may also improve long-term glycaemic control and other metabolic markers. Table 1.11 summarises published chronic oral supplementation studies, most of which investigated fasting parameters, with only a few considering postprandial effects.
Table 1.11. Summary of studies investigating the influence of short & long term oral supplementation with acetate or propionate on metabolic markers. Any values quoted are the mean values

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Study design &amp; protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Venter et al., 1990)</td>
<td>Healthy (n=20) 20 F</td>
<td>Double-blind randomised controlled parallel study Supplementation for 7 wk with 15 capsules daily supplying either: - 7.5 g (78 mmol) Na propionate (n=10) - dibasic Ca phosphate (PL, n=10)</td>
<td>• Propionate sig ↑ HDL-C &amp; ↓ fasting glucose &amp; maximal insulin increment following OGGT relative to PL • Propionate sig ↑ TAG levels relative to baseline (but not to PL)</td>
</tr>
<tr>
<td>(Todesco et al., 1991)</td>
<td>Healthy (n=6) 3M, 3F</td>
<td>2-way randomized crossover study Bread consumed daily for 1 wk, either: - white bread (PL) or - white bread with 9.9 g (103 mmol) propionate</td>
<td>• Propionate sig ↓ glucose AUC by 38.0 % following a 50 g CHO bread challenge • No sig change in serum TAGs, TC, HDL-C, LDL-C</td>
</tr>
<tr>
<td>(White and Johnston, 2007)</td>
<td>T2DM (n=11) 4M, 7 F</td>
<td>Randomised 2-way crossover study At bedtime for 2 d consumed either: - 2 TBS apple cider vinegar or - 2 TBS water (PL)</td>
<td>• Vinegar ↓ fasting glucose with sig treatment x time effect (reduced by 0.15 &amp; 0.26 mmol/l &amp; 2 &amp; 4 % respectively with PL &amp; vinegar)</td>
</tr>
<tr>
<td>(Hlebowicz et al., 2007)</td>
<td>T1DM &amp; diabetic gastroparesis (n=10) 5 M, 5 F</td>
<td>2-way crossover study (not randomised) Tested at start of study (PL), then consumed daily before breakfast 30 ml apple cider vinegar with 20 ml water (experimental)</td>
<td>• Vinegar sig ↓ GER (i.e. significantly delayed gastric emptying)</td>
</tr>
<tr>
<td>(Johnston et al., 2009)</td>
<td>T2DM (n=27) 9 M, 15 F completed 12 wk</td>
<td>Randomised controlled parallel study Daily supplementation for 12 wk with either: - Vinegar pills, 15 mg (0.25 mmol) acetic acid (PL) - 1 pickle, 0.7 g (12 mmol) AcOH (PCK) - 2 TBS vinegar, 1.4 g (23 mmol) AcOH (VIN)</td>
<td>• HbA1C sig ↓ in VIN group (-0.16 %) compared to PL (+0.06 %) and PCK (+0.22 %) • No difference in mean BW &amp; blood indices between groups at baseline • No difference in BW or plasma lipids/insulin between groups post-intervention</td>
</tr>
<tr>
<td>(Kondo et al., 2009)</td>
<td>Obese Japanese (n=155) 97 M, 58 F</td>
<td>Double-blind randomised controlled parallel study Daily supplementation for 12 wk with 500 ml beverage with added apple cider vinegar, either: - None added (PL) - 15 ml vinegar, 0.75 g (12 mmol) AcOH (Low dose) - 30 ml vinegar, 1.5 g (25 mmol) AcOH (High dose)</td>
<td>• Vinegar sig ↓ TAG &amp; TC (both doses) &amp; systolic BP (high dose) relative to baseline • Vinegar sig ↓ TAG (both doses) &amp; TC (low dose) &amp; systolic BP (high dose) relative to PL • Fasting plasma LDL-C, HDL-C, glucose &amp; insulin &amp; HbA1C &amp; HOMA not significantly influenced by vinegar treatment</td>
</tr>
</tbody>
</table>
Daily supplementation with acetate (provided as vinegar) for 12 wk was reported to significantly reduce HbA1C concentrations in T2DM patients (Johnston et al., 2009) but not in obese volunteers (Kondo et al., 2009) relative to PL. However the HbA1C concentrations were already low in the obese volunteers, with a mean baseline HbA1C of 5.29 [SD 0.45] to 5.34 [SD 0.46] %, relative to the clinical target for diabetic patients of <6.5% or <7.0% (Goodall et al., 2007, The Diabetes Control and Complications Trial Research Group, 1993, Landgraf, 2004). HbA1C is a marker of medium-term glycaemic concentrations, used to monitor patients with diabetes, with HbA1C concentrations increasing with increasing plasma glucose concentrations.

Daily vinegar supplementation for 12 wk also significantly reduced fasting TAG and TC concentrations in obese (Kondo et al., 2009) but not in T2DM volunteers (Johnston et al., 2009), reduced SBP in obese volunteers (Kondo et al., 2009) and reduced fasting plasma glucose in T2DM volunteers (White and Johnston, 2007). No effects on fasting plasma glucose, insulin, LDL-C or HDL-C were observed in obese volunteers (Kondo et al., 2009).

Supplementation with 7.5g Na propionate was reported to significantly increase HDL-C and maximum insulin increment (although this was not defined) relative to PL, and reduce fasting glucose and increase fasting TAG relative to baseline. No significant effects on postprandial glucose and insulin AUC following an OGTT or on fasting insulin, TC, LDL-C or NEFA were observed, with the authors concluding the results may indicate improved insulin sensitivity (Venter et al., 1990) - an ambitious claim considering the lack of effect on the postprandial glucose and insulin AUC.

However, daily consumption of bread with 9.9 g added Na propionate for 1 wk significantly decreased the postprandial glucose AUC at an acute 50 g bread challenge, relative to PL. Fasting lipid parameters were not significantly influenced, although as
the supplementation period was short, plasma lipids were unlikely to have been influenced by the intervention (Todesco et al., 1991).

Therefore overall evidence for an effect of chronic SCFA supplementation on metabolic parameters is fairly inconclusive, with limited data available, mostly from parallel rather than crossover studies.

It has been suggested that the observed effects on the glycaemic and insulinaemic response and possibly appetite may arise from oral SCFA delaying the rate of gastric emptying. As summarised in Tables 1.10 and 1.11, various researchers have investigated this with the majority of studies reporting the GER is delayed following acetate (Liljeberg and Bjorck, 1998, Hlebowicz et al., 2007) and propionate (Liljeberg and Bjorck, 1996, Darwiche et al., 2001) relative to PL, with the exception of Hlebowicz and colleagues who found no effect on the GER following an acute challenge with vinegar (Hlebowicz et al., 2008).

However none of the studies investigating the GER used the ‘gold standard’ for GER assessment (scintigraphy), instead using either the paracetamol absorption test (Liljeberg and Bjorck, 1996, Liljeberg and Bjorck, 1998) or real-time ultrasonography (Darwiche et al., 2001, Hlebowicz et al., 2008, Hlebowicz et al., 2007). Recent reviews suggest the ultrasonography method requires further validation and standardisation, although good correlations to scintigraphy and stable isotope methods are reported (Szarka and Camilleri, 2009, Bratten and Jones, 2006). Paracetamol absorption has been found to have moderate to good correlation with scintigraphy in the liquid phase, although further standardisation is suggested for use in research (Willems et al., 2001).

As vinegar and propionate both have a strong taste and smell that may exert an influence during the cephalic phase of ingestion, it is possible that test product
palatability may have triggered the observed effects on the metabolic response and satiety (reviewed below, see 1.10). Breads with propionate and lactic acid had significantly lower acceptability score than PL (Liljeberg et al., 1995) and meals containing PUFA and Na propionate induced higher subjective nausea ratings than meals that were unadulterated (Frost et al., 2003).

1.9.2 Colonic SCFA via NDC and metabolic response

As indicated by the presence of SCFA receptors in the colon, it is conceivable that fermentable NDC may also influence the metabolic response via the generation of colonic SCFA following microflora mediated fermentation.

Focusing on the prebiotic inulin-type fructans, the effects of their ingestion on plasma glucose (Kelly, 2009, Kaur and Gupta, 2002) and lipids (Beylot, 2005, Williams, 1999, Williams and Jackson, 2002, Kelly, 2009, Roberfroid, 2005a, Kaur and Gupta, 2002) have been reviewed. From these reviews, data from studies in rats is promising, indicating inulin-type fructans may decrease postprandial glycaemia and insulinaemia (Kaur and Gupta, 2002) and decrease fasting and postprandial TAGs (Beylot, 2005, Roberfroid, 2005a, Williams and Jackson, 2002, Kaur and Gupta, 2002). However studies in human participants have generated conflicting results.

Table 1.12 summarises findings from chronic intervention studies ranging from 14 d to 6 mo, in which human participants were supplemented with inulin, OF, or a mixture of the two.
Table 1.12. Summary of human clinical studies investigating chronic effects of inulin-type fructan supplementation on the metabolic response. All crossover studies separated intervention periods with a washout unless otherwise stated.³

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Yamashita et al., 1984)</td>
<td>Parallel (n=28) Poorly controlled NIDDM patients</td>
<td>Daily for 14 d: - 8 g FOS (n=18) or - 5 g sucrose (PL, n=10)</td>
<td>FOS sig ↓ fasting glu, TC, LDL-C levels. No sig effects on HDL-C, TAG, NEFA</td>
</tr>
<tr>
<td>(Luo et al., 1996)</td>
<td>Crossover (n=12) 19-32 y Normal weight &amp; healthy</td>
<td>Daily for 4 wk: - 20 g FOS or - 20 g sucrose (PL) Baked in cookies</td>
<td>FOS sig ↓ basal hepatic glu, but total-body glu disposal, or post-absorptive hepatic glu not altered during hyperinsulinemic clamp. No sig effects on fasting glu, insulin, TAG, TC or HDL-C.</td>
</tr>
<tr>
<td>(Pedersen et al., 1997)</td>
<td>Crossover (n=66) 20-36 y Normal weight &amp; healthy</td>
<td>Daily for 4 wk low-fat spread containing: - 14 g inulin Raftiline LS or - no inulin (PL)</td>
<td>No sig effects on fasting plasma TC, LCL-C, HDL-C, TAGs. Glu &amp; insulin not assessed.</td>
</tr>
<tr>
<td>(Davidson et al., 1998)</td>
<td>Crossover (n=21) 30-75 y BMI &lt;32 kg/m²</td>
<td>Daily for 6 wk: - 18 g inulin/OF Raftiline ST or - no inulin (PL)</td>
<td>TC &amp; LDL-C sig ↓ during inulin/OF compared to PL. No sig effects on fasting TAG. Glu &amp; insulin not assessed.</td>
</tr>
<tr>
<td>(Alles et al., 1999)</td>
<td>Crossover (n=20) Patients with T2DM (No washout)</td>
<td>Daily for 20 d: - 15 g FOS or - 4 g glucose (PL) Mixed in yogurt</td>
<td>No sig effects on fasting blood glu, or serum acetate, TC, HDL-C, LDL-C or TAG.</td>
</tr>
<tr>
<td>(van Dokkum et al., 1999)</td>
<td>Crossover (n=12) 23 [SD 3] y Normal weight &amp; healthy</td>
<td>Daily for 21 d: - 15 g inulin - 15 g FOS or - no NDC (PL)</td>
<td>No sig effects on fasting TC, HDL-C, LDL-C. No sig effects on postprandial glucose or insulin following OGTT.</td>
</tr>
<tr>
<td>(Jackson et al., 1999)</td>
<td>Parallel (n=54) 20-32 kg/m² Moderately raised plasma TC (&amp; TAG)</td>
<td>Daily for 8 wk: - 10 g inulin HP (n=27) or - 10 g MD (PL, n=27) Follow up at 12 wk</td>
<td>Inulin sig ↓ fasting insulin at wk 4, but not at wk 8 or wk 12. Inulin sig ↓ fasting TAG over entire study. No sig effects on fasting glu, TC, HDL-C, LDL-C, glu:insulin ratio.</td>
</tr>
<tr>
<td>(Causey et al., 2000)</td>
<td>Crossover (n=12) 27-49 y Patients with mild hypercholesterolemia</td>
<td>Daily for 3 wk: - 20 g inulin or - sucrose (PL) Mixed in ice cream No washout</td>
<td>Inulin sig ↓ fasting TAG, ↑ insulin 1 h following OGTT (p=0.07). No sig effects on fasting insulin, glu, TC, HDL-C, LDL-C or on postprandial glucose or c-peptide following OGTT.</td>
</tr>
<tr>
<td>(Olesen and Gudmand-Høyer, 2000)</td>
<td>Parallel (n=75) 18-70 y Patients with IBS</td>
<td>Daily for 12 wk: - 10 g OF (n=38) or - 10g glu (PL, n=37)</td>
<td>No sig effects on fasting plasma lipids (lipids not specified). Glu &amp; insulin not assessed.</td>
</tr>
<tr>
<td>(Luo et al., 2000)</td>
<td>Crossover (n=10) 57 [SD 2] y Patients with T2DM</td>
<td>Daily for 4 wk: - 20 g FOS or - 20 g sucrose (PL)</td>
<td>No sig effects on HbA1C, fasting glu, insulin, TAG, TC, HDL-C, insulin binding to erythrocytes or on basal hepatic glu production or glu disappearance during ITT.</td>
</tr>
</tbody>
</table>

³MD = Maltodextrin, OF = Oligofructose, glu = glucose, ITT = insulin tolerance test, MTT = mixed meal tolerance test
### Table 1.12 Cont. ¹

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Letexier et al., 2003)</td>
<td>Crossover (n=8) 23-32 y Normal weight &amp; healthy</td>
<td>Daily for 6 wk: - 10 g inulin HP or - 10 g MD (PL)</td>
<td>Inulin sig ↓ fasting TAG. No sig effects on fasting glu, insulin, TC, HDL-C, LDL-C.</td>
</tr>
<tr>
<td>(Giacco et al., 2004)</td>
<td>Crossover (n=30) 46 [SD 10] y Overweight with mild hypercholesterolemia</td>
<td>Daily for 2 mo: - 10.6 g FOS or - 15 g MD (PL)</td>
<td>No sig effects on fasting insulin, glu, NEFA, TC, HDL-C, LDL-C or on postprandial glu, insulin, NEFA, TAG for 5 h following standard high fat meal</td>
</tr>
<tr>
<td>(Boutron-Ruault et al., 2005)</td>
<td>Open pilot study With colorectal adenoma (n=44) vs. no adenoma (n=30)</td>
<td>Daily for 3 mo: - 10 g FOS Outcomes measured at baseline &amp; post-intervention</td>
<td>No sig effects on fasting insulin, glu, TC, TAG or HDL-C post-supplementation in either group compared to baseline (i.e. no proper control group)</td>
</tr>
<tr>
<td>(Daubioul et al., 2005)</td>
<td>Crossover (n=7) Patients with non-alcoholic steatohepatitis</td>
<td>Daily for 8 wk: - 16 g FOS or - 16 g MD (PL)</td>
<td>No sig effects on fasting insulin, glu, NEFA, TC, HDL-C, LDL-C.</td>
</tr>
<tr>
<td>(Forcheron and Boylot, 2007)</td>
<td>Parallel (n=17) Age &amp; BMI not specified</td>
<td>Daily for 6 mo: - 10 g inulin/OF mixture (n=9) or - 10 g unspecified PL (n=8)</td>
<td>Inulin/OF trend for ↓ TC, LDL-C &amp; ↑ HDL-C but not sig different from PL group. No sig effects on fasting plasma glu, insulin, glucagons, NEFA or TAG or on postprandial glu or insulin following OGTT</td>
</tr>
<tr>
<td>(Parnell and Reimer, 2009)</td>
<td>Parallel (n=48, n=39 completed) 20-70 y Overweight &amp; obese</td>
<td>Daily for 12 wk: - 21 g OF Raftilose P95 or - MD equicaloric (PL)</td>
<td>Sig time x diet interaction for postprandial insulin following MTT. Postprandial sig ↓ glu in OF &amp; ↑ glu in PL groups relative to baseline. No sig effects on postprandial serum lipids.</td>
</tr>
<tr>
<td>(Cani et al., 2009)</td>
<td>Parallel (n=10) 21-38 y Normal weight &amp; healthy</td>
<td>Daily for 2 wk: - 16 g Orafti Synergy or - 16 g MD (PL)</td>
<td>Inulin/OF sig ↓ postprandial plasma glu AUC. No sig effects on fasting plasma glu or insulin.</td>
</tr>
</tbody>
</table>

¹MD = Maltodextrin, OF = Oligofructose, glu = glucose, ITT = insulin tolerance test, MTT = meal tolerance test

Most studies examined effects of supplementation with inulin-type fructans on fasting parameters, with mixed findings, particularly for lipid parameters.

Many of the studies found no effect on fasting plasma lipids (Alles et al., 1999, Daubioul et al., 2005, Giacco et al., 2004, Luo et al., 1996, Luo et al., 2000, Olesen and Gudmand-Høyer, 2000, Parnell and Reimer, 2009, Pedersen et al., 1997, van Dokkum et al., 1999). However, supplementation significantly reduced fasting plasma TAG
concentrations in two studies (Causey et al., 2000, Jackson et al., 1999) and concurrently reduced plasma TC and LDL-C concentrations in three studies (Davidson et al., 1998, Forcheron and Beylot, 2007, Yamashita et al., 1984).


Fewer studies have examined effects of chronic supplementation with inulin-type fructans on postprandial metabolites. Postprandial glycaemia was unchanged by supplementation in three studies (Causey et al., 2000, Forcheron and Beylot, 2007, van Dokkum et al., 1999), with only one reporting concentrations to be significantly lower in the OF group and higher in the PL group compared to baseline levels (Parnell and Reimer, 2009). Two studies reported postprandial plasma insulin concentrations were reduced significantly (Parnell and Reimer, 2009) and with a trend approaching significance (p=0.07) (Causey et al., 2000) following OF and inulin treatment respectively, although two other studies found no effect on insulinaemia (Forcheron and Beylot, 2007, van Dokkum et al., 1999). Postprandial lipid responses were not altered by supplementation in the only study to report postprandial lipid responses (Parnell and Reimer, 2009).
Insulin sensitivity following OF supplementation was examined in two studies. Supplementation of 20 g OF per d for 4 wk in healthy volunteers significantly reduced basal hepatic glucose production during a hyperinsulinaemic clamp, although total body glucose disposal and post-absorptive hepatic glucose were not altered (Luo et al., 1996). However in patients with T2DM, no measures were altered during an insulin tolerance test following supplementation with 20 g OF per d for 4 wk (Luo et al., 2000).

Only one study examined acute effects on postprandial metabolites and found 20 g inulin/OF (with no macronutrients supplied) significantly lowered glycaemic AUC and glucose and insulin mean peak concentrations, relative to 20 g fructose (Rumessen et al., 1990). However, fructose is an unsuitable PL as fructose is rapidly absorbed from the GIT. Additionally, 10 g inulin/OF ingested alongside wheat bread (with 50 g starch) significantly lowered the glucose IAUC relative to the same wheat bread with no inulin/OF (Rumessen et al., 1990), due to hypoglycaemia from 90 min postprandially.

1.10 The Cephalic Phase Response

1.10.1 Overview of the Cephalic Phase Response

Physiological responses to eating episodes are divided into three phases, referring to the part of the digestive system being stimulated by food: namely the cephalic, gastric and intestinal phases. The cephalic phase relates to pre-absorptive effects while gastric and intestinal phases relate to post-ingestion effects of food intake (Giduck et al., 1987, Zafra et al., 2006).

The cephalic phase response is initiated by food-related sensory stimuli including the thought, sight, smell, taste and chewing of food, and occurs before and during the first moments of an eating episode (Feldman and Richardson, 1986, Teff, 2000). These
effects arise from vagal cholinergic signalling and have been shown to initiate a variety of autonomic and endocrine responses linked to digestion, absorption and metabolism (summarised in Table 1.13).

Table 1.13. Overview of the main physiological responses triggered during the cephalic phase. Adapted from (Robertson, 2009), (Zafra et al., 2006) and (Power and Schulkin, 2008)

<table>
<thead>
<tr>
<th>Site</th>
<th>Response(s)</th>
<th>Cephalic Function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td>† salivation</td>
<td>• Lubricate food, start starch &amp; fat digestion (amylase &amp; lingual lipase in saliva), dissolve food particles to be transported to taste buds</td>
</tr>
<tr>
<td>Stomach</td>
<td>† gastric acid secretion</td>
<td>• Hydrolysis &amp; breakdown of food</td>
</tr>
<tr>
<td></td>
<td>† gastric motility</td>
<td>•† gastric emptying rate → regulate food passage</td>
</tr>
<tr>
<td></td>
<td>† gastric levels</td>
<td>• Stimulate gastric acid secretion</td>
</tr>
<tr>
<td></td>
<td>† pre-prandial ghrelin levels</td>
<td>• Rise stimulates appetite. However postprandial suppression rapidly follows (i.e. inhibit appetite)</td>
</tr>
<tr>
<td></td>
<td>† leptin mucosal secretion</td>
<td>• May inhibit CHO absorption, promote small peptide absorption, reduce appetite</td>
</tr>
<tr>
<td>Small intestine</td>
<td>† intestinal motility</td>
<td>• Regulate food passage</td>
</tr>
<tr>
<td></td>
<td>† absorption water, glucose</td>
<td>• Feed-forward mechanism</td>
</tr>
<tr>
<td></td>
<td>† motilin</td>
<td>• Stimulate intestinal motility</td>
</tr>
<tr>
<td>Large intestine</td>
<td>† intestinal motility</td>
<td>• Regulate food passage</td>
</tr>
<tr>
<td></td>
<td>† neurotensin</td>
<td>• Unknown</td>
</tr>
<tr>
<td>Pancreas</td>
<td>† insulin release</td>
<td>• Anticipatory metabolic role</td>
</tr>
<tr>
<td></td>
<td>† digestive enzymes</td>
<td>• Digest protein, CHO, fat</td>
</tr>
<tr>
<td></td>
<td>† bicarbonate secretion</td>
<td>• Neutralise stomach acid</td>
</tr>
<tr>
<td></td>
<td>† glucagon release</td>
<td>• Unknown. May † postprandial thermogenesis &amp; or prevent CPIR induced hypoglycaemia</td>
</tr>
<tr>
<td></td>
<td>† PP release</td>
<td>• Cephalic phase role unknown</td>
</tr>
<tr>
<td>Liver</td>
<td>† VLDL levels</td>
<td>• Cephalic phase role unknown</td>
</tr>
<tr>
<td></td>
<td>‡ NEFA levels (suppressed by CPIR)</td>
<td>• May enhance glucose metabolism in insulin sensitive tissues</td>
</tr>
<tr>
<td>Adipose Tissue</td>
<td>† LPL expression (stimulated by CPIR)</td>
<td>• Probably in preparation for imminently incoming nutrients</td>
</tr>
<tr>
<td>Vasculature</td>
<td>† intestinal blood flow</td>
<td>• Probably in preparation for imminently incoming nutrients</td>
</tr>
<tr>
<td></td>
<td>† cardiac output, heart rate</td>
<td>• Cephalic phase role unknown</td>
</tr>
<tr>
<td>Other</td>
<td>† cephalic phase thermogenesis</td>
<td>• May arise from actions of cephalic phase hormones (e.g. insulin, glucagon)</td>
</tr>
</tbody>
</table>
The cephalic response is thought to be a “feed-forward” effect “priming” the GIT and metabolic processes in preparation for imminently incoming nutrients (Robertson, 2009, Zafra et al., 2006). Cephalic initiated responses are more rapid in onset, shorter-lived and lower in magnitude than gastric and intestinal phase responses (Robertson, 2009, Zafra et al., 2006).

The concept of cephalic phase responses were first introduced in the classical experiments of Pavlov, who documented anticipatory salivary and gastric secretions occurring in response to the sight and smell food or even just the expectation of feeding in dogs (Pavlov, 1902).

Experimentally, sham feeding and modified sham feeding (MSF) are the most commonly used methods to investigate cephalic phase responses. In early sham feeding experiments, animals were fitted with a gastric or oesophageal fistulae allowing food to be eaten but not absorbed from the small intestine. These studies have provided much evidence regarding cephalic phase processes, however this model does not allow researchers to differentiate between responses arising from receptors in the mouth, or further down in the oesophagus or stomach (Robertson, 2009, Zafra et al., 2006).

The MSF (or “chew-and-spit”) technique, which involves chewing and tasting but not swallowing food, was developed to investigate cephalic phase responses in humans (Richardson et al., 1977). The MSF technique allows researchers to selectively investigate oral vagal activity, avoiding pharyngeal and oesophageal receptors (Robertson, 2009). However as food is not swallowed the influence of cephalic responses on subsequent nutrient handling is not so clear (Teff, 2000). To overcome this, MSF is often coupled with intragastric nutrient delivery or intravenous glucose (Teff, 2000, LeBlanc, 2000).
1.10.2 Cephalic phase oral responses

Saliva is the first cephalic secretion and is secreted into the oral cavity both prior to eating in response to visual and olfactory stimuli and also once food is being tasted and chewed (Giduck et al., 1987, Robertson, 2009), with taste and chewing inducing higher secretion levels (Mattes, 1997). Indeed, as demonstrated by Pavlov, salivary output increases even at the mere thought of food (Pavlov, 1902), and favourite foods are often described as being “mouth watering” (Mattes, 2000). The quantity of saliva produced in anticipation of a meal has been found to be correlated with hunger ratings and the rated palatability of the food (Wooley and Wooley, 1973).

Saliva acts as a lubricant and protects the oral mucosa during chewing, aiding mastication, bolus formation and swallowing. In addition, saliva contains enzymes such as amylase and lingual lipase for initial digestion of starch and fats respectively (Mattes, 2000, Pedersen et al., 2002). Of importance, saliva acts as a solute so nutrients are dissolved allowing them to be transported to taste buds to stimulate taste receptor cells, thereby facilitating taste perception (Pedersen et al., 2002).

Taste is important in identifying potentially harmful and toxic compounds and, in addition to mastication and swallowing, is the main stimulant of salivary production (Pedersen et al., 2002, Mattes, 2000). The type of gustatory stimulus influences saliva volume and composition, with a sour/acidic (e.g. lemon juice) or bitter (e.g. quinine) taste potently stimulating salivary flow and a sour/acidic taste increasing protein levels in saliva (Davenport, 1982, Robertson, 2009). Increased salivary flow in response to bitter or acidic tastes may have evolved as a protective mechanism to dilute or clear potential toxins, irritants or other dangerous substances from the mouth (Robertson, 2009, Mattes, 2000).
1.10.3 Cephalic phase insulin release (CPIR)

Various studies have demonstrated insulin is secreted from the pancreas in response to taste stimuli (Bellisle et al., 1985, Bruce et al., 1987, Lucas et al., 1987, Teff and Engelman, 1996, Teff et al., 1993a, Teff et al., 1991, Teff et al., 1993b), a phenomenon termed cephalic phase insulin release (CPIR). Similarly, early insulin peaks alongside an increase in C-peptide have been observed during eating episodes within a few min of meal initiation and prior to a postprandial rise in plasma glucose concentrations. During CPIR, insulin concentrations generally rise within 2 min of the oral stimulus, reaching maximal levels after 4 min and returning to baseline by 10 min post-stimulation (Teff, 2000) (Figure 1.9A). The CPIR magnitude and profile are similar post MSF and post actual food ingestion (Teff et al., 1993a).

CPIR appears to be necessary for normal glucose homeostasis. The postprandial plasma glucose AUC following gastric infusion of a glucose solution, thereby bypassing oral stimulation, was significantly lower when preceded by an MSF episode than with no prior cephalic stimulation (Figure 1.9B) (Teff and Engelman, 1996).

The amplitude of CPIR may be related to meal palatability. Higher CPIR concentrations are reported to occur following exposure to foods rated as highly palatable than
following exposure to those with lower palatability ratings (Bellisle et al., 1985, Lucas et al., 1987), although this is not a consistent finding (Teff and Engelman, 1996).

1.10.4 Cephalic gastric secretions

As demonstrated in Pavlov’s experiments, gastric acid is secreted in substantial quantities during the cephalic phase (Pavlov, 1902), accounting for approximately one-third of total secretion levels (Robertson, 2009). Cephalic gastric acid secretion occurs via vagal activation triggering Ach release, in turn stimulating gastric acid production (Robertson, 2009).

The thought of appetising food has been shown to induce gastric acid production, although levels produced are lower than during MSF when food is also seen, smelt, tasted and chewed (Feldman and Richardson, 1986), showing gustation has an important role in cephalic gastric secretion (Zafra et al., 2006). However as it is difficult to separate thoughts of food from tasting food, it is difficult to quantify the influence of taste (Mattes, 1997). In an early human study, gastric acid secretions were found to be lower in response to an MSF unpalatable meal (gruel) in comparison to a palatable meal (freely selected preferred meal) (Janowitz et al., 1950). However in dogs gastric acid secretion was only suppressed when presented with “inedible” meals (Robertson, 2009).

1.10.5 Cephalic phase intestinal motility

Evidence from various studies indicates gastric and intestinal motility is influenced during the cephalic phase. Intragastric delivery of a soup, thereby bypassing the oral cavity, resulted in a significantly higher rate of gastric emptying than when the same soup was administrated orally (Cecil et al., 1998). Furthermore, gastric emptying of a
fat load was significantly faster with prior vagal stimulation via MSF, compared to ingestion alone (Heath et al., 2004).

Oral vagal stimulation has also been shown to influence the migrating motor complex (MMC), which comprises recurring waves of activity propagating motility along the length of the GIT between meals to remove debris and prevent microfloral overgrowth. The MMC occurs in three phases: Phase I (inactive), Phase II (irregular motility similar to postprandial motility) and Phase III (strong, frequent contractions) (Robertson, 2009). Sham feeding during phase III appears to disrupt antroduodenal motility and inhibit the propagation of contractions in the duodenum (Pouderoux et al., 1995) to be replaced by antral contractions (Katschinski et al., 1992).

Colonic motility also appears to be influenced during the cephalic phase, and is stimulated even by the mere smell, sight and thought of food as well as by oral stimulation via sham feeding (Robertson, 2009).

1.10.6 Effects on appetite during the cephalic phase

Sham fed animals fitted with a fistula do not become satiated and continue to eat while food is available, providing the first evidence that intestinally released hormones are necessary for satiation (Robertson, 2006, Robertson, 2009). Furthermore, early studies in humans found that removing cephalic stimuli with nose-clips and topical anaesthesia in the mouth (to block olfactory and taste stimuli respectively) significantly influenced satiation (Geliebter, 1979). It is now known that some gut peptides are released during cephalic phase vagal stimulation, including ghrelin, leptin, gastrin, neurotensin and motilin, whereas others are only released post-ingestively, including CCK, GIP and GLP-1 (Robertson, 2009).
Plasma ghrelin, the ‘hunger hormone’, is elevated during fasting promoting the urge to eat and falls postprandially suppressing the urge to eat (reviewed in section 1.3.1.1). Cephalic stimulation with a palatable meal via MSF results in significantly increased pre-prandial plasma ghrelin concentrations, even during the anticipatory stage of a meal (i.e. the sight, thought and smell) (Simonian et al., 2005, Heath et al., 2004). Alongside this, PP concentrations are also increased during MSF, suggesting efferent vagal activation (Simonian et al., 2005). MSF induced cephalic stimulation appears to accentuate the postprandial ghrelin response when preceding an oral fat load, significantly enhancing the speed and magnitude of suppression, as compared to no MSF prior to eating (Heath et al., 2004), suggesting a role for cephalically stimulated ghrelin in enhancing satiation.

In addition to being expressed in adipose tissue, leptin is also expressed in gastric mucosa and released following vagal stimulation (Sobhani et al., 2002), suggesting gastric-derived leptin is released during the cephalic phase. Leptin receptors are located in jejunal and ileal mucosa, indicating a role for leptin in mediating nutrient digestion and absorption. For example leptin has been shown to enhance small peptide absorption (Sobhani et al., 2002) and may inhibit CHO absorption (Robertson, 2009).

MSF elicited cephalic phase activation appears to reduce subjective hunger ratings in human subjects (LeBlanc and Soucy, 1996, Smeets and Westerterp-Plantenga, 2006b). Subjective appetite ratings for fullness and desire to eat were increased to a significantly greater level following MSF with sandwiches (nutritive material) relative to chewing a latex dummy (non nutritive material) for a similar length of time. However ad libitum EI 85 min post-MSF did not differ significantly between treatments, suggesting only a short time effect of the cephalic phase (Stratton et al., 1999).
Direct administration of nutrients or foods into the GIT allows investigation of responses in the absence of cephalic stimulation. Hunger ratings remained significantly elevated following intragastric infusion of a soup relative to oral ingestion of the same soup (Cecil et al., 1998), suggesting a role for cephalic mechanisms in amplifying gastrointestinal satiety signals. Furthermore, while hunger ratings were reduced to a similar level following intragastric infusion of isocaloric soups high in fat or CHO, oral administration resulted in significantly lower hunger ratings following the high fat soup relative to oral administration of the high CHO soup (Cecil et al., 1999).

Taken together, this data suggests not only do orosensory signals enhance appetite suppression, but information gained at the cephalic phase allows discrimination between nutrients (e.g. fat and CHO). This in turn influences the appetite response, with oral fat loads suppressing appetite to a greater extent than oral CHO loads.

1.11 Influence of palatability on appetite and metabolic response

As reviewed in section 1.10, the cephalic phase of digestion appears to influence subsequent appetite and metabolic processes, with sensory factors governing meal palatability appearing to influence some cephalic responses. It is therefore reasonable to postulate that palatability may influence appetite and the metabolic response.

1.11.1 Defining palatability and sensory-specific satiety

Defining palatability is not straightforward and has been the cause for debate by many researchers with no single definition having yet been assigned (Yeomans, 1998, Ramirez, 1990, Yeomans et al., 2004). Palatability has been defined as the ability of sensory factors to increase food intake, however this can give rise to a circular argument, as it is then not possible to determine if alterations in intake arise from palatability (Yeomans, 1998).
In addition to taste, sensory factors that may influence perceptions of palatability include texture, odour, temperature and appearance (Grinker, 1990). Grinker also noted palatability is influenced by previous exposure and experience of the food, time of day, season, variety (of foods on offer), expectation of the food, metabolic status (e.g. hungry or satiated), location of the meal (e.g. home or restaurant) and nutrient quality of the food, suggesting palatability is not only a function of intrinsic properties but also of exposure and experience (Grinker, 1990).

In a quest to clarify and standardise the definition of palatability, a review by Yeomans concluded current data supports the idea of Le Magnen that palatability should be defined as the “hedonic evaluation of orosensory food cues under standardised conditions” (Le Magnen, 1987, Yeomans, 1998).

The complexity of factors influencing palatability suggest palatability is not a fixed attribute, but is a variable property (Yeomans, 1998), as demonstrated by the observation that ratings of meal pleasantness generally decrease as a meal progresses (Hetherington et al., 1989, Rolls et al., 1981a, Yeomans and Symes, 1999).

Sensory-specific satiety (SSS) refers to the decline in rated pleasantness of the sensory characteristics of a food relative to uneaten foods when consumed to satiety during a single eating episode (Hetherington et al., 1989, Nolan and Hetherington, 2009, Rolls et al., 1981a, Sørensen et al., 2003). It has also been found that sensory ratings can decrease without reaching satiety (Smeets and Westerterp-Plantenga, 2006a). The greatest decline in pleasantness during SSS occurs immediately during the first 20 min of consumption, indicating SSS is related to sensory factors accompanying ingestion rather than post-ingestive or post-absorptive effects (Smeets and Westerterp-Plantenga, 2006a, Rolls et al., 1981a, Sørensen et al., 2003). SSS has been demonstrated to occur
with vagal stimulation via MSF, demonstrating post-ingestive feedback is not necessary for SSS to occur (Nolan and Hetherington, 2009, Smeets and Westerterp-Plantenga, 2006a), further supporting SSS as a sensory phenomenon. The concept of SSS demonstrates the complexity and importance of palatability influences.

1.11.2 Measurement of palatability

Palatability is generally measured with hedonic ratings using continuous VAS scales or fixed point scales (FPS) (Yeomans, 1998, Sørensen et al., 2003). Participants are asked questions such as “How pleasant is the appearance/taste/texture of this food right now?” (Kral et al., 2004), or “How palatable is this meal right now?” (Flint et al., 2000).

However as Yeomans points out, it is important to interpret palatability ratings with caution, as seemingly small differences in the way a question is asked will influence the outcome (Yeomans, 1998). The terms palatability and pleasantness have been used by investigators interchangeably, which may cause difficulties due to differing interpretations of what palatability means (Sørensen et al., 2003). For example, separate ratings to assess meal pleasantness and palatability found that while pleasantness ratings declined from the start to the end of the meal, changes in the palatability were more variable. Two-thirds of participants rated palatability and pleasantness similarly, while one-third rated palatability as being constant from the start to the end of the meal despite pleasantness ratings declining across the meal (Yeomans and Symes, 1999).

This in combination with the different scales (VAS and FPS) used means there is considerable variation in the format of palatability ratings in palatability research.
1.11.3 Palatable food and hedonistic mechanisms

Appetite is regulated by a complex physiological feedback system involving central and peripheral hunger and satiety signals to control E balance (reviewed in section 1.3). However palatable food can override these homeostatic mechanisms by activating the reward system (hedonistic mechanisms), with gratification being the main motivator to eat rather than correcting negative E balance (Saper et al., 2002, Erlanson-Albertsson, 2005). Activation of the reward system triggers a behaviour to “come back for more”, so that in an environment with free access to palatable foods (Kelley et al., 2002), overeating beyond homeostatic requirements can occur (Erlanson-Albertsson, 2005). This overeating is usually characterised by a lengthened eating episode duration due to hedonistic mechanisms over-riding satiation signals (Erlanson-Albertsson, 2005).

The relationship between ingesting palatable food and reward has many similarities to the relationship between drug addiction and reward (Kelley et al., 2002, Erlanson-Albertsson, 2005). As the consumption of palatable food is associated with the release of opioids and dopamine in the reward system, and central administration of opiates and dopamine stimulates food intake, it is argued a vicious cycle may result from the consumption of palatable foods invoking a reinforcing mechanism. In addition, consumption of palatable food may blunt responses to satiety signals, possibly by the reward centre interacting with hypothalamic appetite-controlling neurones (Erlanson-Albertsson, 2005).

One criticism that can be made of the research is that the term palatable has not been defined. For example Erlanson-Albertsson describes palatable food as being “foods high in fat and sugar”, but no assessment or measurement of palatability is actually discussed.
1.11.4 Palatability and appetite

Studies investigating the link between sensory properties of food and appetite suggest sensory properties influence food choice and quantities consumed (Sørensen et al., 2003). A pooled analysis of data from food palatability manipulation studies concluded there was a strong linear relationship between the change in rated palatability and difference in food intake following food manipulation (Figure 1.10) (Yeomans, 2007).

![Figure 1.10. Relationship between change in palatability ratings and food intake when palatability is manipulated. Each data point represents a published study. Taken from (Yeomans, 2007).](image)

As summarised in Table 1.14, investigators have manipulated taste and palatability in various ways, including varying the amount of acid and salt added to sandwiches (Hellemann and Tuorila, 1991), adding citric acid to tomato soup (de Graaf et al., 1999), varying the concentration of oregano added to pasta (Yeomans, 1996, Yeomans et al., 1997), adding cumin to a banana colada frozen yogurt drink (Bobroff and Kissileff, 1986), adding quinine sulphate to impart a bitter taste (Nisbett, 1968) and offering stale popcorn in comparison to fresh.

In general these palatability manipulation studies have found ad libitum intake of the manipulated test product is inversely correlated to product palatability (Bobroff and Kissileff, 1986, de Graaf et al., 1999, Hellemann and Tuorila, 1991, Nisbett, 1968, Yeomans, 1996, Yeomans et al., 1997). The effect on subsequent satiety is less clear.
and has not been extensively investigated. The addition of citric acid to a tomato soup preload did not significantly influence subsequent *ad libitum* intake of a buffet meal regardless of the intermeal time interval (de Graaf et al., 1999).

### Table 1.14. Summary of studies investigating the effect of taste manipulation on satiation

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Design</th>
<th>Test products</th>
<th>Results</th>
</tr>
</thead>
</table>
| (Nisbett, 1968) | Parallel n=14 | Ice-cream:  
  - with added quinine sulphate  
  - no additives (PL)  | *Ad libitum* intake & subjective taste ratings of quinine ice-cream sig ↓ than PL ice cream  
  - Linear relationship between taste ratings and *ad libitum* intake |
| (Bobroff and Kissileff, 1986) |  | Banana colada:  
  - with added cumin  
  - no additives (PL)  | *Ad libitum* intake & subjective taste ratings of cumin colada sig ↓ than PL colada  
  - Time taken to eat cumin colada sig ↑ than for PL colada |
| (Hellemann and Tuorila, 1991) | 12-way crossover n=27 | Sandwiches made using:  
  - 3 types of bread  
  - 4 types of spread each with varying levels of acid & salt | *Number of sandwiches consumed correlated with pleasantness ratings* |
| (Yeomans, 1996) | Parallel n=54 | Pasta & tomato  
  - with oregano 0.27% (palatable) & 0.54% (strong, unpleasant)  
  - no oregano (bland)  | *Ad libitum* intake of palatable was significantly higher than of strong  
  - Eating rate of palatable was significantly faster than of strong |
| (Yeomans et al., 1997) | Crossover n=16 | Pasta & tomato  
  - with oregano 0.27% (palatable)  
  - no oregano (bland)  | *Ad libitum* intake of palatable was significantly ↑ than of bland  
  - Hunger ratings ↑ during initial stages of palatable but not bland |
| (de Graaf et al., 1999) | Crossover n=35 | Tomato soup  
  - with 7.5g (less pleasant) & 15g (unpleasant) citric acid per kg soup  
  - no additives (PL)  | Sig ↓ *ad libitum* intake of ‘less pleasant’ & unpleasant than PL  
  - No effect of taste on *ad libitum* intake at subsequent buffet meal 15 or 90 min following soup preload |
| (Wansink and Kim, 2005) | Parallel n=158 | Popcorn:  
  - 14 d old (Stale)  
  - Fresh (PL)  | *Ad libitum* intake of stale popcorn was sig ↓ than fresh popcorn |

A relatively new area of appetite research is Sensory Specific Satiety (SSS) (defined in 1.11.1), further indicating the importance of sensory properties in influencing satiety. As SSS appears to be specific to sensory properties of an individual food, it is proposed that when a variety of foods are offered together then overall intake should be higher than if a single food item is served alone (Rolls et al., 1981b, Rolls et al., 1984).
Studies appear to support this concept, (reviewed in (Sørensen et al., 2003)). For example, when participants were offered one hors d’oeuvres (no variety) or 3 different types of hors d’oeuvres (variety) (Pliner et al., 1980), or a 4 course meal with different foods in each course (variety) or the same foods in each course (no variety) (Rolls et al., 1984), or served sandwiches with different fillings (variety) or one filling (no variety) (Rolls et al., 1981b, Bellisle and Le Magnen, 1981), or provided three different yogurts that differed in taste, texture and colour (variety) compared to just the participants favourite yogurt flavour (no variety) (Rolls et al., 1981b), to name a few examples, overall intake was higher in the variety condition.

