Gastrointestinal Transit, Appetite and Food Intake: The Role of Dietary Fat

A thesis submitted to the University of Limerick in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract

A high intake of dietary fat has long been implicated in a etiology of obesity, coronary-heart disease and type 2 diabetes mellitus. Fat is the most potent regulator of gastric emptying (progression of food from the stomach to the small intestine). Chronic exposure to fat may cause alterations to the gastrointestinal tract and hence led to down-regulation of the appetite control system. In this thesis, GE was assessed using the $^{13}$C octanoic acid breath test in a healthy adult cohort.

Acute pharmacological and nutritional models demonstrated that 10 mg domperidone and 3 g cinnamon respectively, had no effect on gastric emptying rate or appetite responses to a high-fat meal. Also detailed in this thesis was a methodology for improving the accuracy of the $^{13}$C octanoic acid breath test, through direct measurement of carbon dioxide production rate ($\dot{V}CO_2$).

Investigation of gut sensitivity to specific fatty acids is an area which has been overlooked in previous literature. When adjustments were made for background intake of specific fatty acids, a 5-day high-fat supplemented diet was sufficient to accelerate gastric emptying rate and reduce satiety to a high-fat meal but had no effect on substrate utilisation. After a 5-day deadaptation period to high-fat feeding subjects still had reduced satiety, even though gastric emptying rate was similar to baseline levels.

The findings of this PhD highlight the complexity of appetite regulation, over which control is based on a myriad of factors other than gastrointestinal transit. Furthermore, it was evident from this thesis that the process of adaptation to a high-fat diet may involve mechanisms other than desensitisation to a specific fatty acid. Dietary fat quality may have important implications in the obesity epidemic and associated disease.
Declaration of Originality

I hereby declare that this is entirely my own work and has not been submitted to any other University or higher education institution, or for any other academic award in this University. Where use has been made of the work of other people, it has been fully acknowledged and referenced.

Signed: __________________________

Date: ____________________________
Acknowledgements

First and foremost, I would like to thank my supervisor, Dr Amir Shafat for guiding me through this PhD journey. Thanks so much for your tireless encouragement and enthusiasm over the past four years; without your help this PhD would not have been possible.

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Publications Arising from this Thesis

The data presented in this thesis have formed the basis for the publications listed below:


Markey, O. & Shafat, A. (2011) High Fat Diets Accelerate Gastric Emptying but Deadaptation Depends on Fatty Acid Composition, Obesity, 91, S114 [Abstract]


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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGRP</td>
<td>Agouti related peptide</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine- and amphetamine regulated transcript</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>CON</td>
<td>Control</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>D₂</td>
<td>Dopamine-2 receptor</td>
</tr>
<tr>
<td>DTE</td>
<td>Desire to eat</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure</td>
</tr>
<tr>
<td>EI</td>
<td>Energy intake</td>
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<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>FFT</td>
<td>Fat tolerance test</td>
</tr>
<tr>
<td>FTO</td>
<td>Fat Mass and Obesity Associated gene</td>
</tr>
<tr>
<td>GE</td>
<td>Gastric emptying</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GPR</td>
<td>G-protein</td>
</tr>
<tr>
<td>H₂</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
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<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>HF</td>
<td>High-fat</td>
</tr>
<tr>
<td>HFD</td>
<td>High-fat diet</td>
</tr>
<tr>
<td>IRMS</td>
<td>Isotope ratio mass spectrometer</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LF</td>
<td>Low-fat</td>
</tr>
<tr>
<td>LFD</td>
<td>Low-fat diet</td>
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<tr>
<td>MCTT</td>
<td>Mouth-to-caecum transit time</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>n-3</td>
<td>Omega-3 fatty acids</td>
</tr>
<tr>
<td>OBT</td>
<td>Octanoic acid breath test</td>
</tr>
<tr>
<td>OF</td>
<td>Olive oil and fish powder supplement</td>
</tr>
<tr>
<td>OM</td>
<td>Olive oil and macadamia oil supplement</td>
</tr>
<tr>
<td>OO</td>
<td>Olive oil supplement</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PFC</td>
<td>Prospective food consumption</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PPM</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide YY</td>
</tr>
<tr>
<td>RM</td>
<td>Repeated measures</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td><strong>TCA cycle</strong></td>
<td>Tricarboxylic acid cycle</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>TG</strong></td>
<td>Triglycerides</td>
</tr>
<tr>
<td><strong>VAS</strong></td>
<td>Visual analogue scale</td>
</tr>
<tr>
<td><strong>(\dot{V}\text{CO}_2)</strong></td>
<td>Carbon dioxide production rate</td>
</tr>
<tr>
<td><strong>WFD</strong></td>
<td>Weighed food diary</td>
</tr>
<tr>
<td><strong>WHO</strong></td>
<td>World Health Organisation</td>
</tr>
<tr>
<td><strong>(\alpha)-MSH</strong></td>
<td>(\alpha)-melanocyte-stimulating hormone peptide</td>
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Chapter 1: Introduction

1.1 Introduction

Obesity is a rapidly increasing problem both nationally and internationally and is one of the most substantial threats to the public in the 21st century (Morgan et al., 2008; WHO, 2011). Obesity can be defined as a state of positive energy balance where energy intake (EI) exceeds energy expenditure (EE) (Little et al., 2007) and is related to numerous co-morbidities that increase risk for morbidity and mortality (Flegal et al., 2005). Central or android obesity is commonly associated with the metabolic syndrome, a constellation of metabolic disorders which are risk factors for chronic diseases such as cardiovascular disease (CVD) and type 2 diabetes (T2DM); these factors include insulin resistance, dyslipidemia and hypertension (Grundy, 2004; Gill & Malkova, 2006). In Ireland, 2,000 premature deaths are attributable to obesity per annum and it is estimated to have an economic impact of approximately €4 billion on the Republic (National Taskforce on Obesity, 2005). Current treatment strategies, including behavioural, surgical and pharmacological have failed to curtail the spread of the current obesity epidemic.

Obesity is a multi-faceted condition encompassing genetic, environmental (behavioural) and psychosocial components (Kopelman, 2000). The rapid weight gain evident over the past twenty years in genetically stable populations, potentiate environmental factors (including increased food consumption and sedentary lifestyle) as a major contributor to energy imbalance (British Nutrition Foundation, 1999). This thesis will focus on the salient impact of dietary fat on gastrointestinal (GI) transit, appetite regulation and ultimately, obesity. It is well established that there is a direct link between obesity and high intake of dietary fat (Kleges, 1992; Rolls, 1995). High-fat (HF) feeding is supportive of increased EI or 'high fat hyperphagia' (Little et al., 2007). Consumption of a HF diet (45-50% of energy from fat) for two weeks led to a 15.4% surplus in EI and a significant increase in body mass compared to a medium fat diet (Lissner et al., 1987). Dietary fat is the most energy dense macronutrient (37 kJ) and it has a disproportionately small effect on satiety (Blundell & MacDiarmid, 1997). Satiety refers to the events that occur postprandially to prevent hunger and influences the interval between meals (Camilleri & Grudell, 2007; King et al., 2011). Research has illustrated that following a HF breakfast (803 kcal/3.36 MJ) volunteers were less satiated and consumed more energy in a subsequent ad libitum snack meal provided at 90 minutes postprandially than following an isocaloric high-carbohydrate breakfast (Blundell et al., 1993). Additionally, dietary fat only weakly influences satiation, the process that limits meal...
size and causes meal termination, and this can result in passive overconsumption (Blundell & Gillett, 2001).