Furthermore, when exposed to a cheese sandwich (either ingested or MSF), more sandwiches were consumed subsequently when offered ham (‘variety’) than when offered cheese (‘no variety’) fillings, suggesting that SSS may occur even when the food is only tasted and chewed but not swallowed (via MSF) (Nolan and Hetherington, 2009). However this study did not account for the fact some participants may prefer ham rather than cheese sandwiches, thus confounding the data.

1.11.5 Palatability and metabolic response

Food palatability may also influence the metabolic response. For example, consumption of a highly palatable meal resulted in significantly higher oxygen consumption (LeBlanc and Brondell, 1985, Sawaya et al., 2001) and postprandial plasma insulin (LeBlanc and Brondell, 1985) and glucose (Sawaya et al., 2001) response compared to the same meal blended, then desiccated into a biscuit form (unpalatable meal). Plasma noradrenaline concentrations also increased alongside the palatable, but not the unpalatable blended meal, suggesting vagal involvement (LeBlanc and Brondell, 1985).
Furthermore, as discussed in section 1.10.3, CPIR concentrations may be related to meal palatability, with higher responses occurring following highly palatable foods (Bellisle et al., 1985, Lucas et al., 1987).

Gastric motility also appears to be influenced by food palatability and taste, which will in turn influence the metabolic response. For example, when an orally ingested liquid test meal was preceded by MSF of a modified bitter tasting Slim-Fast bar, gastric emptying was significantly delayed compared to prior MSF of a regular Slim-Fast bar (PL) (Wicks et al., 2005). In a separate study, gastric myoelectric activity was decreased by sham feeding an unappetising food (cold tofu frankfurters) as compared to an appetising food (cooked frankfurters), suggesting a lack of cephalic phase vagal stimulation with the ‘unappetising’ treatment (Stern et al., 2001), however this was a parallel rather than a crossover study. As would be expected, the influence of taste on gastric emptying appears to occur at the cephalic phase of digestion, as demonstrated by intragastric infusion of a bitter solution having no influence on the gastric emptying rate as compared to water (Little, 2009).
1.12 Aims and objectives

The aim of the work presented within this thesis is to elucidate further the role SCFA may have in regulating appetite and the metabolic response in humans, looking at both:

- Oral delivery of SCFA - via the provision of vinegar as a source of acetate and the provision of propionate
- Colonic delivery of SCFA - via the provision of NDC.

Within this, the influence of product palatability, which was over-looked in previous studies, is to be considered. This will be achieved by the following objectives:

A. To investigate the acute effects of including a palatable propionate-rich sourdough bread as a component of one meal on postprandial subjective measures of appetite, prospective consumption at a subsequent ad libitum meal, 24 h intake and postprandial metabolite concentrations compared to a visually and nutritionally identical PL bread of equal palatability (Chapter 3).

B. To examine the acute effects of acetic acid ingestion (sweetened drink containing vinegar) delivered in an unpalatable (Unpal) and more palatable (Pal) form consumed alongside a standard breakfast preload on postprandial subjective measures of appetite, prospective consumption at a subsequent ad libitum meal, 24 h intake and postprandial metabolite concentrations compared to a visually identical PL (sweetened drink with no added vinegar) (Chapter 4).

C. To investigate the acute effects of orosensory stimulation with acetic acid (delivered in a drink containing vinegar) via MSF following a standard breakfast preload on postprandial subjective measures of appetite, prospective consumption at a
subsequent *ad libitum* meal, 24 h intake and postprandial metabolite concentrations as compared to MSF with PL (drink with no added vinegar) (Chapter 5).

D. To evaluate if inulin propionyl ester (IPE), a modified form of inulin HP with propionate tethered to the inulin scaffold, is able to enhance colonic propionate production by investigating the acute dose-response effects of IPE on subsequent colonic propionate production, assessed by determining 24 h urinary SCFA levels during the 24 h period following supplementation (Chapter 6).

E. To investigate the effect of inulin, IPE, and L-Rha following a 1 wk run-in period, as a component of breakfast and lunch, on postprandial subjective measures of appetite and gastrointestinal symptoms, prospective consumption at a subsequent *ad libitum* meal, 24 h intake and postprandial metabolite concentrations and breath hydrogen levels as compared to PL (Chapter 7).
Chapter 2: General methods

The following chapter outlines the general methods followed during the conduct of the Clinical Studies reported in Chapters 3-7 and subsequent analysis of biological samples.

2.1 Screening

At the start of each study, interested participants were invited to attend a screening session at the Clinical Investigation Unit (CIU) in the University of Surrey to assess their suitability for inclusion in the study. During screening, volunteers were asked to complete a Medical and Lifestyle Questionnaire (example questionnaire given in Appendix B) that included a self-certificate medical questionnaire and the Dutch Eating Behaviour Questionnaire (DEBQ, see 2.1.1 and Appendix C)

Anthropometric measurements (height, BW, % body fat and waist circumference) were taken and body mass index (BMI) was calculated. Blood pressure (BP) was measured in triplicate (mean value reported) and a small blood sample was collected by fingerprick and analysed for glucose concentrations using the HemoCue Glucose 201+ (see section 2.6.3.3). In studies where blood samples were collected via an indwelling cannula (Chapters 3 and 7) blood haemoglobin (Hb) concentrations were also measured using the HemoCue Hb 201+ (see section 2.6.9).

2.1.1 Dutch Eating Behaviour Questionnaire (DEBQ)

During screening, participants completed a DEBQ to assess eating behaviour (questionnaire is given in Appendix C). The DEBQ was developed by van Strien and colleagues as a tool to enhance understanding of the complex eating behaviour patterns exhibited by obese individuals (Van Strien et al., 1986). It measures three dimensions of eating behaviour, restraint, emotional and external, and comprises a 33 item
questionnaire, 10 items each to assess restrained and external eating behaviour, and 13 items to assess emotional eating behaviour. Each item has a five-point response format, never, seldom, sometimes, often and very often, scored 1, 2, 3, 4 and 5 respectively for each item except question 26, where a reverse scoring is applied. There is also a "not relevant" category for items presented in a conditional format, designated a score of 0.

The score for each eating behaviour dimension is calculated by determining the sum of the scores from each item regarding that particular eating dimension divided by the number of items assessing that particular dimension. This division factor is reduced by the number of items for which the 'not relevant' response is chosen. For example, the restraint score is calculated as shown below:

\[
Restraint \text{ Score} = \frac{\sum \text{Scores from questions regarding restrained eating}}{10 - \text{number of "not relevant" answers}}
\]

Thus the score for each dimension ranges from 1 to 5, with a higher score implying a stronger tendency towards displaying that eating behaviour dimension.

2.2 Anthropometry, body composition and blood pressure (BP)

2.2.1 Apparatus and equipment

- Harpenden stadiometer
- Tanita TBF-300A body composition analyser (Tanita Europe BV, Netherlands)
- Flexible tape measure
- Omron MX3 BP monitor (Omron Healthcare UK Ltd, Milton Keynes, UK)
2.2.2 Methods

Anthropometric measures and BP were measured at screening sessions and on study days. On study days, these were performed in the morning after a 12 h fast.

Height was measured using a Harpenden stadiometer without shoes. Participants stood erect, facing ahead in a horizontal plane with their back to the stadiometer with their heels, buttocks, shoulder blades and back of the head touching the wall, ankles together, knees straight and arms by their side. The measuring bar was gently lowered to touch the top of the participant's head and height was recorded to the nearest 0.1 cm.

BW and % body fat were measured non-invasively using the Tanita TBF-300A body composition analyser (Tanita Europe BV, Netherlands). Participants were asked to not apply body lotion and to urinate immediately prior to taking measurements, and 1.0 to 1.5 kg was subtracted to allow for the weight of clothes (depending on clothes worn).

The Tanita TBF-300A assesses body composition indirectly by bioimpedance analysis (BIA) through the lower half of the body. A safe electrical signal is sent through the body via the pressure contact foot pad electrodes which participants are asked to step onto in bare feet. The Tanita directly measures BW and the bioelectric impedance (defined as the strength and speed of an electrical signal travelling through the body, measured in Ohms) using the conductive properties of the legs. % body fat, fat free mass and other parameters are then calculated on the basis of measured BW and bioelectric impedance and other variables including age, height, gender and body type (standard or athletic). The use of the Tanita to assess % body fat has been previously validated and found to be highly correlated with the gold standard assessment method, duel energy x-ray absorptiometry (DEXA) in non-obese individuals (Boneva-Asiova and Boyanov, 2008).
Waist circumference was measured with a flexible tape measure, with the participant standing erect and their arms relaxed by their sides. The measurement was taken under clothes and around the navel for standardisation. Participants were asked to breathe out gently and the measurement was recorded at the end of expiration to the nearest 0.5 cm.

BP was measured in triplicate using the Omron MX3 BP monitor (Omron Healthcare UK Ltd, Milton Keynes, UK) with the participant in a relaxed sitting position. The cuff was wrapped tightly but without extensive pressure (so an index finger could be inserted between the cuff and arm) around the upper non-dominant arm. The participant was asked to remain relaxed and refrain from movements during the measurements.

2.3 Subjective and quantitative assessment of appetite and palatability

2.3.1 Visual Analogue Scales to assess appetite and palatability

Effects of the nutrition interventions reported in this thesis on appetite were subjectively assessed by asking participants to complete VAS regarding hunger sensations, prospective food consumption, feelings of fullness, the desire to eat something sweet, salty, savoury or fatty, and for some studies the desire to eat a meal or snack (Hill and Blundell, 1982). The reliability and validity of using VAS as a tool to subjectively assess appetite has been reviewed in Chapter 1 (section 1.4.3)

Participants completed a set of VAS at baseline (in duplicate), and then every 30 min following the test preload until the end of the study, generating a temporal profile for each attribute under investigation. The VAS comprised a 100 mm line with each end representing the most extreme sensation associated with the attribute (Figure 2.1 and Appendix D). Participants were asked to mark the point on the line that they felt best represented their feelings of the attribute under investigation at the time. Each set of
VAS were presented as an individual booklet for each timepoint, with each question presented on a separate page. A new booklet was provided for each timepoint. For analysis, ratings were converted into scores in mm taking the left anchor as the origin.

<table>
<thead>
<tr>
<th>How much do you think you can eat?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nothing at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How full do you feel?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all full</td>
</tr>
</tbody>
</table>

**Figure 2.1.** Example VAS to assess sensations regarding appetite.

VAS were also used to assess palatability of test products used in clinical investigations. These were again presented as booklets with each question on a separate page. Attributes investigated included pleasantness of taste, texture and aftertaste.

### 2.3.2 *Ad libitum* pasta based test meal

Quantitative assessment of appetite was carried out following the “preload-test meal” paradigm (reviewed in Chapter 1, section 1.4.2 and 1.4.4), investigating the impact of the preload under investigation on subsequent EI at an *ad libitum* test meal.

The *ad libitum* test meal used in all appetite studies reported in this thesis comprised a homogenous pasta dish made to a standard recipe. The meal was made by cooking Tesco Fusilli Pasta Twists (400 g dry weight) for 12-15 min which was then drained and mixed with Ragu Original Pasta Sauce (500 g), Tesco Mild Cheddar (100 g) and Tesco
Vegetable Oil (30 g). The dish supplied 9750 kJ (2330 kcal), 81.5 g protein, 339.1 g CHO, 70.0 g fat, 15.9 g fibre and had a mean weight of 1517 [SD 45] g and E density of 6.4 [SD 0.19] kJ/g, which far exceeds a usual portion size. The weight and E density varied due to differing amounts of water absorbed by the pasta during cooking, which was accounted for when calculating EI.

The *ad libitum* test meals were served to participants seated in isolated booths free from distraction. The pasta meal was presented on a tray in a large serving bowl pre-weighed prior to serving along with a smaller dish and a 250 ml glass of tap water. Participants were instructed to serve themselves from the large serving bowl into the smaller dish, and to eat until they were comfortably full. They were informed any leftovers could be taken home to discourage them from over-eating. The amount consumed was determined by re-weighing the meal after the participant had eaten, and the corresponding EI and macronutrient intake was calculated.

### 2.3.3 24 h intake

Food and drink diaries were completed for the remainder of the day on study days to determine 24 h intake and were analysed for EI, macronutrient and fibre intake using WinDiets Professional 2005 (The Robert Gordan University, Aberdeen, UK). The Food Standards Agency (FSA) Food Portion Sizes book was used as a guide when actual quantities were not described (Food Standards Agency, 2002), with a medium portion assumed when the portion size was not given. When actual brands were given, the nutritional values for the products were input into the program and used. In this thesis values for fibre intake are based on non-starch polysaccharide quantities from Windiet.
2.4 Breath hydrogen analysis

2.4.1 Equipment

- Gastrolyzer 2 portable hand held breath hydrogen (H₂) monitor (Bedfont Scientific Ltd, Rochester, UK)
- Disposable T-pieces (Bedfont Scientific Ltd, Rochester, UK)
- Universal disposable cardboard mouthpieces (Bedfont Scientific Ltd, Rochester, UK)
- 200 ppm H₂ in air calibration gas (Bedfont Scientific Ltd, Rochester, UK)

2.4.2 Method

During the NDC supplementation study, breath H₂ concentrations in expired breath were monitored at baseline and at regular time intervals postprandially as outlined in the study protocol (reported in Chapter 7) using a Gastrolyzer 2 breath H₂ monitor (Bedfont Scientific Ltd, Rochester, UK).

Disposable cardboard mouthpieces were connected to the Gastrolyzer via a disposable T-piece, and it was ensured that all connections were pushed firmly in place to prevent sample loss. To operate, the ‘Go’ button on the Gastrolyzer was pressed which initiated a 15 second countdown period. During this time participants were asked to inhale and then hold their breath. At the end of the countdown, participants slowly and gently exhaled into the Gastrolyzer via the cardboard mouthpiece, emptying the lungs as far as possible. The Gastrolyzer then analysed the exhaled breath to determine the breath H₂ concentration (in parts per million, ppm).

The Gastrolyzer was calibrated at least once per month to 200 ppm using 200 ppm H₂ in air calibration gas.
2.5 Blood sampling

2.5.1 Venous blood sample collection

2.5.1.1 Equipment and consumables

- Centrifuge Labfuge 400R (Kendro Laboratory Products, Germany)
- Cannulae Y-can 19G and 21G with syringe valve (Beldigo SA, Marche, Belgium)
- 1 ml, 2 ml, 5 ml, 10 ml, 20 ml syringes (BD Plastipak, Devon, UK)
- White card sample collection trays (Southern Syringe Supplies)
- 1 ml & 2 ml Fluoride oxalate tubes with screw cap (Teklab Ltd, Durham, UK)
- 2 ml, 5 ml & 10 ml Dipotassium EDTA polystyrene tubes with screw cap (Teklab Ltd, Durham, UK)
- 6 ml plain red topped serum tubes (Southern Syringe Supplies)
- 5 ml Lithium Heparin tube with wadded cap (Teklab Ltd, Durham, UK)
- 0.5 ml and 1.5 ml Apex plus microtubes (Alpha Laboratories, Hampshire, UK)
- Apex plus screw caps in various colours (Alpha Laboratories, Hampshire, UK)

2.5.1.2 Reagents

- 500 ml bag sodium chloride (NaCl) 0.9% IV solution (Royal Surrey County Hospital)
- 50 ml vial Trasylol Aprotinin 10,000 KIU / ml (Bayer) – used for study in chapter 3
- 100 mg vial Aprotinin from bovine lung, A1153 2-8 TIU / mg solid (Sigma-Aldrich Company Ltd, Gillingham, Dorset, UK) – used to prepare aprotinin solution (10,000 KIU / ml sodium chloride 0.9% solution) for study in chapter 7
2.5.1.3 Method

Venous blood samples were regularly collected from an indwelling intravenous cannula inserted into an antecubital vein as detailed in individual study protocols during clinical investigation days for the studies in Chapters 3 and 7.

Blood was collected from the cannula by syringe (disposing of the first 1-2 ml to account for dead-space in the cannula and to remove NaCl solution). Following blood collection from the cannula, 2 ml NaCl solution was injected into the cannula to keep the line patent.

Collected blood was immediately decanted into fluoride oxalate tubes for glucose analysis, dipotassium EDTA-coated tubes for insulin, non-esterified fatty acid (NEFA), and triglyceride (TAG) analysis, dipotassium EDTA-coated tubes with added aprotinin (200 kIU/ml whole blood) for gut peptide (for example GLP-1, PYY, PP and Ghrelin) analysis and into plain serum (Chapter 3) or lithium heparin (Chapter 7) tubes for SCFA analysis.

Following collection, blood samples in serum tubes were allowed to clot for one hour at room temperature (RT) prior to separation of the serum. All other collected blood samples were gently mixed then stored at 4 °C to reduce degradation until processed. Samples were centrifuged at 1750 g (3000 rpm) for 10 min to separate the plasma from the red blood cells. Plasma and serum samples were aliquoted into 1.5 ml and 0.5 ml microtubes and stored at -20 °C until analysis. In order to minimize inter-assay variability, samples were batch analysed at the end of each study.

2.5.2 Capillary blood sample collection

2.5.2.1 Equipment and consumables

- Centrifuge Labfuge 400R (Kendro Laboratory Products, Germany)
2.5.2.2 Method

Capillary blood samples were collected by fingerprick at screening sessions and regular time intervals during the clinical investigation days for the studies in Chapters 4 and 5.

For the study detailed in Chapter 4 and during screening, collected blood samples were immediately analysed for blood glucose concentrations using the HemoCue 201+ (see 2.6.3.3). Blood samples were also analysed for blood Hb concentrations at screening using the HemoCue Hb 201+ (see 2.6.9).

For the study detailed in Chapter 5, capillary samples were collected into 300 µl glucose / fluoride microvette tubes for glucose and insulin analysis and were gently mixed then stored at 4 °C until processed. The samples were centrifuged at 1750 g (3000 rpm) for 10 min to separate the plasma from the red blood cells. The plasma was aliquoted into 0.5 ml microtubes and stored at 4 °C until analysed for plasma glucose concentrations using the YSI 2300 Stat Plus Glucose Analyser (see 2.6.3.2). Glucose analysis was carried out on the same day as blood collection. The remaining plasma was stored at -20 °C until analysis of plasma insulin concentrations. To minimize inter-assay variability, samples were batch analysed for insulin levels at the end of the study.

2.6 Blood, plasma and serum biochemical analysis

All frozen plasma samples were defrosted at 4 °C on the day of analysis, vortex mixed and then centrifuged at 1750 g (3000 rpm) for 5 min to separate the plasma from any fibrin that could be present and would otherwise interfere with accuracy of the assay.
All samples batch analysed at the end of the clinical study were re- aliquoted into new microtubes prior to analysis.

2.6.1 Equipment

• ILAB 650 (Instrumentation Laboratory, Milan, Italy)
• YSI 2300 Stat Plus Glucose Analyser (YSI Incorporated, Yellow Springs, Ohio, USA)
• HemoCue Glucose 201 Plus (HemoCue Ltd, Sheffield, UK)
• Microplate Washer AW1 (Anthos Labtec Instruments, Wals, Austria)
• Microplate Luminometer LB 960 Centro (Berthold Technologies, Wildbad, Germany)
• Gamma Counter - Wizard with Multicalc level 4.M software (Wallac International, Turku, Finland)
• HemoCue Hb 201 Plus (HemoCue Ltd, Sheffield, UK)
• Pipetman P20, P100, P200, P1000, P5000 & tips (Gilson Inc, Middleton, USA)
• Positive displacement C250 pipette & tips (Anachem Ltd, Luton, UK)

2.6.2 Consumables and reagents

• LP3 and LP4 test tubes (L.I.P. Ltd, Shipley, UK)
• Hemocue Glucose 201 microcuvettes (HemoCue Ltd, Sheffield, UK)
• Hemocue Hb 201 microcuvettes (HemoCue Ltd, Sheffield, UK)
• GlucoTrol 2.4, 6.0 and 9.7 mmol/l for QC (HemoCue Ltd, Sheffield, UK)
• Glucose (Oxidase) analysis kit (Instrumentation Laboratory Co., Lexington, USA)
• Triglycerides analysis kit (Instrumentation Laboratory Co., Lexington, USA)
• NEFA analysis kit (Randox Laboratories Ltd, Co. Antrim, UK)
• Cholesterol analysis kit (Instrumentation Laboratory Co., Lexington, USA)
• Direct HDL-Cholesterol analysis kit (Instrumentation Laboratory Co., Lexington, USA)
2.6.3 Analysis of plasma and whole blood glucose

Three different methods were adopted to analyse glucose concentrations:

2.6.3.1 ILAB 650

Plasma glucose concentrations of samples collected from the studies in Chapters 3 and 7 were analysed by an enzymatic colorimetric method using the enzyme \( \text{glucose oxidase} \) (Instrumentation Laboratory Co., Lexington, USA) on the ILAB 650 (Instrumentation Laboratory, Milan, Italy). \( \text{Glucose oxidase} \) catalyses the oxidation of glucose to produce gluconic acid and hydrogen peroxide (\( \text{H}_2\text{O}_2 \)).

\[
\text{D-Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Glucose oxidase}} \text{Gluconic Acid} + \text{H}_2\text{O}_2
\]

All \( \text{H}_2\text{O}_2 \) generated then reacts with phenol and 4-aminoantipyrine, catalysed by \( \text{peroxidase} \), leading to the production of red quinoneimine and water.

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

The concentration of red quinoneimine generated is determined by measuring the absorbance at 510 nm. The concentration of red quinoneimine generated is directly proportional to the amount of glucose in the sample.

QCs were included at the start and end of each assay. Intra-assay coefficients of variation (CV) were <2.0 % and <2.5 % and inter-assay CV were 1.3 % and 2.5 % at 5.31 mmol/l and 14.0 mmol/l respectively.
2.6.3.2 YSI 2300 Stat Plus Glucose Analyser

Plasma glucose concentrations of samples collected from the study in Chapter 5 were analysed using the YSI 2300 Stat Plus Analyser (YSI Incorporated, Yellow Springs, Ohio, USA), which uses an immobilized enzyme biosensor. The enzyme glucose oxidase is immobilised between two membrane layers, one comprising polycarbonate and the other cellulose acetate. As the injected sample enters the membrane layer, glucose oxidase catalyses the oxidation of glucose contained in the sample, with $H_2O_2$ generated as a by-product.

\[ D\text{-Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} D\text{-glucono-\delta-lactone} + H_2O_2 \]

Once generated, $H_2O_2$ passes through the cellulose acetate membrane into a platinum anode. This catalyses $H_2O_2$ oxidation to generate electrons. The resultant current is proportional to the glucose concentration of the injected sample.

\[ H_2O_2 \xrightarrow{\text{Platinum anode}} 2H^+ + O_2 + 2e^- \]

A 10 mmol/l QC was included at the start and end of a run of samples for each participant. The intra-assay CV was <2 % and inter-assay CV was 1.2 %.

2.6.3.3 HemoCue Glucose 201 Plus

Glucose concentrations of whole blood capillary samples collected by fingerprick for the study in Chapter 4 and during screening were analysed with the HemoCue Glucose 201 Plus (HemoCue Ltd, Sheffield, UK). The HemoCue Glucose 201 Plus is a portable photometric system which uses a glucose dehydrogenase photometric method.

The first drop of capillary blood drawn following fingerprick was wiped away and the next drop of blood was loaded directly from the fingertip onto a HemoCue microcuvette, with the correct volume of blood being drawn onto the cuvette by
capillary action. Excess blood was wiped from the cuvette, then the cuvette was placed into the cuvette holder.

The microcuvette contains dried reagents saponin, nicotinamide-adenine dinucleotide (NAD), methylthiazolyldiphenyl tetrazolium (MTT), propylene glycol, ammonium chloride and sodium fluoride and an enzyme mix of glucose dehydrogenase, diaphorase and mutarotase. Once blood is introduced to the microcuvette, erythrocytes are haemolysed under the action of saponin to release their contents, including α-D-glucose which undergoes mutarotase catalysed conversion to β-D-glucose. Glucose dehydrogenase (which is specific for β-D-glucose) catalyses β-D-glucose oxidation, generating NADH in quantities equimolar to available glucose.

\[
\beta-D\text{-Glucose} + \text{NAD}^+ \xrightarrow{\text{Glucose dehydrogenase}} \beta-D\text{-Gluconolactone} + \text{NADH}
\]

In the presence of generated NADH, diaphorase catalyses the conversion of MTT to form a coloured formazan which is quantified photometrically at 660 nm and 840 nm. The formazin concentrations are directly proportional to sample glucose concentrations.

\[
\text{MTT} + \text{NADH} \xrightarrow{\text{Diaphorase}} \text{Formazan} + \text{NAD}^+
\]

The HemoCue offers the advantage of being portable, providing instant results and only requiring a very small quantity of blood. The reliability and accuracy of the HemoCue system is reported to be good (Ashworth et al., 1992, Stork et al., 2005, Voss and Cembrowski, 1993), although this finding is not unanimous (Torjman et al., 2001), particularly in neonates (Dahlberg and Whitelaw, 1997).
2.6.4 Analysis of plasma TAG

Plasma TAG concentrations were analysed using an enzymatic colorimetric method (Instrumentation Laboratory Co., Lexington, USA) on the ILAB 650 (Instrumentation Laboratory, Milan, Italy). In this method, \( \text{H}_2\text{O}_2 \) is generated from TAG in 3 steps.

In the first step glycerol is generated from the lipoprotein lipase catalysed hydrolysis of TAG. The glycerol is then phosphorylated with adenosine triphosphate (ATP) to form glycerol-3-phosphate and adenosine diphosphate (ADP), catalysed by glycerol kinase. Glycerol-3-phosphate oxidase then catalyses the oxidation of glycerol-3-phosphate to generate dihydroxyacetone and \( \text{H}_2\text{O}_2 \).

\[
\text{TAG} \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol + FFA} \\
\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-P + ADP} \\
\text{Glycerol-3-P + O}_2 \xrightarrow{\text{Glycerol-3-P oxidase}} \text{Dihydroxyacetone phosphate + H}_2\text{O}_2
\]

Finally peroxidase catalyses the oxidative coupling of 4-chlorophenol and 4-aminophenazone in the presence of the generated \( \text{H}_2\text{O}_2 \) to form a quinoneimine red dye complex. The concentration of the generated quinoneimine complex is quantified by measuring absorbance at 510 nm and is directly proportional to the sample TAG concentration.

\[
\text{H}_2\text{O}_2 + 4\text{-chlorophenol + 4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Red quinoneimine + H}_2\text{O}
\]

QCs were included at the start and end of each assay. Intra- and inter-assay CV were < 2 % and 1.25 % at 1.5 mmol/l and <1 % and 0.99 % at 2.5 mmol/l respectively.
2.6.5 Analysis of plasma NEFA

Plasma NEFA concentrations were analysed using an enzymatic colorimetric method (Randox Laboratories Ltd, Co. Antrim, UK) on the ILAB 650 (Instrumentation Laboratory, Milan, Italy). In this assay, $H_2O_2$ is generated from NEFA in a two-step reaction. In the first step, the coupling of NEFA to Coenzyme A (CoA) is catalysed by Acetyl CoA Synthetase in the presence of ATP. The resultant Acyl CoA is then oxidised, catalysed by Acyl CoA Oxidase, to generate 2,3-trans-Enoyl-CoA and $H_2O_2$.

\[
\text{NEFA} + \text{ATP} + \text{CoA} \xrightarrow{\text{Acetyl CoA Synthetase}} \text{Acyl CoA} + \text{AMP} + \text{PPi}
\]

\[
\text{Acyl CoA} + \text{O}_2 \xrightarrow{\text{Acyl CoA Oxidase}} 2,3\text{-trans-Enoyl-CoA} + H_2O_2
\]

Peroxidase then catalyses the oxidative coupling of N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS) and 4-aminophenazone (4-AAP) in the presence of the generated $H_2O_2$ leading to the formation of a purple adduct.

\[
2H_2O_2 + \text{TOOS} + 4\text{-AAP} \xrightarrow{\text{Peroxidase}} \text{Purple adduct} + 4H_2O
\]

The concentration of the purple adduct is quantified by measuring the absorbance at 550 nm and is directly proportional to the concentration of NEFA in the sample.

QCs were included at the start and end of each assay. Intra- and inter-assay CV were respectively <3 % and 4.3 % at 1.09 mmol/l and <3.5 % and 4.2 % at 1.11 mmol/l.

2.6.6 Analysis of plasma total cholesterol

Plasma total cholesterol concentrations were analysed on the ILAB 650 (Instrumentation Laboratory, Milan, Italy) using a three-step enzymatic colorimetric method (Instrumentation Laboratory Co., Lexington, USA). In the first step, cholesterol
esterase catalyses the hydrolysis of cholesterol ester. In the next step, the resultant free cholesterol is oxidised, catalysed by cholesterol oxidase, to generate H$_2$O$_2$. Finally, peroxidase catalyses the oxidative coupling of 4-aminoantipyridine (4-AA) with phenol in the presence of the generated H$_2$O$_2$, to form quinoneimine, a red dye complex.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{FFA}
\]

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

\[
\text{H}_2\text{O}_2 + 4\text{-AA} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine (red adduct)} + 4\text{H}_2\text{O}
\]

The concentration of generated quinoneimine is directly proportional to the concentration of total cholesterol in the original sample, and is quantified by measuring absorbance at 500 nm.

QCs were included at the start and end of each assay. Intra- and inter-assay CV were < 2 % and 2.2 % at 2.4 mmol/l and <1.5 % and 1.1 % at 5.1 mmol/l respectively.

### 2.6.7 Analysis of plasma HDL-cholesterol

Plasma HDL-cholesterol concentrations were also analysed on the ILAB 650 (Instrumentation Laboratory, Milan, Italy) using an enzymatic colorimetric method (Randox Laboratories Ltd, Co. Antrim, UK) that consists of two distinct reaction steps. In the first step, cholesterol esterase and cholesterol oxidase then catalase catalyse the elimination of chylomicron and VLDL- and LDL-cholesterol.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{FFA}
\]

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

\[
2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2
\]
Following this elimination, surfactants activate the release of HDL-cholesterol. *Cholesterol esterase* then catalyses the hydrolysis of the released cholesterol ester to free cholesterol which is then oxidised to generate $\text{H}_2\text{O}_2$.

*Peroxidase* catalyses the oxidative coupling of 4-aminoantipyridine (4-AA) with N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) in the presence of the generated $\text{H}_2\text{O}_2$, forming a quinine imine dye complex (quinone pigment). During this second step, sodium azide inhibits *catalase* (used in the first step).

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

The concentration of generated quinone pigment is directly proportional to the sample cholesterol concentration, and is quantified by measuring absorbance at 600 nm.

QCs were included at the start and end of each assay. Intra- and inter-assay CV were <2 % and 0.18 % at 0.82 mmol/l, <2 % and 0.21 % at 1.32 mmol/l and <1.5 % and 0.10 % at 1.88 mmol/l respectively.

### 2.6.8 Analysis of plasma insulin

#### 2.6.8.1 Radioimmunoassay

Plasma insulin concentrations for samples collected from the studies in Chapters 3 and 7 were analysed using a Human Insulin Specific radioimmunoassay (RIA) kit (Millipore, St. Charles, Missouri). This assay determines plasma insulin concentrations by the double antibody / PEG technique, utilising human insulin antiserum raised in guinea pig and $^{125}$I-labelled human insulin.

The assay is 100 % specific for human insulin, <0.2 % specific for human proinsulin and des 31-32 split proinsulin, 76 % specific for des 64-65 split proinsulin, 100 % specific for porcine and canine insulin, 62 % specific for bovine insulin and 0.1
specific for rat insulin. There is no cross reactivity with insulin growth factor, glucagon, Somatostatin and PP. The lowest level of detection is 2 μU/ml and the limit of linearity is at a concentration of 200 μU/ml with a 100 μl sample. There is no cross-reactivity with human proinsulin, therefore measuring true insulin concentrations.

This is a two day assay. On day 1, plasma samples were defrosted at 4°C, then vortex mixed and centrifuged at 1750 g (3000 rpm) for 5 min to separate the plasma from any fibrin that could be present and would interfere with accurate pipetting. Samples were set up in duplicate in LP3 tubes in the order outlined in Table 2.1.

The ¹²⁵I-insulin was hydrated by adding the entire contents of the supplied label hydrating buffer (containing Normal Guinea Pig serum as a carrier) and allowed to set at RT for at least 30 min, with occasional gentle mixing.

Table 2.1. Insulin RIA protocol. Based on the Manufacturers information booklet (Millipore, Missouri, USA)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tubes</td>
<td>Tube No</td>
<td>Add assay buffer</td>
</tr>
<tr>
<td>Total Count</td>
<td>1,2</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>3,4</td>
<td>300 μl</td>
</tr>
<tr>
<td>Ref</td>
<td>5,6</td>
<td>300 μl</td>
</tr>
<tr>
<td>Standards</td>
<td>7-20</td>
<td>300 μl</td>
</tr>
<tr>
<td>QC 1</td>
<td>21, 22</td>
<td>300 μl</td>
</tr>
<tr>
<td>QC 2</td>
<td>23, 24</td>
<td>300 μl</td>
</tr>
<tr>
<td>Samples</td>
<td>25-n</td>
<td>300 μl</td>
</tr>
</tbody>
</table>

To start, assay buffer was added to all tubes except Total Count tubes, adding 300 μl to the non-specific binding (NSB) tubes, 200 μl to the zero-standard Reference (Ref) tubes and 100 μl to all remaining tubes, including standards, QC samples and plasma samples,
using an Eppendorf multipipette. The purpose of the NSB tube is to determine non-specific binding of the label to assay tubes.

Next, 100 μl standard, QC or plasma samples were accurately dispensed in duplicate to relevant tubes using a positive displacement pipette. Purified recombinant human insulin standards were provided with the kit in the following concentrations: 2, 5, 10, 20, 50, 100 and 200 μU/ml. When samples were available in only small volumes, 50 μl of sample was added to the tube then an additional 50 μl buffer was also added.

Following this, 100 μl hydrated ¹²⁵I-insulin was added to all tubes using a an Eppendorf multipipette, then 100 μl human insulin antibody was added to all tubes except Total Count tubes and NSB Tubes. The tubes were then covered, vortex mixed and incubated overnight at RT for 20-24 h.

On day 2 of the assay, 1.0 ml cold (4 °C) precipitating (ppt) reagent (Goat anti-Guinea Pig IgG serum containing 3% PEG) was added to all tubes except Total Count tubes. The tubes were covered, vortex mixed and incubated for 20-30 min at 4 °C, then centrifuged for 40 min at 4 °C at 1750 g (3000 rpm).

The supernatant was immediately aspirated under vacuum from all tubes, taking care not to disrupt the solid pellet. The pellet, containing the bound fraction, was counted on a gamma counter (Wallac Wizard 1470 with Multicalc level 4.M software). Insulin concentrations of each plasma and QC sample were determined by the Multicalc software based on the standard curve, Total Counts and NSB readings.

QCs were included at the start and end of each assay. Intra- and inter-assay CV were respectively 10.1 % and 8.4 % for QC level 1 (expected range 5.9 to 12.3 μU/ml) and 5.6 % and 8.1 % for QC level 2 (expected range 20.4 - 42.4 μU/ml).
2.6.8.2 Immunochemiluminometric assay

Plasma insulin concentrations in capillary samples collected for the study reported in Chapter 5 were analysed using a Human Insulin Specific immunochemiluminometric assay (Invitron Ltd, Wyastone Business Park, Monmouth, UK). This is a two-site immunoassay which employs an insulin-specific solid phase antibody immobilised in mitrotitre plate wells, and a soluble chemiluminescent-labelled antibody. This assay requires only small plasma volumes (25μl per well), making it suitable to analyse capillary plasma samples collected by fingerprick.

Plasma samples were defrosted, and all kit components and samples were brought to RT. Samples were vortex mixed then centrifuged at 1750 g (3000 rpm) for 5 min at RT to separate the plasma from any fibrin that could be present. Standards supplied with the kit (ranging from 0 to 200 mU/l) were reconstituted by the addition of deionised water (1 ml), allowed to set for 5 min, then mixed gently to ensure all solids were in solution. Working strength chemiluminescent-labelled antibody solution was prepared by accurately pipetting labelled antibody concentrate (900 μl) into a bottle of antibody diluent, and working strength wash buffer was prepared by diluting 1 part wash buffer concentrate with 9 parts deionised water.

Working strength chemiluminescent-labelled antibody solution (100 μl) was accurately pipetted into each mitrotitre plate well being used, followed by 25 μl standard, QC or sample. All standards, QCs and samples were analysed in duplicate. The plate was sealed with the supplied plate sealers and was incubated at 37 °C for 2 h. Following incubation, the plate sealers were removed and the plate was washed with working strength wash buffer in triplicate using an automatic plate washer, Microplate Washer AW1 (Anthos Labtec Instruments, Wals, Australia). The luminescence of the
bound label was quantified using a microtitre plate luminometer, Microplate Luminometer LB 960 Centro (Berthold Technologies, Wildbad, Germany).

This assay cross-reacts 100% with insulin, 1.2% with intact proinsulin, 1.6% with 32-33 split proinsulin, 0.8% with des 31-32 split proinsulin, 23% with 65-66 split proinsulin and 44% with des 64-65 split proinsulin and does not cross react with C-peptide.

QCs were included at the beginning of each assay. Intra and inter-assay CV were 5.8 % and 22.3 % at 12.5 pmol/l, and 11.3 % and 26.9 % at 162 pmol/l respectively.

2.6.9 Analysis of blood haemoglobin

Hb concentrations of whole blood capillary samples collected by fingerprick during screening were analysed with the HemoCue Hb 201 Plus (HemoCue Ltd, Sheffield, UK), utilising a modified azidemethemoglobin photometric method.

The first drop of capillary blood drawn following fingerprick was wiped away and the next drop of blood was loaded directly from the fingertip onto a HemoCue microcuvette, with the correct volume of blood being drawn onto the cuvette by capillary action. Excess blood was wiped from the cuvette, then the cuvette was placed into the cuvette holder.

The microcuvette contains dried reagents sodium deoxycholate, sodium nitrite and azide. Sodium deoxycholate activates the disintegration of erythrocyte membranes to release Hb. Iron within the Hb is then oxidised from the ferrous to the ferric state under the action of sodium nitrite, to form methemoglobin, which is then coupled with azide to form azidmethemoglobin, a dye complex. The concentration of generated azidmethemoglobin is quantified photometrically at 570 nm and 880 nm and is directly proportional to the Hb concentration in the sample.
2.7 Urinary SCFA biochemical analysis

Dr Douglas Morrison at SUERC analysed the urine samples collected in the study reported in Chapter 6 by GC-MS (gas chromatography-mass spectrometry) to quantify SCFA levels. Prior to analysis the urine samples were thawed defrosted at 4 °C, purified and derivatised as previously described (Morrison et al., 2004) and outlined below.

2.7.1 Equipment

- GC-MS (Hewlett Packard 5890 II GC; Optic II temperature programmable injector; Fisons Instruments A200S autosampler; VG Trio-1000 quadrupole MS with electron ionisation)
- GC column Rtx-5MS 30m x 0.25 mm x 0.25 μm (Thames Restek, Saunderton, UK)
- Solid phase extraction (SPE) manifold (International Sorbent Technology, UK)

2.7.2 Consumables and reagents

- Amicon Centriplus YM-30 centrifugal filter device (Millipore Ltd., Watford, UK)
- SPE Bakerbond SDB-2 disposable cartridges 200 mg, 3 ml capacity (Mallinckrodt Baker UK, Milton Keynes, UK)
- 2 ml crimp-cap and 1 ml tapered vials (Chromacol, Welwyn Garden City, UK)
- 3-methylvaleric acid (3-MV) internal standard (IS) (Sigma Aldrich, Poole, UK)
- tert-butyldimethylsilyl chloride (tBDMS-Cl) (Fluka Chemical Co., Poole, UK)

2.7.3 Sample preparation and GC-MS analysis

Thawed 2.5 ml aliquots of urine or deionised water (for process blank), 4 M NaCl solution (2.5 ml) and IS (400 nM 3-methylvaleric acid solution in 0.15 M NaOH) were added to 25 ml plastic universal bottles and vortex mixed. When necessary 0.15 M
NaOH solution was added to adjust to pH 7-8. This mixture was applied to a centrifugal filter device (Amicon Centriplus YM-30) and centrifuged at 3000 g for 2 h to remove proteins and other high molecular weight materials by ultrafiltration. The resultant ultrafiltrate was acidified with 6M HCl to pH 2-2.5 then loaded onto Bakerbond SDB-2 SPE cartridges (Mallinckrodt Baker, UK) mounted in a SPE manifold (International Sorbent Technology, UK), that were pre-conditioned with 2 x 2 ml methanol (MeOH), then 1 x 2 ml distilled water then 1 x 2 ml 0.1M HCl. SCFA in the urine sample were eluted with 2 x 500 µl MeOH into 100 µl 1M NaOH in 2 ml crimp cap vials (Chromacol, UK) to form stable, non-volatile Na salts of the SCFA. The vial contents were mixed and dried overnight at 55 °C. Vials were capped and stored at RT until ready for analysis.

On the day of analysis the SCFA Na salts were acidified with 4 M HCl (50 µl) then extracted into diethyl ether (400 µl). An aliquot of the ether extract (50 µl) was transferred to a 1 mL tapered vial (Chromacol, UK) containing dry acetonitrile (100 µl) and tBDMS-Cl (Fluka Chemical Co., UK) and heated at 60 °C for 1 h to form SCFA tBDMS esters (which are easily identified by MS).

Samples were analysed by GC-MS on the day of derivatisation following the program sequence previously described (Morrison et al., 2004). The method was calibrated with the use of external SCFA standard solutions relative to IS.

2.8 Estimation of insulin sensitivity

2.8.1 Fasting insulin sensitivity

Fasting insulin sensitivity (S_f) was assessed by Homeostasis Assessment Model (HOMA) using the HOMA2 Calculator Version 2.2 (University of Oxford, Oxford, UK). The HOMA model estimates steady state β-cell function (%B), insulin sensitivity
(%S), and insulin resistance (HOMA IR), which is the reciprocal of %S (100/%S) from paired fasting plasma glucose and plasma insulin (or C-peptide) concentrations (Levy et al., 1998, Matthews et al., 1985, Wallace et al., 2004).

### 2.8.2 Postprandial insulin sensitivity

Postprandial insulin sensitivity (Oral \(S_I\)) was assessed using the minimal model index method (Caumo et al., 2000), which was designed to mathematically describe how exogenous glucose reaches the systemic circulation. It is essentially assessing non-insulin dependent glucose disposal.

In this model, Oral \(S_I\) is estimated following ingestion of CHO, based on the CHO load within the specific meal, basal and postprandial plasma glucose and insulin concentrations at each timepoint and the individual’s BW, using the following equation in Figure 2.2.

#### Figure 2.2. Equation used to estimate Oral \(S_I\).

Taken from (Caumo et al., 2000)

\[
Oral\ S_I = \frac{f \cdot D_{oral} \cdot \Delta g (t)}{AUC [\Delta g (t)]} - GE \cdot \frac{AUC [\Delta g (t)]/g(t)}{AUC [\Delta g (t)]} 
\]

Where: 
- \(f\) = fraction of ingested CHO to reach peripheral circulation as glucose
  (a nominal value of 1 was used for \(f\) for all participants in all studies)
- \(D_{oral}\) = dose of ingested CHO per unit BW (mg/kg BW)
- \(GE\) = glucose effectiveness (fixed at 0.024 dl/kg.min)
- \(AUC\) = area under curve calculated from time zero until test end for glucose \((g(t))\) and insulin \((i(t))\)
This equation is used when postprandial glucose concentrations remain above basal glucose levels, with an adapted equation being used when postprandial glucose concentrations fall below basal levels (Caumo et al., 2000). The model assumes total glucose disposal from the system is equal to the amount of glucose entering the peripheral circulation when basal levels are reached, allowing for first pass extraction by the liver, and that the rate of GE is constant for the entire study period (Caumo et al., 2000).

2.9 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0 (SPSS Inc., Chicago, USA) as described within the methods section of individual chapters, with statistical significance assumed at p≤0.05 unless otherwise stated.

All data was first tested for normality using the Kolmogorov-Smirnov test. Data was assumed to be normally distributed if the p value was not significant. Data was compared using parametric tests when normally distributed and non-parametric tests when not normally distributed, as stated within individual chapters.

The AUC for temporal data such as postprandial plasma metabolites and subjective VAS ratings were calculated using the trapezoidal rule.
Chapter 3A: Sensory evaluation of propionate-rich sourdough bread to assess suitability for use in appetite studies

3.1 Introduction

It has previously been found that product palatability can influence appetite and the metabolic response to nutrients (reviewed Chapter 1, section 1.11). Therefore, in order to eliminate the potential confounder of product palatability in appetite investigations it is necessary to ensure test and placebo (PL) products are equally palatable.

Therefore, prior to commencing the study reported in Chapter 3B, a sensory evaluation of the propionate-rich SOUR bread test product was carried out to determine if the sensory attributes of the bread were acceptable and appropriately matched to the PL bread product. The sensory evaluation was carried out following well established methodology reported in British Standard documents (ISO, 1992, ISO, 2003, ISO, 2004, ISO, 2005b, ISO, 2005a, ISO, 2006) and included the paired test, triangle test, ranking and rating tests and the use of Hedonic Scales. The SOUR bread was initially manufactured using three different levels of starter culture (1, 2 and 3% domani starter culture), so the sensory evaluation tests were designed to investigate the acceptability of the bread products at each level of starter culture.

3.2 Aims

The sensory evaluation therefore aimed to investigate the sensory attributes of a novel propionate-rich SOUR bread made using either 1, 2 or 3% domani starter culture in comparison to a visually identical PL bread (made without starter culture) in order to assess if the novel SOUR bread would be acceptable to volunteers in further studies.
3.3 Methods

3.3.1 Participants

Fourteen healthy participants (four male, ten female) aged between 25 and 45 y were recruited from the student and staff population at the University of Surrey by e-mail advertisement. To assess suitability for inclusion participants completed a Lifestyle and Medical Questionnaire and DEBQ (see section 2.1) prior to participating.

For inclusion, participants were required to have a restraint score of <3.5 (assessed using the DEBQ) and have been stable weight for at least 3 months. Exclusion criteria included currently pregnant or breast feeding, on a weight reducing diet, history of gastrointestinal disorders, history of eating disorders, drug or alcohol abuse in the last 2 y, current habitual smoker or high habitual alcohol intake (>20 units per week).

The mean participant age was 29.1 [SD 6.4] y and mean DEBQ restraint, emotional and external cue scores were 2.3 [SD 0.6], 2.0 [SD 0.5] and 2.7 [SD 0.6] respectively.

3.3.2 Ethics

All participants gave written consent prior to commencing the study. Approval for the study was granted by the University of Surrey Ethics Committee on 14th March 2007 (EC/2007/18/SBMS), and the study took place during March and April 2007 in the CIU at the University of Surrey.

3.3.3 Study protocol

Participants attended in the mid-afternoon at approximately 14.30 h on one occasion following a 2 h fast. The sensory evaluation questionnaires were designed to be self-explanatory, therefore upon arrival following a short brief participants were seated in
individual cubicles free from external noise, smells and other potential distractions, and away from other participants.

Once seated in the cubicle, participants were presented the sensory evaluation questionnaire, which comprised six separate tests (see 3.3.5). Tests were presented to participants three at a time (i.e. Tests 1, 2 and 3, then Tests 4, 5 and 6), with 15 min allowed per three tests. Boiled and cooled water was provided as a palate cleanser to rinse the mouth in between each test. Once all six tests were completed, participants were free to leave the CIU.

Bread samples were identified by a random three digit number between 001 and 999 unique to each sample test to avoid samples in previous tests being identified as the same bread. The numbers used were also unique to each questionnaire for each participant. Samples were presented in plain cake cases labelled with the sample number and placed on disposable paper plates labelled with the corresponding test number.

3.3.4 Test products

The SOUR and PL bread test products were manufactured and supplied by Holgran (a trading name for RHM Group Limited). The SOUR bread was made using a starter culture, Domani, containing microflora that favour the production of propionate. The Domani culture was used at a level of 1 %, 2 % and 3 %, resulting in SOUR bread containing increasing quantities of propionate (Table 3.1). The non-sourdough PL bread was made using yeast.

The SOUR and PL breads were visually identical (white sliced bread) and well matched for energy, macronutrient and fibre content (Chapter 3A, Table 3.7).
Table 3.1. Propionate concentrations in non-sourdough PL bread and SOUR bread made using 1%, 2% and 3% Domani starter culture. Propionate levels determined by analysis by Holgran

<table>
<thead>
<tr>
<th>Bread</th>
<th>Propionic acid</th>
<th>Calcium propionate</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>mmol/kg</td>
<td>mg/kg</td>
</tr>
<tr>
<td>PL</td>
<td>31</td>
<td>0.42</td>
<td>39</td>
</tr>
<tr>
<td>1% SOUR</td>
<td>855</td>
<td>11.55</td>
<td>1077</td>
</tr>
<tr>
<td>2% SOUR</td>
<td>1675</td>
<td>22.64</td>
<td>2111</td>
</tr>
<tr>
<td>3% SOUR</td>
<td>2365</td>
<td>31.96</td>
<td>2980</td>
</tr>
</tbody>
</table>

3.3.5 Sensory evaluation questionnaire


Table 3.2. Procedure for tests used in sensory evaluation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Paired Test</strong> (Test 1)</td>
<td>Participants were presented with 2 bread samples (3% domani SOUR and PL) and asked to indicate which sample they preferred (ISO, 2005b).</td>
</tr>
<tr>
<td><strong>Triangle Difference Test</strong> (Test 2)</td>
<td>Participants were presented with 3 bread samples (two samples of 3% domani SOUR and one sample of PL or vice versa) and asked to indicate which sample differs from the others (ISO, 2004).</td>
</tr>
<tr>
<td><strong>Ranking Test</strong> (Test 3)</td>
<td>Participants were presented with 4 bread samples (1, 2 and 3% domani SOUR and PL) and asked to rank them in order of preference (ISO, 2006).</td>
</tr>
<tr>
<td><strong>Acceptability Test</strong> (Test 4)</td>
<td>Participants were presented with 4 bread samples (1, 2 and 3% domani SOUR and PL) and asked to indicate how much they like each sample using a 9 Point Hedonic Scale (ISO, 2003).</td>
</tr>
<tr>
<td><strong>Rating Tests</strong> (Tests 5 &amp; 6)</td>
<td>Participants were presented 2 bread samples (3% domani SOUR and PL) for each attribute being assessed (Test 5 – aftertaste, and Test 6 - acidic taste) and asked to evaluate the intensity of each attribute using quantitative dynamic response scales (ISO, 2003).</td>
</tr>
</tbody>
</table>
In order to minimise bias, bread samples were presented in different permutations for each participant. For example in the Paired and Rating Test where two samples were presented (SOUR 3% and PL), samples were given in the order SOUR, PL for some and PL, SOUR for others.

### 3.3.6 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0. Differences in the proportions of participants indicating preference between PL and 3 % domani SOUR in the paired test and in proportions of participants correctly identifying the odd sample out in the triangle difference test were compared using the chi-square goodness of fit test.

Ranking and acceptability scores for the PL and 1 %, 2 % and 3 % domani SOUR bread samples were compared by Friedman’s ANOVA analysis. Rating scores for aftertaste and acid taste of PL and 3 % domani SOUR bread samples were compared using paired samples t-test analysis.

Differences were considered significant at a level of p<0.05.
3.4 Results

3.4.1 Paired test

64% of participants (9 out of 14) indicated they preferred the 3 % domani SOUR bread to the PL. This was a non-significant finding when analysed using the chi-square goodness of fit test.

3.4.2 Triangle difference test

The odd sample was correctly identified from a choice of three samples (odd one out plus two identical samples) by 64 % of participants (9 out of 14). This was a non-significant finding when analysed using the chi-square goodness of fit test.

3.4.3 Ranking & acceptability tests

As Table 3.3 shows, the rank scores for each of the three types of SOUR bread (made using 1 %, 2 % and 3 % domani starter culture) and non-sourdough PL bread did not differ significantly between bread types when analysed by Friedman's ANOVA.

Similarly, the acceptability scores for each of the three types of SOUR bread and PL bread did not differ significantly between bread types when analysed by Friedman's ANOVA.

Table 3.3. Sum rank score and mean [SD] acceptability score following sensory evaluation of PL and SOUR (1 %, 2 % & 3 % domani) breads
Comparisons carried out by Friedman's ANOVA analysis (n=14)

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>SOUR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum rank score</td>
<td>36</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Mean acceptability score</td>
<td>5.6 [1.3]</td>
<td>5.9 [1.5]</td>
<td>5.9 [1.7]</td>
</tr>
</tbody>
</table>
3.4.4 Rating test

The ratings for both aftertaste and the level of acidic taste did not differ significantly between the PL non-sourdough bread and SOUR bread made using 3% domani starter culture (Table 3.4).

Table 3.4. Ratings of aftertaste and acidic taste following sensory evaluation of PL and SOUR (3% domani) breads
Comparisons carried out by paired samples t test (n=14)

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>SOUR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>4.9</td>
<td>2.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Acidic taste</td>
<td>3.3</td>
<td>2.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

3.5 Conclusion

The sensory evaluation found no significant differences in preference between PL and 3% domani SOUR breads. There were also no significant differences in identifying the odd one out when presented with two samples of PL and one of 3% domani SOUR or vice versa. Furthermore, the sum rank scores of preference and the mean acceptability scores did not differ significantly between PL and SOUR breads made using 1%, 2% and 3% domani starter. In addition, ratings for level of acid taste and aftertaste did not differ significantly between PL and 3% domani SOUR.

These results indicate that the SOUR bread is equally acceptable to and indistinguishable from PL, and is therefore suitable for use in appetite studies. Furthermore, the SOUR bread made using the highest level of domani starter culture (3%) can be used in these studies.
Chapter 3B: Acute effects of a propionate-rich sourdough bread on appetite, food intake and metabolic response

3.6 Introduction

The provision of Na propionate baked into bread is reported to significantly increase subsequent satiety (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995). Additionally it acutely reduces postprandial glycaemia (Todesco et al., 1991, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995) and insulinaemia (Liljeberg et al., 1995, Liljeberg and Bjorck, 1996, Darwiche et al., 2001) (reviewed in sections 1.8.1 and 1.9.1), suggesting propionate rich bread may be a promising functional food product. Possible mechanisms include delayed gastric emptying (Liljeberg and Bjorck, 1996, Darwiche et al., 2001), and via activation of the SCFA receptors GPR41 and GPR43 expressed in adipocytes which are reported to stimulate leptin release (Covington et al., 2006, Xiong et al., 2004) and inhibit lipolysis (Ge et al., 2008, Hong et al., 2005) respectively when activated (see section 1.7). Postprandial effects on plasma NEFA and TAG have not yet been investigated.

There were however various weaknesses with the study design in previous studies, particularly in investigating effects on appetite. Appetite in humans is regulated by a complex array of stimuli within a psychobiological system (Blundell et al., 1994, Blundell and Halford, 1994), therefore the study design for investigations into effects on appetite require careful consideration. In previous studies (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995), conclusions regarding effects of oral propionate ingestion on satiety were based solely on results from a single bipolar rating scale that used confusing language (reviewed in section 1.8.1), with no quantitative assessment made, thus warranting further investigation. Instead, a set VAS would be more suitable to
assess appetite ratings. VAS are widely used in appetite research and although not without limitations, have been found to generate reproducible results (see section 2.2.3).

The previous studies also did not control for the palatability effects of the test products. Compared to PL, propionate-containing bread had a lower acceptability score (Liljeberg et al., 1995), and a meal with added propionate induced higher subjective nausea ratings (Frost et al., 2003). It is therefore conceivable that the propionate test products triggered differing cephalic phase responses from PL due to their unpleasant taste, resulting in the observed metabolic and appetite effects.

In order to determine if observed effects following propionate ingestion are a physiological effect of propionate in the GIT or if they merely arise due to the orosensory properties of propionate-containing products, it is necessary to control for palatability as far as possible.

A sourdough starter culture (Domani) containing microflora favouring propionate production has been developed, resulting in a sourdough bread (SOUR) naturally rich in propionate. Prior to carrying out the present investigation, the sensory attributes of SOUR were evaluated relative to PL. SOUR was found to be acceptable and indistinguishable from PL (reported in Chapter 3A).

3.7 Aims

The aim of this study was, therefore, to investigate the acute effects of including sourdough bread (SOUR, made with Domani starter culture), as a component of one meal, on appetite VAS ratings, prospective food intake at a subsequent meal, 24 h intake and metabolic response compared to a visually and nutritionally identical placebo bread (PL, made without starter culture) of equal palatability.
3.8 Methods

3.8.1 Sample size calculations

This study was powered to look at the acute effects of consuming a preload containing the SOUR on a main outcome of *ad libitum* intake 3 h postprandially. As it has previously been reported that 17 participants are required to detect a 500 kJ difference in EI in a crossover design study with a power of 0.8 (Arvaniti et al., 2000) provided intake on the previous evening is standardised, we aimed to recruit a similar number to this study.

3.8.2 Participants

The study was conducted in twenty young, healthy participants (nine male, eleven female) aged between 18 and 35 y recruited from the student population at the University of Surrey by e-mail advertisement. To assess suitability for inclusion participants completed a Lifestyle and Medical Questionnaire and DEBQ, and a fasting blood sample was collected by fingerprick to measure blood glucose and Hb levels at an initial screening session (see section 2.1).

For inclusion, participants were required to have a BMI of 19 to 27 kg/m², fasting blood glucose ≤6.0 mmol/l, Hb in the normal range, restraint score of <3.5 (assessed using the DEBQ) and have been stable weight for at least 3 months. Exclusion criteria included currently pregnant or breast feeding, on a weight reducing diet, history of coronary heart disease, diabetes mellitus, gastrointestinal disorders, anaemia, clinical depression and other psychological disorders or of eating disorders, drug or alcohol abuse in the last 2 y, current habitual smoker, high habitual alcohol intake (≥20 units per week) and taking selected current regular medications (excluding contraceptives).
One participant used an asthma inhaler during acute episodes and another participant used migraine pain relief when required. 5 participants were taking oral contraceptives, one used a Mirena coil and one was fitted with a contraceptive implant.

Table 3.5 outlines the baseline participant characteristics. The mean participant age was 25.1 [SD 4.6] y and BMI was 23.1 [SD 2.4] kgm⁻². The mean age, BMI and BP did not differ significantly between genders, while the waist circumference, % body fat and DEBQ restraint scores did.

Table 3.5. Baseline participant characteristics¹

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=20)</th>
<th>Male (n=9)</th>
<th>Female (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age / y</td>
<td>25.1</td>
<td>4.6</td>
<td>18-35</td>
</tr>
<tr>
<td>BMI / kgm⁻²</td>
<td>23.1</td>
<td>2.4</td>
<td>19.0-27.2</td>
</tr>
<tr>
<td>Waist circumference / cm</td>
<td>78.3</td>
<td>3.4</td>
<td>64.3-95.6</td>
</tr>
<tr>
<td>Body fat / %</td>
<td>22.6</td>
<td>8.3</td>
<td>10.0-36.8</td>
</tr>
<tr>
<td>Systolic BP / mmHg</td>
<td>116</td>
<td>9.7</td>
<td>102-133</td>
</tr>
<tr>
<td>Diastolic BP / mmHg</td>
<td>69</td>
<td>5.6</td>
<td>59-77</td>
</tr>
<tr>
<td>DEBQ Restraint</td>
<td>2.1</td>
<td>0.67</td>
<td>1.0-3.4</td>
</tr>
</tbody>
</table>

¹Characteristics that differed significantly (p<0.05) and very highly significantly (p<0.001) between genders are denoted by (*) and (**) respectively.

3.8.3 Ethics

All participants gave written consent prior to commencing the study. Approval for the study was granted by the University of Surrey Ethics Committee on 25th July 2007 (EC/2007/50/SBMS), and the study took place during September to November 2007.
3.8.4 Study protocol

Acute postprandial effects on plasma metabolites and appetite (assessed by subjective ratings and subsequent EI) in response to two breakfast preloads containing either propionate-rich SOUR bread or identical non-sourdough PL bread were investigated using a randomised single-blind crossover study design.

Participants attended on two occasions at least one week apart and were assigned to the experimental condition (SOUR vs PL containing breakfast preload, see 3.8.5 for further details), in a counter-balanced randomly assigned order (using randomizer.org). The breakfast preloads provided a total of 6.0 and 0.1 mmol propionate respectively.

In addition, to control for hormonal variations throughout the menstrual cycle, female participants not using contraceptive methods that regulate hormonal levels (e.g. oral contraceptive, contraceptive implant) attended at approximately the same point of the follicular phase in their menstrual cycle for each study day.

To reduce within-subject variability, participants were instructed to refrain from unaccustomed exercise and alcohol during the 24 h preceding each study day and to approximately replicate their 24 h intake prior to each study day. A standardised evening meal (low fibre ready meal and dessert selected by participant) was provided and 24 h intake was recorded.

All study days took place at the Clinical Investigation Unit (CIU) at the University of Surrey. Participants were required to stay in the CIU for the entire duration of the study, although they were allowed to read, write, work on their computers or watch television (avoiding programmes with food cues).
Figure 3.1 shows a timeline for the study day protocol.

![Figure 3.1. Schematic representation of study protocol]

On each study day, participants arrived at the CIU at approximately 08.00 h following an overnight fast of at least 12 h. Initially anthropometric measurements (% body fat, weight, BMI, waist circumference) and BP were recorded. An intravenous cannula was then inserted into an antecubital vein and two fasting blood samples were taken (at time -30 and -5 min). Two initial VAS to subjectively assess appetite (see 2.3.1) were completed following each blood sample.

Participants were then provided with the breakfast preload (jam sandwiches made with SOUR or PL bread, see 3.8.5) at time = 0 min which they were asked to consume within 15 min. The time taken to consume the test breakfast (satiation time) was recorded. Following breakfast, participants completed VAS regarding the palatability and pleasantness of the breakfast (see 2.3.1).

Blood samples were taken every 15 min for the first hour postprandially and half hourly for the following two hours until lunch. In addition a set of VAS for fullness, hunger, prospective food consumption, desire to eat sweet / savoury / salty / fatty were completed at half hourly intervals. After removing the cannula, a large ad libitum homogenous pasta meal was served in a confined individual booth 180 min following the breakfast preload, and participants were instructed to eat until comfortably full.
Further details about the *ad libitum* buffet meal and the conditions under which it was served are described in Chapter 2 (section 2.3.2).

Once the participants had finished their lunch they were free to leave and were asked to complete a food and drink diary for the remainder of the day until 09.00 h the following morning.

### 3.8.5 Test products and breakfast

The SOUR and PL bread test products were manufactured and supplied by Holgran (a trading name for RHM Group Limited). SOUR bread was made using a starter culture, Domani, containing microflora that favour the production of propionate, while the PL bread was made using yeast. Apart from this, both breads were made using the same ingredients in the same quantities and the breads were visually identical. As shown in Table 3.6, the resultant SOUR bread contained higher levels of propionate (48 mmol per kg) than PL (0.8 mmol per kg).

<table>
<thead>
<tr>
<th>Bread</th>
<th>Propionic acid</th>
<th>Calcium propionate</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg/mmol/kg</td>
<td>mg/kg/mmol/kg</td>
<td>mmol/kg</td>
</tr>
<tr>
<td>PL</td>
<td>31/0.42</td>
<td>39/0.35</td>
<td>0.77</td>
</tr>
<tr>
<td>SOUR</td>
<td>2365/31.96</td>
<td>2980/16.00</td>
<td>47.96</td>
</tr>
</tbody>
</table>

In a sensory evaluation (n=14) no significant differences in preference, odd one out, acceptability, ranking and rating tests were found between the SOUR and PL bread (Chapter 3A), suggesting the SOUR is suitable for use in appetite studies.
The preload breakfast provided on study mornings comprised jam sandwiches made using 126 g of the SOUR or PL bread (equivalent to approximately 3 slices of bread), 24 g Olivio spread and 45 g Tesco strawberry, plum or apricot jam. All ingredients were weighed to the nearest 0.1 g. In addition participants were served either 250 g water or Robinson's Sugar Free orange, lemon or blackcurrant and apple squash. The jam and drink were selected by the participant at screening, and the same jam and drink were served on each occasion.

Table 3.7 summarises the nutritional and propionate content for the test breakfast made using either bread, demonstrating that SOUR and PL breads are well matched for energy, macronutrient and fibre content. The nutritional analysis for the bread was determined by Holgran using samples taken from the actual batch used in the present study.

Table 3.7. Nutritional content of the test breakfast. Samples analysed by Holgran

<table>
<thead>
<tr>
<th></th>
<th>Energy</th>
<th>CHO</th>
<th>Protein</th>
<th>Fat</th>
<th>Fibre</th>
<th>Propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kcal</td>
<td>kJ</td>
<td>g</td>
<td>g</td>
<td>g</td>
<td>mmol</td>
</tr>
<tr>
<td>PL</td>
<td>495</td>
<td>2082</td>
<td>77.1</td>
<td>9.4</td>
<td>16.3</td>
<td>3.5</td>
</tr>
<tr>
<td>SOUR</td>
<td>503</td>
<td>2116</td>
<td>79.0</td>
<td>9.6</td>
<td>16.3</td>
<td>3.2</td>
</tr>
</tbody>
</table>

3.8.6 Blood sampling and analysis

Blood samples were analysed for glucose, TAG, NEFA and total and HDL cholesterol concentrations by an enzymatic calorimetric method using the ILAB 650 analyser (Instrumentation Laboratory, Milan, Italy) and insulin concentrations were analysed by RIA. Sufficient blood was also collected for gut peptide and SCFA analysis although this was not carried out. More detailed descriptions of the blood sample collection and analysis procedures are given in Chapter 2 (sections 2.5 and 2.6).
3.8.7 Food diary analysis

Food and drink diaries were completed on the day before and the day of the study. These diaries were analysed for EI, macronutrient and fibre intake using WinDiets Professional 2005 (The Robert Gordon University, Aberdeen, UK). See Chapter 2 (Section 2.3.3) for further details.

3.8.8 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0. Data was tested for normality using the Kolmogorov-Smirnov test. Differences in anthropometric variables between male and female participants were assessed non-parametrically using the Mann-Whitney test.

Differences in the effects of consuming breakfast made with SOUR compared to PL bread on palatability VAS scores, satiation times, EI at the buffet meal, EI and macronutrient intake during the 24 h preceding the study day and during the 24 h of the study day were assessed by paired samples t-test if normally distributed, and by Wilcoxon signed-ranks test if not normally distributed.

The influence of bread type on subjective appetite VAS ratings and the postprandial metabolic responses were assessed by two-way (bread treatment x time) repeated measures ANOVA and by comparison of the AUC and IAUC using paired samples t-test. The AUC for postprandial plasma metabolites and subjective appetite ratings were calculated using the trapezoidal rule.

Differences were considered significant at a level of p≤0.05.
3.9 Results

3.9.1 Palatability VAS scores

Figure 3.2 shows the mean VAS scores in answer to the questions “How pleasant is the taste of this meal right now?” and “How pleasant is this meal right now?”. Paired samples t-test analysis determined bread type did not significantly influence the perception of the pleasantness of taste (mean score 70 [SD 17] mm for PL and 67 [SD 17] mm for SOUR) nor the palatability (75 [SD 14] mm for PL and 69 [SD 21] mm for SOUR).

![Figure 3.2a: VAS ratings in answer to 'How pleasant is the taste of this meal right now?']

The bread type did not influence the ratings for taste.

Analyses conducted by paired-samples t-test. Results shown as mean with error bars representing the SEM (n=20).

![Figure 3.2b: VAS ratings in answer to 'How palatable is this meal right now?']

The palatability did not differ significantly between bread type.

Analyses conducted by paired-samples t-test. Results shown as mean with error bars representing the SEM (n=20).

The satiation time at breakfast also did not significantly differ between bread type, with participants taking 522 [SD 110] seconds to eat the SOUR breakfast and 504 [SD 133] seconds to eat the PL breakfast.
3.9.2 Subjective appetite ratings

Selected subjective appetite ratings time-course curves as assessed by VAS are shown in Figure 3.3.

Figure 3.3. Subjective appetite ratings following PL ( - • • ) and SOUR ( - ▲ - ) SOUR treatment did not significantly influence any subjective appetite ratings except desire to eat sweet (p=0.024). Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=20).

Two-way repeated measures ANOVA analysis found no significant effects of treatment on hunger, fullness, prospective consumption, desire to eat something savoury, desire to eat something fatty or desire to eat something salty (graph not shown). However, the desire to eat something sweet was found to be significantly lower (p=0.024) following...
the SOUR breakfast compared to PL. In addition, a significant treatment x time interaction was found for the desire to eat something fatty (p=0.010).

Comparison of the AUC (Table 3.8) and IAUC (data not shown) for the VAS appetite ratings from 0 to 180 min found no significant differences between SOUR and PL treatment for all VAS questions except the AUC for the desire to eat something sweet (p=0.022, paired samples t-test, n=20). Differences between SOUR and PL were compared by paired t-test when normally distributed or Wilcoxon signed-ranks test when not normally distributed.

Table 3.8. AUC (0-180 min) for VAS appetite ratings after PL and SOUR preloads
No significant differences between preloads found except for the desire to eat something sweet. Analysis was carried out by paired-samples t-test or Wilcoxon signed-ranks test as appropriate. Values are mean [SD] (n=20).

<table>
<thead>
<tr>
<th>VAS rating</th>
<th>PL</th>
<th>SOUR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean [SD]</td>
<td>Mean [SD]</td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>7193 [2564]</td>
<td>6449 [2519]</td>
<td>NS</td>
</tr>
<tr>
<td>Fullness</td>
<td>8279 [3178]</td>
<td>8834 [2503]</td>
<td>NS</td>
</tr>
<tr>
<td>Prospective consumption</td>
<td>8186 [2927]</td>
<td>7755 [2718]</td>
<td>NS</td>
</tr>
<tr>
<td>Desire for sweet</td>
<td>7483 [3998]</td>
<td>5745 [3596]</td>
<td>0.022</td>
</tr>
<tr>
<td>Desire for savoury</td>
<td>9521 [2944]</td>
<td>8370 [3847]</td>
<td>NS</td>
</tr>
<tr>
<td>Desire for fatty</td>
<td>6503 [4025]</td>
<td>5377 [3842]</td>
<td>NS</td>
</tr>
<tr>
<td>Desire for salty</td>
<td>6722 [4209]</td>
<td>5939 [4158]</td>
<td>NS</td>
</tr>
</tbody>
</table>

When the data was sub-divided by gender, no significant treatment effects were found within either sub-group for any of the appetite ratings.
3.9.3 Quantitative appetite assessment

3.9.3.1 Intake at the ad libitum test meal

Figure 3.4 shows the mean EI of the ad libitum pasta meal 3 h following the test breakfast preload. The mean intake following PL breakfast preload (527 [SD 188]g, 3438 [SD 1225] kJ) did not differ significantly from the mean intake following the SOUR breakfast (531 [SD 163] g, 3469 [SD 1078] kJ), when analysed by paired sample t-test. Differences in intake were also non significant when sub-divided by gender.

![Figure 3.4. EI of ad libitum pasta test meal provided 3 h postprandially](image)

Bread type did not significantly influence intake within the overall sample (n=20) or within male (n=9) or female (n=11) sub-groups. Analyses were carried out by paired samples t-test. Results shown as mean with error bars representing the SEM.

Additionally, the mean satiation time at the ad libitum lunch did not differ between PL (639 [SD 170] seconds) and SOUR (632 [SD 164] seconds) treatment. Again differences in satiation time were also non significant when sub-divided by gender.
3.9.3.2 Intake during 24 h period following preload

The mean EI and macronutrient intake for the entire study day (including food provided during the study morning) following PL and SOUR based preloads is shown in Table 3.9. Mean EI following the SOUR breakfast was over 500 kJ lower than following PL, however this difference was non significant. When intake from alcohol was removed from the analysis, the difference in EI between treatments remained non significant. There were also no significant differences between treatments for macronutrient and alcohol intakes when analysed using paired-samples t-test for parametric data and Wilcoxon signed-ranks test for non-parametric data.

Table 3.9. Energy and macronutrient intake 24 h after PL and SOUR preloads
No significant effect of bread treatment on energy and macronutrient intake was found. Analysis was carried out by paired-samples t-test or Wilcoxon signed-ranks test as appropriate (n=20).

<table>
<thead>
<tr>
<th>PL</th>
<th>SOUR</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>9751 2076</td>
<td>9208 2375</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>78.8 22.3</td>
<td>71.1 30.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>75.0 19.2</td>
<td>71.9 22.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CHO (g)</td>
<td>320.0 77.2</td>
<td>307.2 65.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>15.7 4.5</td>
<td>15.4 3.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>4.8 12.5</td>
<td>5.2 20.4</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

There was also no difference in % contribution of macronutrients and alcohol to the total 24 h EI between PL and SOUR (Table 3.10).

Table 3.10. % contribution to EI by each macronutrient and alcohol 24 h after PL and SOUR preloads. No significant effect of bread treatment was found when analysed by paired-samples t-test (n=20).

<table>
<thead>
<tr>
<th>PL</th>
<th>SOUR</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Fat %EI</td>
<td>30.3 4.9</td>
<td>28.7 6.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Protein %EI</td>
<td>12.9 2.1</td>
<td>13.1 1.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CHO %EI</td>
<td>55.2 7.1</td>
<td>56.9 7.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Alcohol %EI</td>
<td>1.5 4.0</td>
<td>1.2 5.0</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
3.9.4 Postprandial plasma metabolites

3.9.4.1 Glycaemic response

The mean postprandial glycaemic response following consumption of the PL and SOUR breakfast is shown in Figure 3.5. Ingestion of the SOUR breakfast led to a higher mean plasma glucose peak than PL at 30 min. A second increase in plasma glucose occurred at 120 min after both breads. Two-way repeated measures ANOVA analysis found no significant treatment effects of SOUR compared to PL and no significant time x treatment effect.

![Figure 3.5. Postprandial plasma glucose concentrations](image)

No significant influence of SOUR treatment found by two-way repeated measures ANOVA analysis. Results shown as mean with error bars representing the SEM (n=20).

Comparison of the AUC and IAUC by paired t-test analysis also found no significant difference between SOUR and PL treatment (data not shown).

Similarly no significant differences were found when sub-divided by gender.
3.9.4.2 Insulinaemic response

As shown in Figure 3.6, the mean postprandial plasma insulin response following ingestion of the SOUR preload remained elevated above PL from 45 to 90 min postprandially. Two-way repeated measures ANOVA analysis indicated there was a treatment x time effect with a trend approaching significance ($p=0.061$) for the entire 180 min, which was significant for the 60 min postprandially ($p=0.033$). However the overall treatment effect of SOUR treatment was non significant compared to PL.

![Plasma insulin concentrations](image)

**Figure 3.6. Postprandial plasma insulin concentrations**
No significant effect of SOUR treatment found by two-way repeated measures ANOVA analysis, although there was a treatment x time interaction with a trend approaching significance ($p=0.061$). Results shown as mean with error bars representing the SEM (n=20).

Comparison of the AUC and IAUC by paired t-test or Wilcoxon signed-ranks test analysis also as appropriate found no significant difference between SOUR and PL treatment (data not shown).

Similarly no significant differences were found when sub-divided by gender.
3.9.4.3 NEFA response

The mean postprandial plasma NEFA response is shown in Figure 3.7. Two-way repeated measures ANOVA analysis found a significant treatment effect (p=0.025), although the treatment x time effect was not significant.

![Figure 3.7. Postprandial plasma NEFA concentrations](image)

No significant effect of SOUR treatment was found by two-way repeated measures ANOVA analysis. Results shown as mean with error bars representing the SEM (n=20).

As shown in Table 3.11, the mean AUC for the postprandial NEFA response was significantly higher following SOUR than for PL (p=0.007), although the IAUC did not differ between treatments for the overall sample. When subdivided by gender these differences were significant for the female sub-group (p=0.048, n=11) and with a trend approaching significance for the male sub-group (p=0.054, n=9) (data not shown).

<table>
<thead>
<tr>
<th>PL</th>
<th>SOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC 180</td>
<td>37.3</td>
</tr>
<tr>
<td>IAUC 180</td>
<td>-56.2</td>
</tr>
</tbody>
</table>

Table 3.11. AUC and IAUC of NEFA response following PL and SOUR preloads
AUC following SOUR preload was significantly higher than PL (paired-samples t-test). The IAUC did not differ between treatments (Wilcoxon signed-ranks test) (n=20).
3.9.4.3 TAG response

As shown in Figure 3.8, the postprandial plasma TAG response was not significantly influenced by bread treatment when analysed by two-way repeated measures ANOVA. The treatment x time interaction was also non significant.

![Figure 3.8. Postprandial plasma TAG concentrations]

Bread treatment did not significantly influence postprandial TAG concentrations when analysed by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=20).

Additionally, comparison of the AUCs and IAUCs by paired t-test analysis also found no significant difference between bread types (data not shown).

Similarly there were also no significant differences when sub-divided by gender.
3.9.5 Indices of insulin sensitivity

As shown in Table 3.12, the fasting insulin sensitivity, β-cell function and insulin resistance as estimated by Homeostasis Model Assessment (HOMA) (Levy et al., 1998, Wallace et al., 2004, Matthews et al., 1985) (see section 2.8.1 for further details) were not significantly different at the start of each study day.

Postprandially, neither the oral insulin sensitivity as estimated using the minimal model method (Caumo et al., 2000) (see section 2.8.2 for further details) nor the insulin to glucose AUC ratio (for 0-180 min) differed significantly between SOUR and PL.

Table 3.12. Indices of insulin sensitivity at baseline and following consumption of PL or SOUR preload

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>SD</th>
<th>SOUR</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA % S</td>
<td>78.6</td>
<td>22.8</td>
<td>75.2</td>
<td>20.1</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA % B</td>
<td>122.8</td>
<td>21.1</td>
<td>122.8</td>
<td>23.9</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>1.4</td>
<td>0.46</td>
<td>1.5</td>
<td>0.44</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin:glucose AUC</td>
<td>7.5</td>
<td>3.08</td>
<td>8.0</td>
<td>3.18</td>
<td>NS</td>
</tr>
<tr>
<td>Oral S1</td>
<td>2.18</td>
<td>1.21</td>
<td>1.82</td>
<td>0.97</td>
<td>NS</td>
</tr>
</tbody>
</table>

1  HOMA %S, fasted oral insulin sensitivity, HOMA %B, β-cell function and HOMA IR, insulin resistance all estimated by homeostasis model assessment.
2  AUC 0-180 mins
3  Oral S1 values are x 10⁻³ dl glucose / kg.min / μU insulin.ml. Calculated by minimal model.

Comparisons were carried out by paired t-test when normally distributed and by Wilcoxon signed-ranks test when non-parametric.
3.9.6 Intake 24 h preceding study

As shown in Table 3.13, the mean energy, macronutrient and fibre intake during the 24 h period prior to participating in each study day did not differ significantly between PL and SOUR treatment when analysed by Wilcoxon signed-ranks test.

Additionally, the food diaries indicated that all participants had adhered to the required 10-12 h fast prior to coming in for the study day, had consumed the supplied standard meals and had not consumed any alcohol during the 24 h period preceding the study.

Table 3.13. Energy and macronutrient intake 24 h prior to study day
No significant differences were found between treatments when analysed by Wilcoxon signed-ranks test (n=20).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th></th>
<th>SOUR</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>7666</td>
<td>1856</td>
<td>7422</td>
<td>1654</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>77.9</td>
<td>26.9</td>
<td>73.2</td>
<td>28.5</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>64.8</td>
<td>18.4</td>
<td>66.8</td>
<td>14.5</td>
<td>NS</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>228.7</td>
<td>48.5</td>
<td>216.7</td>
<td>14.5</td>
<td>NS</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>13.2</td>
<td>4.3</td>
<td>12.4</td>
<td>4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>
3.9.7 Anthropometric and clinical measures on each study leg

The mean baseline anthropometric measurements, clinical measurements and fasting plasma results taken at the start of each study day are summarised in Table 3.14. None of these parameters differed significantly between treatments.

Table 3.14. Fasting plasma, anthropometric & clinical measures on each study day
No significant difference between treatments was found by paired-sample t-test analysis (n=20).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>SOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Fasting plasma metabolites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC / mmol/l</td>
<td>4.0</td>
<td>0.6</td>
</tr>
<tr>
<td>HDL-C / mmol/l</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>TAG / mmol/l</td>
<td>0.97</td>
<td>0.35</td>
</tr>
<tr>
<td>NEFA / mmol/l</td>
<td>0.52</td>
<td>0.25</td>
</tr>
<tr>
<td>Glucose / mmol/l</td>
<td>5.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Insulin / mmol/l</td>
<td>56.4</td>
<td>19.6</td>
</tr>
<tr>
<td>Anthropometrics/Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI / kgm⁻²</td>
<td>22.6</td>
<td>2.4</td>
</tr>
<tr>
<td>% body fat / %</td>
<td>22.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Waist circumference / cm</td>
<td>78.2</td>
<td>8.3</td>
</tr>
<tr>
<td>BP systolic / mmHg</td>
<td>116</td>
<td>9</td>
</tr>
<tr>
<td>BP diastolic / mmHg</td>
<td>68</td>
<td>6</td>
</tr>
</tbody>
</table>
3.10 Discussion

The present investigation found a mixed meal that included a palatable propionate-rich SOUR bread providing 6.0 mmol propionate did not improve the postprandial insulin response compared to a non-sourdough PL bread. Indeed it may have a detrimental effect on insulinaemia as evidenced by a treatment x time effect with a trend approaching significance (p=0.061), which was significant for the first 60 min (p=0.033). The postprandial NEFA response AUC was also significantly higher following SOUR (p=0.007), however postprandial glycaemic and TAG responses were not significantly influenced by bread type.

A further important finding was that appetite was not significantly influenced following ingestion of the propionate-rich SOUR bread as part of a mixed meal preload, with no effects on ad libitum EI 3 h postprandially, on postprandial 24 h intake or on subjective appetite ratings except the desire to eat sweet.

To our knowledge, this is the first study in which propionate has been delivered as part of a meal in a palatable form that would be considered a 'real' unmodified food that can be purchased and incorporated into the diet, albeit delivering propionate in lower quantities than previous studies. The amount of propionate in the SOUR bread in present study was however what could be naturally incorporated without influencing product palatability and represents what is commercially available.

These findings are in contrast to previous studies that found ingestion of Na propionate baked into bread acutely reduced rather than increased the glycaemic (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Todesco et al., 1991) and insulinaemic (Darwiche et al., 2001, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995) response and significantly increased subjective satiety ratings relative to PL (see in section 1.9.1).
One of the major differences between the present study and previous studies (Darwiche et al., 2001, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Todesco et al., 1991) is that the unpleasant taste of Na propionate was not controlled for in previous studies and was therefore a potential cofounder. It may be postulated that the results from the previous studies were elicited by the unpleasant taste of the Na propionate rather than a physiological effect of propionate. There is now a large body of evidence suggesting that even at the cephalic (pre-absorptive) phase of ingestion metabolic responses can occur that can influence metabolic handling at later stages. For example vagal stimulation via MSF has been shown to initiate insulin release (CPIR) (Bellisle et al., 1985, Bruce et al., 1987, Lucas et al., 1987, Teff and Engelman, 1996, Teff et al., 1991, Teff et al., 1993b, Robertson et al., 2001, Just et al., 2008) and gastric secretions (Feldman and Richardson, 1986, Pavlov, 1902, Robertson, 2009, Zafra et al., 2006), to suppress NEFA release (Robertson et al., 2001) and alter intestinal motility (Heath et al., 2004, Katschinski, 2000, Pouderoux et al., 1995) amongst others (reviewed in Chapter 1, section 1.10). Postprandial lipaemia is also enhanced when preceded by vagal stimulation (Robertson et al., 2002) and CPIR appears to be necessary for normal glucose homostasis (Teff and Engelman, 1996).