Although it has been shown that an increased intake of dietary fat can lead to overconsumption and weight gain (Lissner et al., 1987), it should be noted that acute ingestion of fat has strong stimulatory effects on the GI tract (Little et al., 2007). Gastric emptying (GE) is the rate at which food progresses from the stomach into the small intestine. Compared to other macronutrients, acute exposure to dietary fat in the small intestine potently inhibits GE and suppresses appetite and subsequent EI (Woodward, 1957; Welch et al., 1985; Cecil et al., 1999). A recent study illustrated that ingestion of a HF meal can even delay GE of a subsequent meal, without affecting satiety (Clegg et al., 2010). It appears likely that the appetite control system only has weak inhibitory mechanisms to prevent overconsumption of dietary fat (Blundell et al., 1993). Chronic exposure to fat (HF feeding) leads to down-regulation of the appetite control system through alterations of the GI tract (French et al., 1995; Little et al., 2007). A rat study illustrated that chronic intermittent infusion of palm oil into the distal small intestine reduced the delay in stomach to caecum transit time (Brown et al., 1994). It was originally postulated by Hunt et al. (1975) that an enhanced GE rate may reduce the satiating effect of food, thus leading to overeating and the onset of obesity. In humans, 14 days of HF feeding has been shown to reduce the delay in GE and mouth to caecum transit time (MCTT) compared to a low fat (LF) diet (Cunningham et al., 1991). Recently published data demonstrated that a 3-day high fat supplemented diet rich in sunflower oil was sufficient to accelerate GI transit (Clegg et al., 2011).

Independent of its influence on the appetite control system, GE rate has the potential to alter metabolic variables in the postprandial period. It is well established that GE rate is inversely associated with postprandial glycaemia in healthy adults (Horowitz et al., 1993). Postprandial glycaemic and insulin responses of healthy adults were lowered by adding 50 g of fat to a test meal (Cunningham & Read, 1989). Accelerated GE, through exposure to a HF diet (HFD) may lead to heightened postprandial glycaemia and lipaemia (Darwiche et al., 2001) and thus led to increased risk for development of atherosclerosis, coronary artery disease and T2DM (Castiglione et al., 2002).

1.2 Rationale for the thesis

The role of a HF diet in the aetiology of obesity is well documented. It is now emerging that fat quality (e.g. fatty acid chain length, degree of saturation) of the diet, as well as quantity of fat per se may have a salient role to play in the development of obesity and lifestyle diseases
(MacIntosh et al., 2003; Khor, 2004). Research has indicated that the GE of a HF test meal is dependent on its fatty acid composition (Robertson et al., 2002). The current PhD thesis aims to explore the effect acute and chronic exposure to dietary fatty acids on the GI tract in a young, healthy population. It is envisaged that this body of work will help to gain a greater understanding of how specific fatty acids affect GI function influence regulation of appetite, food intake and metabolic variables in the postprandial state. GE is a variable outcome measure with inter- and intra-individual variability (Brophy et al., 1986). This thesis will also examine the association background intake of specific nutrients and GE, in an attempt to explain some of the inter-subject variability observed in GE rate. The $^{13}$C octanoic acid breath test (OBT) was originally devised as a non-invasive, indirect measurement of GE by Ghoos et al. (1993). Factors such as physical activity and food intake can affect carbon dioxide ($\dot{V}CO_2$) production rate during the OBT. However the majority of research relies on an assumption of constant resting $\dot{V}CO_2$ production rate. A further aim of this thesis is to quantify difference in $^{13}$C OBT results using directly measuring $\dot{V}CO_2$ production rate from throughout the test with a constant directly measured and predicted resting values of $\dot{V}CO_2$ production rates (Shreeve et al., 1970).

1.3 Thesis Outline

This thesis comprises of seven chapters. Chapters are presented in manuscript format. Each study chapter consists of an abstract, introduction, methodology, results, discussion and conclusion section. The following chapter reviews the literature applicable to this area (Chapter 2). Acute studies are then presented using (i) pharmacological and (ii) nutritional models (Chapter 3 and 4). The relationship between habitual diet and specific dietary fatty acids in background diet and GE will also be discussed in these chapters. Subsequently, a technical chapter exploring an assumption of the $^{13}$C OBT is discussed (Chapter 5). Findings presented in preceding chapters culminated in the decision to design a 5-day HF supplemented diet varying in fatty acid composition (Chapter 6). Finally, a synopsis of thesis findings is presented, along with some suggestions for future research (Chapter 7).

Each thesis chapter is outlined below:

Chapter 2 reviews the literature relating to the significance of GE in health and disease. The role of GE of dietary fat on (dys)regulation of appetite, postprandial glycaemia and lipaemia,
and the development of obesity and its co-morbidities are discussed. Mechanisms relating to the impact of acute and chronic exposure to dietary fat in the GI tract are explored. Finally, various methodological issues relating to procedures employed in this thesis are reviewed.

Chapter 3 presents a pharmacological model which was designed to examine the effect of acute administration of 10 mg of domperidone on the GE rate of a HF solid meal and appetite sensations in healthy adults. In addition, the relationship between intake of specific macronutrients and fatty acids in background diet and GE is explored.

Chapter 4 details a nutritional model which aimed to look at the effect of acute ingestion of 3 g cinnamon on GE rate of a HF test meal, as well as appetite responses and postprandial metabolic variables in a cohort of healthy adults. The association between background intake of macronutrients/fatty acids and GE is also examined.

The observational findings of Chapter 3 and 4, in relation to the relationship between background fatty acid intake and GE aided the decision to design a HF intervention study with supplements that varied in fatty acid composition.

Chapter 5 describes a study that supports the importance of directly quantifying carbon dioxide (\(\dot{\text{V}}\text{CO}_2\)) production rate throughout the 13C OBT for accurate assessment of 13C percentage dose recovered and GE parameters.

Chapter 6 examined the effect of a 5-day HF diet supplemented with specific fatty acids on GE, mouth to caecum transit time (MCTT), appetite, food intake and substrate utilisation. Another novel aspect of this chapter was examining whether a subsequent 5-day period is sufficient to reverse the effects of HF feeding on GE and other aforementioned parameters.

Chapter 7 summarises the main findings of this body of work and suggests some directions for future research in the area.
Chapter 2: Literature Review

2.1 Introduction

This chapter addresses the current scientific knowledge and identifies gaps in the literature relating to studies contained in this thesis. The obesity epidemic will be discussed followed by a detailed look at the role of acute and chronic dietary fat intake on the GI tract, appetite (dys) regulation and EI. Scope for future targeted studies will also be outlined. Finally, the methods employed in this thesis will be discussed.

2.2 Obesity

2.2.1 Prevalence of Obesity

The prevalence of obesity is rapidly increasing and has reached epidemic proportions worldwide (WHO, 2011). By 2015, the World Health Organisation (2011) predicts that approximately 2.3 billion adults will be considered overweight (Body mass index (BMI) > 25 kg·m⁻²) and more than 700 million obese (BMI > 30 kg·m⁻²). According to National data from 2008, it is estimated that 26% and 23% of Irish males and females 20 years and older are obese (OECD, 2010).