It is therefore reasonable to postulate that the sensory properties of a food could influence subsequent metabolic responses, a possibility that has been investigated by various researchers. For example when product palatability was modified by the addition of a bitter taste (quinine), subsequent gastric emptying was significantly delayed compared to the same product without added quinine (Wicks et al., 2005). Additionally, gastric myoelectric activity was decreased when volunteers were sham fed an unappetising food (cold tofu frankfurters) as compared to an appetising food (cooked frankfurters), suggesting a lack of cephalic phase vagal stimulation with the
‘unappetising’ treatment (Stern et al., 2001). It is highly likely that altered gastric motility would influence postprandial metabolite responses.

Furthermore, eliciting cephalic phase response by MSF has been found to increase ghrelin concentrations (Heath et al., 2004, Simonian et al., 2005) and reduce subjective hunger ratings (LeBlanc and Soucy, 1996, Smeets and Westerterp-Plantenga, 2006b) in human subjects. It is therefore also reasonable to postulate that unpleasant tasting food could also influence subsequent satiety. Yeomans found a strong linear relationship between the change in rated palatability and differences in food intake when data was pooled from studies in which palatability was manipulated (Yeomans, 2007). For example, intake and taste ratings of an ice-cream adulterated with a bitter tastant were significantly lower than the unadulterated ice cream (Nisbett, 1968). This suggests an unpleasant taste influences satiation.

Based on this it can be postulated that the reduced postprandial insulinaemic and glycaemic responses (Darwiche et al., 2001, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Todesco et al., 1991) and delayed gastric emptying (Darwiche et al., 2001, Liljeberg and Bjorck, 1996) and increased satiety (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995) observed in previous studies following oral propionate ingestion arose as a result of the unpleasant taste of the Na propionate breads. Indeed acceptability ratings for the Na propionate breads used in a previous studies were significantly lower than PL (Liljeberg et al., 1995). By contrast, in the present study prior sensory evaluation determined participants were unable to distinguish the propionate rich SOUR bread from the PL bread. This could also explain why the findings of the present study do not replicate the results from these previous studies.
Furthermore, as discussed in the introduction, conclusions from previous studies that propionate-containing bread can increase subsequent satiety were based solely on results from a single bipolar rating scale (Liljeberg and Bjorck, 1996, Liljeberg et al., 1995), and a quantitative assessment of appetite was not made. The validity of their conclusions can therefore be questioned. The bipolar scale was anchored by extreme hunger at one end and extreme satiety at the other. However although satiety and hunger could be considered to lie at polar opposites, satiety is not simply the absence of hunger. It is therefore inappropriate to have satiety and hunger on the same rating scale (Mattes et al., 2005). In the present study our conclusions were based on a combination of data generated from appetite VAS, EI at an ad libitum meal and 24 h intake, which is a more valid way to investigate appetite based on current thinking (see section 1.4).

It still remains to be explained why in the present study the propionate-rich SOUR bread may be detrimental to postprandial insulinaemia. To our knowledge this is the first time that such an observation has been made following oral administration of propionate in humans. A possible explanation is that the orally ingested propionate elicited a gluconeogenic response in the present study.

Wolever and colleagues reported rectal administration of a propionate solution significantly increased subsequent blood glucose levels in man while rectal acetate and saline infusions did not. As these solutions were rectally infused, the taste of propionate was eliminated, and as no CHO source was given, the observed increase in glucose must have occurred due to the infused propionate. The authors of this study postulate propionate is gluconeogenic in humans, based on the fact that animal studies appear to suggest propionate is converted to glucose via succinate and oxaloacetate (Wolever et al., 1991). However gastric infusion of a propionate solution administered at levels
similar to that generated by 30g fibre per day did not influence hepatic glucose production (Laurent et al., 1995), although the sample size for this study was low (n=6).

Propionate is well known to be gluconeogenic in ruminants, and is the primary glucose source via hepatic metabolism. As much lower quantities of propionate are supplied to the non-ruminant liver, propionate is not an important gluconeogenic precursor in non-ruminants, although the non-ruminant liver is capable of propionate metabolism (Allen et al., 2009).

Based on this it may be postulated that in the present study, propionate was absorbed from the GIT and traversed to the liver via the portal vein. In the liver it may have been metabolised to glucose, which in turn could have enhanced insulin secretion. However, if gluconeogenesis explains the observed effects on insulin response in the present study, why did this not occur in previous studies using Na propionate bread? It is possible that in previous studies while gluconeogenesis may have occurred, the cephalic phase effects arising from the sensory properties of the Na propionate bread out-weighed gluconeogenic effects.

Postprandial plasma NEFA concentrations were also significantly influenced by treatment (p=0.025) in the present study, with a significantly higher AUC with SOUR treatment than PL (p=0.007). However this result needs to be interpreted with caution, as the mean fasting NEFA concentrations were higher than PL, although not significantly so, and by 120 min postprandially concentrations were almost identical between treatments. This is suggestive that SOUR may have suppressed NEFA concentrations to a greater degree than did PL. Plasma TAG concentrations were not significantly influenced. To our knowledge this is the first study to investigate the postprandial effects of oral propionate ingestion as part of a mixed meal on postprandial lipid responses.
It should also be considered that the SOUR bread in the present study delivered lower quantities of propionate (6.0 mmol) than in the previous studies (Darwiche et al., 2001, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Todesco et al., 1991), which may be an alternative explanation for a lack of effect in the present study. The amount delivered in these studies is not given and not clear, but by calculation it seems to be approximately 15 mmol and 45 mmol from low and high dose breads respectively. These quantities were sufficient to significantly reduce acceptability rating scores (45 mmol) (Liljeberg et al., 1995) and increase nausea ratings (30 mmol) (Frost et al., 2003), thus representing a product that could not be marketed.

In the present study we did not analyse gut peptide concentrations due to the observed lack of effect on subjective and quantitative proxy measures of appetite. We had also intended to analyse serum SCFA concentrations to confirm propionate levels had been modulated after ingesting SOUR, giving a more complete picture of events taking place. However analysis of serum SCFA concentrations is not straightforward, largely due to the low concentrations present in the peripheral circulation, and we were unable to successfully set up and validate the method for their analysis.

Finally, some recently published studies have found sourdough products can acutely reduce postprandial glycaemia and insulinaemia (De Angelis et al., 2007, Lioger et al., 2009, Maioli et al., 2008, Najjar et al., 2009), satiety ratings (Lioger et al., 2009) and GLP-1 and GIP concentrations (Najjar et al., 2009) relative to PL. In these studies a lactobacilli sourdough starter was used which promotes lactic acid production rather than Domani starter used in the present study that contains propionate producing microflora. The differing organic acid compositions of the resulting sourdough products
may explain the differences in observed results, suggesting the findings from the present study are specific to the SCFA propionate.

3.11 Conclusion

In summary, we found oral ingestion of a propionate-rich palatable sourdough bread (providing 6.0 mmol propionate) did not influence appetite, postprandial glucose or TAG concentrations and that it may even worsen postprandial insulinemia, in contrast to previous findings. We propose that the results from the previous studies (Darwiche et al., 2001, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Todesco et al., 1991) may have resulted from the unpleasant taste of the test bread rather than physiological effects of propionate. The increased postprandial insulin concentrations in the present study may have arisen from a gluconeogenic effect of propionate in the liver.

Alternatively it is possible that the lack of effect was simply due to a lower dose of propionate being provided than in the previous studies. However, while the bread used in this study was rated as being palatable (see Chapter 3A) and is commercially available, the Na propionate breads used in previous studies containing higher doses were rated as not acceptable and associated with increased nausea.

To our knowledge, this is the first study in which propionate has been delivered as part of a meal in a palatable form using a test product that would be considered a ‘real’ unmodified food that can be purchased and incorporated into the diet. Prior sensory evaluation confirmed that participants were unable to distinguish the PL from SOUR and that the SOUR was no less preferable than the PL bread (see Chapter 3A).

Overall, our results do not support the promotion of propionate-rich sourdough bread as a functional food product (Diplock et al., 1999), as we do not consider it likely to reduce disease risk or confer additional health benefits.
Chapter 4: Acute effects of acetic acid supplementation on appetite, food intake and glycaemic response

4.1 Introduction

Following on from the study reported in the previous chapter, which considered the influence of oral ingestion of the SCFA propionate on the postprandial metabolic response and satiety, the present study was designed to investigate the oral provision of acetic acid (provided within vinegar) on postprandial glycaemia and satiety.

The provision of vinegar (as a source of acetic acid) has been linked with a reduced acute glycaemic and insulinaemic response in healthy volunteers (Brighenti et al., 1995, Liljeberg and Bjorck, 1998, Ostman et al., 2005, Sugiyama et al., 2003, Johnston and Buller, 2005, Liljeberg and Bjorck, 1996) and in individuals with insulin resistance and T2DM (Johnston et al., 2004), as reviewed in Chapter 1 (sections 1.8.1 and 1.9.1). Possible mechanisms for this include delayed gastric emptying (Liljeberg and Bjorck, 1998, Hlebowicz et al., 2007) and a decreased rate of starch digestion (Ogawa et al., 2000). Oral ingestion of vinegar has also been reported to significantly increase subsequent satiety (Hlebowicz et al., 2008, Ostman et al., 2005), and supplementation for 12 wk significantly lowered BMI, % body fat and waist and hip circumference (Kondo et al., 2009). The SCFA receptor proteins GPR41 and GPR43 may also be implicated in modulating effects (see Chapter 1, section 1.7).

However, there were problems in the study design of the previous studies investigating oral vinegar provision, particularly in investigating appetite. In common with previous propionate studies, studies that found a significant effect of vinegar ingestion on satiety (Hlebowicz et al., 2008, Ostman et al., 2005) based their conclusions on results generated from a single bipolar rating scale using confusing language with no complementary quantitative assessment of appetite. Ostman and co-workers (2005)
additionally excluded data from participants whose satiety ratings were lower postprandially than at fasting, which could be argued to be data manipulation.

As with the propionate studies, palatability effects of vinegar were also not controlled for. While the acceptability of the vinegar test products were not rated, both studies used large quantities of vinegar (between 18g and 28g) which were supplied to participants soaked in bread. When tested in our laboratories during the study development stage, vinegar served in this way was found to be highly unpleasant and nauseating to eat. It is therefore possible that the observed effects on satiety and the metabolic response were a cephalic phase effect due to the unpleasant orosensory properties of the test products, particularly in view of the results from our previous study that found no effect on satiety with oral ingestion of the SCFA propionate in a palatable form (see Chapter 3), although far lower quantities of SCFA were used in that study.

The present study was therefore designed to further investigate the influence of vinegar ingestion on appetite using validated methodology (appetite VAS ratings, food intake at a subsequent ad libitum meal and 24 h intake), and to explore the influence of the palatability of the test products. Palatability effects were assessed by supplying vinegar within a drink (sugar-free orange squash) in a more palatable form (diluted across the entire drink) and a less palatable form (diluted in part of the drink, thus supplying the vinegar in a more concentrated form). We hypothesised that reduced product palatability, whilst controlling for the amount of acetic acid consumed, would result in increased satiety and reduced postprandial glycaemia.
4.2 Aims
The primary aim was to investigate the acute effects of acetic acid ingestion (in a sweetened drink containing vinegar) delivered in an unpalatable (Unpal) and more palatable (Pal) form when consumed alongside a standard breakfast preload on subsequent appetite ratings, prospective food intake at a subsequent meal, 24 h intake and plasma glucose response compared to a visually identical PL (sweetened drink without added vinegar).

4.3 Methods
4.3.1 Sample size calculations
This study was a pilot study. As it has previously been reported that 17 participants are required to detect a 500 kJ difference in EI in a crossover design study with a power of 0.8 (Arvaniti et al., 2000) provided intake on the previous evening is standardised, we aimed to recruit a similar number to this study.

4.3.2 Participants
Sixteen healthy participants (3 male, 13 female) aged between 21 and 33 y were recruited from the student population at the University of Surrey by e-mail advertisement. To assess suitability for inclusion participants completed a Lifestyle and Medical Questionnaire and DEBQ, and a blood sample was collected by fingerprick to measure fasting blood glucose levels at an initial screening session (see section 2.1).

For inclusion, participants were required to have a BMI of 19 to 27 kgm⁻², fasting blood glucose ≤6.0 mmol/l, restraint score of ≤3.5 (assessed using the DEBQ) and have been stable weight for at least 3 months. Exclusion criteria included currently pregnant or breast feeding, on a weight reducing diet, history of coronary heart disease,
diabetes mellitus, gastrointestinal disorders, anaemia, clinical depression and other psychological disorders or of eating disorders, drug or alcohol abuse in the last 2 y, current habitual smoker, high habitual alcohol intake (>20 units per week) and taking selected current regular medications (excluding contraceptives).

Nine participants were taking oral contraceptives and one was fitted with a contraceptive implant, but no other medications were prescribed to participants.

Table 4.1 outlines the baseline participant characteristics. The mean participant age was 22.2 [SD 3.0] y and BMI was 22.1 [SD 2.4] kg/m². None of the characteristics significantly differed between genders except the waist circumference and % body fat.

Table 4.1: Baseline participant characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (n=16)</th>
<th>Male (n=3)</th>
<th>Female (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age / y</td>
<td>22.2 3.0 21-33</td>
<td>22.0 1.0 21-23</td>
<td>22.2 3.3 21-33</td>
</tr>
<tr>
<td>BMI / kg/m²</td>
<td>22.1 2.4 18.5-27.0</td>
<td>23.9 3.5 20.1-27.0</td>
<td>21.7 2.1 18.5-25.1</td>
</tr>
<tr>
<td>Waist circumference / cm</td>
<td>74.6 8.6 62.4-94.0</td>
<td>88.0 7.9 79.0-94.0</td>
<td>71.5 5.2 62.4-80.0</td>
</tr>
<tr>
<td>Body fat / %</td>
<td>23.6 6.3 9.7-33.9</td>
<td>15.3 4.9 9.7-18.8</td>
<td>25.5 4.9 15.1-33.9</td>
</tr>
<tr>
<td>Systolic BP / mmHg</td>
<td>122 13.3 103-147</td>
<td>134 18.2 113-147</td>
<td>119 11.1 103-137</td>
</tr>
<tr>
<td>Diastolic BP / mmHg</td>
<td>74 9.6 61-94</td>
<td>73 9.4 62-80</td>
<td>74 10.0 61-94</td>
</tr>
<tr>
<td>DEBQ Restraint</td>
<td>1.9 0.7 1.1-3.5</td>
<td>2.0 0.5 1.5-2.4</td>
<td>1.9 0.7 1.1-3.5</td>
</tr>
<tr>
<td>DEBQ Emotion</td>
<td>2.6 0.7 1.7-4.1</td>
<td>2.9 1.1 1.7-3.9</td>
<td>1.9 0.7 1.8-4.1</td>
</tr>
<tr>
<td>DEBQ External</td>
<td>2.6 0.7 1.3-3.8</td>
<td>1.7 0.4 1.3-2.1</td>
<td>2.5 0.5 2.0-3.8</td>
</tr>
</tbody>
</table>

*Characteristics that differed significantly (p<0.05) between genders are denoted by (*)

4.3.3 Ethics

All participants gave written consent prior to commencing the study. The study was approved by the University of Surrey Ethics Committee on 11th October 2007 (EC/2007/76/FHMS), and took place from November 2007 to December 2008.
4.3.4 Study protocol

Acute postprandial effects on blood glucose and appetite (assessed by subjective ratings and subsequent EI) in response to a standard breakfast preload alongside either an unpalatable (Unpal) or more palatable (Pal) vinegar-containing drink or PL drink were investigated using a randomised single-blind crossover study design.

Participants attended on three occasions at least 2 d apart and were assigned to the experimental condition (Unpal vs. Pal vs. PL, see 4.3.5 for further details), in a counter-balanced randomly assigned order.

To reduce within-subject variability, participants were instructed to refrain from unaccustomed exercise and alcohol during the 24 h preceding each study day and to approximately replicate their 24 h intake prior to each study day. A standardised evening meal (low fibre ready meal and desert selected by participant) was provided and 24 h intake was recorded.

All study days took place at the CIU at the University of Surrey. Participants were requested to stay in the CIU during the study, although they could to read, write, work on their computers or watch television (avoiding programmes with food cues).

Figure 4.1 shows a timeline for the study day protocol.

Figure 4.1. Schematic representation of study protocol
On each study day, participants arrived at the CIU at approximately 08.00 h following an overnight fast of at least 12 h. Upon arrival, two initial fasting capillary blood samples were taken by fingerprick at time = -30 and -5 min and analysed for glucose concentrations (see 2.3.6), and participants were asked to complete two initial VAS to subjectively assess appetite, nausea and other attributes unrelated to appetite (see 2.3.1) following each blood sample.

Participants were then provided with the standard breakfast preload (jam sandwiches) alongside the test drinks (Unpal vinegar, Pal vinegar or PL) divided between 2 glasses (see 4.3.5) at time = 0 min. It was requested that participants ingested the breakfast within 15 min and consume the first of the two test drinks alongside the first sandwich quarter and the remaining drink before completing the breakfast. Following breakfast, participants completed VAS regarding the palatability and pleasantness of the breakfast and drinks (see 2.3.1).

Blood samples were taken every 15 min for the first hour following breakfast and half hourly for the following 2 h until lunch. In addition a set of VAS for fullness, hunger, prospective food consumption, desire to eat sweet / savoury / salty / fatty and nausea plus additional VAS unrelated to appetite such as calmness, happiness, tiredness, boredom and anxiety were completed at half hourly intervals. Participants were not informed this study was investigating effects on appetite which is why the additional VAS questions were included to distract from the purpose of the study.

180 min following breakfast, a large ad libitum homogenous pasta meal was served in a confined individual booth, and participants were instructed to eat until comfortably full. The difference in intake following PL, Pal and Unpal test drinks was determined. Further details about the ad libitum buffet meal and the conditions under which it was served are described in Chapter 2 (section 2.3.2).
Once the participants had finished their lunch they were free to leave and were asked to complete a food and drink diary for the remainder of the day until 09.00 h the following morning.

### 4.3.5 Test breakfast

On the study mornings participants were provided with a test breakfast comprising jam sandwiches cut into quarters and the test drink that the participant had been randomised to on that occasion (ingredients given in Table 4.2), either:

- **a)** Placebo drink with no added vinegar - PL
- **b)** More palatable drink with 25 g added vinegar (= 25 mmol acetic acid) - Pal
- **c)** Unpalatable drink with 25 g added vinegar (= 25 mmol acetic acid) – Unpal

All test drinks were made using Robinson’s Sugar Free Orange Squash, and the vinegar used was Tesco White Wine Vinegar which contains 6% acetic acid.

#### Table 4.2. Ingredients used to make the test drinks.

Once prepared, drinks (a) PL and (b) Pal were equally divided between 2 glasses (175g per glass) and chilled. (c) Unpal drinks were prepared separately (Drink 1 and 2) and were kept at RT until serving.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>a) PL</th>
<th>b) Pal</th>
<th>c) Unpal</th>
<th>Drink 1</th>
<th>Drink 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squash / g</td>
<td>75</td>
<td>75</td>
<td></td>
<td>25</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Vinegar / g</td>
<td>-</td>
<td>25</td>
<td></td>
<td>25</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Water / g</td>
<td>275</td>
<td>250</td>
<td></td>
<td>100</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>Total amount / g</td>
<td>350</td>
<td>350</td>
<td></td>
<td>150</td>
<td>200</td>
<td>350</td>
</tr>
</tbody>
</table>

On the first study morning participants were provided with 7 quarters of jam sandwiches (equivalent to three and a half slices bread, with each slice weighing 38 g) for the test breakfast. Participants were asked to consume a minimum of 4 full quarters (equivalent to 2 slices of bread). On subsequent study days participants were provided
with the same number of quarters as consumed on the first study occasion. This procedure allowing participants to eat to their desired intake has been previously reported (Westatrate and van Amelsvoort, 1993) and used in our group (Bodinham et al., 2010).

The sandwiches were made using 8 g Olivio spread and 16 g Tesco strawberry jam per 38 g slice of Kingsmill Everyday Soft Bread. Table 4.3 summarises the nutritional content of the test breakfast for 4, 5, 6 and 7 quarters (equivalent to 2, 2.5, 3 and 3.5 slices of bread respectively).

<table>
<thead>
<tr>
<th>Number sandwich quarters</th>
<th>Number slices bread</th>
<th>Portion size (g)</th>
<th>Energy kcal</th>
<th>Energy kJ</th>
<th>Protein g</th>
<th>CHO g</th>
<th>Fat g</th>
<th>Fibre g</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
<td>76</td>
<td>16</td>
<td>32</td>
<td>345</td>
<td>1454</td>
<td>6.9</td>
<td>54.5</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>95</td>
<td>20</td>
<td>40</td>
<td>431</td>
<td>1817</td>
<td>8.7</td>
<td>68.2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>114</td>
<td>24</td>
<td>48</td>
<td>518</td>
<td>2180</td>
<td>10.4</td>
<td>81.7</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>133</td>
<td>28</td>
<td>56</td>
<td>605</td>
<td>2543</td>
<td>12.3</td>
<td>95.4</td>
</tr>
</tbody>
</table>

4.3.6 Blood sampling and glucose analysis

Capillary blood samples were taken by fingerprick (for method, see section 2.5.2) and analysed immediately using a HemoCue Glucose 201+ system (for method, see section 2.6.3.3). Participants were invited to a session to familiarise them with the fingerprick method prior to commencing the study, to minimise effects on appetite during the study.

4.3.7 Food diary analysis

Food and drink diaries were completed on the day before and the day of the study. These diaries were analysed for EI, macronutrient and fibre intake using WinDiets Professional 2005 (The Robert Gordan University, Aberdeen, UK). See Chapter 2 (Section 2.3.3) for further details.
4.3.8 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0. Data was tested for normality using the Kolmogorov-Smirnov test. Differences in anthropometric variables between male and female participants were assessed non-parametrically using the Mann-Whitney test.

Differences in the effects of vinegar treatment on palatability VAS scores, EI at the buffet meal, EI and macronutrient intake during the 24 h preceding the study day and during the 24 h of the study day were assessed by one-way repeated measures ANOVA with post-hoc Bonferroni if normally distributed, and by Friedman’s ANOVA with post-hoc Wilcoxon signed-ranks test if not normally distributed.

The influence of vinegar treatment on postprandial subjective VAS ratings and glycaemic response was assessed by two-way (vinegar treatment x time) repeated measures ANOVA. The AUC and IAUC were compared by one-way repeated measures ANOVA with post-hoc Bonferroni. The AUC for postprandial plasma metabolites and subjective appetite and nausea ratings were calculated by the trapezoidal rule.

As well as analysing the blood glucose response over the entire study morning (180 min), analysis was carried out for the first 120 min to allow direct comparison with previous investigations.

Data was pooled from all study days to examine the relationship of palatability VAS ratings and nausea AUC with subjective appetite AUC, EI 3 h postprandially and 24 h EI by correlation analysis. Analysis was carried out by 2-tailed Pearson’s product-moment (parametric) and Spearman’s rho (non-parametric) as appropriate.

Differences were considered to be significant at p≤0.05 except the post-hoc Wilcoxon signed-ranks test, where significance was set at a level of p≤0.0167 (=0.05/3).
4.4 Results

4.4.1 Palatability VAS ratings

Figure 4.2 shows the mean palatability VAS ratings. Vinegar treatment significantly influenced the rated pleasantness of taste of the drinks (p<0.001) (Figure 4.2a) and palatability of breakfast (p<0.001) (Figure 4.2b) with one-way repeated measures ANOVA analysis. The mean ratings for both were highest for PL and lowest for Unpal.

Post-hoc bonferroni analysis revealed the taste ratings for both the Pal and Unpal drinks were significantly lower than PL (p<0.001), and the Unpal drink was rated as less pleasant than the Pal drink with a trend approaching significance (p=0.055). For the breakfast palatability, the ratings were significantly lower when consumed with Pal and Unpal drinks (p=0.020 and 0.002 respectively) than with PL, and the Unpal treatment rating was significantly lower than during Pal treatment (p=0.022, n=16).

Figure 4.2a: VAS ratings in answer to ‘How pleasant was the taste of the drink?’
Vinegar treatment significantly reduced the pleasantness of the drink (p<0.0001). Columns showing the same symbol denote significant differences between treatments: ¤p<0.0001, ¢p<0.0001, ~p=0.055.
Analyses conducted by one-way repeated measures ANOVA with post-hoc Bonferroni. Results shown as mean with error bars representing the SEM (n=16).

Figure 4.2b: VAS ratings in answer to ‘How palatable was the breakfast?’
Vinegar treatment significantly influenced the breakfast palatability (p<0.0001). Columns showing the same symbol denote significant differences between treatments: ¤p=0.020, ¢p<0.002, ~p=0.022.
Analyses conducted by one-way repeated measures ANOVA with post-hoc Bonferroni. Results shown as mean with error bars representing the SEM (n=16).
4.4.2 Subjective appetite ratings

As summarised in Figure 4.3, vinegar treatment significantly influenced postprandial subjective appetite ratings for fullness (p<0.0001, n=16), hunger (p=0.045, n=16) and prospective food consumption (p=0.036, n=16) when analysed by two-way repeated measures ANOVA analysis. Additionally, the desire to eat something sweet was influenced with a trend towards significance (p=0.058, n=16), and a significant vinegar treatment x time interaction was observed for prospective consumption (p=0.009). Subjective ratings for the desire to eat something savoury, salty and fatty were not significantly influenced by vinegar treatment (data not shown).

Figure 4.3. Subjective appetite ratings following PL (- ● -), Pal (- ■ -) and Unpal (- ▲ -) drinks.

Vinegar treatment significantly influenced (a) fullness (p<0.0001), (b) hunger (p=0.045) and (c) prospective consumption (p=0.036) and influenced (d) desire to eat something sweet with a trend towards significance (p=0.058). Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=16).
Figure 4.4 shows the AUC of subjective appetite ratings from 0 to 180 min. Vinegar treatment significantly lowered the AUC for fullness (p<0.0001), prospective food consumption (p=0.048) and desire to eat something sweet (p=0.048) by one-way repeated measures ANOVA analysis. In addition, hunger was lowered with a trend towards significance (p=0.067). AUC for the desire to eat something savoury, salty and fatty were not significantly influenced by vinegar treatment (data not shown).

Post-hoc analysis found the AUC for fullness was significantly lower for PL than for both Pal (p=0.023) and Unpal (p=0.007), and the AUC for the desire to eat something sweet was significantly higher for PL than Unpal (p=0.035). No other significant differences by post-hoc analysis were found for the other appetite ratings.

Figure 4.4. AUC (0-180 min) for subjective appetite ratings
Vinegar treatment significantly influenced the AUC for (a) fullness (p<0.0001) and (c) prospective consumption (p=0.048) and influenced (b) hunger (p=0.067) and (d) desire to eat something sweet (p=0.051) with a trend towards significance. Columns showing the same symbol denote significant differences between treatments by post-hoc analysis for: a) fullness ^p=0.023 (PL vs Pal) and *p=0.007 (PL vs Unpal) and d) desire to eat something sweet for $p=0.055 (Pal vs Unpal).

Analyses were carried out by one-way repeated measures ANOVA with post-hoc Bonferroni. Results shown as mean with error bars representing the SEM (n=16).
4.4.3 Quantitative appetite assessment

4.4.3.1 Intake at the ad libitum test meal

When analysed for the overall sample (n=16), the mean intake ad libitum intake of pasta did not differ between treatments as shown in Figure 4.5.

![Figure 4.5. Intake of ad libitum pasta meal in (a) grammes & (b) kJ](image)

Intake did not differ significantly between treatments. Results shown as mean with error bars representing the SEM (n=16).

However, box-plot analysis of intake of the buffet meal provided 3 h postprandially identified one outlier in the dataset, with an intake value >3 SD over the mean intake during Unpal treatment (Figure 4.6). The ad libitum intake of the pasta meal was therefore also re-analyses following the removal of all data from this participant, with analyses carried out on the remaining 15 participants.

![Figure 4.6. Dot-plot graph of ad libitum EI 3 h postprandially](image)

An outlier was identified with a value >3SD over the mean. Results shown as mean with error bars representing the SD (n=16).
The mean *ad libitum* intake of pasta after removal of the outlier (n=15) is shown in Figure 4.6. The mean intake following Unpal was the lowest (2797 [SD 888] kJ), followed by Pal (3034 [SD 851] kJ), with the highest mean intake occurring with PL treatment (3430 [SD 1010] kJ). Following removal of the outlier, vinegar treatment significantly influenced intake of the *ad libitum* meal both in grammes and in kJ (p=0.022 for both g and kJ, n=15) when analysed by Friedman’s ANOVA test. Post-hoc Wilcoxon analysis (with the level of significance assumed at p=0.0167) found the difference in mean intake between PL and Unpal approached significance (p=0.023 for both g and kJ, n=15), although there was no significant difference between PL and Pal or Pal and Unpal.

![Figure 4.7. Intake of *ad libitum* meal in (a) grammes & (b) kJ with outlier removed](image)

Vinegar treatment significantly influenced the intake (p=0.022 for both g and kJ) with post-hoc identifying a difference approaching significance between intake following PL and Unpal (*p=0.023 for both g and kJ).

Analyses were carried out by Friedman’s ANOVA with post-hoc Wilcoxon. Results shown as mean with error bars representing the SEM (with outlier removed, n=15).
4.4.3.2 Intake during 24 h period following preload

The mean 24 h energy and macronutrient intake post ingestion of PL, Pal and Unpal preloads is shown in Table 4.4. Data from two participants was excluded due to incomplete 24 h intake dietary records, therefore analyses were carried out on the remaining 14 participants.

As Table 4.4 shows, the highest intake of energy and of all macronutrients was following PL, followed by Pal then Unpal. Energy (p=0.021, n=14) and CHO (p=0.010, n=14) intakes were both significantly influenced by vinegar treatment when analysed by one-way repeated measures ANOVA. The significance for EI remained when alcohol was removed from the contributions to EI (p=0.010, n=14). There was also a vinegar treatment effect with a trend towards a lower intake of fat and protein following Unpal (p=0.059).

Table 4.4. EI and macronutrient intake 24 h after PL, Pal and Unpal treatment

Vinegar treatment was found to significantly influence energy (p=0.021) and CHO intake (p=0.010) and to influence fat and protein intakes with a trend towards significance (both p=0.059). All analyses carried out by one-way repeated measures ANOVA with post-hoc Bonferroni (n=14).

<table>
<thead>
<tr>
<th></th>
<th>PL Mean</th>
<th>PL SD</th>
<th>Pal Mean</th>
<th>Pal SD</th>
<th>Unpal Mean</th>
<th>Unpal SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>^9515</td>
<td>2560</td>
<td>8565</td>
<td>2710</td>
<td>^7475</td>
<td>2092</td>
<td>0.021</td>
</tr>
<tr>
<td>EI excluding alcohol (kJ)</td>
<td>*9001</td>
<td>1992</td>
<td>~8259</td>
<td>2316</td>
<td>*7081</td>
<td>1954</td>
<td>0.010</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>72.2</td>
<td>22.9</td>
<td>66.5</td>
<td>25.8</td>
<td>53.7</td>
<td>22.5</td>
<td>0.059</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>70.2</td>
<td>10.6</td>
<td>*70.9</td>
<td>19.8</td>
<td>59.4</td>
<td>19.1</td>
<td>0.059</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>#308.3</td>
<td>75.9</td>
<td>273.5</td>
<td>79.5</td>
<td>#242.7</td>
<td>63.3</td>
<td>0.010</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>17.4</td>
<td>40.6</td>
<td>12.4</td>
<td>22.3</td>
<td>13.2</td>
<td>21.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Mean values marked with the same symbol denote significant differences between treatments following post-hoc analysis *p<0.05, ^p<0.02, *p<0.005, or trend approaching significance ~p=0.054.

Post-hoc analysis revealed EI and CHO intake was significantly lower following Unpal than PL treatment (p=0.017 and p=0.004 respectively). Furthermore, protein intake was
lower with a trend towards significance following Pal than PL (p=0.023). When alcohol was removed from the contribution to El, El following Unpal was significantly lower than PL (p=0.002) and lower than Pal with a trend towards significance (p=0.054).

As shown in Figure 4.8, there was no difference in % contribution of macronutrients and alcohol to the total 24 h EI between PL, Pal and Unpal treatment when analysed by one-way repeated measures ANOVA.

Figure 4.8. % contribution to 24 h EI by each macronutrient and alcohol
No significant difference between treatments was found for any of the macronutrients or alcohol by one-way repeated measures ANOVA analysis. Results shown as mean with error bars representing the SEM (n=14).
4.4.4 Postprandial glycaemic response

The mean postprandial glycaemic response following the different treatments is shown in Figure 4.9. Vinegar ingestion alongside the standard breakfast resulted in a lower and later glucose peak than PL. A significant effect of vinegar treatment on the glycaemic response was found for 180 min (p=0.022, n=16) and 120 min (p=0.003, n=16) postprandially by two-way repeated measures ANOVA analysis. In addition there was a significant vinegar x time interaction (p<0.0001 and p=0.012 for 180 and 120 min postprandially respectively, n=16) and a significant effect of time (p<0.0001 for both 180 and 120 min postprandially, n=16).

![Figure 4.9. Postprandial blood glucose concentrations](image)

**Figure 4.9. Postprandial blood glucose concentrations**

Vinegar treatment significantly influenced the glycaemic response (p=0.022, two-way repeated measures ANOVA analysis). Results shown as mean with error bars representing the SEM (n=16).

The AUC and IAUC for the blood glucose response from 0 to 120 and to 180 min is shown in Table 4.5. Vinegar treatment significantly influenced the AUC (both 0 to 120 and 180 min) and the IAUC from 0 to 120 min, when analysed by one-way repeated measures ANOVA.
Post-hoc analysis found that the AUC and IAUC from 0 to 120 min following Pal was significantly lower than PL ($p=0.006$ and $0.011$) and the AUC from 0 to 180 min was lower following Pal than following PL with a trend towards significance ($p=0.079$). There was no significant difference between Unpal and PL or between Pal and Unpal AUC and IAUC.

Table 4.5. AUC & IAUC for blood glucose response 120 and 180 min postprandially following PL, Pal and Unpal. Vinegar treatment significantly influenced the AUC 180 and 120 and IAUC 120. All analyses carried out by one-way repeated measures ANOVA with post-hoc Bonferroni (n=16).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Pal</th>
<th>Unpal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>AUC 180</td>
<td>1019.6</td>
<td>84.8</td>
<td>956.9</td>
</tr>
<tr>
<td>120</td>
<td>727.5</td>
<td>75.6</td>
<td>662.1</td>
</tr>
<tr>
<td>IAUC 180</td>
<td>184.9</td>
<td>71.9</td>
<td>140.7</td>
</tr>
<tr>
<td>120</td>
<td>*171.0</td>
<td>73.4</td>
<td>*118.0</td>
</tr>
</tbody>
</table>

$^1$AUC & IAUC mean values marked with the same symbol denote significant differences between treatments ($^p=0.006$, $^{*p}=0.011$) or a trend approaching significance ($^{*p}=0.079$)
4.4.5 Subjective nausea ratings

Figure 4.10 shows the VAS ratings for nausea. Vinegar treatment was found to significantly influence nausea ratings (p=0.001) when analysed by two-way repeated measures ANOVA. In addition there was a significant vinegar x time interaction (p<0.0001).

![Figure 4.10. Postprandial VAS ratings for feelings of nausea](image)

Vinigar treatment significantly influenced nausea (p=0.001, two-way repeated measures ANOVA analysis). Results shown as mean with error bars representing the SEM (n=16).

The mean AUC and IAUC from 0 to 180 min for the nausea appetite ratings are shown in Figure 4.11. A significant effect of vinegar treatment on both the AUC (p=0.001) and the IAUC (p=0.009) was found by one-way repeated measures ANOVA analysis.

Post-hoc bonferroni analysis found the AUC and IAUC for nausea were significantly higher following Pal than PL (p=0.024 and p=0.037 respectively) and following Unpal than PL (p=0.007 and p=0.014 respectively). The mean nausea AUC and IAUC following Unpal was higher than following Pal, although this difference was not significant.
Figure 4.11. Subjective nausea ratings AUC and IAUC
Vinegar treatment significantly influenced nausea ratings (a) AUC \( (p=0.001) \) and (b) IAUC \( (p=0.009) \). Values marked with the same symbol denote significant differences between treatments \( (p<0.05) \).

Analyses were carried out by one-way repeated measures ANOVA with post-hoc Bonferroni. Results shown as mean with error bars representing the SEM \( (n=16) \).
4.4.6 Correlations of palatability and nausea with appetite parameters

Table 4.6 summarises the relationship of palatability ratings and nausea AUC with appetite parameters (postprandial subjective appetite AUC and actual EI). Correlation analysis was carried out on pooled data from all study days using Pearson’s product-moment correlation to examine relationships with breakfast palatability VAS ratings and nausea AUC (normally distributed) and Spearman rho correlation to examine relationships with drink pleasantness VAS ratings (not normally distributed).

A large inverse correlation was found between breakfast palatability ratings and the nausea AUC ($r=-0.557, n=48, p<0.001$) and a medium inverse correlation was found between drink pleasantness ratings and the nausea AUC ($r=-0.408, n=48, p=0.004$).

Table 4.6. Correlations of palatability & nausea measures with appetite measures. Bivariate correlation coefficients for palatability VAS ratings and postprandial nausea AUC compared to postprandial appetite VAS AUC & quantitative appetite measures (1-5).

<table>
<thead>
<tr>
<th></th>
<th>3VAS AUC</th>
<th>2Palatable Rating</th>
<th>2Nausea AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fullness</td>
<td>Hunger</td>
<td>Prospective consumption</td>
</tr>
<tr>
<td></td>
<td>-0.528 &lt;0.001</td>
<td>0.313 0.030</td>
<td>0.316 0.029</td>
</tr>
<tr>
<td></td>
<td>Hunger</td>
<td>0.540 &lt;0.001</td>
<td>0.613 &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Prospective consumption</td>
<td>0.640 &lt;0.001</td>
<td>-0.387 0.007</td>
</tr>
<tr>
<td></td>
<td>Desire to eat savoury</td>
<td>0.613 &lt;0.001</td>
<td>-0.138 NS</td>
</tr>
<tr>
<td></td>
<td>Desire to eat sweet</td>
<td>0.618 &lt;0.001</td>
<td>-0.148 NS</td>
</tr>
<tr>
<td></td>
<td>Desire to eat fatty</td>
<td>0.391 0.006</td>
<td>0.052 NS</td>
</tr>
<tr>
<td></td>
<td>Desire to eat salty</td>
<td>0.402 0.005</td>
<td>0.093 NS</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
<td>-0.408 0.004</td>
<td>-0.557 &lt;0.001</td>
</tr>
</tbody>
</table>

Energy Intake

4 Ad libitum test meal EI | 0.449 0.002 | 0.452 0.002 | -0.313 0.037
5 24 h EI | 0.428 0.005 | 0.262 NS | -0.279 0.073

1 Correlation analysis carried out by Spearman rho correlation
2 Correlation analysis carried out by Spearman’s product moment correlation
3 Analyses for the AUC VAS were carried out on the entire dataset ($n = 3 \times 16 = 48$)
4 Analyses for ad libitum test meal EI were carried out after outlier was removed ($n = 3 \times 15 = 45$)
5 Analyses for 24 h EI was carried out after missing data from 2 participants was removed ($n = 3 \times 14 = 42$)
Breakfast palatability ratings were additionally significantly and positively correlated with all appetite AUC except fullness with medium to large correlations (ranging from r=0.391 to 0.618, n=48, p = <0.001 to 0.006) and were significantly and negatively correlated to fullness AUC (r=-0.447, n=48, p=0.001) when examined by Pearson correlation. The breakfast palatability ratings were also significantly and positively correlated with the ad libitum test meal EI (r=0.449, n=45, p=0.002) and 24 h EI (r=0.428, n=42, p=0.005).

Correlations between drink pleasantness ratings and subjective appetite AUC were not as strong. Spearman rho analysis found a strong inverse significant correlation with the fullness AUC (r=-0.528, n=48, p<0.001) and medium positive significant correlation with hunger, prospective intake and desire to eat savoury (r=0.313 to 0.394, p=0.006 to 0.030). There was also a significant medium positive correlation between the drink pleasantness ratings and *ad libitum* test meal EI (r=0.449, n=45, p=0.002) and 24 h EI (r=0.487, n=42, p=0.001).

Selected correlations are displayed in Figure 4.12.

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Figure 4.12. Correlations of 1. drink taste pleasantness and 2. breakfast palatability ratings with (a) fullness AUC, (b) nausea VAS and (c) *ad libitum* test meal EI
4.4.7 Intake 24 h preceding study

As shown in Table 4.7, differences between the mean energy and macronutrient intake during the 24 h period prior to participating in each study day were not significant as determined by one-way repeated measures ANOVA analysis.

Additionally, the food diaries indicated that all participants had adhered to the required 10-12 h fast prior to coming in for the study day and had not consumed any alcohol during the 24 h period preceding the study.

Table 4.7. Energy and macronutrient intake 24 h prior to study days.
No significant differences were found between treatments for energy and macronutrient intake during the 24 h time-period preceding study days by one-way repeated measures ANOVA analysis (n=16).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th></th>
<th>Pal</th>
<th></th>
<th>Unpal</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>7335</td>
<td>1449</td>
<td>7612</td>
<td>2700</td>
<td>7527</td>
<td>2500</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>62.0</td>
<td>21.4</td>
<td>63.0</td>
<td>24.2</td>
<td>59.4</td>
<td>23.4</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>75.1</td>
<td>20.6</td>
<td>71.8</td>
<td>18.7</td>
<td>71.8</td>
<td>20.9</td>
<td>NS</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>229.7</td>
<td>50.8</td>
<td>247.7</td>
<td>110.8</td>
<td>250.9</td>
<td>111.3</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>
4.5 Discussion

The present study found that ingestion of 25 mmol acetic acid (supplied in 25 g white wine vinegar containing 6 % acetic acid) alongside a mixed meal significantly increases satiety postprandially. Vinegar treatment significantly increased subjective fullness ratings (p<0.0001) and decreased prospective consumption (p=0.036) and hunger ratings (p=0.045) and significantly lowered the EI over the 24 h period following the vinegar preload (p=0.021). Additionally, EI of ad libitum test meal provided 3 h postprandially was significantly lower (p=0.022) following the removal of an outlier. These findings agree with previous studies that had concluded vinegar ingestion significantly increased satiety (Hlebowicz et al., 2008, Ostman et al., 2005), with a dose-response effect on satiety ratings having been observed (Ostman et al., 2005). However in these previous studies, the methodology to investigate effects on satiety was weak and the statistical tests used were inappropriate for the data, making the conclusions questionable. In the present study, appetite was investigated with the use of subjective VAS ratings and measurement of prospective food intake of a pre-weighed ad libitum meal. To our knowledge this is the first time that the influence of vinegar ingestion on appetite has been investigated in this way.

Similar doses of vinegar were supplied in the previous studies (18, 23 and 28 g vinegar supplying 18, 23 and 28 mmol acetic acid respectively (Ostman et al., 2005) and 28g vinegar supplying 28 mmol acetic acid (Hlebowicz et al., 2008)) to the present study (25g vinegar supplying 25 mmol acetic acid). All studies also used similar types of vinegar (white wine vinegar containing 6% acetic acid).

The present study also found ingestion of vinegar alongside a mixed meal significantly reduced the glycaemic response (p=0.022). The influence of vinegar ingestion on
postprandial glycaemia has been extensively investigated (Brighenti et al., 1995, Liljeberg and Bjorck, 1998, Ostman et al., 2005, Sugiyama et al., 2003, Johnston and Buller, 2005, Liljeberg and Bjorck, 1996), and findings from the present study agree with previous results.

In addition to investigating the influence of vinegar ingestion on subsequent appetite using more robust methodology, we also aimed to determine if palatability had an influence on outcomes.

Ideally to investigate the role of palatability, palatability ratings for PL and Pal treatments would not differ significantly. However both the Pal and Unpal drinks were rated as significantly less pleasant than PL, and both the Pal and Unpal breakfasts were rated as significantly less palatable than PL, which means that we were not successful in making Pal to a similar level of acceptability as PL. The Unpal breakfast was however rated significantly less palatable than Pal and the Unpal drink was less pleasant than Pal with a trend towards significance, so we successfully made Pal more acceptable than Unpal treatment. It is interesting to note there were large differences between mean ratings for pleasantness and palatability, indicating that the wording of questions can greatly influence responses, which has previously been indicated in the literature (Sørensen et al., 2003, Yeomans, 1998).

It was found that EI decreased in the order PL, Pal and Unpal for the entire 24 h of the study day. Furthermore, following the removal of an outlier EI of the *ad libitum* test meal provided 3 h postprandially also decreased in the order PL, Pal and Unpal, although it may be argued that this is not an appropriate analytical approach. Post-hoc analysis found that while the difference in EI between PL and Unpal treatments approached significance for the *ad libitum* meal (p=0.023, Wilcoxon analysis) and was significant for 24 h intake (p=0.017, Bonferroni analysis), differences between PL and
Pal and between Pal and Unpal were not significant. When alcohol was removed from contributions to 24 h EI, in addition to a significant difference between PL and Unpal (p=0.002) EI differed between Pal and Unpal with a trend approaching significance.

So what are the implications of these findings? The Pal and Unpal drinks delivered exactly the same dose of vinegar (25 g, equivalent to 25 mmol acetic acid) in exactly the same total volume of liquid alongside an identical breakfast. As Unpal treatment decreased subsequent EI (at both the ad libitum meal 3 h postprandially and over the entire 24 h period) while the Pal drink did not, this provides support for the hypothesis that preload palatability had an influence on subsequent appetite.

Added to this, post-hoc analysis of appetite ratings in the present study found the fullness AUC was significantly higher following Unpal than PL and higher with a trend towards significance following Pal than PL. As the palatability ratings for Unpal were significantly lower than Pal, this still fits with our hypothesis that palatability may influence appetite, although it does not exclude the possibility that the acetic acid itself may have a physiological effect. No other post-hoc differences between PL, Pal and Unpal for appetite ratings were found, except the desire to eat sweet AUC (significantly higher following Pal than Unpal), despite vinegar treatment also significantly influencing hunger and prospective consumption. The lack of significant findings may simply be due to the study having insufficient power to detect significant differences in the post-hoc analyses. Flint and co-workers suggest at least 20 participants should be recruited for a crossover study in order to detect a 5mm difference in mean 4.5h fullness ratings (Flint et al., 2000).

When data was pooled from all the study days, medium to strong correlations were found between the palatability VAS ratings and all appetite VAS AUC, ad libitum test meal EI and 24 h EI. Drink pleasantness ratings were correlated to fullness, hunger,
prospective intake and desire to eat savoury AUC and to ad libitum test meal and 24 h EI. While correlations do not imply causality and do not indicate which variable is causing the other to change (Field, 2009), these findings are certainly interesting in supporting our hypothesis that decreased palatability of test products is associated with decreased appetite/increased satiety.

It has previously been shown that palatability manipulation may influence appetite. For example, the addition of citric acid to soup was found to increase satiation in a dose-response manner, with ad libitum intake of the soup and palatability ratings both significantly decreasing with increasing levels of citric acid (de Graaf et al., 1999). However the relationship is not straightforward, as this study found EI at a postprandial ad libitum buffet meal was not significantly influenced by treatment, leading the authors to conclude there was no effect on subsequent satiety. Other previous studies have also found an influence of palatability on satiation (Hellemann and Tuorila, 1991, Nisbett, 1968, Yeomans, 1996, Yeomans et al., 1997). The lack of effect of palatability on satiety in the study by de Graaf and co-workers (1999) is in contrast to the findings of the present study where we did find an influence on satiety. However the fact that researchers have found an influence of palatability on satiation (Hellemann and Tuorila, 1991, Nisbett, 1968, Yeomans, 1996, Yeomans et al., 1997, de Graaf et al., 1999) adds some weight to our hypothesis that palatability has an influence on appetite.

With this in mind, it may be interesting to additionally investigate ad libitum intake of vinegar-containing test products to determine the influence of vinegar on satiation. The test drinks used in the present study would not be suitable due to the minimal energy content of the drinks, but this could be investigated for example with the addition of vinegar to soup at different level of concentration.
Conversely, Freeland and Wolever have recently reported that rectal and intravenous infusion of acetate (thereby bypassing cephalic responses) increased postprandial PYY and GLP-1 concentrations providing some evidence that acetate may have a physiological influence on appetite aside from a cephalic response (Freeland and Wolever, 2010). However the study was only small (n=6), no subjective or quantitative appetite measures were reported and it needs to be remembered that rectal infusion stimulates a different part of the GIT in comparison to oral delivery. Blood was not collected for gut peptide analysis in the present study so it is not possible to directly compare results.

Looking now at the metabolic results, the postprandial glycaemia AUC was highest following PL, followed by Unpal then Pal. Post-hoc analysis found the AUC following Pal was significantly lower than PL during the first 120 min but did not differ significantly between PL and Unpal and between Pal and Unpal. The difference between Pal and PL was not significant during the full 180 min (p=0.079), despite a significant overall treatment effect of vinegar (p=0.047).

A possible explanation for Pal significantly lowering glycaemia while Unpal did not is that participants were observed to consume the Unpal drink as quickly as possible to 'get it over with', whereas the Pal drink took much longer to drink as it was spread throughout a larger volume, meaning participants were consuming the vinegar drink for the entire duration of the test breakfast with Pal but not Unpal treatment. Interpretation of these results in relation to palatability effects is therefore difficult, as Unpal did not significantly lower glycaemia while Pal did. We therefore feel our results are inconclusive regarding the influence of vinegar treatment on the glycaemic response.
One limitation of the study was that as blood analyses were carried out at the point of sample collection on each study day (the HemoCue 201+ analyser provides immediate results from a single drop of blood), error will have been introduced due to day-to-day variability in the analysis. QCs were however run at the start of each study day and the same HemoCue analyser was used on each occasion. As the main study outcome was appetite, we chose to use the HemoCue system as the amount of blood required was minimal, thus reducing the invasiveness of the blood collection procedure and reducing confounding influences of this procedure on appetite.

Another limitation in the present study is that we did not control for the effects of hormonal variations throughout the menstrual cycle. However, of the 13 female participants, 9 were using oral contraceptives and one was fitted with an implant, so only three participants were not using hormonal preparations that would have helped to moderate hormonal fluctuations.

We also did not carry out a sensory evaluation of the test products prior to carrying out the present investigation, which could have helped us in improving the palatability of the Pal treatment in relation to PL, although making a palatable product that contains vinegar in the quantities being investigated is challenging and probably impossible.

4.6 Conclusion

To our knowledge this is the first study that has investigated the influence of oral vinegar ingestion on subjective appetite VAS ratings and on quantitative measures of appetite, and the first study to attempt to investigate the influence of palatability on these outcome measures.

Our results indicate the unpleasant taste of vinegar test products influence postprandial appetite as indicated by effects on subjective appetite ratings and
quantitative appetite measures being greater following Unpal treatment compared to Pal treatment. In addition there were medium to strong correlations between palatability ratings and appetite parameters. It is not possible to ascertain if palatability is the sole cause of observed effects in the present study as we were only partially successful in producing a more palatable (Pal) and less palatable (Unpal) vinegar containing test product. Furthermore correlations do imply causality. The influence of palatability on postprandial glycaemia was less clear.

Further work is required to investigate the influence of the palatability in appetite and metabolic responses following vinegar ingestion.
Chapter 5: Orosensory effects of vinegar on appetite, food intake and metabolic response

5.1 Introduction

In the previous study (Chapter 4) it was reported that oral ingestion of vinegar (as a source of the SCFA acetic acid) acutely reduced glycaemia and increased satiety postprandially, and that these effects may have arisen from the palatability of the vinegar-containing test products.

However results from the previous study were not conclusive, mainly due to the difficulty in producing a vinegar-containing test product that does not differ in palatability from a non-vinegar containing PL. We produced a more ‘palatable’ and an ‘unpalatable’ vinegar-containing test drink that delivered equimolar quantities of acetate. While our ‘unpalatable’ test drink was rated as having a significantly less pleasant taste than the ‘palatable’ test drink, the ‘palatable’ drink also had a significantly lower taste rating than PL, so it was not possible to separate the orosensory properties of the test product from additional physiological effects acetate may have.

Therefore although we found significant correlations between the palatability of the breakfast and appetite, our findings from the previous study were not conclusive.

Following on from this, the present study was designed to investigate the orosensory effects (taste, smell, thought, etc) of vinegar on appetite and postprandial glycaemia and insulinaemia. A method that can be adopted to investigate orosensory effects of a product is the modified sham feeding (MSF), or “spit-and-chew” technique, which has been used extensively to investigate cephalic phase responses (see Chapter 1, section 1.10). In this technique, the food under investigation is tasted and chewed but not swallowed, instead being expectorated, thus separating the orosensory properties of the food from physiological effects that it’s constituents may have following ingestion.
5.2 Aims

The aim of this study was, therefore, to investigate the acute effects of orosensory stimulation with acetic acid (delivered in a drink containing vinegar, VIN) following a standard breakfast preload on appetite VAS ratings, prospective food intake at a subsequent *ad libitum* test meal 3 h postprandially, 24 h intake and glycaemic and insulinaemic response as compared to stimulation with PL (drink with no added vinegar). Orosensory stimulation was achieved using the MSF technique.

5.3 Methods

5.3.1 Sample size calculations

This study was a pilot study. As it has previously been reported that 17 participants are required to detect a 500 kJ difference in EI in a crossover design study with a power of 0.8 (Arvaniti et al., 2000) provided intake on the previous evening is standardised, we aimed to recruit a similar number to this study.

5.3.2 Participants

Fourteen healthy participants (6 male, 8 female) aged between 20 and 42 y were recruited from the student population at the University of Surrey by word of mouth during June to July 2009. To assess suitability for inclusion participants completed a Lifestyle and Medical Questionnaire and DEBQ, and a blood sample was collected by fingerprick to measure fasting blood glucose concentrations at an initial screening session (see section 2.1).

For inclusion, participants were required to have a BMI of 19 to 28 kgm$^2$, fasting blood glucose $\leq 6.0$ mmol/l, restraint score of $\leq 3.5$ (assessed using the DEBQ) and have been stable weight for at least 3 months. Exclusion criteria included currently
pregnant or breast feeding, on a weight reducing diet, history of coronary heart disease, diabetes mellitus, gastrointestinal disorders, anaemia, clinical depression and other psychological disorders or of eating disorders, drug or alcohol abuse in the last 2 y, current habitual smoker, high habitual alcohol intake (≥20 units per week) and taking selected current regular medications (excluding contraceptives). One participant was prescribed an asthma inhaler to use during acute episodes, but no other medications were prescribed to participants.

Table 5.1 outlines the baseline participant characteristics. The mean participant age was 27.5 [SD 5.4] y and BMI was 22.7 [SD 3.2] kgm². None of the characteristics significantly differed between genders except the % body fat, systolic and diastolic BP and fasting blood glucose.

Table 5.1: Baseline participant characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=14)</th>
<th>Male (n=6)</th>
<th>Female (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age / years</td>
<td>27.5</td>
<td>5.4</td>
<td>20-42</td>
</tr>
<tr>
<td>BMI / kgm²</td>
<td>22.7</td>
<td>3.2</td>
<td>18.1-28.2</td>
</tr>
<tr>
<td>Waist circumference / cm</td>
<td>81.4</td>
<td>9.4</td>
<td>68.0-95.0</td>
</tr>
<tr>
<td>% Body fat</td>
<td>21.7</td>
<td>8.9</td>
<td>10.3-33.9</td>
</tr>
<tr>
<td>*Systolic BP / mmHg</td>
<td>108</td>
<td>10.9</td>
<td>95-127</td>
</tr>
<tr>
<td>**Diastolic BP / mmHg</td>
<td>67.7</td>
<td>5.6</td>
<td>60-78</td>
</tr>
<tr>
<td>DEBQ Restraint</td>
<td>2.2</td>
<td>0.7</td>
<td>1.2-3.2</td>
</tr>
<tr>
<td>DEBQ Emotion</td>
<td>1.9</td>
<td>0.7</td>
<td>1.0-3.1</td>
</tr>
<tr>
<td>DEBQ External</td>
<td>2.9</td>
<td>0.4</td>
<td>2.1-3.7</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>4.7</td>
<td>0.4</td>
<td>4.3-5.5</td>
</tr>
</tbody>
</table>

Characteristics that differed significantly (*p<0.05) and very highly significantly (**p<0.001) between genders are denoted by (*) and (**) respectively.
5.3.3 Ethics

All participants gave written consent prior to commencing the study. Approval for the study was granted by the University of Surrey Ethics Committee on 29th September 2008 (EC/2008/70/FHMS), and the study took place from June until August 2009 in the Clinical Research Centre (CRC) at the University of Surrey.

5.3.4 Study protocol

Acute postprandial effects on appetite (assessed by subjective ratings and subsequent EI) and plasma glucose and insulin in response to a standard milkshake preload followed by a MSF phase with either a vinegar-containing (VIN) or PL drink were investigated using a randomised balanced crossover study design. Participants attended on two occasions separated by at least 2 d and were assigned to the experimental condition (VIN or PL, see 5.3.5 for further details), in a counter-balanced randomly assigned order.

To reduce within-subject variability, participants were instructed to refrain from unaccustomed exercise and alcohol during the 24 h preceding each study day and to approximately replicate their 24 h intake prior to each study day. A standardised evening meal (low fibre ready meal and desert selected by participant) was provided and 24 h intake was recorded.

All study days took place at the CRC at the University of Surrey. Participants were requested to stay in the CRC during the study, although they were allowed to read, write or work on their computers.
Figure 5.1 shows a timeline for the study day protocol.

![Timeline](image)

**Figure 5.1: Schematic representation of study protocol**

On each study day participants arrived at the CRC at approximately 08.30 h following an overnight fast of at least 12 h. Upon arrival, anthropometric measurements (% body fat, weight, BMI) were taken. Two fasting capillary blood samples were then collected by fingerprick (time -30 and -5 min) and two initial VAS to subjectively assess appetite, nausea and other attributes unrelated to appetite (see 2.3.1) were completed following each blood sample.

Participants were then provided the breakfast preload (Nurishment milkshake, see 5.3.5), which they were asked to consume within 5 min. At t = 6 min, the MSF phase was commenced (see 5.3.5 for details) with either VIN or PL treatment, which took approximately 5 min. Following breakfast, participants completed VAS regarding the pleasantness of MSF drink and the milkshake (see 2.3.1).

Blood samples were taken every 15 min for the first hour following breakfast and half hourly for the following 2 h until lunch. In addition a set of VAS for fullness, hunger, prospective food consumption, desire to eat meal / snack / sweet / savoury / salty / fatty and nausea plus additional VAS unrelated to appetite such as calmness, happiness, tiredness, boredom and anxiety were completed at half hourly intervals. Participants
were not informed this study was investigating effects on appetite which is why the additional VAS questions were included to distract from the purpose of the study.

180 min following the start of the preload milkshake, a large *ad libitum* homogenous pasta meal was served in a confined individual booth, and participants were instructed to eat until comfortably full. The difference in intake following VIN and PL treatment was determined. Further details about the *ad libitum* buffet meal and the conditions under which it was served are described in Chapter 2 (section 2.3.2).

Once the participants had finished their lunch they were free to leave and were asked to complete a food and drink diary for the remainder of the day until 09.00 h the following morning.

### 5.3.5 Test breakfast and MSF drinks

The standard breakfast preload provided at $t = 0$ min was 420 g Nurishment milkshake (chocolate, vanilla or strawberry flavour). Participants were given the same flavour on each visit and were asked to drink the milkshake within 5 min. Each milkshake supplied 1804 kJ (428 kcal), 21.0 g protein, 60.0 g CHO and 12.6 g fat.

At $t = 6$ min (i.e. 6 min following the start of the milkshake preload), participants were asked to commence the MSF phase randomised to on that occasion, either PL (water with no added vinegar) or VIN (water with added vinegar at a level of 16.7 g vinegar / 100 g total volume, equivalent to 160 mmol/l acetic acid which is the same concentration as the Unpal drink used in Chapter 4). The vinegar used was Tesco White Wine Vinegar (6% acetic acid). The ingredients used to make the test drinks are summarised in Table 5.2.
### Table 5.2: MSF test drinks

Once prepared, the drink was divided equally between 10 small taster cups

<table>
<thead>
<tr>
<th>Product</th>
<th>a) PL</th>
<th>b) VIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tesco White Wine Vinegar (6% acetic acid)</td>
<td>-</td>
<td>30g</td>
</tr>
<tr>
<td>Tap water</td>
<td>180g</td>
<td>150g</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>180g</strong></td>
<td><strong>180g</strong></td>
</tr>
</tbody>
</table>

The MSF drinks were divided equally between 10 small taster cups. During the MSF phase of the study, participants were asked to sip from one cup at a time and to hold the drink in their mouth for 25 seconds without swallowing any of the provided drink. They then expectorated the drink into a pre-weighed receptacle and 5 seconds later were asked to sip from the next cup and so on. This sequence continued until all 10 cups had been sham fed, which took approximately 5 min. The pre-weighed receptacle was then re-weighed in order to determine the recovery following the MSF sequence. The MSF protocol is outlined in Figure 5.2.

### Figure 5.2: MSF protocol

![MSF protocol diagram](image)

**Key**
- 25 seconds – hold MSF drink in mouth
- 5 seconds – expectorate the MSF drink

### 5.3.6 Blood sampling and analysis

Capillary blood samples (approximately 300μl per timepoint) were collected by fingerprick into fluoride microvette tubes (Starstedt Ltd, Beaumont, Leicester, UK) (see section 2.5.2). Glucose concentrations were analysed immediately on fresh plasma samples using the YSI 2300 Stat Plus Glucose Analyser (YSI Incorporated, Yellow Springs, Ohio, USA) (see section 2.6.3.2). The remaining plasma was stored at -20°C until analysed for insulin concentrations using an immunochemiluminometric ELISA.
kit (Invitron Ltd, Wyastone Business Park, Monmouth, UK) (see section 2.6.8.2). Samples from the same participants were analysed on the same plate.

Participants were invited to a session to familiarise them with the fingerprick method prior to commencing the study, to minimise effects on appetite during the study.

5.3.7 Food diary analysis

Food and drink diaries were completed on the day before and the day of the study. These diaries were analysed for EI, macronutrient and fibre intake using WinDiets Professional 2005 (The Robert Gordon University, Aberdeen, UK) (see section 2.2.3).

5.3.8 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0. Data was tested for normality using the Kolmogorov-Smirnov test. Differences in anthropometric variables between male and female participants were assessed non-parametrically using the Mann-Whitney test.

Differences in the effects of VIN compared to PL on palatability VAS scores, EI at the buffet meal, EI and macronutrient intake during the 24 h preceding the study day and during the 24 h of the study day were assessed by paired samples t-test if normally distributed, and by Wilcoxon signed-ranks test if not normally distributed.

The influence of VIN treatment compared to PL on postprandial subjective VAS ratings, glycaemia and insulinaemia was assessed by two-way (VIN treatment x time) repeated measures ANOVA. The AUC and IAUC were compared by paired samples t-test or Wilcoxon signed-ranks test as appropriate. The AUC was calculated using the trapezoidal rule.

Differences were considered significant at a level of $p<0.05$. 

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5.4 Results

5.4.1 Palatability VAS scores

Figure 5.3 shows the mean VAS scores in answer to the questions “How pleasant was the taste of the MSF drink?” and “How pleasant was the taste of the milkshake?”.

The MSF drink containing vinegar (Figure 5.3a) was rated as significantly less pleasant than PL (p<0.0001) when analysed by paired t-test, with mean scores of 22.4 [SD 22.5] mm and 73.0 [18.7] mm respectively.

By contrast, VIN treatment did not significantly influence the perception of the pleasantness of taste of the milkshake relative to PL (Figure 5.2b).

![Figure 5.3a. VAS ratings in answer to 'How pleasant was the taste of the MSF drink?' VIN significantly reduced the pleasantness of the MSF drink relative to PL (p<0.0001). Analyses conducted by paired samples t-test. Results shown as mean with error bars representing the SEM (n=14).](image1)

![Figure 5.3b. VAS ratings in answer to 'How pleasant was the taste of the milkshake?' VIN did not significantly influence pleasantness of the milkshake relative to PL. Analyses conducted by paired samples t-test. Results shown as mean with error bars representing the SEM (n=14).](image2)
5.4.2 Recovery of the MSF drink following sham feeding

Recovery of the VIN drink following sham feeding was found to be significantly higher than PL recovery (p=0.001) when analysed by the Wilcoxon signed-ranks test (Table 5.3). The mean recovery was 97 [SD 5] % following PL and 107 [SD 4] % following VIN, and recovery of drinks was >90 % in all cases except one.

Table 5.3. % recovery of the MSF drinks following sham feeding protocol

Recovery of the vinegar MSF drink was found to be significantly higher than recovery of the PL MSF drink (n=14)

<table>
<thead>
<tr>
<th>P No</th>
<th>% Recovery</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PL</td>
<td>VIN</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>99</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>99</td>
<td>116</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>104</td>
</tr>
<tr>
<td>5</td>
<td>98</td>
<td>107</td>
</tr>
<tr>
<td>6</td>
<td>99</td>
<td>104</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>111</td>
</tr>
<tr>
<td>8</td>
<td>98</td>
<td>114</td>
</tr>
<tr>
<td>9</td>
<td>101</td>
<td>106</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>101</td>
</tr>
<tr>
<td>11</td>
<td>99</td>
<td>104</td>
</tr>
<tr>
<td>12</td>
<td>91</td>
<td>109</td>
</tr>
<tr>
<td>13</td>
<td>101</td>
<td>107</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>108</td>
</tr>
</tbody>
</table>

\[\text{MEAN} \quad ^{*}97 \quad ^{*}107\]

\[\text{SD} \quad 5 \quad 4\]

\(1\) Mean % recovery differed significantly between treatments (*p=0.001, Wilcoxon signed-ranks test)
5.4.3 Subjective appetite ratings

The mean VAS rating time-course curves were lower following VIN for all appetite ratings except fullness (Figure 5.4).

Figure 5.4. Subjective appetite ratings following PL (- ○ -) and VIN (- ▲ -)
VIN treatment did not significantly influence any VAS ratings over the entire study period. A significant treatment effect over part of the study was found for (a) hunger (0-150 min, p=0.044), (b) desire to eat (0-90 min, p=0.050) & (d) prospective consumption (0-60 min, p=0.034). Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=14).

VIN treatment did not have a significant influence when looking at the entire acute study period (from 0 to 180 min) when analysed by two-way repeated measures ANOVA. There was however a significant treatment x time effect for hunger (p=0.009) and the desire to eat something salty (p=0.002).
VIN treatment reduced the mean AUC and IAUC from 0 to 180 min for all appetite ratings except fullness (Table 5.4). However this was only to a significant level for the IAUC for hunger (p=0.024), desire to eat a snack (p=0.010) desire to eat salty (p=0.006) and with a trend towards significance for the AUC for hunger (p=0.052).

Table 5.4. AUC and IAUC from 0-180 min and AUC from 0-90 min for selected VAS appetite ratings after PL and VIN treatment

AUC and IAUC were lower for all ratings following VIN. This was significant over entire study duration for desire to eat a snack AUC and for hunger and desire to eat salty IAUC and trend towards significance for hunger AUC. Analysis was carried out by paired-samples t-test or Wilcoxon signed-ranks test as appropriate (n=20).

<table>
<thead>
<tr>
<th>VAS rating</th>
<th>PL</th>
<th>VIN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>AUC (0-180)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>9013</td>
<td>3452</td>
<td>0.052</td>
</tr>
<tr>
<td>Prospective consumption</td>
<td>9089</td>
<td>3275</td>
<td>NS</td>
</tr>
<tr>
<td>Desire to eat a meal</td>
<td>9198</td>
<td>3482</td>
<td>NS</td>
</tr>
<tr>
<td>Desire to eat a snack</td>
<td>8867</td>
<td>4109</td>
<td>NS</td>
</tr>
<tr>
<td>Desire to eat salty</td>
<td>7680</td>
<td>3966</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAUC (0-180)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>553</td>
<td>3331</td>
<td>0.024</td>
</tr>
<tr>
<td>Prospective consumption</td>
<td>-46</td>
<td>2446</td>
<td>NS</td>
</tr>
<tr>
<td>Desire to eat a meal</td>
<td>-670</td>
<td>2550</td>
<td>NS</td>
</tr>
<tr>
<td>Desire to eat a snack</td>
<td>516</td>
<td>2245</td>
<td>NS</td>
</tr>
<tr>
<td>Desire to eat salty</td>
<td>-73</td>
<td>1535</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (0-90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prospective consumption</td>
<td>-376</td>
<td>1422</td>
<td>0.041</td>
</tr>
<tr>
<td>Desire to eat a meal</td>
<td>-596</td>
<td>1409</td>
<td>0.045</td>
</tr>
</tbody>
</table>

While it is not statistically appropriate to do so, it is interesting to note that during the earlier part of the study, two-way repeated measures ANOVA found VIN treatment significantly reduced hunger ratings from 0 to 150 min (p=0.044) and the desire to eat from 0 to 90 min (p=0.050). Prospective consumption was influenced with a trend towards significance from 0 to 90 min (p=0.051). Significant treatment x time interactions were also observed for desire to eat a snack (p=0.024) from 0 to 150 min. VIN significantly reduced the AUC for hunger (p=0.028) from 0 to 150 min and for prospective consumption (p=0.045) and desire to eat a meal (p=0.041) from 0 to 90 min.
5.4.4 Quantitative appetite Assessment

5.4.4.1 Intake at the ad libitum test meal

Figure 5.5 shows the mean intake of the ad libitum pasta meal 3 h following the milkshake preload. The mean intake was higher following MSF with VIN (562 [SD 290] g, 3636 [SD 1909] kJ) than following MSF with PL (534 [SD 180] g, 3392 [SD 1119] kJ) treatment. Comparative analysis using the paired sample t-test determined the difference in intakes were non significant.

![Figure 5.5. Intake of ad libitum pasta meal in (a) grammes and (b) kJ](image)

No significant influence of VIN treatment on pasta meal intake in grammes or kJ. Analyses were carried out by paired samples t-test. Results shown as mean with error bars representing the SEM (n=14).
5.4.4.2 Intake during 24 h period following preload

The mean energy and macronutrient intake for the entire study day following PL and VIN treatment is shown in Table 5.5. Mean EI following VIN treatment (9576 [SD 2079] kJ) was higher than following PL (9394 [SD 2866] kJ), however this difference was non significant. There were also no significant differences between treatments for macronutrient and alcohol intakes when analysed using paired-samples t-test for parametric data and Wilcoxon signed-ranks test for non-parametric data.

Table 5.5. EI and macronutrient intake 24 h following PL and VIN treatment
No significant effect of VIN treatment was found. Analysis was carried out using paired-samples t-test or Wilcoxon signed-ranks test when appropriate (n=14).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>VIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>9304</td>
<td>2866</td>
</tr>
<tr>
<td>EI excluding alcohol (kJ)</td>
<td>9054</td>
<td>2379</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>74.7</td>
<td>31.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>93.8</td>
<td>31.0</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>281.5</td>
<td>55.3</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>11.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>8.5</td>
<td>31.8</td>
</tr>
</tbody>
</table>

As shown in Table 5.6, there was also no difference in % contribution of macronutrients and alcohol to the total 24 h EI between PL and VIN treatment when analysed by paired-samples t-test.

Table 5.6. % contribution to EI by each macronutrient and alcohol 24 h after PL and VIN treatment
No significant effect of VIN treatment was found. Analysis carried out by paired-samples t-test (n=14).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>VIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Fat %EI</td>
<td>29.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Protein %EI</td>
<td>16.9</td>
<td>2.9</td>
</tr>
<tr>
<td>CHO %EI</td>
<td>52.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Alcohol %EI</td>
<td>1.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>
5.4.5 Postprandial plasma metabolites

The mean postprandial glycaemic and insulinaemic responses for the different treatments are shown in Figure 5.6. No influence of VIN treatment on either the glycaemic or insulinaemic responses was found when analysed by two-way repeated measures ANOVA. Additionally the treatment x time interactions were non significant.

![Image of Figure 5.6](image)

**Figure 5.6. Postprandial plasma (a) glucose & (b) insulin concentrations following PL (- ○ -) and VIN (- △ -) treatment**

No significant influence of VIN treatment on either metabolite was found. Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=14).

Comparison of the AUC and IAUC using the paired t-test analysis also determined there was no significant difference between VIN and PL treatment for both postprandial plasma glucose and insulin concentrations (data not shown).
5.4.6 Indices of insulin sensitivity

As shown in Table 5.7, the fasting insulin sensitivity, β-cell function and insulin resistance as estimated by Homeostasis Model Assessment (HOMA) (Levy et al., 1998, Wallace et al., 2004, Matthews et al., 1985) (see section 2.8.1 for further details) were not significantly different at the start of each study day.

Postprandially, neither the Oral $S_I$ as estimated using the minimal model method (Caumo et al., 2000) (see section 2.8.2 for further details) nor the insulin to glucose AUC ratio (for 0-180 min) differed significantly between VIN and PL.

Table 5.7. Indices of insulin sensitivity at baseline and following milkshake preload\(^{1,2,3}\)

No significant difference between VIN and PL treatments was found by paired-samples t-test / Wilcoxon signed ranks comparison as appropriate (n=14).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>VIN</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>HOMA % S</td>
<td>243.33</td>
<td>38.99</td>
<td>236.34</td>
<td>64.88</td>
</tr>
<tr>
<td>HOMA % B</td>
<td>58.01</td>
<td>16.55</td>
<td>65.88</td>
<td>30.66</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.45</td>
<td>0.11</td>
<td>0.52</td>
<td>0.32</td>
</tr>
<tr>
<td>Insulin:glucose AUC (^{(1)})</td>
<td>3.46</td>
<td>1.91</td>
<td>3.23</td>
<td>2.06</td>
</tr>
<tr>
<td>Oral $S_I$ (^{(2)})</td>
<td>37.03</td>
<td>25.16</td>
<td>53.66</td>
<td>71.54</td>
</tr>
</tbody>
</table>

\(^{1}\) HOMA %S, fasted oral insulin sensitivity, HOMA %B, β-cell function and HOMA IR, insulin resistance all estimated by homeostasis model assessment.

\(^{2}\) AUC 0-180 mins

\(^{3}\) Oral $S_I$ values are x $10^3$ dl glucose / kg.min / μU insulin.ml. Calculated by minimal model.

Comparisons were carried out by paired t-test when normally distributed and by Wilcoxon signed-ranks test when non-parametric.
5.4.7 Subjective nausea ratings

Figure 5.7 shows the VAS ratings for nausea. Analysis by two-way repeated measures ANOVA found that although the mean nausea ratings were higher following VIN than following PL, this difference was not significant. Additionally the treatment x time interactions were non significant.

Comparison of the AUC and IAUC by paired-samples t-test also found no significant difference between treatments (data not shown).

![Figure 5.7. Postprandial VAS ratings for feelings of nausea](image)

No significant influence of VIN treatment was found by two-way repeated measures ANOVA analysis. Results shown as mean with error bars representing the SEM (n=14).

Comparison of the fasting nausea ratings by paired-samples t-test analysis determined that the nausea ratings were significantly higher during the VIN condition than the PL condition.
5.4.8 Intake 24 h preceding study

As shown in Table 5.8, the mean energy, macronutrient and fibre intake during the 24 h period prior to participating in each study day did not differ significantly between PL and VIN treatment when compared by paired-samples t-test or Wilcoxon signed-ranks test as appropriate.

Additionally, the food diaries indicated that all participants had adhered to the required 10-12 h fast prior to coming in for the study day, had consumed the supplied standard meals and had not consumed any alcohol during the 24 h period preceding the study.

Table 5.8: Energy and macronutrient intake 24 h prior to study day
No significant differences were found between treatments for the energy and macronutrient intake during the 24 h time-period preceding the study days when analysed by paired-samples t-test or Wilcoxon signed-ranks test as appropriate (n=14)

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th></th>
<th></th>
<th>VIN</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>6649</td>
<td>1617</td>
<td>6845</td>
<td>1639</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>55.4</td>
<td>20.6</td>
<td>58.1</td>
<td>23.2</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>69.7</td>
<td>20.5</td>
<td>68.2</td>
<td>18.4</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO (g)</td>
<td>202.7</td>
<td>59.9</td>
<td>211.0</td>
<td>73.0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>16.3</td>
<td>7.6</td>
<td>16.3</td>
<td>7.7</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.5 Discussion

The present study found that orosensory stimulation with a vinegar containing drink following a standard breakfast preload did not significantly alter postprandial glycaemia, insulinaemia and oral insulin sensitivity nor did it alter subsequent EI 3 h postprandially or EI over the entire 24 h period following the preload. Some postprandial subjective appetite VAS ratings were however significantly altered. In particular hunger was significantly reduced, indicating orosensory stimulation with vinegar may have transiently reduced appetite.

Of note, for all appetite ratings except fullness, the mean temporal curves following VIN treatment ran below the PL curves, and the mean AUC and IAUC were lower following VIN treatment than PL. When looking at the entire study duration (0 to 180 min) these differences were only significant for hunger (treatment x time effect: p=0.009, AUC: p=0.052, IAUC: p=0.024), desire for a snack (AUC: p=0.010) and desire to eat salty (treatment x time: p=0.002, IAUC: p=0.006). However this is a pilot study and the study was underpowered to detect differences in VAS ratings (Flint et al., 2000).

We did find significant differences when looking at earlier parts of the study for prospective consumption (0 to 90 min), the desire to eat a meal (0 to 90 min), and the desire to eat a snack (0 to 150 min). It can be argued that statistically this is not the most appropriate way to look at the data due to the influence of the data from later time-points, so these findings should be interpreted with caution. However these results are suggestive that there was a transient influence of the orosensory properties on subjective appetite ratings, but that the orosensory effects did not last for the entire 180 min of the study, therefore having no impact on EI at the buffet meal.
Therefore from our results it seems that the orosensory properties of vinegar-containing products do appear to have some influence on appetite. This influence is relatively short-lived and the effects on appetite are not as strong as when vinegar-containing products are ingested. It is possible that there may be an additive effect of the taste of acetate in the oral cavity plus physiological effects of SCFA in the GIT upon ingestion. However the lack of effect on appetite of oral ingestion of propionate delivered in a palatable form (Chapter 3) in which there are no taste effects in the oral cavity does not back this idea up, although in this study the quantity of propionate ingested (Chapter 3, 6.0 mmol propionate) was much lower than when vinegar was ingested (Chapter 4, 25 mmol acetic acid).

It is also possible that effects on appetite following ingestion of vinegar are partially modulated by feelings of nausea. Nausea ratings in the present study were not significantly reduced by MSF with VIN relative to MSF with PL, in contrast to when VIN was ingested in our previous study (Chapter 4). It is probable that merely stimulating the oral cavity does not trigger the same level of nausea as triggered by ingesting the vinegar. If this is the case then this could explain why influences on appetite and the metabolic response were not as strong as the responses in the previous study.

A further interesting possibility is that when vinegar is ingested, as well as activating taste cells in the oral cavity, it is ‘tasted’ further down the GIT. It is now known that taste molecules are expressed in the GIT (Young et al., 2009, Kokrashvili et al., 2009b), which suggests it is possible that the taste of ingested food can be sensed in the gut. It has further been shown that taste-signalling proteins are expressed on enteroendocrine L cells in the small intestine, the same cells that express the appetite gut peptides GLP-1 and PYY (Kokrashvili et al., 2009b, Kokrashvili et al., 2009a, Martin et al., 2009),
suggesting taste sensing in the GIT may have a role in modulating appetite and insulin release. Indeed, luminal sugars have been demonstrated to act on taste signalling proteins in L cells to trigger GLP-1 release (Kokrashvili et al., 2009b). The effect of SCFA on taste receptors has not been reported, however it is possible that they could also be 'tasted' by the gut lumen.

An important difference between the present study and the previous studies investigating the oral supplementation with SCFA (Chapters 3 and 4) is that we provided volunteers with a milkshake preload whereas in other studies participants were given solid foods. We chose to use milkshake because it can be consumed much quicker than a solid meal, allowing the MSF phase of the study to commence at the earlier phase of digestion and absorption of the preload. However this also meant that the gut transit time of the liquid breakfast preload in the present study was faster than in the previous studies following solid breakfast preloads, as reflected in a sharper and faster glycaemic and insulinaemic response following the liquid preload. Furthermore, the impact on subjective appetite was much less pronounced following the liquid preload than solid preloads in the previous studies, which is unsurprising as it was recently reported that a semi-solid dessert is significantly more satisfying than an equicaloric drink (Zijlstra et al., 2009). In addition, consuming a liquid rather than a solid bypasses the need for mastication (which is the main reason that it is quicker to consume a liquid rather than a solid), which will alter cephalic responses.

Overall the use of a liquid preload could make it more difficult to alter appetite and metabolic responses than when a solid preload is used, and it is possible that we may have observed stronger effects if the study had been carried out using a solid preload. It is also possibly the reason for the shorter-lived influence of MSF with vinegar on appetite.
Additionally, the timing of vagal stimulation via MSF may have influenced the findings. We chose to have the MSF phase after the preload rather than before so that the taste effects would occur at the stage of digestion and absorption. However it has previously been found that vagal stimulation with an appetising meal gave rise to more significant effects when applied prior to a fat load than when applied after a fat load (Robertson et al., 2002).

Finally, it may have been worthwhile having a shorter study duration, as the effects on subjective appetite ratings occurred earlier in the study. For example if we had carried out a 90 minute postprandial study we may have observed effects on EI at the buffet meal as more VAS appetite ratings were significantly altered at the point.

5.6 Conclusion

In conclusion, the results from this pilot study indicate orosensory stimulation with a vinegar-containing product may transiently reduce subsequent appetite ratings, however these effects are not as strong as effects when vinegar is ingested (Chapter 4). This is suggestive that while the taste of the vinegar-product in the oral cavity does influence appetite, this may not be the only reason for a reduced appetite following vinegar ingestion. Possible additional reasons for ingested vinegar having a stronger influence on appetite include feelings of nausea or ‘tasting’ in the upper GIT or both.

Further work is therefore required in order to ascertain the role of palatability in influencing appetite. For example the oral cavity could be bypassed via gastric infusion of an acetate solution vs. saline alongside a standard oral preload to determine if observed effects on appetite and the metabolic response occur when there is no orosensory stimulation with acetic acid, thereby just observing the physiological effects of SCFA in the GIT.
Chapter 6: Acute effects of supplementation with inulin propionyl ester on propionate production

6.1 Introduction

The previous studies in this thesis reported in Chapters 3-5 have considered the delivery of specific SCFA (propionate in Chapter 3 and acetate in Chapters 4 and 5) to the upper GIT via oral ingestion. An alternate approach to deliver SCFA to the body is by ingestion of fermentable NDC to deliver SCFA further along the GIT to the colon.

A method to deliver specific SCFA to the colon is being developed in which specific SCFA (e.g. propionate) are tethered to an NDC scaffold such as inulin by chemical esterification (Figure 6.1).

![Figure 6.1. Diagrammatic representation of inulin propionyl ester (IPE)](image)

Propionate molecules are esterified to an inulin scaffold (NDC) which resist digestion, thereby delivering propionate to the colon.

NDC SCFA esters such as an inulin acyl ester or starch acyl ester overcome the problems associated with the other modes of delivery to the body (e.g. via gastric infusion or oral delivery), as it non-invasively places the specific SCFA at the site of interest (the colon) and overcomes palatability problems that occur when SCFA are added to food items.

Investigations are underway to determine if NDC esters are able to deliver specific SCFA to the colon. To be suitable, NDC SCFA esters need to be able to resist digestion in the upper GIT in order to reach the colon for fermentation. Lactulose monacetate was found to resist digestion in vitro when incubated in conditions modelling the
stomach (pH 1.2) and the small intestine (pancreatic enzymes), suggesting this NDC ester is able to resist digestion in the upper GIT (Morrison et al., 2005). This is backed up by in vivo data from a recent study where ileostomy patients (n=7) were provided with 20 g cooked acetylated, propionylated, butyrylated starch as a single dose on separate occasions. Analysis of 24 h stomal effluent determined significant quantities (73-76%) of each starch acyl ester were recovered intact, implying these esters are able to resist digestion in the small intestine (Bajka et al., 2006).