2.2.2 Obesity and Its Co-morbidities

Obesity is a long-term state of positive energy balance where EI exceeds energy EE (Weiser et al., 1997). It is a multi-system disease which results from a ‘failure of normal homeostatic mechanisms regulating food intake, fat storage, and energy utilisation’ (Kaplan, 1998). Android or abdominal obesity is frequently associated with the ‘metabolic syndrome’, a term coined to describe a constellation of metabolic disturbances which are risk factors for the development of CVD and T2DM (Reaven, 1988; Eckel et al., 2005; Zimmet et al., 2001). There is controversy over the exact definition of the syndrome and this has subsequently resulted in numerous definitions of the syndrome (WHO, 1999; NCEP:ATP I II, 2 001; International Diabetes Federation, 2005). Despite lack of agreement over the definition of the syndrome, importance is placed on the presence of the following combination of abnormalities: android obesity, dyslipidemia, dysglycaemia and hypertension (WHO, 1999; International Diabetes Federation, 2005). Obesity is also known to increase the risk of developing osteoarthritis, breast, colon and endometrial cancer and gastrointestinal complications such as gastroesophageal reflux disease, non-
alcoholic steatohepatitis and gallstones (Van Itallie, 1985; Bray, 1996; Kaplan, 1998). Therefore, obesity represents a significant social and economic burden. The economic burden of obesity is attributable to (1) direct costs-increased medical costs associated with the treatment of obesity-related diseases and (2) indirect costs-premature death and reduced productivity due to absenteeism (Branca et al., 2007). Fry and Finley (2005) estimated that the overall cost of obesity (direct and indirect costs) for the 15 EU member States in 2002, was €32 billion per annum. Two thousand premature deaths in Ireland are attributable to obesity per annum and it was projected to have an economic impact of approximately €4 billion on the Republic (National Taskforce on Obesity, 2005).

2.2.3 Aetiology of Obesity

Obesity is a multi-faceted condition encompassing genetic, environmental and psychosocial features (Kopelman, 2000). The genetic factors that can lead to obesity are poorly understood. However, Walters and colleagues (2010) have recently shown that a highly penetrant form of obesity was caused by deletions on the 16p11.2 chromosome. Previously, Frayling et al. (2008) identified the fat mass and obesity associated (FTO) gene which may lead to T2DM through its influence on BMI. The authors showed that 16% of adults who had inherited the risk allele were approximately 3 kg heavier and had a 1.67-fold increased odds of developing obesity compared to their counterparts who had not inherited the allele. Although excess adiposity may be a familial trait, the effect of the genotype on the development of obesity may be ‘attenuated’ or ‘exacerbated’ by environmental factors (Kopelman, 2000).

Rapid weight gain in recent years is suggestive of the influential role that environmental factors play in the development of adverse weight profiles. Physical inactivity is a major determinant of obesity. There is an inverse association between physical activity levels and degree of adiposity (Hunter et al., 1996). Additionally, the occurrence of overweight was shown to be significantly greater in sedentary males (14%) and females (21%) compared to active males (7%) and females (8%) (Rissanen et al., 1991). Waller and colleagues (2008) published the results of a 30-year longitudinal twin study which examined the relation between long-term physical activity, waist circumference and weight gain. It was shown that from 1975 through 2005, that mean waist circumference and weight gain were 5.4 kg and 8.4 cm less in 42 active compared to their inactive co-twins.
The role of sedentary behaviour (television viewing, sitting, video games) is emerging as another environmental factor that can lead to the development and maintenance of obesity. Epidemiological findings suggest that sedentary behaviour is related to obesity, independent of physical activity levels (Jakes et al., 2003; Stamatakis et al., 2009).

2.2.3.1 Role of High Dietary Fat Intake

This review will focus on one of the prominent environmental factors contributing to the onset of obesity - the over-consumption of HF, energy dense foods (Little et al., 2007). Epidemiological evidence suggests that there is a direct relation between intake of dietary fat and adverse weight profiles (Rolls, 1995; Golay & Bobbioni, 1997). In countries where the occurrence of obesity is rapidly increasing, in the region of 45% of daily energy intake is provided by dietary fat (Golay & Bobbioni, 1997). Furthermore, research has illustrated a positive association between preference for fatty food consumption and adiposity (Mela & Sacchetti, 1991). Miller and colleagues (1990) have also shown that obese individuals consumed more dietary fat than their lean counterparts suggesting that diet composition rather than energy intake per se is related to adiposity.

When compared to a medium fat diet, consumption of a HF diet (45-50% of energy from fat) for 2-wks led to a 15.4% surplus in EI and a significant increase in body mass (Lissner et al., 1987). Little et al. (2007) suggest that consuming a HF diet supports a greater energy intake which is termed ‘high fat hyperphagia.’ This is in agreement with the ‘protein lever hypothesis,’ proposed by Simpson and Raubenheimer (2005), which states that dietary fat and carbohydrates must be over-consumed in order to keep protein intake tightly regulated. It is clear from the aforementioned research that dietary fat intake has a significant role to play in overconsumption of energy and development of obesity.

2.3 Digestion and Absorption of Dietary Lipids

2.3.1 Introduction to Dietary Lipids

The term lipid or originates from the Greek term lipos meaning ‘fat’; it describes any naturally occurring, non-polar substance that is nearly or completely insoluble in water.
but is soluble in non-polar solvents (Bohinski, 1983). According to the British Nutrition Foundation (1999), dietary sources of lipid include seed oils of plants and storage fats of mammals and fish. 90-95% of fat derives from triglycerides (TG) while phospholipids and cholesterol are the minor components of fat (Gurr et al., 2002, p170).

According to the length of their chain, free fatty acids (FFAs) are classified as:

- short-chain (2-4 carbon atoms)
- medium-chain (6-10 carbon atoms)
- long-chain (12-26 carbon atoms) fatty acids (Montogomery et al., 1996).

2.3.2 Digestion of Lipids

The small intestine is the predominant site for digestion of lipids. Digestion of lipids is dependent on the emulsification of TG and the formation of micelles, with bile salts and FFAs acting as the emulsifying agents (Figure 2.1). Bile salts, which are derived from cholesterol, are synthesized in the liver and secreted in bile to form covalent conjugates including sodium glycocholate and sodium taurocholate. Bile salts are amphipathic molecules that mainly possess a non-ring structure and a highly polar acidic group. Micelles form an oil-in-water emulsion by the non-polar tails of the amphipathic molecules stabilising small groups of non-polar molecules, especially TG. The polar ends of the molecules face outwards into the aqueous surroundings of the small intestinal contents. The repulsive action of the outward facing polar groups has a tendency to lead to greater emulsification of the lipid droplets (Gurr et al., 2002, p173).