In addition to resisting digestion in the upper GIT, it is also necessary that the SCFA can be cleaved from the NDC SCFA ester once the NDC SCFA ester reaches the colon. In vitro incubation of lactulose mono-acetate in human batch faecal fermenters resulted in an increased acetate production, implying faecal microflora are able to release the acetate from the NDC ester. Furthermore, the provision of acetylated, propionylated or butyrylated starch to rats for 14 d led to increased acetate, propionate and butyrate pools respectively in the caecum when compared to a control non-acylated starch (Annison et al., 2003). In rats, acylated starch also significantly increased overall large bowel SCFA pools (Annison et al., 2003, Bird et al., 2006, Morita et al., 2005) and butyrylated starch significantly increased portal venous plasma butyrate levels (Bajka et al., 2006).

There is only limited evidence in healthy humans. The provision of lactulose mono-(13C)acetate alongside 20 g OF to human volunteers (n=2) resulted in 85 % of the (13C)acetate (marked by 13CO2 production) being released alongside breath H2 production from colonic fermentation. This suggests that the majority (~85%) of the lactulose mono-acetate was delivered to the colon, with less than 15% absorbed from the small intestine (Morrison et al., 2005), particularly remembering some 13C is lost via equilibration with body bicarbonate pools.
In a separate study, Morrison and co-workers (2007) provided volunteers (n=7) with 2 g inulin mono-(13C)-butyryl ester alongside 20 g OF. It was found >90 % of the butyrate was released for oxidation only once it had reached the colon, as indicated by >90 % of the 13CO2 being released following a rise in breath H2, suggesting >90 % (13C)-butyrate was released following colonic fermentation. The data further indicated most of the butyrate was cleaved from the inulin (Morrison and Preston, 2007).

The evidence to date indicates esterified NDC may be able to deliver SCFA to the colon for use in clinical studies, such as those investigating the effects of specific SCFA on appetite. The present study was designed to evaluate if inulin propionyl ester (IPE), an inulin acyl ester with propionate as the tethered SCFA, is able to enhance colonic propionate production in humans, using 24 h urinary SCFA levels as a non-invasive proxy measure of colonic SCFA production.

Urinary SCFA levels have been used in initial stable isotope studies to determine the contribution of exogenous acetate to whole-body acetate flux, with similar results to those obtained from regular blood sampling (Morrison et al., 2004). This initial study therefore indicates 24 h urinary SCFA levels can non-invasively assess colonic SCFA production.

**6.2 Aims**

This study therefore aimed to evaluate if IPE was able to enhance colonic propionate production by investigating the acute dose-response effects of IPE on subsequent colonic propionate production. Colonic propionate production was assessed by measuring 24 h urinary SCFA levels during the 24 h period following supplementation, to act as a non-invasive marker for large bowel SCFA production (Morrison and Preston, 2007).
6.3 Methods

6.3.1 Participants

The study was conducted in twelve healthy participants (6 male, 6 female) aged between 24 and 44 y recruited from the student and staff population at the University of Surrey by e-mail and poster advertisement during April 2007. Suitability for inclusion was assessed using a Lifestyle and Medical Questionnaire (see section 2.1).

For inclusion, participants were required to have a BMI of 19 to 28 kgm\(^2\) and have been a stable weight for at least 3 months. Exclusion criteria included currently pregnant or breast feeding, on a weight reducing diet, history of coronary heart disease, diabetes mellitus, gastrointestinal disorders, anaemia, clinical depression and other psychological disorders or of eating disorders, drug or alcohol abuse in the last 2 y, current habitual smoker, high habitual alcohol intake (≥20 units per week) and taking selected current regular medications (excluding contraceptives).

Three participants were taking oral contraceptives, but no other medications were prescribed to participants.

Table 6.1 outlines the baseline participant characteristics. The mean participant age was 32.1 [SD 5.8] y and BMI was 21.9 [SD 2.4] kgm\(^2\). None of the characteristics significantly differed between genders.

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=12)</th>
<th>Male (n=6)</th>
<th>Female (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD  Range</td>
<td>Mean  SD  Range</td>
<td>Mean  SD  Range</td>
</tr>
<tr>
<td>Age / years</td>
<td>32.1  5.8  24 - 44</td>
<td>32.2  5.4  27 - 42</td>
<td>32.0  6.7  24 - 44</td>
</tr>
<tr>
<td>BMI / kgm(^2)</td>
<td>21.9  2.4  18.5 - 25.6</td>
<td>22.0  2.1  19.2 - 24.7</td>
<td>21.7  2.8  18.5 - 25.6</td>
</tr>
</tbody>
</table>
6.3.2 Ethics

All participants gave written consent prior to commencing the study. Approval for the study was granted by the University of Surrey Ethics Committee on 26th March 2007 (EC/2007/19/SBMS), and the study took place from April to July 2007 in the CIU at the University of Surrey.

6.3.3 Study protocol

The study was performed as a randomised double-blind balanced crossover study to investigate the acute dose-response effects of IPE on subsequent colonic SCFA production as assessed by 24 h urinary propionate levels.

Participants attended on five occasions at least 1 wk apart and were assigned to the experimental condition (see 6.3.4 for further details) in a counter-balanced randomly assigned order, either:

- No inulin or IPE supplement
- 15 g inulin
- 5 g IPE + 10 g inulin
- 10 g IPE + 5 g inulin
- 15 g IPE

To reduce within-subject variability, participants were instructed to refrain from unaccustomed exercise and alcohol during the 24 h preceding each study day, to avoid high fibre food choices (advice was provided) and to approximately replicate their 24 h
intake prior to each study day. A standardised evening meal (low fibre ready meal and
desert selected by participant) was provided and 24 h intake was recorded.

On each study day participants arrived at the CIU at approximately 08.30 h
following an overnight fast of at least 12 h. Upon arrival participants were provided
with a test breakfast containing the inulin / IPE dosage they had been randomised to for
that visit mixed into a flavoured mousse (Angel Delight see 6.3.4 for further details).

Following breakfast, participants were supplied with a low fibre packed lunch prepared
in-house with choices selected at screening, and also an evening meal (low fibre ready
meal and desert selected by participant). They were supplied with the same lunch and
evening meal on each study day and were asked to complete a food and drink diary to
monitor compliance. They were then free to leave and were asked to collect all urine
passed during the 24 h period and dropped off the 24 h urine collections the following
morning (see 6.3.5). All food and drink diaries were analysed using Windiet.

6.3.4 Test breakfast and products

The inulin (Orafti Beneo Inulin HP, with a DPave >23) was donated by Orafti Active
Food Ingredients. The IPE was manufactured and donated by Dr Douglas Morrison and
Dr Tom Preston at the Scottish Universities Environmental Research Centre (SUERC).
Prior to serving, it was crushed to a fine powder with the use of a pestle and mortar.

On study mornings participants were provided with a standard breakfast of 30g Rice
Krispies served with 125 ml semi-skimmed milk and a mousse containing the assigned
inulin and/or IPE dose (either no supplement, 15 g inulin, 5 g IPE + 10 g inulin, 10 g
IPE + 5 g inulin or 15 g IPE).
The mousse was made by whisking together Angel Delight powder (22 g butterscotch, 20 g raspberry or 22 g chocolate flavour as chosen by participant) mixed with the assigned inulin and/or IPE dose with 100ml semi-skimmed milk. The mousse was left to set at RT for 5 minutes and then stored at 4 °C until served. In total the breakfast supplied 1353 kJ, 10.4 g protein, 52.7 g CHO, 8.0 g fat and 0.8 g fibre (excluding the added NDC supplement) when a chocolate Angel Delight was served.

6.3.5 Urine collection and processing

Participants were supplied with two 3 L Urisafe Urine Collection Containers (VWR International) with no added preservative, as preservatives interfere with the SCFA analysis. In addition female participants were supplied with 1 L polypropylene beakers (Fisher Scientific UK) to help with the urine collection.

Participants were requested to collect all urine passed during the 24 h period following the test breakfast until the following morning. The following morning participants dropped off their 24 h urine collections at the CIU which was stored at 4 °C until processing. The urine was always processed on the day of receipt. To process, the 24 h urine was pooled and 10 ml aliquots were removed and stored at -20°C until ready to process for analysis. The total volume of the 24 h urine was measured using a 500 ml measuring cylinder and recorded.

6.3.6 Urinary analysis

Dr Douglas Morrison at SUERC analysed the urine samples by GC-MS to quantify SCFA levels (Morrison et al., 2004) as described in section 2.7. All analyses were carried out blinded to treatment.
6.3.7 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0. The urinary data was not normally distributed as assessed by the Kolmogorov-Smirnov test, so data was analysed non-parametrically. Dose-response effects were analysed by comparison of mean values at each dose using the Friedman’s ANOVA test and also by correlation using the Spearman’s rho test to compare the IPE / inulin dose to urinary results.

Differences were considered significant at a level of $p \leq 0.05$. 
6.4 Results

6.4.1 Dose response effects on 24 h urinary propionate parameters

Figure 6.2 shows the dose response effects of supplementing with inulin and IPE on total 24 h urinary propionate levels for all participants (n=12) and also with an outlier removed (n=11). 24 h urinary propionate production did not differ significantly between each dose for the overall sample (n=12) nor when the outlier was removed (n=11) by Friedman’s ANOVA analysis. Furthermore doses did not correlate with 24 h propionate production by one-tailed Spearman’s rho analysis for the overall sample ($R^2=0.016$, $n=12$) nor when the outlier was removed ($R^2=0.008$, $n=11$).

![Figure 6.2. Total urinary propionate levels (mmol/24 h) at each IPE dose (a) for all participants (n=12), (b) after excluding an outlier (n=11) and (c) showing the outlying value (n=12).

No significant effect of treatment on 24 h urinary propionate was found by Friedman’s ANOVA and the dose was not significantly correlated to 24 h urinary propionate levels for all participants (n=12) and with the outlier removed (n=11). In = inulin.
6.4.2 48 h nutritional intake

The mean nutritional intake on the study day and the day before each study day is shown in Table 6.2A and B respectively. Participants achieved a low mean fibre intake, consuming 10.0 [SD 2.6] to 10.6 [SD 2.9] g and 8.7 [SD 1.6] to 9.1 [SD 1.6] g on the day before and the day of each study day respectively.

Table 6.2. Mean [SD] energy and macronutrient intake on (A) study day and (B) day before study for each dose level

No significant differences between energy and macronutrient intake were found between each dose level for either day. Analysis carried out using one-way repeated measures ANOVA or Friedman's ANOVA analysis as appropriate (n=12).

(A) Study day

<table>
<thead>
<tr>
<th>Dose (g)</th>
<th>IPE</th>
<th>Inulin</th>
<th>Energy (kJ)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fibre (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0</td>
<td>8727 [1213]</td>
<td>74.0 [17.5]</td>
<td>72.4 [12.9]</td>
<td>283.8 [42.0]</td>
<td>8.7 [1.6]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 15</td>
<td>8561 [1256]</td>
<td>72.2 [18.5]</td>
<td>71.0 [11.6]</td>
<td>279.3 [46.1]</td>
<td>8.8 [1.8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 10</td>
<td>8680 [1336]</td>
<td>73.1 [19.1]</td>
<td>71.7 [12.7]</td>
<td>283.7 [44.9]</td>
<td>8.8 [1.7]</td>
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<td></td>
</tr>
<tr>
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<td>8570 [1211]</td>
<td>71.7 [18.0]</td>
<td>70.6 [11.9]</td>
<td>281.5 [44.6]</td>
<td>8.8 [1.7]</td>
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<td></td>
</tr>
</tbody>
</table>

P value NS NS NS NS NS

(B) Day before study day

<table>
<thead>
<tr>
<th>Dose (g)</th>
<th>IPE</th>
<th>Inulin</th>
<th>Energy (kJ)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fibre (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0</td>
<td>7445 [1582]</td>
<td>66.2 [22.2]</td>
<td>69.1 [10.1]</td>
<td>230.8 [58.0]</td>
<td>10.2 [2.8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 15</td>
<td>7775 [2360]</td>
<td>70.3 [30.3]</td>
<td>74.3 [15.4]</td>
<td>236.7 [77.4]</td>
<td>10.3 [2.7]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 0</td>
<td>7768 [1937]</td>
<td>70.9 [28.5]</td>
<td>70.8 [15.2]</td>
<td>237.4 [62.9]</td>
<td>10.0 [2.6]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P value NS NS NS NS NS

Energy, macronutrient and DF intakes did not differ significantly between dose levels both on the day before and the day of each study morning when analysed by dose level by within subjects one-way repeated measures ANOVA (when normally distributed) or Friedman's ANOVA analysis (when not normally distributed).
6.5 Discussion

The results from the current data are inconclusive. There did not appear to be an effect on 24 h urinary propionate levels with increasing doses of IPE. However from the current dataset it is not possible to determine if this lack of effect is due to IPE being ineffective at enhancing colonic propionate levels, or if it is due to 24 h urinary propionate not being sufficiently sensitive to reflect colonic SCFA levels.

Certainly this dataset does not disprove the hypothesis that the IPE can enhance propionate delivery to the colon. One possibility is that the lack of effect arose from participants having a ‘naive gut’, as the colonic microflora are unlikely to have previously encountered IPE (and in many cases even inulin) in these quantities. It is therefore possible that a run-in period is required whereby a daily dose of the inulin / IPE is given to participants over a period of a week or two to introduce the NDC to the colonic microflora. It could also be worthwhile giving an increased dose, for example 20 g rather than 15 g. Additionally there is much inter-individual variation in colonic microflora, which will have introduced biological noise that may have been reduced with a greater sample size.

Another problem with the present study was the fact that it was not possible to assess complete urine collection. A commonly utilised tool to assess completeness is to measure para-aminobenzoic acid (PABA) excretion when ingested as 3 x 80 mg doses during the day of urine collection (Bingham and Cummings, 1983). It was not used in the present study because PABA may interfere with SCFA metabolism, which would in turn influence the primary study outcome. Creatinine levels were used instead, however PABA is a more sensitive and reliable indicator (Bingham and Cummings, 1985). Therefore error may have been introduced due to incomplete 24 hour urinary collection.
Furthermore, the propionate concentrations being measured in the 24 h urine samples were low (in the µmol range), so it is possible that at these low levels, any actual effects on propionate levels are lost in the background propionate ‘noise’. Propionate will not have been generated in the first few hours following breakfast, as the orocaecal transit time for inulin generally takes approximately 5-6 h (Geboes et al., 2003), so an improvement could be to commence urine collection partway through day (for example 4-5 h postprandially), to avoid diluting the collection with urine that will not contain propionate generated from fermentation. It is also uncertain exactly what the metabolic fate of propionate is once it has reached the general circulation, and it is possible that metabolic demands for propionate vary from day-to-day, which may mask any effects of enhanced propionate levels in the urine.

6.6 Conclusion

In summary, the results from the present study were inconclusive as it was not possible to determine if the lack of effect on 24 h urinary propionate levels arose from either:

• The IPE not being able to effectively raise colonic propionate production
• The dose of IPE being inadequate to modulate colonic propionate production
• The urinary propionate analytical method not being sufficiently sensitive to detect urinary propionate in the concentrations present in urine
• 24 h urinary propionate not being a sufficiently sensitive proxy measure of colonic propionate production.

The only way by which it is practically possible to determine if the IPE is able to enhance propionate production in humans is by taking postprandial peripheral blood samples throughout the day to monitor the appearance of SCFA in the bloodstream.
Chapter 7: The influence of don-digestible carbohydrate supplements on appetite, food intake and metabolic response

7.1 Introduction

In the previous chapters, the effects of orally delivered SCFA on appetite and the metabolic response were reported. Following on from this, the present study was designed to investigate the effects of colonically delivered SCFA, particularly propionate, via fermentable NDC.

The SCFA activated G-coupled protein receptors GPR43 and to a lesser extent GPR41 are co-expressed in colonic enteroendocrine cells with the anorexigenic ‘ileal brake’ gut peptide PYY (Karaki et al., 2008, Tazoe et al., 2008), providing a rationale that colonically derived SCFA may be more effective than orally delivered SCFA in influencing appetite and the metabolic response. Propionate is the most potent ligand for GPR41 (Tazoe et al., 2008). As sensitivity to stimulation in vitro for PYY secretion is in the order propionate ≥ butyrate >> acetate (Plaisancié et al., 1996) GPR41 is therefore implicated as the responsible receptor for modulating PYY despite being present in the colon in lower quantities than GPR43. This is suggestive of a role for colonic propionate in influencing PYY release and therefore appetite and metabolic effects. This is a possibility that the present study aimed to investigate.

A novel NDC has been developed in which propionate molecules are tethered to an inulin scaffold (inulin propionyl ester, IPE) to maximise the delivery of propionate to the colon, making it a suitable product to investigate physiological effects of colonic propionate. The effectiveness of IPE in modulating propionate production was evaluated, although findings from this study were inconclusive (Chapter 6).
In addition, the non absorbable sugar L-rhamnose (L-Rha) also appears to be suitable to investigate effects of colonic propionate. L-Rha is reported to favour propionate production during \textit{in vitro} fermentation (Fernandes et al., 2000), with increased serum concentrations occurring approximately 6 h following an acute challenge (Vogt et al., 2004b). While acute (Vogt et al., 2004a) and chronic (Vogt et al., 2006) effects of L-Rha ingestion on metabolites have been previously investigated, albeit not extensively, effects on appetite have not.

L-Rha is a naturally occurring deoxy monosaccharide that is found in polysaccharides of gums and mucilages (Schaffer, 1980), being found in foods such as oranges, french beans, winter cabbage and carrots (Cummings and Englyst, 1987). When ingested, it resists digestion and absorption in the small intestine, to reach the colon intact so it is available for colonic fermentation (Vogt et al., 2004b). L-Rha cannot be categorised as a DF as it is a monosaccharide, so it will therefore be referred to as an NDC in this thesis.

Effects of supplementing with inulin-type fructans on appetite (reviewed in section 1.8.2) have been more extensively investigated. However data is not conclusive, with contradictory results due to variable dosages, choice of inulin-type fructan, and confounding factors from study design limitationss. Furthermore, few studies have investigated effects of high DP inulin (e.g. inulin HP) on appetite. Previous studies have also explored the influence of inulin-type fructan supplementation on plasma metabolites (reviewed in section 1.9.2), although only a few considered postprandial effects, with some indication supplementation may reduce postprandial insulinaemia (Causey et al., 2000, Parnell and Reimer, 2009), although this finding was not universal (Forcheron and Beylot, 2007, van Dokkum et al., 1999). There is therefore still a need to further investigate effects of high DP inulin on appetite and the metabolic response.
7.2 Aims

7.2.1 Original aims (4 way crossover)

The present study originally aimed to investigate the effects of IPE, following a 1 wk run-in period, as a component of breakfast and lunch, on postprandial appetite and metabolic response, compared to a high DP inulin (inulin HP), L-Rha (positive control) and no NDC (PL, negative control).

However it was not viable to continue using the IPE due to supply and QC difficulties that occurred during the study. The study was therefore continued without the IPE leg, as the work was still novel particularly in terms of investigating the effects of L-Rha on appetite and satiety. The results are therefore presented as a 3 way crossover study.

7.2.2 Aims of present study (3 way crossover)

The present study therefore aimed to investigate the effect of the fermentable NDC L-Rha and inulin HP following a 1 wk run-in period, as a component of breakfast and lunch, on postprandial subjective measures of appetite and gastrointestinal symptoms, prospective consumption at a subsequent *ad libitum* meal, 24 h intake and postprandial metabolite concentrations and breath H2 concentrations as compared to PL.
7.3 Methods

7.3.1 Sample size calculations

This study was a pilot study.

7.3.2 Participants

Sixteen healthy participants (8 male, 8 female) were initially recruited to this study from the student population at the University of Surrey by e-mail advertisement during September 2008 to May 2009. Of these, one participant was excluded after the first study day due to commencing antibiotic therapy, one participant was excluded partway through the study due to poor compliance with study instructions, and one participant was removed from analyses at the end of due to poor compliance with fasting on one study visit evidenced by plasma TAG results.

Therefore at the end of the study, analyses were carried out on thirteen participants (5 male, 8 female) aged between 19 and 32 y.

To assess suitability for inclusion participants completed a screening Lifestyle and Medical Questionnaire and DEBQ, and a fasting blood sample was collected by fingerprick to measure fasting blood glucose and Hb concentrations (see section 2.1).

For inclusion, participants were required to have a BMI of 19 to 27 kgm$^{-2}$, fasting blood glucose ≤6.0 mmol/l, Hb in the normal range, DEBQ restraint score of <3.7 and have been stable weight for at least 3 months. Exclusion criteria included currently pregnant or breast feeding, on a weight reducing diet, history of coronary heart disease, diabetes mellitus, gastrointestinal disorders, anaemia, clinical depression and other psychological
disorders or of eating disorders, drug or alcohol abuse in the last 2 years, current habitual smoker, high habitual alcohol intake (≥20 units per week) and taking selected current regular medications (excluding contraceptives).

Six participants were taking oral contraceptives and one was fitted with a contraceptive implant, but no other medications were prescribed to participants.

Table 7.1 outlines the baseline participant characteristics (n=13). The mean participant age was 22.9 [SD 3.6] y and BMI was 22.1 [SD 1.6] kgm⁻². None of the characteristics significantly differed between genders except the waist circumference, % body fat and systolic BP.

Table 7.1. Baseline participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=13)</th>
<th>Male (n=5)</th>
<th>Female (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age / years</td>
<td>Mean 22.9 SD 3.6 Range 19-32</td>
<td>Mean 22.6 SD 3.3 Range 19-28</td>
<td>Mean 23.1 SD 4.0 Range 19-32</td>
</tr>
<tr>
<td>BMI / kgm⁻²</td>
<td>22.1 SD 1.6 Range 19.5-24.7</td>
<td>23.0 SD 1.5 Range 21.4-24.7</td>
<td>21.5 SD 1.5 Range 19.5-24.2</td>
</tr>
<tr>
<td>Waist circumference / cm</td>
<td><strong>75.5 SD 6.9 Range 67.5-86.0</strong></td>
<td><strong>82.9 SD 3.1 Range 78.0-86.0</strong></td>
<td><strong>70.5 SD 2.3 Range 67.5-73.0</strong></td>
</tr>
<tr>
<td>Body fat / %</td>
<td>21.9 SD 6.5 Range 11.4-29.4</td>
<td>15.5 SD 3.4 Range 11.4-19.6</td>
<td>26.0 SD 4.1 Range 16.6-29.4</td>
</tr>
<tr>
<td>Systolic BP / mmHg</td>
<td>115 SD 8.5 Range 103-130</td>
<td>120 SD 6.5 Range 114-130</td>
<td>110 SD 7.5 Range 103-121</td>
</tr>
<tr>
<td>Diastolic BP / mmHg</td>
<td>68 SD 7.7 Range 60-84</td>
<td>67 SD 5.6 Range 63-77</td>
<td>69 SD 9.3 Range 60-84</td>
</tr>
<tr>
<td>DEBQ Restraint</td>
<td>2.1 SD 0.8 Range 1.1-3.6</td>
<td>2.2 SD 0.9 Range 1.4-3.6</td>
<td>2.1 SD 0.7 Range 1.1-2.8</td>
</tr>
<tr>
<td>DEBQ Emotion</td>
<td>2.3 SD 0.6 Range 1.5-3.5</td>
<td>2.4 SD 0.2 Range 2.2-2.7</td>
<td>2.3 SD 0.7 Range 1.5-3.5</td>
</tr>
<tr>
<td>DEBQ External</td>
<td>3.5 SD 0.5 Range 3.0-4.4</td>
<td>3.8 SD 0.6 Range 3.0-4.4</td>
<td>3.4 SD 0.3 Range 3.0-3.7</td>
</tr>
<tr>
<td>Fasting blood glucose / mmol/l</td>
<td>4.3 SD 0.5 Range 3.6-5.0</td>
<td>4.4 SD 0.5 Range 3.6-5.0</td>
<td>4.2 SD 0.4 Range 3.7-5.0</td>
</tr>
<tr>
<td>Hb / g/dl</td>
<td>15.6 SD 1.3 Range 12.8-17.1</td>
<td>14.9 SD 1.5 Range 13.0-17.1</td>
<td>14.4 SD 1.3 Range 12.8-17.0</td>
</tr>
</tbody>
</table>

*Characteristics that differed significantly between genders are denoted by *p<0.05 and **p<0.001*
7.3.3 Ethics

All participants gave written consent prior to commencing the study. Approval for the study was granted by the University of Surrey Ethics Committee on 19th August 2008 (EC/2008/53/FHMS), and the study took place from September 2008 until April 2009 in the CIU at the University of Surrey.

7.3.4 Description of test products

Two different NDC supplements were used in the 3-way crossover study. A high DP inulin (Orafti Beneo Inulin HP, which has a DP_{ave} > 23) was donated by DKSH Great Britain Limited, and L-Rha (food grade L-(+)-Rhamnose Monohydrate) was purchased from Vitanutrition Limited. These were supplied within carrier products (sugar free jelly and Angel Delight) as described in Sections 7.3.7 and 7.3.9. During the PL leg, the carrier product only was consumed.

7.3.5 Study protocol

The study was performed as a randomised single-blind balanced crossover study. It was initially a 4-way crossover study where participants commenced four separate study periods in which they were randomised to either IPE, non-esterified inulin HP, L-Rha (positive control) or PL (negative control, no fibre). Once the IPE leg was removed this became a 3-way crossover study with participants being randomised to either inulin HP, L-Rha or PL (control, no fibre).

Following screening, all participants who had not previously participated in an appetite study involving cannulation were invited to attend an initial ‘familiarisation’ study morning in order to familiarise them with the techniques being used (see 7.3.6).
The overall study plan for the 3-way crossover is shown in Figure 7.1.

Participants were then randomised to commence the first study period. The timeline for each study period is given in Figure 7.2. Each study period comprised a 6 d run-in period during which time participants were asked to incorporate the supplement they were randomised to into their usual diet (see 7.3.7). On Day 7 of the study period, participants attended a study day at the CIU (see 7.3.8).

Following a washout period of at least one week, participants then commenced the next study period. Subsequent study periods were also separated by at least one week to allow for washout.
In addition, in order to control for hormonal variations throughout the menstrual cycle, female participants not using contraceptive methods that regulate hormonal levels (e.g. oral contraceptive, contraceptive implant) attended at approximately the same point in their menstrual cycle during the early (luteal phase) for each study day.

**7.3.6 Initial familiarisation study morning**

All participants that had not previously participated in an appetite study with cannulation attended an initial study morning in order to reduce the confounding effects on appetite assessment that can arise from the unfamiliar procedures being used. All data and samples collected on these study morning were disposed of. Participants were told that this was a baseline study morning and were not informed of the true purpose. All study mornings took place in the CIU at the University of Surrey.

The day before the study morning, participants were asked to refrain from unaccustomed exercise and alcohol and to fast overnight for 12 h. Upon arrival at the CIU, various anthropometric measurements (% body fat, weight, BMI) were taken after which an intravenous cannula was inserted into an antecubital vein and a small initial blood sample was taken. VAS to subjectively assess appetite and gastrointestinal symptoms were also completed (see section 2.3.1) and participants were also asked to collect two baseline breath samples to assess breath H₂ concentrations (see section 2.4).

Participants were then provided with a standard breakfast (croissants with jam alongside an Angel Delight mousse – see section 7.3.9). Following breakfast, VAS were completed and breath samples were collected every 30 min and a few small blood samples were collected. Three hours following breakfast the cannula was removed, and participants were provided with a large pasta ad libitum meal which they were asked to
eat until they were comfortably full (see section 2.3.2). Once the participants had finished their lunch they were free to leave.

**7.3.7 Run-in period (Days 1-6 of each study period)**

During each 6 d run-in period, participants were asked to incorporate the NDC supplement (inulin or L-Rha) or PL that they were randomised to into their diet. Table 7.2 shows the daily quantities supplied for each supplement.

![Table 7.2. Daily dose of inulin and L-Rha provided during run-in period (Days 1-6) and on study day (Day 7). Provided as a split dose within 2 jellies per d during run-in.](image)

<table>
<thead>
<tr>
<th>Day</th>
<th>Inulin</th>
<th>L-Rha (98%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>11.2</td>
<td>12.8</td>
</tr>
<tr>
<td>3</td>
<td>14.9</td>
<td>17.0</td>
</tr>
<tr>
<td>4-6</td>
<td>22.4</td>
<td>25.5</td>
</tr>
<tr>
<td>7 (study day)</td>
<td>22.4</td>
<td>25.5</td>
</tr>
<tr>
<td>Total (g/week)</td>
<td>123.2</td>
<td>140.5</td>
</tr>
</tbody>
</table>

The daily dose provided was steadily increased during Days 1-3 to reach the target dose by Day 4. This daily stepped increase in dose has been used by another group who increased OF doses by 5 g per d for the first 3 d (Alles et al., 1999). In the present study, doses of the different supplements were matched by pentose/hexose equivalents (see Appendix G) and were chosen to match the dose provided in previous clinical studies using L-Rha (Vogt et al., 2004a, Vogt et al., 2004b).

The daily supplement doses were incorporated into two jellies (Hartley’s Sugar Free Jelly in strawberry, raspberry, lemon and lime, blackcurrant and orange flavours) which participants were asked to consume alongside either breakfast or lunch and with their evening meal. When randomised to PL, participants were provided with jellies
containing no additional supplement. Sugar-free jelly was chosen as the vehicle to provide the supplement as did not contribute greatly to EI, they disguised the supplement (or PL) randomised to and they made it easy for the participant to consume the supplement as no additional preparation (such as mixing into a drink) was required.

Compliance was monitored with a daily diary in which participants were asked to note if and when they consumed the jelly and to score the pleasantness of taste of the jellies daily (page from diary shown in Appendix H). Using the same diary, gastrointestinal symptoms were also monitored (page from diary shown in Appendix H). The diary was designed to be easily completed by participants. Food intake was also monitored with the use of a 4 d food and drink diary from Days 3 to 6 (see Section 2.3.3).

To reduce within-subject variability, participants were instructed to refrain from unaccustomed exercise and alcohol during the 24 h preceding each study day (Day 6), to consume a low fibre diet and to approximately replicate their 24 h intake prior to each study day. Participants were provided with low fibre food items (e.g. white bread, low fibre breakfast cereal) and a low fibre diet sheet (see Appendix H) which gave an overview of the main foods to avoid, low fibre alternatives and some lunch suggestions.

A standardised evening meal (pre-selected low fibre ready meal and desert) was provided and 24 h intake was recorded.

7.3.8 Study day (Day 7 of study period)

Study days took place at the CIU at the University of Surrey and participants were required to stay in the CIU for the entire duration of the study, although they were
allowed to read, write, work on their computers or watch television (avoiding programmes with food cues).

Figure 7.3 gives the timeline for the study day.

![Timeline for the study day](image)

On each study day, participants arrived at the CIU at approximately 08.00 h following an overnight fast of at least 12 h. Initially anthropometric measurements (% body fat, weight, BMI) were taken, then an intravenous cannula was inserted into an antecubital vein and two fasting blood samples were taken (time -30 and -5 min). Two initial VAS to subjectively assess appetite (see section 2.3.1) were completed following each blood sample, and an initial VAS to subjectively assess gastrointestinal symptoms was completed following the first blood sample. Additionally participants were asked to collect two baseline breath samples to assess breath H₂ concentrations (see section 2.4).

At time = 0 min, participants were provided with the breakfast preload, croissants with jam, sugar free squash or water and an Angel Delight mousse containing the NDC supplement under investigation or PL (see 7.3.9), which they were asked to consume within 15 min. Following breakfast, participants completed VAS regarding the palatability and pleasantness of the mousse (see section 2.3.1).
Blood samples were taken every 15 min for the first hour postprandially and at 30 min intervals for the following two hours until lunch. In addition a set of VAS for fullness, hunger, prospective food consumption, desire to eat meal / snack / sweet / savoury / salty / fatty and nausea were completed after blood samples at half hourly intervals. Gastrointestinal symptom VAS were completed at hourly intervals and breath H₂ samples were collected hourly for the first hour and half hourly thereafter.

Three hours following breakfast, a standard lunch was served, comprising sandwiches with a choice of fillings, crisps, sugar free squash or water and an Angel Delight mousse containing the NDC supplement under investigation or PL (see 7.3.9).

Following lunch, blood samples were collected every 15 min for the first hour and then half hourly for the next 3 h, appetite VAS were completed half hourly, gastrointestinal symptom VAS were collected hourly and breath samples were collected half hourly. Four hours following lunch (7 h following breakfast) the cannula was removed. Participants were provided a large pasta ad libitum test meal which they were asked to eat until they were comfortably full (see section 2.3.2). Once the participants had finished they were free to leave and were asked to complete a 2 d food diary for the rest of the day and for the next day (see section 2.3.3).

7.3.9 Study day meals

The standard breakfast provided on the initial study morning (see 7.3.6) and on the study day (see 7.3.8) comprised 66 g Tesco All Butter Mini Croissants (equivalent to 2 small croissants) filled with 28 g Tesco strawberry or plum jam with either 250 g water or Robinson’s Sugar Free orange, lemon or blackcurrant and apple squash (made using 42 g squash and 208 g water). In addition participants were given an Angel Delight
made by whisking together one-third of a packet Angel Delight (22 g butterscotch or chocolate, 20 g strawberry or raspberry), 100 ml semi-skimmed milk and two-thirds of the daily NDC dose randomised to (14.9 g inulin or 16.8 g L-Rha) or PL (no NDC supplement). On the initial study morning no NDC supplement was added. Table 7.3 shows the nutritional content of the test breakfast with different Angel Delight flavours.

<table>
<thead>
<tr>
<th>Angel Delight used</th>
<th>Energy kcal</th>
<th>Energy kJ</th>
<th>CHO g</th>
<th>Sugar g</th>
<th>Protein g</th>
<th>Fat g</th>
<th>Fibre g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate</td>
<td>495</td>
<td>2074</td>
<td>10.5</td>
<td>65.9</td>
<td>36.4</td>
<td>21.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Butterscotch</td>
<td>496</td>
<td>2078</td>
<td>10.2</td>
<td>66.0</td>
<td>37.5</td>
<td>21.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Strawberry</td>
<td>489</td>
<td>2049</td>
<td>10.2</td>
<td>64.1</td>
<td>35.7</td>
<td>21.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Raspberry</td>
<td>487</td>
<td>2039</td>
<td>10.1</td>
<td>64.5</td>
<td>36.3</td>
<td>20.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

The standard study day lunch comprised sandwiches cut into quarters made using Kingsmill Everyday medium sliced bread, Olivio spread and a choice of chicken (Tesco Light Choices Roast Chicken), ham (Tesco Light Choices Smoked Ham) or edam cheese (Tesco Edam Cheese Slices) filling. Participants were also given crisps selected for their low fibre levels (choice of Frazzles, Tesco Ready Salted Crunchy Sticks, Tesco Salt and Vinegar Twirls or Tesco Onion Rings), Angel Delight (made with one-third packet Angel Delight and 100 ml semi skimmed milk, same as per breakfast), but this time with one-third of the daily NDC dose added (7.5 g inulin, 8.4 g L-Rha and no fibre for PL) and a drink (water or sugar free squash, same as per breakfast).

On the first study day participants were provided with 8 sandwich quarters (i.e. made using four slices of bread, with each slice bread weighing 38 g) and 20 g crisps and were asked to consume at least 4 full sandwich quarters (equivalent to 2 slices of bread) and as many crisps and they would like. They were required to consume the entire
Angel Delight provided. Thereafter the participants were provided the same number of quarters of sandwiches and quantity of crisps as consumed on the first study occasion. This procedure allowing participants to eat to their desired intake on the first visit then giving the same on subsequent occasions has been previously reported (Westatrate and van Amelsvoort, 1993) and used in our group (Bodinham et al., 2010).

Table 7.4: Nutritional content of the test lunch. For 4, 6 or 8 quarters sandwich containing either ham, chicken or cheese filling plus 20g Frazzles and chocolate Angel Delight. Quantity of fibre excluding the NDC supplements.

<table>
<thead>
<tr>
<th>Bread quarters (slices)</th>
<th>Filling</th>
<th>Portion size (g)</th>
<th>Energy kcal</th>
<th>Energy kJ</th>
<th>Protein g</th>
<th>CHO g</th>
<th>Fat g</th>
<th>Fibre g</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 quarters (2 slices)</td>
<td>Ham</td>
<td>76 12 44</td>
<td>534 2245</td>
<td>22.3</td>
<td>67.2</td>
<td>21.1</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>76 12 44</td>
<td>539 2265</td>
<td>22.9</td>
<td>67.3</td>
<td>21.3</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>76 6 32</td>
<td>556 2332</td>
<td>20.6</td>
<td>67.1</td>
<td>24.3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>6 quarters (3 slices)</td>
<td>Ham</td>
<td>114 18 66</td>
<td>679 2853</td>
<td>30.6</td>
<td>84.3</td>
<td>26.6</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>114 18 66</td>
<td>685 2883</td>
<td>31.5</td>
<td>84.4</td>
<td>26.9</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>114 9 48</td>
<td>711 2982</td>
<td>28.0</td>
<td>84.1</td>
<td>31.3</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>8 quarters (4 slices)</td>
<td>Ham</td>
<td>152 24 88</td>
<td>823 3461</td>
<td>38.8</td>
<td>101.3</td>
<td>32.0</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>152 24 88</td>
<td>832 3500</td>
<td>40.1</td>
<td>101.5</td>
<td>32.5</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>152 12 64</td>
<td>867 3633</td>
<td>35.5</td>
<td>101.1</td>
<td>38.4</td>
<td>4.9</td>
<td></td>
</tr>
</tbody>
</table>

For the ham and chicken sandwiches, 22 g ham/chicken and 6 g spread were used per 38 g slice of bread and for the cheese sandwiches 16 g edam and 3 g spread were used per 38 g slice of bread, with one slice of bread equating to 2 sandwich quarters. Table 7.4 summarises the nutritional content of the test lunch.

The choice of jam for breakfast, sandwich filling and flavour of crisps for lunch and drink and Angel Delight flavours for breakfast and lunch were selected by participants at screening, and the same choices were served on each occasion. All food ingredients were weighed to the nearest 1 g except the Angel Delight and fibre supplements which were weighed to the nearest 0.1 g.
7.3.10 Blood sampling and analysis

Blood samples were analysed for glucose, TAG, NEFA and total and HDL cholesterol concentrations by an enzymatic calorimetric method using the ILAB 650 analyser (Instrumentation Laboratory, Milan, Italy) and insulin concentrations were analysed by RIA. More detailed descriptions of the blood sample collection and analysis procedures are given in Chapter 2 (see section 2.5 and 2.6 respectively).

7.3.11 Statistical analyses

All statistical analyses were conducted using SPSS for Windows 16.0 (SPSS Inc., Chicago, USA). Data was tested for normality using the Kolmogorov-Smirnov test. Differences in anthropometric variables between male and female participants were assessed non-parametrically using the Mann-Whitney test.

Differences in the effects of treatment on palatability VAS scores, intake at the buffet meal, EI and macronutrient mean intake during the days preceding the study day and intake during the 24 h of the study day, HOMA estimates, insulin sensitivity index estimates and participant characteristics on each study day were analysed parametrically by one way repeated measures ANOVA with post-hoc Bonferroni or non-parametrically using the Friedman test with post-hoc Wilcoxon signed ranks test.

Time course data was analysed by two-way (treatment x time) repeated measures ANOVA of all timepoints and also by comparison of the AUC and IAUC using one way repeated measures ANOVA with post-hoc Bonferroni when parametrically distributed or Friedman test with post-hoc Wilcoxon signed ranks test when non-parametrically distributed. Time course data included postprandial subjective appetite and gastrointestinal symptom ratings, plasma metabolites (glucose, insulin, NEFA,
TAGs) and breath $H_2$ concentrations on the study day, and daily taste and gastrointestinal symptom ratings during the run-in period.

Time course data collected on the study day was considered for the entire study duration (from 0 to 420 min) and also separately following breakfast (from 0 to 180 min) and lunch (from 180 to 420 min). The AUC for postprandial plasma metabolites and subjective appetite ratings were calculated for each individual using the trapezoidal rule and the IAUC was calculated by subtracting the area below basal levels from the AUC.

Differences were considered to significant at $p \leq 0.05$ with the exception of the post-hoc Wilcoxon signed-ranks test, where significance was set at a level of $p \leq 0.0167 (=0.05/3)$. 
7.4 Results

7.4.1 Subjective appetite ratings

Selected subjective appetite ratings time-course curves as assessed by VAS are shown in Figure 7.4.

No significant treatment effects were found for the entire study duration, during the morning (0-180 min) and after lunch (180-420 min). A treatment x time interaction was found during the morning with a trend approaching significance for a) hunger (p=0.070) and to a significant level for d) meal desire (p=0.008), and during the afternoon for f) desire to eat sweet (p=0.036). Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).

No significant treatment effects of NDC supplementation were found for any of the VAS appetite ratings when analysed by two-way repeated measures ANOVA. This was...
the case when considering the overall study day (from time 0 to 420 min) and when considering the morning (from time 0 to 180 min) and afternoon (from time 180 to 420 min) separately. A significant treatment x time interaction was found during the morning (0-180 min) for meal desire (p=0.008) and approaching significance for hunger (p=0.070), and during the afternoon (180-420 min) for desire to eat sweet (p=0.036). No other significant interactions were found for the VAS appetite ratings.

Comparison of the AUC and IAUC of the subjective appetite ratings (selected data in Table 7.5) determined there were no significant differences between inulin, L-Rha and PL treatment for all VAS questions, except the desire to eat sweet. Post-hoc analysis found the desire to eat sweet AUC after lunch (180-420 min) was significantly lower following L-Rha than PL (p=0.048). Differences were compared by one-way within subjects repeated measures ANOVA with post-hoc Bonferroni when normally distributed and Friedman test with post-hoc Wilcoxon when not normally distributed.