The TGs are hydrolyzed by pancreatic lipase in the intestinal lumen, leading to the generation of fatty acids and 2-monoglycerides (Johnson, 2001, p 1693). Monoglycerides are amphipathic molecules that aid in emulsification and helps in the action of bile salts. Very small molecules known as mixed micelles (~4-6 nm in diameter) are gradually formed; these contain bile salts and other molecules including fatty acids and monoglycerides. They have the ability to diffuse through the unstirred aqueous layer positioned on top of the enterocytes and thus, can transport non-esterified fatty acids and monoglycerides to the surface of the absorptive cells (Gurr et al., 2002, p173).
Figure 2.1 The Digestion and Absorption of Dietary Fat in the small intestine

Lipid droplets entering the small intestine from the stomach are subjected to the action of pancreatic lipase, phospholipase A₂ and cholesterol esterase, which hydrolyse triglycerides (TG) to produce monoglycerides and fatty acids (FA), phospholipids (PL) to produce lysophospholipids and fatty acids; and cholesterol esterase (CE) to liberate cholesterol (C) and fatty acids. These are emulsified with bile salts (from the gall bladder) to produce a micellar suspension (the mixed micelles) from which components are absorbed across the epithelial cell (enterocyte) membranes. Short- and medium-chain fatty acids pass through into the circulation (into the hepatic portal vein, and bile salts are reabsorbed, along with further cholesterol, in the lower part of the small intestine. Within the enterocyte, the components are reassembled, and packaged into chylomicrons, the largest of the lipoprotein particles. The chylomicrons are secreted into small branches of the lymphatic system, the lacteals (Gurr et al., 2002, p171).

2.3.3 Absorption of Lipids

Lipid absorption in humans commences in the distal duodenum and terminates in the jejunum. Non-esterified long-chain fatty acids and monoglycerides pass slowly across the brush-border membrane and uptake depends on the number of molecules in contact with the enterocyte membrane and absorption is diffusion limited. Bile salts are not absorbed in the proximal small intestine; instead, they are reabsorbed in the ileum. Subsequently, they are recirculated in the hepatic portal vein to the liver and are re-secreted in the bile in a process known as entero-hepatic circulation.
The digestion products can come upon two main obstacles to their absorption. Mixed micelles are water soluble and easily pass through the unstirred water layer. Mixed micelles increase the concentration of fatty acids at the border membrane by 100 to 1000-fold. The unstirred water layer, maintained by the epithelial cells (mucosa), has an acidic pH. This helps to uncharge the non-esterified fatty acids so that they can leave the micelles and move into the membrane of the epithelial cells. Another barrier to absorption is the brush-border membrane. It appears likely that fatty acids are aided in their movement into the cell membrane with specific fatty acid binding proteins (e.g. plasma fatty acid translocase and fatty acid translocase- with a cell surface receptor called CD36) (Gurr et al., 2002, p173).

2.3.3.1 Absorption of Short and Medium-Chain Fatty Acids

Short and medium-chain fatty acids show less ‘affinity’ for the enterocyte membrane, are not soluble in water and are not bound to specific proteins. The medium-chain acly-CoA synthetase which is necessary for their esterification is not present within the enterocytes (Frayn, 1996). Thus, the fatty acids can enter the capillary plasma in the form of non-esterified fatty acids without the necessity for chylomicrons. They are carried to the liver via the hepatic portal vein weakly bound to albumin. Fatty acid uptake to the liver ranges from 80 to 100% of the portal flux and the left-over portion is discharged into the bloodstream where it becomes available to the peripheral tissues (Guillot et al., 1993).

2.3.3.2 Absorption of Long-Chain Fatty Acids

Within the enterocyte, long-chain fatty acids (LCFAs) and monoacylglycerols are re-esterified to form new triglycerides molecules. This occurs predominantly by the monoacylglycerol esterification pathway which involves the synthesis of triglycerides from 2-monoacylglycerol and coenzyme A (CoA) activated fatty acids. Triglycerides are reassembled in the enterocyte before they are exported to systemic circulation. LCFAs as well as monoacylglycerides are reesterified into TG in the smooth endoplasmic reticulum. Cholesterol and fats soluble vitamins are also transferred and undergo reesterification. Reassembled lipids are coated with apoproteins (especially apolipoprotein B which is synthesised in the rough endoplasmic reticulum) for transfer from the enterocyte. The apoproteins become glycosylated in the Golgi apparatus and coat the reesterified lipids to form particles called ‘chylomicrons’. Chylomicrons consist of
a T G core (80 – 90 % of weight) surrounded by phospholipids (8 – 9 % of weight), cholesterol esters, fat soluble vitamins as well as apoproteins (trace amounts). The chylomicron particles are used as a means to transfer dietary lipids to other parts of the body via the basolateral membrane in a process known as exocytosis. Chylomicrons are range from 750 – 5000 Å in diameter and thus, are too large to cross intracellular junctions. This dietary lipids incorporated into chylomicrons depart from the intestinal villi through the lacteals, lymph vessels, which have larger junctions (Barrett, 2006, p271-3). They make their way from the main branches of the lymphatic system, up the thoracic duct and into circulation (Frayn, 1996).

2.4 Peripheral Regulation of Appetite

2.4.1 Introduction to Appetite Regulation

Appetite regulation is a complex system, orchestrated by GI signals (hormones, neuropeptides, and autonomic nervous system), metabolic cues from hormones, peptides and absorbed nutrients as well as the hypothalamus (Mathus-Vliegen et al., 2005). Appetite includes a minimum of two separate components, satiation and satiety (Camilleri & Grudell, 2007). Satiation limits meal size, causes meal termination and describes the feeling of fullness directly after a meal (Capasso & Izzo, 2008; Blundell & Gillett, 2001). Satiation is the consequence of synchronized neural and hormonal signals that are generated by the stomach as a result of ‘food-induced mechanical and chemical stimuli’ (Capasso & Izzo, 2008). On the other hand, satiety controls meal frequency; it refers to events that occur postprandially to prevent hunger and results in an individual not consuming food for a certain period (Camilleri & Grudell, 2007). In other words, satiety delays the onset of the next meal, while satiation terminates the current meal.

Blundell & Halford (1994) conceived that appetite control is representative of three levels of events and processes:

1) Psychological events (hedonic sensations, cravings, hunger sensations) and behavioural operations (meals, snacks, energy & macronutrient intake)
2) Peripheral physiology and metabolic events
3) Neurotransmitter and metabolic interactions in the brain
Although it is recognised that appetite is controlled by many factors, the main scope of this review will be on the role of the GI tract in the peripheral physiological regulation of appetite. The GI tract is the ‘portal of entry of all nutrients’ and it has a pivotal role to play in appetite regulation (Capasso & Izzo, 2008).

### 2.4.2 Acute Effect of Dietary Fat on Appetite

It is well established that there is a hierarchal influence of macronutrients on early satiety (Rolls, 2000). Dietary fat is less satiating compared to iso-energetic quantities of other macronutrients (Jebb, 2007). Dietary fat contains 36.7 kJ·g⁻¹ of energy compared to carbohydrate (CHO) and protein which both contain 16.7 kJ·g⁻¹. If the quantity of food is responsible for the regulation of food intake, it can be hypothesised that the energy density of food can affect satiation and satiety and this may help to explain excess consumption of energy-dense dietary fat (Rolls, 2000).

Blundell et al. (1993) established that in comparison to a HF breakfast (803 kcal/3.36 MJ) test breakfast and a combination control (439 kcal/1.84 MJ), an iso-energetic high-carbohydrate meal suppressed subjective satiety sensations as assessed by visual analogue scale (VAS) but had no influence on subsequent food intake for the remainder of the day. A second experiment by this research group was designed using identical breakfasts but was followed by a snack test meal 90 minutes postprandially. The snack meal was deliberately given to subjects within a time period where the original breakfast meal would be exerting an effect based on the results of the previous study. Results indicated that subjects were significantly more satiated following the ingestion of the CHO supplemented breakfast than the HF breakfast. Even after the HF breakfast was significantly greater than after consumption of the high-carbohydrate breakfast (Blundell et al., 1993). Furthermore, Cotton et al. (1994) conducted a study where a light breakfast (440 kcal/1.84 MJ) was supplemented with either 40 g fat (362 kcal/1.51 MJ) or 90 g carbohydrate (365 kcal/1.51 MJ). It was found that in comparison to the control (light breakfast), the breakfast supplemented with CHO reduced hunger sensations and ad libitum food intake 4.5-h following the test breakfast while the HF breakfast had no effect on satiety. On the contrary, Sepple and Read (1990) found that a HF breakfast reduced subsequent food intake compared to its LF counterpart of equal mass. However, Blundell and Macdiarmid (1997) suggested that the presence of protein within the HF meal could have been responsible for the more satiating effect of this meal.

A weakness of some studies examining the effect of macronutrients on satiety is that preloads are iso-caloric but not of equal mass (Blundell et al., 1993; Cotton et al., 1994;
Holt et al., 1999; Johnson & Vickers, 1993) or vice-versa (Sepple & Read, 1990). Taking meal mass into consideration, Rolls and colleagues (1994) examined the effects of yoghurt supplement preloads varying in macronutrient composition (control, medium-CHO, high-CHO, medium-fat and HF) on satiety. Joule for joule, it was shown that the HF supplements suppressed food intake less at a buffet meal than a high-CHO preload.

Holt and colleagues (1999) established that a breakfast (486 kcal/2 MJ) high in fibre and CHO was more satiating and lead to lower food intake than after consumption of an isoenergetic HF breakfast.

Research by Johnson and Vickers (1993) and Stubbs et al. (1996) have established that protein is the most satiating macronutrient. While the macronutrient content of a snack (239 kcal/1 MJ) was shown to have no effect on ad libitum energy or macronutrient intake at a dinner meal, consumption of a HF or high-CHO snack prompted the request for dinner by 35 and 26 minutes less than a high-protein snack respectively (Marmonier et al., 2000).

Acute oral administration of macronutrients has a different effect on satiety to identical preloads infused directly into the small intestine. Two experiments were conducted where iso-caloric HF and LF soups of equal mass or volume were introduced intragastrically or orally ingested (Cecil et al., 1999). When given intragastrically, similar sensations of hunger and fullness and subsequent food intake were reported, whereas oral ingestion of the HF soup increased satiety and reduced food intake compared to the LF soup. The authors surmised that orosensory stimulation plays an important role in the regulation of appetite (Cecil et al., 1999). On the contrary, Clegg and Shafat (2010) have illustrated that consumption of a HF breakfast test meal led to an average surplus in energy (324 kcal) and fat (15 g) intake compared to a LF iso-energetic meal. Overall, it appears that short-term intake of dietary fat is less satiating per kJ than carbohydrates and protein.

2.4.2.1 Physiochemical properties of fatty acids

The degree of fatty acid saturation may also have important implications for physiological processes related to appetite and eating behaviour (Lawton et al., 2000). Lawton and colleagues explored the effect of degree of saturation, with constant fatty acid chain length (fatty acids predominantly C18) on post-ingestive satiety of fat. Compared to MUFAS, SFAs and PUFAs tended to have a greater influence on satiety. Unlike the 80 g
fat that was incorporated into the test meals by Lawton and colleagues, 30 g fat might not have been a large enough quantity to affect satiety in the studies of MacIntosh et al., 2003 and C asas-Agustench et al., 2008 and Strik et al., 2010. Therefore, in order to elucidate the role of fat in appetite regulation, a HF meal containing 40 – 60 g fat must be used.

2.4.3 Gastrointestinal Function

2.4.3.1 Stomach

The stomach is a hollow, J-shaped organ which can be broken down into the fundus, gastric corpus, antrum and pylorus. The stomach can be divided into three separate regions. The proximal compartment of the stomach, which consists of the fundus and the proximal corpus, acts as a reservoir for the ingested food. The distal compartment of the stomach consists of

the antrum and gastric corpus and generates motor activity for the mixing and grinding of the ingested food to chyme. The pylorus controls the entry of chyme from the stomach into the duodenum in the small intestine via the pyloric sphincter (Tack, 2005).

2.4.3.2 Gastric Distension

Evidence suggests that gastric distension, along with the presence of nutrients in the small intestine, contributes to satiety (Geliebter et al., 1988; Read et al., 1994). Gastric distension is believed to influence appetite through its effect on stretch and tension receptors (Grundy, 2002).

The inverse relationship between an increase in postprandial sensations of hunger and time for 90 % of a meal to empty from the stomach suggests that return of hunger is partly explained by a decrease in gastric distension (Sepple & Read, 1989). It has also been shown that there is a close relation between feelings of fullness and antral distension (Jones et al., 1997). Using a water-filled balloon to imitate gastric distension, Geliebter (1988) demonstrated that food intake was significantly reduced when water volume was greater than or equal to 400 ml in healthy and obese adults. In a second experiment by Geliebter (1988), it was noted that compared to their lean counterparts, obese individuals had a mean gastric capacity for approximately 800 ml more water.
when the balloon was filled gradually at a rate of 100ml·min⁻¹. Beckoff and co-workers (2001) have confirmed that *ad libitum* food intake is inversely associated with volume of gastric contents from a previous meal. Horner and colleagues (2011) speculated that an accelerated GE rate, and therefore reduced gastric distension might lead to over-eating. However, the authors also noted that gastric distension was not the only factor that affected appetite (Horner et al., 2011); the presence of nutrients in the gut also has an important role to play in satiation and satiety (Read et al., 1994).

2.4.3.3 Gastric Emptying

GE is the rate at which a meal is emptied from the stomach into the small intestine. The process is regulated by the proximal and distal compartments of the stomach, the duodenum and extrinsic adjustments by the central nervous system and distal gut factors (Horowitz et al., 1994; American Gastroenterological Association, 2004; Figure 2.1).

The antrum grinds and sieves solids and pumps chyme into the duodenum against the resistance of the pylorus. Timing of antral contractions is dependent on the frequency of electrical tonic pyloric slow waves (usually ~ 3 waves/min), which are generated by the gastric pacemaker and move distally from the proximal stomach to the pylorus. Upon ingestion of a meal, the proximal stomach relaxes to accommodate solids. Nutrient interaction with receptors in the small intestine is responsible for the generation of neural and hormonal feedback that retards GE through processes including: relaxation of the fundus, suppression of antral contraction and stimulation of tonic pyloric and phasic contractions (Rayner & Horowitz, 2005). Phasic and tonic pyloric pressure waves have a fundamental role to play in GE as emptying can only take place when the pylorus is open (Horowitz et al., 1994).
Figure 2.2 Motor events during normal gastric emptying. The fundus relaxes to accommodate the meal, while the antrum grinds and sieves solids, pumping the resultant chyme into the duodenum against resistance generated by phasic and tonic pyloric contractions. The presence of nutrients in the small intestine generates neurohumoral feedback on gastric motor function, enhancing fundic relaxation and pyloric contraction, while suppressing antral motility, with the effect of slowing further emptying to a closely regulated rate (From: Rayner & Horowitz, 2005).
2.5 Gastrointestinal Peptides involved in the Regulation of Gastrointestinal Transit, Appetite and Energy Intake

The presence of dietary fat in the small intestine stimulates the secretion of GI peptides, including cholecystokinin (CCK) (Liddle et al., 1985; Moran & McHugh, 1982), glucagon-like peptide-1 (GLP-1) (Näslund et al., 2001), peptide YY (PYY) (MacIntosh et al., 1999) and suppresses ghrelin (Cummings et al., 2001). All of these peptides are involved in short-term regulation of E I by controlling satiation, satiety and intake of energy at a subsequent meal (Little, 2006) and are shown in Figure 2.3.