Table 7.5. AUC for selected appetite VAS ratings during the morning (0-180 min) and afternoon (180-420 min)

No significant differences were found between treatments for except a trend towards significance for the desire to eat sweet IAUC. Analyses carried out by one-way within subjects repeated measures ANOVA or Friedman’s as appropriate (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>AUC (0-180)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>8254</td>
<td>2838</td>
<td>8997</td>
<td>2560</td>
</tr>
<tr>
<td>Meal desire</td>
<td>9170</td>
<td>2646</td>
<td>9265</td>
<td>2247</td>
</tr>
<tr>
<td>Sweet desire</td>
<td>8022</td>
<td>3173</td>
<td>7017</td>
<td>3271</td>
</tr>
<tr>
<td>AUC (180-420)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>8106</td>
<td>3304</td>
<td>8947</td>
<td>3118</td>
</tr>
<tr>
<td>Meal desire</td>
<td>9907</td>
<td>3704</td>
<td>9825</td>
<td>2951</td>
</tr>
<tr>
<td>Sweet desire</td>
<td>*10086</td>
<td>4145</td>
<td>8175</td>
<td>4726</td>
</tr>
</tbody>
</table>

1 Mean values marked with the same symbol denote significant differences between treatments following post-hoc analysis *p=0.048
7.4.2 Quantitative appetite Assessment

7.4.2.1 Intake at the *ad libitum* test meal

Figure 7.5 shows the mean intake of the *ad libitum* pasta meal provided 4 h following the test lunch (and 7 h following the test breakfast). No significant differences in mean intake between PL (4204 [SD 1666] kJ), inulin (4089 [SD 1680] kJ) and L-Rha (4053 [SD 1538] kJ) were found by one-way repeated measures ANOVA analysis.

![Figure 7.5. Intake of *ad libitum* pasta meal in a) grammes and b) kJ](image)

No significant influence of NDC treatment on intake in grammes or the El was found. All analyses carried by one-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).
7.4.2.2 Intake following breakfast & lunch preloads

Table 7.6 shows the mean energy and macronutrient 24 h intake on the study days, including all study food and drink in addition to all consumed following the study. No significant differences between treatments were found for energy and macronutrient intakes when analysed by within subject one-way repeated measures ANOVA.

Table 7.6. EI and macronutrient intake 24 h after PL, inulin and L-Rha treatment. No significant differences between treatments were found. Analysis was carried out using within subjects one-way repeated measures ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL Mean</th>
<th>SD</th>
<th>Inulin Mean</th>
<th>SD</th>
<th>L-Rha Mean</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>12368</td>
<td>3128</td>
<td>11667</td>
<td>1930</td>
<td>12383</td>
<td>2656</td>
<td>NS</td>
</tr>
<tr>
<td>EI excluding alcohol (kJ)</td>
<td>11964</td>
<td>2162</td>
<td>11210</td>
<td>1765</td>
<td>11500</td>
<td>1245</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>101.5</td>
<td>15.2</td>
<td>93.3</td>
<td>16.6</td>
<td>103.2</td>
<td>16.4</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>101.3</td>
<td>20.9</td>
<td>96.4</td>
<td>20.5</td>
<td>97.5</td>
<td>15.5</td>
<td>NS</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>382.8</td>
<td>80.4</td>
<td>368.6</td>
<td>55.7</td>
<td>368.3</td>
<td>56.0</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>13.7</td>
<td>43.8</td>
<td>11.4</td>
<td>27.3</td>
<td>23.1</td>
<td>54.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Comparison of the % contribution of macronutrients and alcohol to EI found no significant difference between treatments when analysed by within subjects one-way repeated measures ANOVA (Table 7.7).

Table 7.7. % contribution to EI by each macronutrient and alcohol 24 h after PL, inulin and L-Rha treatment. No significant differences between treatments were found. Analysis was carried out using within subjects one-way repeated measures ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL Mean</th>
<th>SD</th>
<th>Inulin Mean</th>
<th>SD</th>
<th>L-Rha Mean</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat %EI</td>
<td>31.6</td>
<td>3.3</td>
<td>30.3</td>
<td>2.9</td>
<td>32.9</td>
<td>2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Protein %EI</td>
<td>13.9</td>
<td>1.8</td>
<td>13.8</td>
<td>1.3</td>
<td>13.6</td>
<td>1.5</td>
<td>NS</td>
</tr>
<tr>
<td>CHO %EI</td>
<td>52.4</td>
<td>3.5</td>
<td>53.2</td>
<td>3.6</td>
<td>51.1</td>
<td>3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol %EI</td>
<td>2.0</td>
<td>6.2</td>
<td>2.8</td>
<td>5.9</td>
<td>2.3</td>
<td>3.7</td>
<td>NS</td>
</tr>
</tbody>
</table>
7.4.2.3 Intake during run-in period

Table 7.8 shows the mean EI and macronutrient intake over the 4 d preceding the study (Days 3 to 6 of run-in period), whilst loading up on inulin or L-Rha supplements or PL. No significant differences were found between intakes following the different treatments when analysed by within subject one-way repeated measures ANOVA.

Table 7.8. EI and macronutrient intake during the 4 d preceding the study day (Days 3-6 of run-in period). No significant differences between treatments were found. Analysis was carried out using within subjects one-way repeated measures ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>9078</td>
<td>1857</td>
<td>8730</td>
</tr>
<tr>
<td>EI excluding alcohol (kJ)</td>
<td>8822</td>
<td>1901</td>
<td>8266</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>82.9</td>
<td>26.1</td>
<td>75.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>79.4</td>
<td>18.1</td>
<td>72.5</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>268.5</td>
<td>59.9</td>
<td>257.3</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>8.7</td>
<td>12.0</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Comparison of the % contribution of macronutrients and alcohol to EI also found no significant difference between treatments when analysed by within subject one-way repeated measures ANOVA (Table 7.9).

Table 7.9. % contribution to EI by each macronutrient and alcohol during the 4 d preceding the study day (Days 3-6 of run-in period). No significant differences between treatments were found. Analysis was carried out using within subjects one-way repeated measures ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Fat %EI</td>
<td>33.5</td>
<td>5.9</td>
<td>31.9</td>
</tr>
<tr>
<td>Protein %EI</td>
<td>14.7</td>
<td>3.1</td>
<td>14.1</td>
</tr>
<tr>
<td>CHO %EI</td>
<td>49.0</td>
<td>5.2</td>
<td>49.3</td>
</tr>
<tr>
<td>Alcohol %EI</td>
<td>2.9</td>
<td>4.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>
7.4.3 Postprandial plasma metabolites

7.4.3.1 Glycaemic response

Figure 7.6 shows the mean postprandial glycaemic response following consumption of either the inulin or L-Rha supplements or PL. Two-way repeated measures ANOVA analysis found no significant treatment effect when analysed over the entire study duration (T=0-420 min), just during the morning (T=0-180 min), nor when analysed after lunch (T=180-420 min, p=0.070). Furthermore, no significant time x treatment effects were observed.

Comparison of the AUC and IAUC (Table 7.10) by one-way within subject repeated measures ANOVA found a difference between treatments approaching significance for the AUC after lunch (T=180-420 min, p=0.061). However, post-hoc bonferroni analysis found no significant differences between the 3 groups. There were no significant differences between the AUC and IAUC during the overall study day (T=0-420 min) or the study morning (T=0-180 min).
Table 7.10. AUC & IAUC for plasma glucose response during the morning (0-180 min), afternoon (180-420 min) and full study day (180-420 min)

No significant differences were found between treatments for the AUC and IAUC except with a trend towards for the AUC during the afternoon (180-420 min). Analyses were carried out by one-way within subjects repeated measures ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-180</td>
<td>970</td>
<td>173</td>
<td>970</td>
</tr>
<tr>
<td>180-420</td>
<td>1295</td>
<td>145</td>
<td>1386</td>
</tr>
<tr>
<td>0-420</td>
<td>2265</td>
<td>303</td>
<td>2356</td>
</tr>
<tr>
<td>IAUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-180</td>
<td>102</td>
<td>152</td>
<td>88</td>
</tr>
<tr>
<td>180-420</td>
<td>193</td>
<td>123</td>
<td>213</td>
</tr>
<tr>
<td>0-420</td>
<td>239</td>
<td>270</td>
<td>299</td>
</tr>
</tbody>
</table>
7.4.3.2 Insulinaemic response

The mean postprandial plasma insulin response is shown in Figure 7.7. There was a significant influence of NDC treatment found during the morning (T=0-180 min, p=0.051), although the treatment effect was not significant during the afternoon (T=180-420 min) or for the overall study day (T=0-180 min). In addition, a significant NDC treatment x time interaction was found during the morning (T=0-180 min, p=0.024), afternoon (T=180-420 min, p=0.026) and for the entire study day (T=0-420 min, p=0.009).

![Figure 7.7. Postprandial plasma insulin response](image)

**Figure 7.7. Postprandial plasma insulin response**

Plasma insulin concentrations were significantly influenced by NDC treatment during the morning (0-180 min, p=0.051), but not after lunch (180-420 min) or for the entire study duration (0-420 min). A significant treatment x time interaction was also found for the entire study duration (0-420 min, p=0.009), during the morning (0-180 min, p=0.024) and after lunch (180-420 min, p=0.026).

Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).

Table 7.11 summarises the IAUC values for the postprandial insulin response curve (AUC values not given). The mean AUC and IAUC were highest during PL and lowest following L-Rha. As AUC and IAUC were not normally distributed, analyses were carried out using Friedman’s ANOVA with post-hoc Wilcoxon Signed Rank Test.
The IAUC for the entire study day (T=0-420 min) differed between treatments with a trend approaching significance (p=0.058), with L-Rha treatment lowering the IAUC with a trend approaching significance (p=0.023) relative to PL by post-hoc analysis. The AUC for the entire study day did not differ significantly between treatments.

During the morning, there was a significant treatment effect on the IAUC (T=0-180 min, p=0.050 and T=0-120 min, p=0.037). Post-hoc analysis found the IAUC was significantly lower following L-Rha than PL (p=0.011) from 0-120 min and lower with a trend towards significance following L-Rha than PL (p=0.033) from 0-180 min. There were no significant differences between PL and inulin nor between L-Rha and inulin.

During the afternoon the IAUC differed with a trend towards significance (p=0.058) during the first 180 min postprandially (180-360 min), although the differences were not significant from 180-420 min. Post-hoc analysis found no significant differences between treatments.

Table 7.11. IAUC for plasma insulin response during morning (0-180 min), afternoon (180-420 min) and full study day (180-420 min)
Analyses carried out by Friedman’s ANOVA with post-hoc Wilcoxon (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>0-120</td>
<td>5374</td>
<td>3080</td>
<td>4426</td>
</tr>
<tr>
<td>0-180</td>
<td>#6255</td>
<td>4363</td>
<td>5357</td>
</tr>
<tr>
<td>180-360</td>
<td>6982</td>
<td>4163</td>
<td>6477</td>
</tr>
<tr>
<td>180-420</td>
<td>6610</td>
<td>4100</td>
<td>6359</td>
</tr>
<tr>
<td>0-420</td>
<td>15119</td>
<td>12128</td>
<td>13756</td>
</tr>
</tbody>
</table>

1 Mean values marked with the same symbol denote significant differences between treatments following post-hoc Wilcoxon analysis (*p=0.011) or a trend approaching significance (*p=0.033, ^p=0.023)
7.4.3.3 TAG response

The mean postprandial plasma TAG response is shown in Figure 7.8. No significant treatment effects were found when analysed over the entire study duration (T=0-420 min), during the morning (T=0-180 min), nor after lunch (T=180-420 min) when analysed by two-way repeated measures ANOVA. However, there was a significant time x treatment effect for the full study day over the first 300 min (T=0-300 min, p=0.036) and for the study morning (T=0-180 min, p=0.006).

![Figure 7.8. Postprandial plasma TAG response](image)

**Figure 7.8. Postprandial plasma TAG response**

No significant treatment effects were found for the entire study duration, during the morning (0-180 mins) and after lunch (180-420 mins). A significant treatment x time interaction was found during the morning (0-180 min, p=0.006) and for the full study day until 300 min following breakfast (0-300 min, p=0.036) Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).

Between treatment comparisons of the AUC and IAUC for the postprandial TAG response curve using one-way within subject repeated measures ANOVA found no significant differences (data not shown).
7.4.3.4 NEFA response

The mean postprandial plasma NEFA response is shown in Figure 7.9. Two-way repeated measures ANOVA analysis found no significant treatment effects when analysed over the entire study duration (T=0-420 min), just during the morning (T=0-180 min), or when analysed after lunch (T=180-420 min). There was however a significant time x treatment effect during the study afternoon (T=180-420 min, p=0.046), although time x treatment effects were not seen for the entire study day or during the study morning.

![Figure 7.9. Postprandial plasma NEFA response](image)

Figure 7.9. Postprandial plasma NEFA response
No significant treatment effects were found for the entire study duration, during the morning (0-180 mins) and after lunch (180-420 mins). A significant treatment x time interaction was found during the afternoon following lunch (180-420 min, p=0.046). Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).

Table 7.12 summarises the AUC and IAUC values for the postprandial NEFA response curve. Between treatment comparisons using one-way within subject repeated measures ANOVA found no significant differences in treatment effects on AUC and IAUC for the entire study day (T=0-420 min) or the study morning (T=0-180 min). The IAUC after lunch (T=180-420 min) differed between treatments with a trend approaching
significance (p=0.075), with the lowest mean IAUC following L-Rha, although post-hoc Bonferroni analysis found this difference was not significant.

Table 7.12. AUC & IAUC for plasma NEFA response during morning (0-180 min), afternoon (180-420 min) and full study day (180-420 min)
Analyses carried out by one-way within subjects repeated measures ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-180</td>
<td>40.4</td>
<td>11.6</td>
<td>41.3</td>
</tr>
<tr>
<td>180-420</td>
<td>45.3</td>
<td>11.0</td>
<td>46.3</td>
</tr>
<tr>
<td>0-420</td>
<td>85.7</td>
<td>20.3</td>
<td>87.6</td>
</tr>
<tr>
<td>IAUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-180</td>
<td>66.0</td>
<td>28.6</td>
<td>67.8</td>
</tr>
<tr>
<td>180-420</td>
<td>28.2</td>
<td>35.7</td>
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</tr>
<tr>
<td>0-420</td>
<td>162.4</td>
<td>75.9</td>
<td>166.9</td>
</tr>
</tbody>
</table>
7.4.4 Insulin sensitivity

As shown in Table 7.13, the fasting insulin sensitivity, β-cell function and insulin resistance as estimated by HOMA (Levy et al., 1998, Wallace et al., 2004, Matthews et al., 1985) (see section 2.8.1 for further details) were not significantly different at the start of each study day.

Table 7.13: Indices of fasting insulin sensitivity following consumption of inulin or L-Rha supplements or PL during a 6 d run-in

No significant difference between treatments found by Friedman’s ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>HOMA %S</td>
<td>89.6</td>
<td>46.2</td>
<td>83.0</td>
<td>37.2</td>
</tr>
<tr>
<td>HOMA %B</td>
<td>124.9</td>
<td>35.2</td>
<td>125.0</td>
<td>33.1</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>1.4</td>
<td>0.5</td>
<td>1.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

HOMA %S, fasted oral insulin sensitivity, HOMA %B, β-cell function and HOMA IR, insulin resistance all estimated by homeostasis model assessment.

Postprandially, neither the Oral $S_t$ as estimated using the minimal model method (Caumo et al., 2000) (see section 2.8.2) nor the insulin to glucose AUC ratios differed significantly between treatments when compared by Friedman’s ANOVA analysis.

Table 7.14. Indices of postprandial insulin sensitivity following ingestion of inulin or L-Rha supplements or PL

No significant difference between treatments found by Friedman’s ANOVA (n=13).

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Insulin:glucose AUC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-180</td>
<td>8.6</td>
<td>5.2</td>
<td>7.5</td>
<td>3.4</td>
</tr>
<tr>
<td>180-420</td>
<td>8.7</td>
<td>6.1</td>
<td>7.7</td>
<td>5.5</td>
</tr>
<tr>
<td>0-420</td>
<td>8.6</td>
<td>5.7</td>
<td>7.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Oral $S_t$ 1

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>0-180</td>
<td>21.7</td>
<td>16.5</td>
<td>26.3</td>
<td>17.6</td>
</tr>
<tr>
<td>180-420</td>
<td>18.0</td>
<td>19.8</td>
<td>19.2</td>
<td>7.5</td>
</tr>
</tbody>
</table>

1 Oral $S_t$ values are x 10^2 dl glucose / kg.min / µU insulin.ml. Calculated by minimal model.
7.4.5 Breath hydrogen concentrations

The mean postprandial breath H$_2$ concentrations are shown in Figure 7.10. Two-way repeated measures ANOVA analysis found a significant effect of NDC supplementation for the entire study day duration (T=0-420 min, p<0.001), during the morning (T=0-180 min, p=0.010) and during the afternoon (T=180-420 min, p<0.001). There was also a significant NDC treatment x time interaction for the entire study day, the morning and the afternoon (p<0.001 for all cases).

![Figure 7.10. Postprandial breath H$_2$ concentrations](image)

Breath H$_2$ concentrations were significantly influenced by NDC treatment during the entire study duration (0-420 min, p<0.001), during the morning (0-180 min, p=0.010) and after lunch (180-420 min, p<0.001). Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).

Comparison of the AUC of the postprandial breath H$_2$ AUC (Figure 7.12a) by Friedman’s ANOVA analysis found a significant difference between treatments for the entire study day (T=0-420 min, p<0.001), for the study morning (T=0-180 min, p=0.023, data not shown) and for the study afternoon (T=180-420 min, p<0.001, data not shown). Post-hoc Wilcoxon analysis found consumption of both inulin and L-Rha significantly increased the AUC over the entire study duration (T=0-420 min, p=0.001 and 0.009 respectively), during the morning (T=0-180 min, p=0.023 and 0.033 respectively).
respectively) and after lunch (T=180-420 min, p=0.001 and 0.006 respectively) relative to PL. There was no significant difference between the AUC for inulin and L-Rha.

Comparison of mean fasting breath H$_2$ concentrations (Figure 7.12b) by Friedman’s ANOVA analysis found a significant difference between treatments, with post-hoc Wilcoxon finding the mean fasting breath H$_2$ concentrations were significantly higher with inulin treatment (24 [SD 23] ppm) than PL (7 [SD 7] ppm, p=0.011) and than L-Rha (5 [SD 7] ppm, p=0.002). The fasting breath H$_2$ concentrations did not differ significantly between PL and L-Rha.
7.4.6 Palatability

7.4.6.1 VAS ratings on study day

Figure 7.13 shows the mean VAS scores regarding the taste, texture and aftertaste of the Angel Delight mousses containing inulin or L-Rha or PL at breakfast and lunch.

Figure 7.13. VAS ratings regarding palatability of the mousse
Analyses were carried out by one-way repeated measures ANOVA with post-hoc bonferroni. Results shown as mean with error bars representing the SEM (n=13).

1. ‘How pleasant was the taste of this mousse?’ NDC treatment significantly influenced VAS score at breakfast (p=0.011). Post hoc analysis found a difference with a trend approaching significance between PL and L-Rha (p=0.059). There was no significant influence at lunch.

2. ‘How pleasant was the texture of this mousse?’ NDC treatment influenced VAS rating significantly at lunch (p=0.050) and with a trend towards significance at breakfast (p=0.073). No significant differences found by post-hoc analysis except PL and L-Rha differed with a trend approaching significance (p=0.056) at breakfast.

3. ‘Did the mousse have an unpleasant aftertaste?’ No significant influence of NDC treatment on the aftertaste VAS score was found at breakfast and at lunch.

NDC treatment significantly influenced the rated taste pleasantness of the mousses at breakfast (p=0.011), but not at lunch when analysed by one-way repeated measures ANOVA analysis. L-Rha had the lowest mean score while PL had the highest at both breakfast and lunch (58 [SD 21], 73 [SD 20] and 75 [SD 21] mm at breakfast and 69
Post-hoc bonferroni analysis found the taste ratings for the L-Rha mousse was lower with a trend approaching significance than PL at breakfast (p=0.059), but no other post-hoc significant differences were found.

The rated pleasantness of the mousse texture was significantly influenced by NDC treatment at lunch (p=0.050) and influenced with a trend towards significance at breakfast (p=0.073). Again, L-Rha had the lowest mean score at both breakfast and lunch (59 [SD 21], 72 [SD 21] and 69 [SD 21] mm at breakfast and 63 [SD 20], 72 [SD 22], 73 [SD 21] mm at lunch for L-Rha, PL and inulin respectively). However again post-hoc analysis found no significant differences between treatments at breakfast or lunch except a trend approaching significance between PL and L-Rha (p=0.056) at breakfast.

The mean VAS score regarding any aftertaste following consumption of the mousse ("Did the mousse have an unpleasant aftertaste?") was not significantly influenced by NDC treatment at either mealtime.
7.4.6.2 Daily taste ratings during run-in period

Figure 7.13 shows the mean daily taste ratings for the jellies (PL, inulin or L-Rha) supplied during the run-in period, where 1 indicated 'like extremely' and 9 indicated 'dislike extremely'.

It was found that neither the addition of inulin nor of L-Rha influenced taste ratings over time to a significant level as compared to PL.

![Figure 7.13. Daily taste ratings for jellies given during run-in. NDC treatment did not significantly influence the taste. Analysis was carried out by one-way repeated measures ANOVA with post-hoc bonferroni. Results shown as mean with error bars representing the SEM (n=13).]
7.4.7 Gastrointestinal symptoms

7.4.7.1 VAS ratings on study day

Selected subjective gastrointestinal symptom ratings time-course curves as assessed by VAS on the study days are shown in Figure 7.14. Two-way repeated measures ANOVA analysis revealed that the only gastrointestinal symptom influenced by NDC treatment on the study days was the urge to defecate (p=0.013). Furthermore, no significant treatment x time interactions were observed for any symptom.

For all gastrointestinal symptoms rated, the mean scores were low at all timepoints (all were less than 25mm) and for all treatments, and the baseline scores did not differ significantly between treatments.

Figure 7.14 Selected subjective gastrointestinal symptom ratings following PL (- • - ), inulin (- ■ - ) and L-Rha (-▲-) treatment

The urge to defecate was significantly influenced with NDC treatment (p=0.013). No other subjective gastrointestinal symptom ratings were significantly influenced by treatment. Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=13).
Table 7.15 summarises the AUC values for the postprandial gastrointestinal symptom ratings, which were compared by Friedman’s ANOVA analysis with post-hoc Wilcoxon as the data was not normally distributed.

The urge to defecate was significantly influenced by NDC treatment (p=0.046), with the AUC following L-Rha treatment being significantly higher than PL (p=0.007) and higher than inulin with a trend towards significance (p=0.039). The PL and inulin AUC did not differ significantly.

The AUC for stomach discomfort was also significantly influenced by NDC treatment for the first 315 min of the study (p=0.046), with post hoc analysis finding the AUC with L-Rha treatment was higher than PL with a trend approaching significance (p=0.041). The difference between PL and inulin and between inulin and L-Rha was not significant.

Table 7.15. AUC for all gastrointestinal and headache symptom ratings over entire study day until pasta meal

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>AUC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urge to defecate</td>
<td>*1879</td>
<td>2000</td>
<td>2811</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1571</td>
<td>1800</td>
<td>1154</td>
</tr>
<tr>
<td>Stomach discomfort</td>
<td>^1595</td>
<td>1912</td>
<td>2054</td>
</tr>
<tr>
<td>Bloating</td>
<td>2122</td>
<td>2455</td>
<td>3258</td>
</tr>
<tr>
<td>Flatulence</td>
<td>2431</td>
<td>2325</td>
<td>4331</td>
</tr>
<tr>
<td>Heartburn</td>
<td>1284</td>
<td>1012</td>
<td>1963</td>
</tr>
<tr>
<td>Burp/belch</td>
<td>3190</td>
<td>2650</td>
<td>2926</td>
</tr>
<tr>
<td>Headache</td>
<td>2613</td>
<td>3277</td>
<td>2934</td>
</tr>
</tbody>
</table>

1AUC & IAUC mean values marked with the same symbol denote significant differences between treatments (*p=0.007) or a trend approaching significance (^p=0.039, ^p=0.041)

2 Data for AUC from 0-315 min
7.4.7.2 Daily ratings during run-in period (Days 1-6)

Selected subjective daily gastrointestinal symptom time-course curves as assessed by a gastrointestinal symptom diary during the run-in period are shown in Figure 7.15. Data is shown for only 12 participants due to missing data for one participant.

![Figure 7.15. Selected daily gastrointestinal symptom ratings during 6 d run-in period with PL (-•-), inulin (-■-) and L-Rha (-▲-) treatment](image)

Flatulence ratings were significantly influenced by NDC treatment (p=0.010), but none of the other subjective gastrointestinal symptom ratings were significantly influenced. Analyses were carried out by two-way repeated measures ANOVA. Results shown as mean with error bars representing the SEM (n=12).

The ratings for flatulence were significantly influenced by treatment (p=0.010) as determined by two-way repeated measures ANOVA analysis. Treatment x time interactions were significant for flatulence ratings (p=0.044) and approaching significance for stomach pain ratings (p=0.073). There were no significant treatment or treatment x time influences for any other subjective ratings.

Table 7.16 shows the main daily ratings for gastrointestinal symptoms during Days 4 to 6 of the run-in, when participants were consuming the target dose of NDC supplements. The mean flatulence ratings were significantly influenced by treatment when analysed by Friedman’s ANOVA (p=0.007), with the mean rating during inulin supplementation being significantly higher than PL (p=0.011) and L-Rha (p=0.012). Stomach pain was
also influenced by treatment with a trend toward significance (p=0.069), with the mean rating during inulin supplementation being higher than during L-Rha supplementation with a trend approaching significance (p=0.034). None of the other daily gastrointestinal symptoms were influenced by treatment.

Table 7.16. Mean daily gastrointestinal symptom ratings during Days 4-6 of run-in period

Analysed carried out by Freidman’s ANOVA analysis with post-hoc Wilcoxon (n=12).

<table>
<thead>
<tr>
<th>Symptom</th>
<th>PL Mean</th>
<th>PL SD</th>
<th>Inulin Mean</th>
<th>Inulin SD</th>
<th>L-Rha Mean</th>
<th>L-Rha SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flatulence</td>
<td>1.4</td>
<td>0.6</td>
<td>2.2</td>
<td>0.9</td>
<td>1.5</td>
<td>0.5</td>
<td>0.007</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>1.3</td>
<td>0.5</td>
<td>1.5</td>
<td>0.6</td>
<td>1.1</td>
<td>0.1</td>
<td>0.069</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1.2</td>
<td>0.3</td>
<td>1.4</td>
<td>0.7</td>
<td>1.3</td>
<td>0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Constipation</td>
<td>1.0</td>
<td>0.1</td>
<td>1.0</td>
<td>0.0</td>
<td>1.1</td>
<td>0.0</td>
<td>NS</td>
</tr>
<tr>
<td>Belching</td>
<td>1.3</td>
<td>0.4</td>
<td>1.5</td>
<td>0.6</td>
<td>1.3</td>
<td>0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Nausea</td>
<td>1.1</td>
<td>0.2</td>
<td>1.2</td>
<td>0.3</td>
<td>1.1</td>
<td>0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Acid</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Heartburn</td>
<td>1.0</td>
<td>0.1</td>
<td>1.0</td>
<td>0.1</td>
<td>1.0</td>
<td>0.0</td>
<td>NS</td>
</tr>
<tr>
<td>Rumbling</td>
<td>1.2</td>
<td>0.4</td>
<td>1.5</td>
<td>0.6</td>
<td>1.7</td>
<td>0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Bloating</td>
<td>1.3</td>
<td>0.5</td>
<td>1.7</td>
<td>0.8</td>
<td>1.4</td>
<td>0.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean values marked with the same symbol denote significant differences between treatments (*p=0.012, ^p=0.011) or a trend approaching significance (\(P=0.034\))
7.4.8 Compliance with study protocol

Compliance expressed as the mean reported proportion of jellies consumed over the 6 d run-in period was 98 [SD 5], 94 [SD 10] and 99 [SD 2] % of the supplied jellies for PL, inulin and L-Rha respectively.

According to the food diaries, no alcohol was consumed on the day before the study day, the provided ready meals were consumed and all participants fasted overnight for 12 h.

Table 7.17 shows the mean energy and macronutrient 24 h intake on the day before the study day (Day 6 of run in period). No significant differences were found between treatments except CHO intake which was significantly lower with the L-Rha treatment (p=0.027) as compared to PL when analysed by within subject one-way repeated measures ANOVA with post-hoc Bonferroni.

Table 7.17. EI and macronutrient intake on day preceding study day (Day 6 of run-in period)\textsuperscript{1,2}

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>8847</td>
<td>1851</td>
<td>8647</td>
<td>2378</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>82.6</td>
<td>29.8</td>
<td>80.5</td>
<td>35.8</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>79.8</td>
<td>21.5</td>
<td>73.4</td>
<td>21.8</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>*266.3</td>
<td>65.5</td>
<td>268.1</td>
<td>69.7</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>13.9</td>
<td>3.0</td>
<td>12.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0.0</td>
<td>-</td>
<td>0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Data for fibre excluding fibre from supplements
\textsuperscript{2} Mean values marked with same symbol denote significant differences between treatments (*p<0.027)
7.4.9 Participant characteristics

Table 7.18 shows the mean weight, BMI, % body fat and fasting blood metabolite concentrations on each study leg.

<table>
<thead>
<tr>
<th></th>
<th>PL</th>
<th>Inulin</th>
<th>L-Rha</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.8</td>
<td>10.5</td>
<td>67.2</td>
<td>10.4</td>
</tr>
<tr>
<td>BMI (kgm⁻²)</td>
<td>22.1</td>
<td>1.4</td>
<td>22.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.6</td>
<td>6.7</td>
<td>21.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>4.8</td>
<td>0.4</td>
<td>4.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.82</td>
<td>0.54</td>
<td>4.00</td>
<td>0.49</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.44</td>
<td>0.31</td>
<td>1.46</td>
<td>0.32</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0.59</td>
<td>0.20</td>
<td>0.61</td>
<td>0.28</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1.00</td>
<td>0.40</td>
<td>1.03</td>
<td>0.31</td>
</tr>
<tr>
<td>Insulin (pmol/ml)</td>
<td>63.2</td>
<td>24.5</td>
<td>66.1</td>
<td>26.7</td>
</tr>
</tbody>
</table>

None of the anthropometric or fasting plasma metabolite concentrations differed significantly between each treatment when analysed by one way within-subjects repeated measures ANOVA. The total cholesterol concentrations did differ with a trend towards significance (p=0.072), however post-hoc bonferroni found no significant differences between treatments.
7.5 Discussion

The present investigation found that NDC supplementation (with 25.5 g L-Rha or 22.4 g inulin HP) served as a split dose alongside a mixed meal served at breakfast (two-thirds dose) and lunch (one-third dose) and following a 6 d run-in period did not influence quantitative and subjective appetite measures, except a treatment x time interaction for the desire to eat a meal (p=0.008, during morning) and desire to eat sweet (p=0.036, after lunch) to a significant level, and for hunger (p=0.070, during morning) with a trend approaching significance. This suggests that neither 25.5 g L-Rha nor 22.4 g inulin HP appear to influence appetite when served as a split dose alongside a mixed meal served at breakfast (two-thirds dose) and lunch (one-third dose) following a 6 d run-in period.

To our knowledge this is the first study that has examined the influence of L-Rha on appetite regulation. Furthermore there are a number of design limitations in previous investigations into the role of inulin-type fructans on appetite regulation, and effects on inulin HP have not been extensively investigated (reviewed in section 1.8.2).

The present investigation also found that NDC supplementation resulted in significant treatment x time interactions for postprandial plasma insulin (entire study day, p=0.009), NEFA (following lunch, p=0.046) and TAG (following breakfast, p=0.006) concentrations. This was accompanied by a significant treatment effect and treatment x time interaction for postprandial breath H₂ levels (p<0.001 for the entire study duration), indicating the NDC had reached the colon and was being fermented.

Post-hoc Wilcoxon analysis of the insulin IAUC indicated the postprandial insulin response was significantly lower with L-Rha treatment relative to PL, evidenced by the IAUC for L-Rha and PL differing significantly for first 120 min (p=0.011), and with a trend approaching significance for first 180 min (p=0.033), and entire study
duration (p=0.023). No post-hoc differences between inulin and the other treatments were found, suggesting inulin did not alter the postprandial insulin response.

The differences between treatment groups for postprandial NEFA and TAG responses were less clear, with post-hoc analysis of AUC and IAUC not finding any significant differences. Looking at the timecourse curve, L-Rha appeared to suppress the rise in NEFA concentrations prior to lunch, and concentrations remained suppressed following lunch relative to PL and inulin. However, the postprandial TAG timecourse was elevated both following L-Rha and particularly following inulin supplementation relative to PL, suggesting a negative impact of NDC supplementation on postprandial TAG concentrations.

Focusing first on the metabolic findings, our findings are in contrast to previous reports which found no effects of L-Rha supplementation on postprandial metabolites. However, there is only limited previous data regarding the influence of L-Rha on metabolic parameters, with one published study examining acute (Vogt et al., 2004b) and one examining chronic (Vogt et al., 2006) effects, both from the same group. Both were 3-way crossover studies delivering a daily dose of 25 g L-Rha and comparing effects to supplementation with 25 g lactulose and 25 g glucose. The acute study only collected data on the postprandial glycaemic response (n=22), and found the AUC for the entire study day (0 to 12 h) did not differ between treatments (Vogt et al., 2004b). In the chronic study, participants (n=11) attended an acute challenge study day after 4 wk supplementation during which it was found the AUC for the entire study day (0 to 12 h) did not differ between treatments for postprandial plasma insulin, glucose, NEFA and TAG responses (Vogt et al., 2006).

However these previous studies (Vogt et al., 2006, Vogt et al., 2004b) were not appropriately designed to investigate postprandial effects on plasma insulin, glucose and
NEFA concentrations, all of which change rapidly following a meal, particularly during the first 1-2 h. In both studies postprandial blood samples were collected only hourly, meaning these rapid changes were not monitored, thereby limiting interpretation. Furthermore, glucose was used for the control arm of the studies, which would be rapidly digested and absorbed from the upper GIT and can therefore be argued as an unsuitable control for comparison to L-Rha.

Therefore to our knowledge, the present study is the first to examine effects of L-Rha on postprandial metabolites with regular blood sampling following each meal, allowing more detailed analysis of metabolic profiles, and explaining why our results contrast from previous findings.

It may be postulated that the observed suppression of postprandial plasma insulin and NEFA following L-Rha supplementation in the present study arose via propionate mediated activation of GPR41 and/or GPR43 in adipose tissue and colonic mucosa (see section 1.7). L-Rha is reported to modulate colonic propionate production (Fernandes et al., 2000), accompanied by a rise in serum propionate (Vogt et al., 2004b), and propionate is the most potent ligand for GPR41. It is therefore conceivable that propionate generated from colonic fermentation of L-Rha interacted with GPR41 and GPR43, which are co-expressed with PYY, to initiate ileal brake mechanisms (see section 1.7.1 and 1.7.3). This would slow the rate of absorption of digested nutrients into the bloodstream, thereby reducing insulin production rates. However as significant effects were seen following breakfast, and breath H₂ data indicates L-Rha did not appear to reach the colon until 90-120 min postprandially this theory does not provide an explanation for effects immediately following breakfast. It is possible colonic propionate concentrations at the start of the study morning were raised relative to usual levels due to the 6 d run-in supplementation period. Effects on plasma NEFA
suppression may have arisen from propionate mediated activation of GPR43 in adipocytes, which has been shown in vitro to inhibit lipolysis (see section 1.7.2).

The lack of effect of inulin supplementation on postprandial metabolites in the present study agrees with findings from some previous studies, but not others. As reviewed in section 1.9.2, only a few studies have examined postprandial effects of inulin-type fructans (Causey et al., 2000, Forcheron and Beylot, 2007, Parnell and Reimer, 2009, van Dokkum et al., 1999, Rumessen et al., 1990), with variable findings. Of these, only one included an inulin-type fructan as part of the acute challenge mixed test meal preload (Parnell and Reimer, 2009), and one included the inulin-type fructan alongside bread (Rumessen et al., 1990) thus allowing direct comparison with the present study. The other studies used an OGTT challenge following a supplementation period (Causey et al., 2000, Forcheron and Beylot, 2007, van Dokkum et al., 1999).

Parnell and Reimer reported that the inclusion of 21 g OF as part of a mixed meal acute challenge following a 12 wk supplementation period significantly reduced postprandial plasma insulin and glucose concentrations (in contrast to findings from the present study), with no effect on postprandial lipid responses (in agreement with findings from the present study). In the present study a slightly higher dose of inulin-type fructan (22.4 g) than supplied in the Parnell and Reimer study (21 g) was used. However there were a number of contrasts between the present study and the Parnell and Reimer study, in terms of type of inulin-type fructan used (inulin HP vs. OF) and the duration of the supplementation period (6 d vs. 12 wk), both of which may explain the differing findings. This suggests it is possible that when consumed habitually and included as part of a mixed meal, OF may improve postprandial insulinaemia (Parnell and Reimer, 2009). The long term effects of inulin HP supplementation on postprandial metabolic responses are unknown. Inulin HP has the benefit of being associated with less adverse
GIT symptoms than OF (Bruhwyluer et al., 2008), therefore long term effects of inulin HP could warrant further investigation.

Rumessen and colleagues found an acute challenge with a 10 g OF/inulin mixture alongside wheat bread significantly lowered the plasma glucose IAUC in comparison to bread with no OF/inulin (Rumessen et al., 1990). This evidence along with results from Parnell and Reimer and from the present study suggest the presence of the low DP inulin-type fructans (OF) rather than high DP inulin (as used in the present study) may be necessary to alter postprandial metabolic response.

Focusing now on the lack of effect on appetite in the present study, the only previously published data on the effect of inulin HP on appetite was an acute crossover study (n=33). In this study, 24 h EI was significantly lower following a muffin and sausage pattie preload containing 24 g inulin HP than following PL (no NDC) (Archer et al., 2004), which is in contrast to the present study, although rated satiety was not influenced. However subjective appetite ratings were only monitored for 225 min postprandially, which is insufficient to assess fermentation effects, and were measured using a single continuous scale questionnaire rather than VAS. Quantitative appetite effects were assessed solely with a 24 h weighed food record with no measurements of intake of an ad libitum test meal. Furthermore the preloads were not isoenergetic, as the PL had a higher fat and therefore E content than the inulin preload.

Therefore although the lack of effect of inulin HP on 24 h intake in the present study did not agree with the findings of Archer and colleagues, as the preloads in the Archer study were not isoenergetic, it is difficult to differentiate effects of inulin HP from those of fat and E content. This suggests the findings from the present study are more valid. However the present study had a smaller sample size, and may be underpowered for EI and macronutrient intake assessment.
One other study may have investigated a high DP inulin, as the type of inulin was not specified in the paper (Perrigue et al., 2009) (discussed below). However that study investigated a much lower dose of inulin (8 g) than the present study and only monitored effects for 2 h following the preload, therefore the results are not comparable to the present study.

Quantitative appetite measures assessed in the present study included EI at an ad libitum buffet meal provided 7 h postprandially, total 24 h EI on the study day and mean 24 h intake over 3 d during the run-in, none of which were altered by treatment with 25.5 g L-Rha or 22.4 g inulin HP.

As far as we are aware the present study is the first to appropriately measure quantitative effects on appetite by measuring ad libitum intake of a test meal following a high DP inulin-containing preload using the “preload-test meal” paradigm in a controlled setting (reviewed in section 1.4.2) (Stubbs et al., 1998).

Only two other studies have followed the “preload-test meal” method to investigate effects of inulin-type fructans on appetite (Perrigue et al., 2009, Peters et al., 2009), both acute studies. Peters and colleagues reported ad libitum EI at a test meal 4 h following a OF-containing meal replacement bar preload did not differ from PL (Peters et al., 2009). However, Peters and colleagues used a low DP inulin-type fructan in a much lower dose (8 g) than the present study (14.9 + 7.5 g). The overall energy content of the test preload was only 190 kcal, which is lower than a usual breakfast EI and may override effects on appetite. Also, the buffet meal was served much earlier than in the present study, thereby missing the peak stages of fermentation, and participants were allowed to leave the clinical unit in between the preload and the test meal.
The other study to follow a “preload-test meal” method found *ad libitum* EI at a test meal 2 h following a low and high E yogurt preload (180 and 440 kcal respectively) with 6 g added inulin (DP not specified) was significantly lower than when no preload was supplied (PL) but did not differ from EI following the yogurts with no inulin (Perrigue et al., 2009). However, this study was not designed to investigate fermentative effects of inulin, as the test meal was supplied only 2 h following the preload, giving insufficient time for inulin to reach the colon. Also the dose used was very low compared to the present study.

Effects of inulin-type fructans on *ad libitum* intake at a buffet meal has also been investigated by Cani and colleagues (Cani et al., 2006a, Cani et al., 2009). However neither of these followed the “preload-test meal” paradigm and instead measured intake following a 2 wk supplementation period with no acute inulin-type fructan challenge on the study days. Therefore neither study design is comparable to that in the present study.

Looking in more detail now at the subjective appetite data, as stated above, no significant treatment effects were found for any appetite measures, and treatment x time interactions were only found during the morning for desire to eat a meal (p=0.008) and hunger (p=0.070) and following lunch for desire to eat sweet (p=0.036), with hunger (p=0.070, during morning). This lack of effect for both inulin HP and L-Rha agrees with previous investigations into subjective appetite effects following an inulin HP preload (Archer et al., 2004). However the present investigation was underpowered for subjective VAS data, due to the fairly low sample size (n=13). Flint and colleagues suggest 20 participants should be recruited to a crossover study to detect a 5 mm difference in mean 4.5 h fullness ratings. However, as the p values were far from significance for many of the parameters examined it is unlikely that a larger sample size will have detected further significant differences.
As discussed earlier in this thesis (see section 1.11) an important consideration for appetite investigations is test product palatability. The L-Rha mousse was rated as less acceptable than the inulin and PL mousses, with the L-Rha products having the lowest rating scores both for taste and texture at breakfast and lunch. There was a significant treatment influence on taste ratings at breakfast ($p=0.011$), but not at lunch and the texture rating was significantly influenced at lunch ($p=0.050$) and influenced with a trend towards significance at breakfast ($p=0.073$). Aftertaste was not influenced by treatment. However the mean rating scores were still fairly high for the L-Rha mousses 58 [SD 21] and 69 [SD 19] for taste, and 59 [SD 21] and 63 [SD 20] for texture for breakfast and lunch respectively, with 0 being not at all pleasant and 100 being extremely pleasant. This suggests that although the L-Rha mousses were considered less pleasant than the inulin and PL mousses, they were still not considered to be unpleasant. Furthermore, daily taste ratings of the jellies provided during the run-in were not significantly influenced by addition of NDC supplement. However it is still possible that the lower palatability of the L-Rha mousse may have influenced appetite.