2.5.1 Cholecystokinin

Cholecystokinin (CCK) is located predominantly in the I cells of the duodenal and jejunal mucosa of the GI tract and is secreted in response to nutrients, especially dietary fat in the small intestine (Liddle et al., 1985; Belinger & Degen, 2004). In 1973, it was discovered that CCK influenced appetite and energy intake in rats (Gibbs et al., 1973). CCK uses a neural pathway to the brainstem to signal its satiety effects to the hypothalamus via CCK receptors vagal afferents (Huda et al., 2006). Afferent vagal fibres innervating the stomach and the proximal intestine contain CCK receptors, such as CCK1 (CCK-A) which has a dominant role in appetite control (Asin et al., 1992). CCK triggers afferent fibres that are receptive to chemical composition and volume of gastrointestinal contents and feedback is provided to the hypothalamus on the nutrient composition of the meal (Schwartz et al., 1993). Vagotomy or disruption of vagal afferent signaling from the upper GI tract stops the satiety effects that CCK imposes on the hypothalamus (Smith et al., 1981). CCK may also act at local level to enhance mechanical signals of gastric and intestinal distension to the hypothalamus (Moran & Kinzig, 2004). CCK helps to aid nutrient absorption from the gut by stimulating gall bladder contraction, pancreatic enzyme secretion and inhibiting GE (Liddle et al., 1985; Moran & McHugh, 1982). CCK is quickly released from the proximal intestine and has only short bioactivity duration; this makes CCK’s control of eating restricted to finishing a single meal (Moran, 2006). Peripheral administration of CCK has been shown to reduce meal size and the extent of meal time in human and rodent models (Gibbs et al., 1973; Kissileff et al., 1981; Moran & Schwartz, 1994; Muurahainen et al., 1988). West and colleagues (1984) highlighted that long-term, intermittent administration of CCK-8 in free-feeding rats reduced meal size by at least 44%. CCK-8 infusion lead to increase meal regularity by at least 162% and consequently, no decrease in overall energy intake was observed. In comparison to lean controls, fasting CCK levels have been found to be
decreased in females with anorexia nervosa and increased in obese females in comparison to lean controls (Baranowska et al., 2000).

2.5.2 Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is secreted from the L cells in the distal small intestinal mucosa, especially in response to fat ingestion (Feinle et al., 2003). GLP-1 also functions as an incretin; it enhances insulin secretion and suppression of glucagon after feeding (Kreymann et al., 1987). Rat studies have shown that GLP-1 infusion has an inhibiting effect on GE (Tolessa et al., 1998) and food intake (9-39) (Turton et al., 1996). When infused intravenously, GLP-1 delays GE in humans (Delgados-Aros et al., 2002; Flint et al., 2001), this effect is related to relaxation of the proximal stomach (Delgados-Aros et al., 2002) as well as inhibited antral and duodenal activity and tonic and phasic pyloric wave stimulation (Schirra et al., 2000). Exogenous administration of GLP-1 has been shown to inhibit GE in healthy adults (Delgados-Aros et al., 2002; Nuack et al., 1997) as well as obese (Flint et al., 2001; Näslund et al., 1998) and T2DM patients (Meier et al., 2003). Research by Verdich et al. (2001) and Näslund et al. (1999) has illustrated that intravenous GLP-1 administration reduces appetite and food intake. However, opposing results have been demonstrated by others (Brennan et al., 2005; Long et al., 1999).

2.5.3 Peptide YY

Peptide YY (PYY) is secreted from the L cells in the ileum and large intestine, predominantly after ingestion of long-chain fatty acids (Pappas et al., 1986). Secretion of this peptide has also been recorded to occur in response to neurohumoral signals from the proximal gut, especially CCK (Lin et al., 2003). When intravenously infused, PYY inhibits meal-stimulated gastric, pancreatic secretion, delays GE and intestinal transit in a dose-dependent manner in healthy adults (Savage et al., 1987). Intravenous infusion of PYY has been reported to suppress EI for up to 12 hours in healthy and obese individuals suggesting that PYY may play a role in longer-term appetite regulation (Batterham et al., 2003; Batterham et al., 2002). However, more recent research suggests that PYY infusion may only cause suppression of EI at very high concentrations and this may be associated with feelings of nausea in subjects (Degen et al., 2005).

2.5.4 Ghrelin

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor is mainly produced in the fundic mucosa (Kojima et al., 1999). Unlike the previously mentioned
peptides, ghrelin is suppressed following food intake (Parker et al., 2005; Brennan et al., 2007; Kojima et al., 1999). Studies on rodents have illustrated that ghrelin has an important role to play in energy homeostasis; the hormone acts peripherally to stimulate EI and increase adiposity (Wren et al., 2001; Tschop et al., 2000). Previous studies have shown that ghrelin levels decrease after the consumption of food in lean individuals (Shiiya et al., 2002; Tschop et al., 2001). Ghrelin may have a role to play in meal initiation; Cummings et al. (2001) has demonstrated that circulating levels of ghrelin rise prior to and fall after food consumption in healthy individuals. When compared to healthy, lean subjects, 24-h plasma ghrelin profiles of obese and anorexic individuals illustrated higher and lower plasma levels respectively (Shiiya et al., 2002). The state of nutrition is a determinant of circulating ghrelin in humans where ghrelin secretion is ‘up-regulated’ and ‘down-regulated’ in a state of negative and positive energy balance respectively (Valera Mora et al., 2005). In comparison to their lean counterparts, ghrelin and leptin concentrations did not fall after consumption of a test meal in obese adults (English et al., 2002). The lack of postprandial suppression of ghrelin in obese patients could lead to increased EI and could show the involvement of ghrelin in the pathogenesis of obesity.
Figure 2.3 A hypothetical model of the relative contributors of gastric distension, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin (all episodic signals influences by gastric emptying) to satiation to satiety. Time (x-axis) and magnitude (y-axis) are dependent on the meal characteristics. Gut peptides are particularly sensitive to specific macronutrients (e.g., CCK to fat and protein (Liddle, 1997); GLP-1 to carbohydrate and fat (Belinger & Degen, 2006); PYY primarily to fat (Belinger & Degen, 2006); ghrelin suppression to protein and carbohydrate (Foster-Schubert et al., 2008) and peak at different time points. Ghrelin is the only peptide implicated in meal initiation, rising before a meal and falling approximately 60 min after a meal (Cummings et al., 2001). During a meal and shortly after, gastric distension and CCK (peaking within 15 min (Boyd et al., 2003) are important determinants of satiation. The release of GLP-1 and PYY occurs in a biphasic pattern and can remain elevated for several hours after a meal (Cummings & Overduin, 2007; Adrian et al., 1985). As the stomach continues to empty, it is likely that the exposure of nutrients to the distal small intestine has an increasingly greater contribution to satiety. These episodic signals may also be modulated by tonic signals such as leptin and insulin, which are responsive to energy availability and body adiposity. (From: Horner et al., 2011).
2.6 Central Regulation of Appetite

The hypothalamus and its intricate arrangement of nuclei and neuronal circuits have a fundamental role to play in appetite regulation. The arcuate nucleus (ARC) of the hypothalamus is the site of two neuronal populations, namely orexigenic (involved in food stimulation) and anorexigenic neuropeptides (involved in suppression of food intake). The aforementioned neuropeptides signal from the ARC to the paraventricular nucleus (PVN) as well as the other hypothalamic nuclei including the dorsomedial nucleus, lateral hypothalamic area and the ventromedial hypothalamic nucleus (VMN) (Suzuki et al., 2010).

2.6.1 Orexigenic Neuropeptides

2.6.1.1 Neuropeptide Y

Neuropeptide Y (NPY), a 36-amino acid neuropeptide is one of the most potent orexigenic in the central nervous system. When administered chronically into the hypothalamic PVN, NPY leads to continuous hyperphagia and adiposity; whereas an NPY antagonist caused a reduction of energy intake (Kalra et al., 1999). NPY concentrations increase rapidly before meals in the PVN and elevated concentrations are maintained in the absence of food; this illustrates that NPY has an important role to play in the central control of meal initiation (Neary et al., 2004). The majority of NPY neuropeptides (~90%) are involved in the co-excretion of a gut-related peptide (Meister, 2007).

2.6.1.2 Agouti related peptide

Agouti related peptide (AgRP), an endogenous antagonist of melanocortin MC3 and MC4 receptors, is also involved in the food intake stimulation. In contrast to the comparatively short-lived effect of NPY, central administration of AgRP to rodents results in increased food intake for up to 7 days (Rossi et al., 1998). Long-term administration of AgRP causes unremitting hyperphagia as well as onset of obesity (Small et al., 2001). Both NPY and AgRP neurones are stimulated by ghrelin (Nakazato et al., 2001) and repressed by leptin and insulin (Kalra et al., 1999).
2.6.2 Anorexigenic Neuropeptides

2.6.2.1 Melanocortins

The melanocortins, derived from the pre-cursor molecule pro-opiomelanocortin (POMC) through the production and release of α-melanocyte-stimulating hormone peptide, are classified as one of the prominent anorexogens (Neary et al., 2004). The α-melanocyte-stimulating hormone peptide (α-MSH) is the main endogenous antagonist that activates MC3 and MC4 melanocortin receptors. The stimulatory influence of ArRP on food intake is inhibited by α-MSH (Rossi et al., 1998). The MC4 receptor is noted to have a critical influence on body weight regulation. Huszar and co-workers (1997) have demonstrated that the MC4 knockout mouse is insensitive to α-MSH and demonstrates hyperphagia and a high level of adiposity. It should also be noted that mutations of the MC4 receptor is evident in 4% of adults who were diagnosed with severe childhood-onset of obesity (Farooqi et al., 2000). Peripheral administration of PYY 3-36 increases expression of POMC and inhibits NPY (Batterham et al., 2002).

2.6.2.2 Cocaine- and amphetamine regulated transcript

Cocaine- and amphetamine regulated transcript (CART) neuropeptides are highly expressed in hypothalamic nuclei and have a role to play in the regulation of feeding behaviour (Lambert et al., 1998). Lambert and colleagues (1998) demonstrated repressed nocturnal and fasting-induced feeding in rodents after CART administration. Expression of CART is regulated by numerous peripheral appetite-related peptides including leptin, ghrelin and CCK (Murphy, 2005; de Lartigue et al., 2007).

CART peptides are also located in the ventral tegmental area and nucleus accumbens; these parts of the brain which contribute to the mesolimbic dopamine pathway and are related to reward and reinforcement (Dallvechia-Adams et al., 2002). CART controls mesolimbic dopaminergic activity (Yang et al., 2004) and attenuates feeding (Yang et al., 2005); this highlights the complex association between CART, mesolimbic dopaminergic, and the brain's reward/reinforcement pathways (Jones & Kuhar, 2006).
2.7 The Role of Gastric Emptying in the Regulation of Postprandial Glycaemia and Lipaemia

Independent of its influence on appetite regulation, rate of delivery of nutrients to the small intestine can also influence metabolic variables in the postprandial period. Healthy adults are in the postprandial state for approximately 17-h of a 24-h period (Williams, 1997). It has been established that non-fasting triglyceride (TG) levels are independently associated with cardiovascular events (Bansal et al., 2007). Elevated postprandial TG concentrations are associated with greater risk of developing atherosclerosis than elevated fasting concentrations (Patsch et al., 2002; Karpe et al., 1994). Similarly, blood glucose response 2-h postprandially is a more accurate predictor of mortality from CVD than fasting blood glucose (DECODE, 2001). Accelerated GE, through exposure to a HF diet may lead to heightened postprandial glycaemia and lipaemia (Darwiche et al., 2002) and thus increased risk for development of atherosclerosis, coronary artery disease and T2DM (Castiglione et al., 2002).

GE accounts for 34% of the variance in peak plasma glucose following a 75 g oral glucose tolerance test in healthy adults and in patients with early stage T2DM (Horowitz et al., 1993). Horowitz et al. (1993) showed that plasma glucose concentrations at 120 min postprandially were inversely associated with GE ($r = -0.56; p < 0.05$). Postprandial glycaemic and insulin responses of healthy adults were lowered by adding 50 g of fat to a test meal (Cunningham & Read, 1989). Upper GI tract function, especially GE rate is a fundamental regulator of postprandial glycaemic concentrations (Rayner et al., 2001). According to Kolterman et al. (1996), changing GE rate could be used as a mode to optimise glycaemic control in diabetic patients.

Short-term intake of a 3-day HF supplemented diet rich in PUFA derived from sunflower oil led to a reduction in postprandial TG and cholesterol levels in healthy adults even though GE was accelerated; this suggests that short-term intake of a HF diet is not detrimental to cardiovascular health’ (Clegg et al., 2011). However, it should be noted that these findings could be related to the fatty acid composition of the HF supplement (90 g fat·day$^{-1}$) and test meal (both rich in sunflower oil). Thomsen and colleagues (2003) have previously shown that a HF unsaturated meal rich in olive oil induced significantly lower TG responses than a meal rich in saturated fats derived from butter in T2DM patients. Robertson et al. (2004) conducted a 21-day mixed HF supplemented diet (88 g fat·day$^{-1}$) pilot study in six healthy adults. They found that TG concentrations to an oral fat tolerance test were only significantly elevated following 14-21 days of dietary supplementation, whereas GE half-time was significantly accelerated.
after 7 days of the HFD. The authors suggested that alterations in absorptive function occurred over the 21-day period were mediated by a faster entry of fat into the small intestine (Robertson et al., 2004).

Given the high prevalence rates of T2DMs in today’s society; pharmacological and nutritional models that may alter GE and hence influence metabolic perturbations such as postprandial glycaemia and lipaemia merit further investigation.

2.8 Gastric Emptying of Liquids and Solids

Numerous factors including energy and macronutrient content, volume, mass, particle size, viscosity and pH of stomach contents can influence GE rate (Sherwood, 2004, p 606).