Data collected on gastrointestinal symptoms during the study suggest the inulin and L-Rha supplements were fairly well tolerated. Daily symptom ratings for flatulence were significantly higher following inulin than PL ($p=0.012$) and L-Rha ($p=0.001$), and stomach pain was also influenced by treatment with a trend approaching significant ($p=0.069$), with a higher AUC following inulin than L-Rha ($p=0.034$) and PL (NS). None of the other ratings (for diarrhoea, constipation, belching, nausea, acid reflux, heartburn, rumbling stomach or bloating) were influenced by treatment.

On study days, the mean VAS rating scores for GIT symptoms were low (<25 mm) for all parameters and only the AUC for urge to defecate ($p=0.046$) and stomach discomfort ($p=0.046$) were influenced significantly by treatment. Both were highest
following L-Rha, although this was only significant for urge to defecate (p=0.007) relative to PL. None of the ratings were significantly influenced by inulin, although stomach discomfort was higher with a trend approaching significance (p=0.039, post-hoc Wilcoxon).

These findings are in agreement with a recent study that found inulin-type fructans consumed at a level of 5-20g per day for 2 weeks were well tolerated (Bruhwiler et al., 2008).

Compliance with consuming jellies during the run-in period was good according to the monitoring diaries. Ideally compliance would have been assessed by monitoring the number of jelly containers returned, however logistically this was not practical. The fact that daily gastrointestinal symptoms and fasting breath H2 concentrations were higher with inulin treatment suggests participants had consumed the jellies.

7.6 Conclusion

In summary, the current investigation found that supplementation with 25.5 g L-Rha supplied as a split dose alongside a mixed meal at breakfast (two-thirds dose) and lunch (one-third dose) following a 6 d run-in period acutely reduced the postprandial insulinaemic response, significantly so for 2 h following breakfast (p=0.011, post-hoc Wilcoxon), and with a trend approaching significance for the entire 7 h study day (p=0.023, post-hoc Wilcoxon) relative to PL. It also appears to suppress postprandial NEFA concentrations, evidenced by a significant treatment x time interaction (p=0.046, following lunch). This was accompanied by a significant increase in postprandial breath H2 concentrations, indicative that colonic fermentation occurred.

It was also found that 22.4 g of a high DP inulin supplement (inulin HP) did not significantly alter any of postprandial metabolic measures monitored.
The present study is the first to examine effects of L-Rha on postprandial metabolites with regular blood sampling. This is in contrast to previous studies that took samples at hourly intervals and found no effect of L-Rha on postprandial metabolites (Vogt et al., 2006, Vogt et al., 2004b). We postulate that observed effects may have been mediated by propionate generated during colonic fermentation of the L-Rha activating GPR41 and GPR43 SCFA receptors in the colonic mucosa and in adipocytes to modulate PYY production and suppress lipolysis respectively.

The other important finding from the present study was that neither the L-Rha nor the inulin HP supplements appeared to influence appetite, as assessed using subjective (VAS) and quantitative (ad libitum intake 7 h postprandially, 24 h intake, mean daily intake during run-in) measures, except VAS ratings for the desire to eat a meal (p=0.008, during morning) and desire to eat sweet (p=0.036, after lunch). This lack of effect may be explained due to the study being underpowered, however as the p values were far from significant for most parameters, a larger sample size is unlikely to have detected further significant differences.

To our knowledge, this is the first study to investigate effects of L-Rha supplementation on appetite, and is also the first to investigate quantitative effects of high DP inulin on appetite with the “preload-test meal” paradigm for 7 h postprandially.

Overall, the findings from the present study are suggestive that L-Rha may influence postprandial metabolites, in particular plasma insulin and NEFA. This finding may warrant further investigation to look at effects of supplementation on insulin sensitivity measured in a clinical setting. However, our findings do not support a role for L-Rha nor inulin HP in regulating appetite, although it is possible that this finding is in part due to a short supplementation period and a low sample size.
Chapter 8. General discussion

The clinical studies described within this thesis were designed to investigate the role of SCFA in modulating appetite and the metabolic response in different parts of the GIT. Three studies investigated the role of orally delivered SCFA to the upper GIT (Chapters 3-5) and one study investigated the role of colonically derived SCFA generated via fermentation of NDC (Chapter 7). Work was also carried out to determine if tethering specific SCFA to an NDC scaffold can modulate colonic production of that specific SCFA following ingestion (Chapter 6). The discussion herein will draw together the main findings from this work.

8.1 Overview of main findings

8.1.1 Orally delivered SCFA

Three of the clinical studies reported within this thesis were designed to investigate the role of orally delivered SCFA on appetite regulation (Chapters 3-5), following on from previous work primarily carried out by Bjorck and colleagues. These previous studies found supplementation with SCFA (propionate, acetate and lactate) acutely reduced glycaemia and insulinaemia and increased satiety postprandially (Darwiche et al., 2001, Hlebowicz et al., 2007, Hlebowicz et al., 2008, Johnston and Buller, 2005, Johnston et al., 2004, Liljeberg and Bjorck, 1998, Liljeberg and Bjorck, 1996, Liljeberg et al., 1995, Ostman et al., 2005, Sugiyama et al., 2003) (reviewed in Chapter 1, sections 1.10.1 and 1.11.1). However conclusions regarding effects of oral SCFA on satiety were solely based on results from a single bipolar rating scale, with no quantitative assessment of appetite, thus warranting further investigation. Furthermore, palatability of test products were not controlled for.
In the first study, reported in Chapter 3, it was found that a palatable propionate-rich sourdough bread delivering 6.0 mmol propionate ingested as part of a standard breakfast did not acutely influence postprandial appetite or plasma glucose or TAG levels and that it may even worsen postprandial insulinaemia, contrary to previous findings.

By contrast, the next study, reported in Chapter 4, found that supplementation with 25 mmol acetic acid (supplied within white wine vinegar) alongside a standard breakfast significantly increased satiety postprandially, indicated by both postprandial subjective appetite VAS ratings and quantitative measures of appetite, and significantly reduced postprandial glycaemia. However, the results indicated the unpleasant taste of the vinegar-containing product may have influenced these outcomes, possibly via cephalic phase mechanisms. This was evidenced by effects on subjective appetite ratings and quantitative appetite measures being greater following Unpal treatment compared to Pal treatment. Nausea ratings were additionally significantly higher following Unpal than Pal. There also were medium to strong correlations between palatability ratings and appetite parameters. These findings in combination with the lack of effect on satiety following oral ingestion of propionate in a palatable form (study reported in Chapter 3) is also suggestive of a role for palatability, although far lower quantities of SCFA were used in the study reported in Chapter 3. However it was not possible to ascertain if palatability was the sole cause of observed effects.

Following on from these findings, the study reported in Chapter 5 aimed to determine the role of palatability of SCFA-containing products in modulating effects on appetite and the metabolic response with the use of the MSF technique. This study found that orosensory stimulation with a vinegar-containing drink (as a source of acetate) following a milkshake preload did not significantly alter postprandial glycaemia.
insulinaemia or quantitative appetite measures relative to PL, although it did reduce postprandial VAS ratings, some significantly. Of note, hunger, the desire for a snack and the desire to eat something salty were significantly reduced over the entire study duration, and prospective consumption and the desire to eat a meal were significantly reduced over the first 90 min of the study. This led to the conclusion that while orosensory stimulation with an acetate-containing product may transiently reduce subsequent appetite ratings, these effects are not as strong as when vinegar is ingested. This suggests that while the unpleasant taste of the vinegar-product in the oral cavity appears to influence appetite at least transiently, this may not be the only reason for a reduced appetite following vinegar ingestion. Possible additional explanations may include increased nausea following ingestion or 'tasting' of the acetate by taste receptors located in the upper GIT.

It is also of course possible that SCFA have a physiological effect in addition to the effect of palatability, and that the the lack of effect in the first study (Chapter 3) was due a lower dose of propionate being delivered in comparison to previous studies, meaning it did not reach the required threshold for an effect.

To explore this possibility further, it is necessary to supply higher quantity of SCFA in a palatable form, which is a difficult, if not impossible proposition.

8.1.2 Colonically delivered SCFA

In addition to considering the effects of orally delivered SCFA, the effects of colon-derived SCFA on appetite and the metabolic response were investigated as reported in Chapter 7. It was initially planned to investigate the effects of colon-derived propionate via the provision of propionate tethered to an insulin scaffold (inulin propionyl ester
(IPE)) as part of a 4-way crossover study in comparison to the NDC L-Rha and inulin HP and to PL.

Unfortunately part way into this study, it was necessary to stop running the IPE part of the investigation due to supply and QC problems and the investigation became a 3-way crossover study (25.5 g L-Rha vs. 22.4 g inulin HP vs. PL). The study was still novel as there is a lack of good quality data investigating the effects of high DP inulin-type fructans on appetite and the metabolic response. There is also no published data investigating the role of L-Rha, an NDC previously demonstrated to enhance colonic propionate production (Fernandes et al., 2000, Vogt et al., 2004b), in appetite regulation.

This study found no effect of supplementation with the either of the fermentable NDC investigated (L-Rha and high DP inulin) on satiety. This was the case for subjective and quantitative measures of appetite. These finding therefore do not support a case for colonically derived SCFA influencing appetite.

There was however the interesting finding that supplementation with L-Rha significantly suppressed the postprandial plasma insulin and NEFA response relative to PL. This finding was in contrast to previous reports that found no effect of L-Rha supplementation on postprandial plasma metabolites (Vogt et al., 2006, Vogt et al., 2004b) when supplied in a similar dosage to the study reported in this thesis. The lack of effect in those previous studies was explained following closer examination of the data which revealed that postprandial blood sampling was only hourly thereby missing the rapid changes that occur following a meal (Vogt et al., 2006, Vogt et al., 2004b).
Supplementation with the high DP inulin did not significantly alter postprandial plasma metabolites. This is in contrast to published data in which an acute challenge with a meal including inulin-type fructans was reported to significantly lower postprandial glycaemia (Parnell and Reimer, 2009, Rumessen et al., 1990) and insulinaemia (Parnell and Reimer, 2009). However in both these studies OF was included, suggesting a low DP inulin-type fructan may be necessary to alter the postprandial metabolic response.

Prior to commencing this investigation, the effectiveness of IPE in enhancing propionate production was explored in a separate dose-response study reported in Chapter 6, in which 24 h urinary propionate levels were measured as a proxy indication of body propionate levels. However the findings from this study were inconclusive. There were no obvious dose-response effects, and it was not clear if the lack of effect was due to weaknesses in the analytical method or if the IPE was ineffective in enhancing propionate production. It had therefore been planned to collect serum samples to acutely monitor the appearance of propionate following ingestion of IPE, however due to the problems with the IPE in the subsequent study this was not possible.

8.2 Study limitations

There were a number of limitations with the studies reported in this thesis.

Of importance are the methods used to investigate appetite. Eating behaviour is regulated by a complex and multidimensional array of factors, as reviewed in Chapter 1 (section 1.3). Therefore experimentally it is not straightforward to assess effects on appetite. The use of a battery of VAS regarding different aspects of appetite completed in a controlled laboratory setting used in conjunction with quantitative measures of appetite are considered to be one of the more validated and robust methods to
investigate effects on appetite. However these methods are not without limitation, not least in terms of the validity of findings when applied to a free-living setting and also the difficulty in controlling for the influence of psychological factors such as a participant’s mood on a study day. Furthermore Stubbs and colleagues consider that VAS are not necessarily sensitive to relatively modest manipulations in an intervention (Stubbs et al., 2000). This therefore raises the question does a lack of effect on rated appetite with the use of VAS imply a lack of effect on appetite? However in the absence of any better methods, the use of appetite VAS in conjunction with quantitative appetite measures still remains the best method to investigate appetite.

A potential confounder in the studies reported in this thesis is that blood samples were being collected at the same time as appetite being measured, which will have influenced appetite. However as the studies are all crossover studies, it is probable that the influence of this effect will have been minimised, apart from when there were particular difficulties with blood sampling (e.g. difficulties in obtaining blood and the need to manipulate or replace cannulae in order to collect blood).

Furthermore, postprandial gut peptides concentrations were not measured in the studies reported in Chapters 3 and 7, despite blood samples being collected for this purpose. It would have added to the data enabling a fuller picture of effects, however the high cost of analysis could not be justified in view of the lack of effect on subjective and quantitative appetite measures.

The studies reported in this thesis were also under-powered for assessment of subjective and quantitative appetite measures, although not greatly so. Data from previous work by our group suggests 22 participants are required to detect a significant difference in ad libitum intake at 80% power with significance assumed at a level of p≤0.05 (Bodinham
et al., 2010). Arvanti and colleagues (2000) have also reported that 17 participants are required to detect a 500 kJ difference in a crossover design study with a power of 0.8.

In terms of subjective appetite assessment Flint and co-workers (2000) suggest at least 20 participants should be recruited to detect a 5 mm difference in mean 4.5 h fullness ratings (Flint et al., 2000).

However, it is likely that more participants than this would be required in three-way crossover studies, such as the studies reported in Chapters 4 and 7.

Data generated from diet diaries is also not without limitations. These include under-reporting due to forgetting / not bothering to write foods down and not wishing to seem greedy, altering food choices to appear more healthy or to make it easier to note in the food diary, difficulties for the investigator in interpreting portion sizes, inadequate descriptions of food choices (although this was minimised in the studies reported in this thesis by going through the food diaries with participants when possible) and limitations in food composition databases in terms of choice and differences between recipes for seemingly the same foods. Therefore again data generated from diet diaries need to be interpreted with these limitations in mind.

Looking in more detail at the fermentable NDC crossover study reported in Chapter 7, it would have been preferable to have had a longer run-in period than 6 d prior to each study day. The rationale for the 6 d run-in was to allow the GIT to adapt to the dose given on study days, and to identify any participants with tolerance problems. A longer run-in period would have been required to result in physiological alterations within the gut. However the main limiting factor in the crossover study was the quantity of L-Rha available, as it was expensive, so it was not possible to offer a longer run-in without reducing the sample size.
A further limitation in this study was that it was necessary to drop the IPE leg, so the effectiveness of the IPE to modulate plasma propionate production alongside a suppressed appetite and metabolic response was not investigated as had initially been planned.

An original aim of the studies reported in Chapters 3 and 7 was to monitor the postprandial appearance of SCFA into the peripheral circulation to determine if the propionate-rich sourdough bread and the IPE, L-Rha and inulin HP modulated plasma concentrations of propionate and other SCFA. Attempts were made to set up an analytical method for plasma SCFA (findings not reported). However it unfortunately proved difficult to set up and validate an accurate method within the time and budget available, largely due to difficulties in analysing plasma SCFA in the very low concentrations that occur in peripheral blood. It was therefore not possible to determine if and when plasma SCFA concentrations were altered by the intervention.

Finally, all the clinical studies reported in this thesis included female participants. Efforts were made to control for the hormonal changes that occur throughout the menstrual cycle in the studies reported in Chapters 3 and 7. This was not possible for the studies reported in Chapters 4 and 5 due to practical and logistical considerations. However, as study days were very close to each other in both these studies, the influence of these effects were minimised. Furthermore, a large proportion of the female participants were using oral contraceptives or the implant in the study reported in Chapters 4, which would have regulated the hormonal levels.

A further consideration is the how representative the recruited participants were of the general population. For example, a number of the participants were nutrition
students who have a particular interest in food, which may have biased the appetite results.

8.3 Overall conclusions

Overall, the data reported within this thesis does not support a role for orally delivered or colonically derived SCFA in regulating appetite, with no effects on appetite following ingestion of a meal made with a palatable sourdough bread delivering 6 mmol propionate (Chapter 3) nor following supplementation with fermentable NDC (Chapter 7). However the data was not entirely conclusive, as supplementation with 25 mmol acetate significantly increased satiety, with a greater effect following delivery in an unpalatable form relative to a more palatable form (Chapter 4). Discrepancies between findings from the oral SCFA supplementation studies may be explained by product palatability or the SCFA dose or both, and further work is required to investigate this.

Data from the MSF study (Chapter 5) suggests that while the unpleasant taste of the vinegar-product in the oral cavity has a transient effect, this may not be the only reason for a reduced appetite following vinegar ingestion. Possible additional explanations include increased nausea following ingestion or ‘tasting’ of the acetate by taste receptors located in the upper GIT. It is also possible that the MSF vinegar drink was not sufficiently ‘unpleasant’ to participants as nausea ratings were not high compared to oral delivery of the vinegar drink, making data from the MSF study inconclusive.

Further work is required to determine unpick the role of palatability relative to the dose of SCFA being provided.

The picture for metabolic effects of SCFA is less clear. While oral supplementation with acetate significantly reduced the acute glycaemic response (Chapter 4), palatable
propionate-rich sourdough bread did not significantly alter postprandial glycaemia and appeared to worsen postprandial insulinaemia (Chapter 3). Again these discrepancies may be explained by product palatability or the SCFA dose or both, and further work is required to investigate this. MSF with a vinegar-drink did not significantly alter postprandial glycaemia and insulinaemia (Chapter 5), suggesting palatability did not have an influence, although this study was not entirely conclusive due to the lack of effect of the intervention on nausea ratings.

Colon-derived SCFA had differing metabolic effects depending on the NDC source. Supplementation with the propionate-generating NDC L-Rha significantly suppressed postprandial plasma insulin and NEFA concentrations, while inulin HP had no effect.

8.4 Future work

Looking first at the studies reported in this thesis, if the budget was available it would enhance interpretation of the data if postprandial plasma concentrations of appetite related gut peptides were analysed in the plasma samples collected for the studies reported in Chapters 3 and 7. This would allow an evaluation of physiological measures of appetite as well as subjective and quantitative measures.

Furthermore, if a suitable method is developed, it would be interesting to quantify postprandial plasma SCFA levels in samples collected in the same studies (in Chapters 3 and 7), to determine if the interventions successfully modulated SCFA concentrations.

Finally, as it was necessary to drop the IPE leg in the fermentable NDC study (Chapter 7), this is still an area that remains to be investigated.
Looking now at studies to follow on from findings in the present thesis, as L-Rha was found to significantly suppress postprandial plasma insulin and NEFA concentrations (Chapter 7), further work could be carried out to investigate this. For example effects on insulin sensitivity in participants with the metabolic syndrome measured either by intravenous glucose tolerance test (IVGTT) or insulin tolerance test (ITT) following chronic supplementation. In addition an acute challenge study day could be included to monitor postprandial effects on plasma glucose, insulin and C-peptide.

It also appears that low DP inulin (OF) is more likely to suppress postprandial insulinaemia and glycaemia than the high DP inulin (inulin HP) used in the study reported in this thesis (Chapter 7). However despite a fairly large number of published studies exploring metabolic effects of inulin-type fructans, the majority of these previous studies have only reported effects on fasting parameters following a period of supplementation (reviewed in section 1.9.2). Only a limited number investigated postprandial effects (Causey et al., 2000, Forcheron and Beylot, 2007, Parnell and Reimer, 2009, Rumessen et al., 1990, van Dokkum et al., 1999), of which only two carried out an acute challenge (Parnell and Reimer, 2009, Rumessen et al., 1990). This lack of data therefore warrants further investigation to measure postprandial effects on plasma glucose, insulin and lipids following an acute challenge.

As data on the role of palatability of oral SCFA in postprandial effects on appetite and the metabolic response is inconclusive, due to the differences in doses used in the SCFA supplementation studies (Chapters 3 and 4) and also as the vinegar intervention in the MSF did not significantly influence nausea ratings, further work is required. It is not an easy task to supply a large SCFA dose in a palatable, non-invasive form.
One possibility is to bypass the mouth, thereby eliminating orosensory stimulation, thereby just observing the physiological effects of SCFA in the GIT. This could be achieved via gastric infusion of an SCFA solution (propionate or acetate) vs. saline alongside a standard oral preload. A less invasive option is to encapsulate SCFA and ingest these capsules alongside a mixed meal compared to ingestion of PL capsules. However a large quantity of capsules would need to be ingested to deliver similar quantities of SCFA used in previous studies.

Alternatively, the MSF study reported in Chapter 5 could be repeated, this time using a solid mixed meal (rather than a liquid milkshake) preload, with the MSF phase occurring prior to ingestion of the meal. The study duration could also be shorter (possibly 2 h postprandially) to determine if there are orosensory influences earlier in the postprandial phase. It would be worthwhile strengthening the vinegar-containing drink to attempt to adversely influence nausea ratings.

A further interesting and easy study that could be carried out is to investigate the satiating effects of SCFA, for example by adding increasing concentrations of vinegar to a soup and measuring the *ad libitum* intake of the soup.
References


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Appendices

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Appendix A: Publications, presentations and awards

Publications
Darzi J, Frost GS and Robertson MD (2010) Do short chain fatty acids have a role in appetite regulation? *Proceedings of the Nutrition Society*, accepted for publication


Presentations
06/2010 Due to present at Postgraduate Symposium of Nutrition Society Summer Meeting 2010 (Oral)
06/2009 Nutrition Society Summer Meeting 2009 (Poster)
05/2009 European Conference on Obesity (ECO) 2009 (Poster)
09/2008 Joint University of Reading / University of Surrey Food Biosciences Annual Research Day (Poster)
06/2008 Nutrition Society Summer Meeting 2008 (Oral)
03/2008 Maastrict University Nutrient Sensing Course (Poster)
09/2007 Joint University of Reading / University of Surrey Food Biosciences Annual Research Day (Oral)

Awards
- Abstract was shortlisted for presentation at the Postgraduate Symposium of Nutrition Society Summer Meeting 2010
- Awarded ASO Bursary to attend European Conference on Obesity 2009
Medical and Lifestyle Questionnaire

Study: The Effects of Fibre Supplementation on Appetite and Metabolic Response

Please find below a short health and lifestyle questionnaire that will be used as part of the screening process for this study.
Please note you are not obliged to complete all sections, and non-completion will be without prejudice. All information will be kept strictly confidential.

A) Participant Details

Date: ........................................
Name: .............................................................................
Gender: Male / Female
Address: ............................................................................
......................................................................................
......................................................................................
Date of Birth: ........../........... Age: ...................................
Ethnicity: .............................................................................
E-mail: .............................................................................
Phone Number: Day ......................................
               Evening ......................................
               Mobile ......................................
URN: .............................................................................
GP Name: ...........................................................................
GP Address: ........................................................................

B) Self-Certificate Medical Questionnaire

Please tick all of the following that apply:

☐ I have no prior/present history of Coronary Heart Disease, Angina or Stroke
☐ I have no prior/present history of Type 1 or Type 2 diabetes
☐ I have no prior/present history of anaemia
☐ I have no prior/current history of gastrointestinal disorders (for example Crohn’s disease, Coeliac disease, Irritable Bowel Syndrome)
☐ I have no prior/present history of liver disease
☐ I have no prior/present history of endocrine disorders
☐ I have no prior/present history of, nor am I currently being treated for, clinical depression and/or other psychological disorders
☐ I have no prior/present history of eating disorders, including anorexia or bulimia nervosa
☐ I have no prior/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 (excluding the contraceptive pill)
☐ I am not currently taking or have been taking any supplements such as vitamins, minerals or fish oils in the last 6 months
☐ I am not currently pregnant
☐ I am not currently breastfeeding

Have you had any other conditions or recent hospital visits

☐ YES  ☐ NO

If you answered yes, could you provide details? ..............................................................

Please list any other medications (including pills, tablets, contraceptives, inhalers etc) that you are taking. Either prescribed by your GP or purchased over the counter ..........................................................................................................................

Signed: .................................................. Date: ........../.........../.........
C) Food and lifestyle

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. a) Are you allergic to any foods? □ YES □ NO
   b) If you answered yes to question 2.a, please state what foods you are allergic to

3. a) Do you have any religious dietary requirements? □ YES □ NO
   b) If you answered yes to question 3.a, please state your dietary requirements

4. a) Do you take any dietary supplements such as vitamins, minerals or fish oils? □ YES □ NO
   b) If you answered yes to question 4.a, please state which type and how often

5. a) Are you currently on a weight-reducing diet or other dietary restrictions? □ YES □ NO
   b) If you answered yes to question 5.a, please give details

6. a) Do you smoke? □ YES □ NO
   b) If you answered yes to question 6.a, approximately how many do smoke per day?

7. a) Do you drink alcohol? □ YES □ NO
   b) If you answered yes to question 7.a, approximately how much do you consume in a week (as a guide, 1 unit = 1 measure of spirits / 1 small glass of wine / 1/2 pint of beer)

D) History of Blood Donation & Clinical Study Participation

1. a) Have you ever donated blood? □ YES □ NO
   b) If you answered yes to question 1.a, when did you last donate blood?

2. a) Have you ever taken part in a Clinical Study? □ YES □ NO
   b) If you answered yes to question 2.a, when did the study (or studies) take place?
   c) If you answered yes to question 2.a, can you give some information about what the study (or studies) were investigating?
   c) If you answered yes to question 2.a, did the studies involve donating blood? □ YES □ NO

Thank you for your time in completing this questionnaire.
All information will be kept strictly confidential at all times.
### Appendix C: Dutch Eating Behaviour Questionnaire

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

<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>If you have put on weight, do you eat less than you usually do?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have a desire to eat when you are irritated?</td>
<td></td>
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<tr>
<td>If food tastes good to you, do you eat more than you usually do?</td>
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</tr>
<tr>
<td>Do you try to eat less at meal times than you would like to eat?</td>
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</tr>
<tr>
<td>Do you have a desire to eat when you have nothing to do?</td>
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</tr>
<tr>
<td>Do you have a desire to eat when you are depressed or discouraged?</td>
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<tr>
<td>If food smells and looks good, do you eat more than you usually eat?</td>
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<td></td>
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</tr>
<tr>
<td>How often do you refuse food or drink offered because you are concerned about your weight?</td>
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</tr>
<tr>
<td>Do you have a desire to eat when you are feeling lonely?</td>
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</tr>
<tr>
<td>If you see or smell something delicious, do you have a desire to eat it?</td>
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<td></td>
</tr>
<tr>
<td>Do you watch exactly what you eat?</td>
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<td></td>
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</tr>
<tr>
<td>Do you have a desire to eat when somebody lets you down?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>If you have something delicious to eat, do you eat it straight away?</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Do you deliberately eat foods that are slimming?</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Do you have a desire to eat when you are cross?</td>
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<tr>
<td>Do you have a desire to eat when you are approaching something unpleasant to happen?</td>
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</tr>
<tr>
<td>If you walk past the baker do you have a desire to buy something delicious?</td>
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</tr>
<tr>
<td>When you have eaten too much, do you eat less than usual the following days?</td>
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</tr>
<tr>
<td>Do you get a desire to eat when you are anxious, worried or tense?</td>
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</tr>
<tr>
<td>If you walk past a snack bar or café, do you have a desire to buy something delicious?</td>
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<tr>
<td>Do you deliberately eat less in order not to become heavier?</td>
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<tr>
<td>Do you have a desire to eat when things are going against you, or things have gone wrong?</td>
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<tr>
<td>If you see others eating, do you have also the desire to eat?</td>
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<td></td>
</tr>
<tr>
<td>How often do you try not to eat between meals because you are watching your weight?</td>
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</tr>
<tr>
<td>Do you have a desire to eat when you are frightened?</td>
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<td></td>
</tr>
<tr>
<td>Can you resist eating delicious food?</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How often in the evening do you try not to eat because you are watching your weight?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have a desire to eat when you are disappointed?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you eat more than usual when you see other eating?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you take your weight into account when you eat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have a desire to eat when you are emotionally upset?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>When preparing a meal are you inclined to eat something?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have a desire to eat when you are bored or restless?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix D: VAS to assess appetite and nausea

Participants were given a VAS answer booklet with each question presented on a separate page.

Answer the following questions by placing a vertical mark through the line for each question. Mark the line according to how you feel at this moment. Regard both the ends of the lines as indicating the most extreme sensations you have ever felt.

I am not hungry at all | How hungry do you feel? | I have never been more hungry

Not at all full | How full do you feel? | Extremely full

Nothing at all | How much do you think you can eat? | A lot

Not at all strong | How strong is your desire to eat a meal? | Extremely strong

Not at all strong | How strong is your desire to eat a snack? | Extremely strong

No, not at all | Would you like to eat something salty/savoury/sweet? | Yes, very much

Not at all nauseous | How nauseous do you feel? | Extremely nauseous
**Appendix E: VAS to assess gastrointestinal symptoms**

Participants were given a VAS answer booklet with each question presented on a separate page.

Answer the following questions by placing a vertical mark through the line for each question. Mark the line according to how you **feel at this moment**. Regard both the ends of the lines as indicating the **most extreme sensations you have ever felt**.

<table>
<thead>
<tr>
<th>Question</th>
<th>No, none at all</th>
<th>Yes, a lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you experienced any flatulence in the past hour?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you experienced any diarrhoea in the past hour?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you experience any bloating in the past hour?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you needed to belch/burp in the past hour?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you experienced any stomach discomfort in the past hour?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you had the urge to defecate in the past hour?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you experienced heartburn over the past hour?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F: Example Sensory Evaluation Questionnaire

### 3. Random Test

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Date</th>
<th>Explanation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>214</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 4. Acceptability Test

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Date</th>
<th>Explanation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 1. Paired Test

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Date</th>
<th>Description</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>699</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2. Tasted Difference Test

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Date</th>
<th>Description</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>511</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Instructions:**
- **Paired Test:** Take the paired samples provided, working from left to right. If you are not sure, please make your best guess. You may use the term "Comment" to note any issues.
- **Random Test:** Select the sample you prefer and write the code in the corresponding box.
- **Acceptability Test:** Circle the code number under "Comment" if you need to make any comments.
- **Tasted Difference Test:** Place the sample on the left and note any differences. Please select the sample that differs from the other and identify it by placing a cross in the corresponding box.
5. Rating Test A

Assessor No. 1 Name:.............................................. Date:.........../

Instructions:
Taste the two samples provided beginning with the one on your left. Please evaluate attributes for each sample as instructed below.

Indicate the intensity of aftertaste for each sample by ticking the appropriate box:

<table>
<thead>
<tr>
<th>No aftertaste</th>
<th>Strong aftertaste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
</tbody>
</table>

6. Rating Test B

Assessor No. 1 Name:.............................................. Date:.........../

Instructions:
Taste the two samples provided beginning with the one on your left. Please evaluate attributes for each sample as instructed below.

Indicate the intensity of acidic taste for each sample by ticking the appropriate box:

<table>
<thead>
<tr>
<th>Not acidic</th>
<th>Extremely acidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Sample 2</td>
</tr>
<tr>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
</tbody>
</table>
Appendix G: Calculations for pentose/hexose equivalents used for NDC dose in Chapter 7

It has been estimated by Cani and colleagues (2004) that there are 25 hexose & pentose units per mole of inulin HP and there are 1 hexose/pentose units per mole of L-Rha monohydrate.

Based on this, the daily dose of inulin HP for the study reported in Chapter 7 was calculated to match the hexose & pentose equivalents of the L-Rha dose given in previous studies (Vogt et al., 2004a, Vogt et al., 2004b, Vogt et al., 2006). In these previous studies, participants were supplied with 25 g L-Rha monohydrate.

The dose for inulin HP was calculated by first determining the molar hexose equivalents per 1 g of NDC, knowing the molecular mass ($M_r$) is 4073 g / mole:

Molar pentose & hexose equiv per 1 g = \( \frac{1}{M_r} \) * hexose & pentose equivs per mole

\[ \rightarrow \text{Molar pentose & hexose equiv per 1 g inulin HP} = \frac{1}{4073} \times 25 \]

\[ = 0.00614 \text{ molar equivs / g} \]

The dose of inulin HP required to match a 25 g dose of L-Rha monohydrate ($M_r = 182.11$) was then determined by the following calculation:

\[ \text{g inulin HP dose required to match a 25 g dose of L-Rha} = \left( \frac{\text{molar pentose & hexose equiv per 1 g inulin HP}}{\text{molar pentose & hexose equiv per 1 g L-Rha}} \right) \times 25 \]

\[ = \left( \frac{0.00614}{1/182.11} \right) \times 25 = 24.3 \text{ g} \]

The daily dose of L-Rha monohydrate supplied in the study reported in Chapter 7 was actually 25.5 g to compensate for the L-Rha provided being 98 % pure.
GASTROINTESTINAL SYMPTOM DIARY

The Effects of Fibre Supplementation on Appetite and Metabolic Response

instructions:

Please could you complete this gastrointestinal symptom diary every evening before going to bed with reference to symptoms experienced that day.

If you have any queries please contact Julia by e-mailing j.darzi@surrey.ac.uk or calling 01483 686393.

<table>
<thead>
<tr>
<th>Day X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date: <strong>/</strong>/___ Day of the week: ____________</td>
</tr>
<tr>
<td>Have you experienced any of the following symptoms today, and if so, to what level? Please complete each evening before going to bed with reference to symptoms experienced that day. Please tick the appropriate box for each question.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptom</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain and/or discomfort in the stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea (Frequent loose or liquid bowel movements)</td>
<td></td>
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</tr>
<tr>
<td>Constipation (Hard faeces that are difficult and sometimes painful to expel)</td>
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</tr>
<tr>
<td>Belching / burping (The release of gas from the mouth)</td>
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</tr>
<tr>
<td>Flatulence / passing wind (The release of gas from the rectum)</td>
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</tr>
<tr>
<td>Nausea and/or feeling sick</td>
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<tr>
<td>Acid regurgitation (The appearance of stomach contents into the throat or mouth)</td>
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</tr>
<tr>
<td>Heartburn (Burning pain in the middle of the chest due to acid regurgitation)</td>
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<tr>
<td>Rumbling stomach (Stomach growling and noisy due to the movement of gas in the stomach)</td>
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<tr>
<td>Bloating (Tight and full feeling stomach)</td>
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<td></td>
</tr>
</tbody>
</table>

Please could you also indicate below when each fibre jelly was consumed?

<table>
<thead>
<tr>
<th>Jelly</th>
<th>Did you eat the jelly?</th>
<th>What time did you eat the jelly?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes / No</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Please circle correct answer</td>
<td></td>
</tr>
</tbody>
</table>

Please indicate on a scale of 1 to 9 how much you liked the taste of the jellies today?

<table>
<thead>
<tr>
<th>Taste</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste very much</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Taste moderately</td>
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<td></td>
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<tr>
<td>Taste slightly</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither like nor dislike</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste slightly</td>
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<td></td>
<td></td>
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<tr>
<td>Taste moderately</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Taste very much</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste extremely</td>
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<td></td>
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</tbody>
</table>

Appendix H: Daily Compliance and Gastrointestinal Symptom Diary
## LOW FIBRE DIET SHEET

**The Effects of Fibre Supplementation on Appetite and Metabolic Response**

### Guidelines:

You have been requested to follow a low fibre diet the day before coming in for the study day.

This booklet provides guidance on what foods to avoid and what foods to eat during this time. On the back page there are some lunch suggestions.

If you have any queries please contact Julia by e-mailing J.darzi@surrey.ac.uk or calling 01483 686393.

<table>
<thead>
<tr>
<th>Food Type</th>
<th>☑ High Fibre - Avoid</th>
<th>☑ Low Fibre - Good choice</th>
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<tbody>
<tr>
<td><strong>Breads &amp; Cakes</strong></td>
<td>* Wholemeal, Wholegrain, Brown, Granary bread / rolls / pitta</td>
<td>☑ White bread / rolls / pitta</td>
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<tr>
<td></td>
<td>* Any with added seeds</td>
<td>☑ Naan</td>
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<td></td>
<td>* Any with added fibre (e.g. Hovis Best of Both, Kingsmill 50%0)</td>
<td>☑ Crumpets</td>
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<td></td>
<td>* Any with added fruit or vegetables</td>
<td>☑ Croissant</td>
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<td></td>
<td>* Wholewheat tortillas</td>
<td>☑ Plain bagels</td>
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<td>* Plain scone</td>
<td>☑ Plain scone</td>
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<tr>
<td><strong>Breakfast Cereals</strong></td>
<td>* Kellogg’s - All bran, Bran Flakes, Start, Fruit ‘n’ Fibre, Frosted Wheats, Raisin Wheats, Honeynut Loops, Rice Krispies Multigrain, Special K Sustain, Special K Medley, Raisin Wheats, Optivita</td>
<td>* Kellogg’s - Rice Krispies, Cornflakes, Coco Pops, Frosties, Special K, Crunchy Nut Cornflakes, Honey Comflakes, Pop Tarts</td>
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<td></td>
<td>* Nestle - Shredded Wheat, Cheerios, Shreddies, Clusters, Arts &amp; More</td>
<td>☑ Sugar Puffs</td>
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<td></td>
<td>* Weetabix, Oatabix</td>
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<td></td>
<td>* Musli, Granola, Alpen, Jordans</td>
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<td></td>
<td>* Porridge, Rolled Oats, Wheatgerm, Ready Break, Hot Oat Cereal</td>
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<td></td>
<td>* Cereal Bars</td>
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<td><strong>Rice, pasta &amp; other grains</strong></td>
<td>* Brown, wholegrain or wild rice</td>
<td>☑ Any polished white rice (e.g. basmati, easy cook, long grain, arborio, risotto, Thai Jasmine)</td>
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<td></td>
<td>* Brown or Wholewheat pasta</td>
<td>☑ Any dried, fresh or canned pasta (except brown or wholegrain)</td>
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<td></td>
<td>* Bulgar wheat, Pearl Barley, Quinoa, Millet, Buckwheat, Oatmeal, Oatmeal/Porridge</td>
<td>☑ Any noodles, Couscous</td>
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<td></td>
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<td>☑ Gnocchi</td>
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<tr>
<td><strong>Potatoes &amp; other starchy carbs</strong></td>
<td>* Boiled, roast, baked, fried potatoes with skin intact</td>
<td>☑ Boiled, mashed, roast, baked, fried potatoes with skin removed</td>
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<td></td>
<td>* Potato wedges, Potato croquettes, Hash Browns, Chips</td>
<td>☑ Potato waffles</td>
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<td></td>
<td>* Yam, sweet potato, plantain</td>
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<td><strong>Soups</strong></td>
<td>* Canned or fresh ready-prepared soup with &lt;0.7g fibre per 100g</td>
<td>☑ Canned or fresh ready-prepared soup with &lt;0.7g fibre per 100g including &quot;cream of&quot; soups</td>
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<td></td>
<td>* Hommeade soup made with added vegetables or pulses</td>
<td>☑ Homemade soup without added vegetables or pulses</td>
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* Or Supermarket Own Brand equivalent
<table>
<thead>
<tr>
<th>Food Type</th>
<th>High Fibre - Avoid</th>
<th>Low Fibre - Good choice</th>
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<tbody>
<tr>
<td>Fruit &amp; Veg</td>
<td>Fresh, frozen, canned fruit</td>
<td>Fruit juice</td>
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<td></td>
<td>Fresh, frozen, canned vegetables including baked beans</td>
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<td>Dried fruit</td>
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<td>Salad</td>
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<td>Pulses – e.g. lentils, chick peas, kidney beans, haddock beans, etc</td>
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<td>Milk &amp; Milk Products</td>
<td>Probiotic yogurts and fromage frais with added fibre or probiotics fibre (e.g. Muller Vitality, Danone Activa, Danone Shape Lasting Satisfaction, Rora Pro-Avic, Weight Watchers Fromage Frais)</td>
<td>Dairy milk</td>
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<td></td>
<td>Soya desserts (e.g. Alpro Soya Yout, Provenil &amp; Yogurt)</td>
<td>Yogurts and fromage frais with no added fibre or probiotics</td>
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<td>Various soya milks - Alpro Soya, Teosan, Sainsbury's, Waitrose, Auda</td>
<td>Any cheese</td>
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<td>Biscuits with added fruits, flapjacks</td>
<td>Water biscuits, cream crackers, Carrs Cheese Melts, rice cakes</td>
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<td>Kipprolle, Ryvita, Krackernest, Oat Biscuits</td>
<td>Bounty, Aero, Drifter, Milly Bar, Rolos, Smarties, Kit Kat, Milky Way, Munchies, Blue Riband, Chocolate Fingers, McVitie's Gold Medallions, Earl Grey Biscuits, etc.</td>
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<td>Other Snacks</td>
<td>Twiglets, Walkers Crisps, Squares, French Fries, Real McCoy's, Nik Naks, Hula Hoops Multigrain, Razzles, Wheat Crunchies, Supermarket Own Potato Crisps, Kettle Chips</td>
<td>Discos, Hale Hoops, Quavers, Mini Cheddars, Skips, Wotsits/Cheese Pulls, Monster Munch, Chips Ahoy/Crunchy Sticks, Quavers/Cheese Twists, Cheese Balls</td>
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<td>Tortilla Chips, Nachos, Doritos, Ryvita Limpus, Sunblites, Pringles</td>
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<td>Popcorn, Pretzels</td>
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This list is by no means exhaustive.  
If you are in any doubt about particular products that you would like to eat, please contact Julia at i.darzi@surrey.ac.uk to check suitability.

Low Fibre Lunch Suggestions

Please find below a few ideas for your lunch:

1. **Sandwiches** - Make with white bread, white pita bread or plain bagel, without salad.
   - Meats: Ham & mustard; ham & pickle (small amount of pickle); chicken or turkey roll; roast beef & horseradish; corned beef; salami; chicken & bacon mayonnaise; pate; coronation chicken
   - Fish filling suggestions: Tuna mayonnaise; tuna pate; smoked mackerel pate; salmon & cream cheese
   - Vegetarian filling suggestions: Brie & cranberry jelly; cheese & onion mayonnaise; egg mayonnaise; plain omelette; cheddar & pickle (small amount of pickle); cottage cheese; cream cheese; emmental cheese; goats cheese & pesto

2. **Hot Snacks**
   - Pilchards, Sardines or Mackerel in tomato sauce on toast (made with white bread)
   - Spaghetti Hoops in tomato sauce on toast (made with white bread)
   - Scrambled, fried or poached eggs on toast (made with white bread)
   - Cheese on toast!
   - Plain, herb, cheese or ham omelette with white bread, white roll or toast (made with white bread)
   - Welsh rarebit
   - Noodles with stir-fried chicken (no vegetables)
   - Jacket potato (remember not to eat the skin, only the flesh) with:
     - Tuna mayonnaise, cottage cheese, cheddar cheese (can grill to melt), coronation chicken, cheese mayonnaise (grated cheese mixed with mayonnaise), chicken curry, cheese & bacon, egg mayonnaise
   - Soup with white bread or roll (refer to soup list for further suggestions):
     - Cream of tomato, cream of chicken, cream of mushroom, oxtail, French onion
   - Pasta with pesto & olive oil