The emptying rate of liquids (Figure 2.2) from the stomach is more rapid than the rate of solid GE. Previous studies have described the pattern of liquid emptying from the stomach as ‘exponential’ (Horowitz et al., 1983; Wright et al., 1983). However, Hopkins (1966) suggested that the pattern is more accurately described by relating the square root of the volume of the liquid remaining to time (Freeman & Balan, 2007, p183). Liquid GE is dependent on fundal tone, since the pressure gradient between the stomach and the duodenum in the small intestine controls this type of emptying (Horowitz et al., 1993). Energy dense liquids empty more slowly from the stomach than liquids of lower energy density (Freeman & Balan, 2007, p183).

Solid GE (Figure 2.4) is described as an early lag phase, the time before the first part of the meal enters the small intestine, followed by a linear pattern of emptying (Horowitz et al., 1983; Wright et al., 1983). After ingestion, food enters the fundus immediately. The fundus fills with food until meal completion and subsequently, it empties in a linear pattern. During the fundic filling phase, food leaves and begins to fill the antral area of the stomach. Prior to emptying into the duodenum, the antrum fills to a certain level. Therefore, complete antral filling does not take place until after meal completion so that there is a delay before food empties from the stomach. The lag phase is a ‘reflection of redistribution of food from the fundus to the antrum’ (Sheiner et al., 1980) and also the time taken for solid food to be reduced to small particles (Horowitz et al., 1983). Solid GE is dependent on factors including fundal tone, stomach volume and antral motility. Once GE commences, it is linear because of the capacity of the antrum to sustain a constant volume, similar amounts enter from the fundus and depart from the antrum.
After the ingestion of a meal containing solid and liquid components, Houghton (1988) observed that the solid component of the meal remained in the fundus until nearly 80% of the liquid component had emptied. The emptying of a semi-solid food is ‘generally intermediate’ between liquid and solid GE, but its pattern is dependent on the nutrient content and volume of the meal (Freeman & Balan, 2007, p183).

![Figure 2.4 Gastric Emptying Curves for Solids and Liquids in Healthy Individuals (From: Freeman & Balan, 2007, p183)](image)

2.9 Measurement of Gastric Emptying Rate

The earliest gastric emptying study recorded dates back to early Roman times. Holy Roman Emperor Frederic II killed and disembowelled two men in order to satisfy his curiosity on the effect of rest and vigorous exercise on the GE of a large meal (McDowall, 1955). In present times, less drastic techniques are employed to calculate gastric emptying in humans. Numerous techniques are used for the calculation of GE; all of which have their strengths and limitations (Table 2.1). A more detailed account of the most common techniques, namely radiology, intubation or aspiration of gastric contents, ultrasonography, the paracetamol absorption test and the $^{13}$COBT will be discussed below. The gastric emptying technique of choice is dependent on a number of factors such as the properties of the test meal, necessity for measurement precision, ethical considerations, facilities available and the degree of invasiveness tolerable to a patient or subject (Maughan & Leiper, 1996).
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<td><strong>Scintigraphy</strong></td>
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<td><strong>Ultrasonography</strong></td>
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<tr>
<td><strong>Electrical Impedance Tomography</strong></td>
<td>Non-invasive</td>
<td>Need for gastric acid suppression</td>
</tr>
<tr>
<td><strong>Paracetemol Absorption test</strong></td>
<td>Safe</td>
<td>Indirect measurement</td>
</tr>
<tr>
<td></td>
<td>Economical</td>
<td>Liquid GE only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Affected by drug absorption rate</td>
</tr>
<tr>
<td><strong>Magnetic Resonance Imaging</strong></td>
<td>Provides imaging of gastric function</td>
<td>Expensive machinery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Operator expertise</td>
</tr>
<tr>
<td><strong>$^{13}$C Octanoic Acid Breath test</strong></td>
<td>Non-invasive</td>
<td>Indirect measurement</td>
</tr>
<tr>
<td></td>
<td>Reliable</td>
<td>Does not provide imaging of gastric function</td>
</tr>
<tr>
<td></td>
<td>Sage</td>
<td>Requires normal small intestine, liver &amp; lung function</td>
</tr>
</tbody>
</table>
2.9.1 Radiology

Radiological observation was used in the early 1900's (Cannon, 1911). However, the technique was deserted due to radiation exposure and the non-physiological nature of the barium meal. Barium is a dense, heavy liquid; liquid and solids consumed normally by humans have a density similar to water (Holder, 2005, p188). Later, Feldman and colleagues (1984) employed radio-opaque markers to study gastric emptying. Kondo (2000) reported that this technique was beneficial for examining the GE of indigestible solids, which may 'represent the integrity of the interdigestive migrating motor complex'.

2.9.2 Aspiration of Gastric Contents

The double sampling gastric aspiration was first introduced by George (1968). It is a direct, invasive method and allows for the collection of serial measurements and the time course of liquid gastric emptying to be followed. It involves introducing a standard volume of a non-absorbable marker such as polyethylene glycol or phenol red in a test drink into the stomach through a nasogastric tube (Chaudhuri et al., 1975). Gastric contents are completely aspirated at set times in the postprandial period. The technique also allows for simultaneous measurement of gastric secretion volume which helps to calculate the exact rate of gastric evacuation (George, 1968). Aspiration works on the principle that the volume of liquid in a container can be determined by calculating the rise in concentration of a dye produced by the addition of a small concentrated measure of the same dye (ibid). Weaknesses associated with intubation include its inability to measure solid GE and introduction of the naso-gastric tube may cause patient discomfort such as nausea or emesis.

2.9.3 Scintigraphy

Since 1966, scintigraphy has been recognised as the 'gold standard' or 'reference method' for measuring GE in humans (Kondo, 2000) as it offers a physiologic, non-invasive and quantitative measurement of GE (Griffith et al., 1966; Abell et al., 2007; Szarka & Camilleri, 2009). The technique involves radiolabeling the liquid or solid part of a meal. Solid phase scans typically involve labelling an egg meal with 99m technetium-sulfur colloid. Following ingestion of the labelled meal, a gamma camera is placed over the abdominal region to record radioactivity at frequent intervals—usually every 10 minutes for 3-4 h postprandially (Holder, 2005, p188). Scintigraphy is used to calculate gastric counts, which have been found to directly relate to the amount of food remaining in the stomach, without the need for 'geometric assumptions' regarding the shape of the stomach (Maurer et al., 2002, p173). Another advantage of scintigraphy is
that it has the ability to measure liquid and solid phases of GE simultaneously (dual labelled scintigraphy) if the liquid component of the test meal is labelled with a different radioactive marker (Bromer et al., 2002). Scintigraphy is not without its limitations; the use of scintigraphy requires operator expertise and also has the disadvantage of emitting significant radiation. This makes it difficult to use the technique for repeated measurements over a short time period, especially in vulnerable populations such as children and pregnant women (Galmiche et al., 1998). Additional weaknesses of the technique include factors such as Cramer scatter, count attenuation and overlap imaging (Perri et al., 1998).

2.9.4 Ultrasonography

Ultrasonography has also been suggested as a non-invasive reproducible measure of liquid and semi-solid GE (Irvine et al., 1993; Dumitrascu et al., 1995). GE rates using ultrasonography correlate well with scintigraphy data (Hveem et al., 1996; Marizo et al., 1989). Ultrasonography is an indirect measure of gastric emptying by examining gastric size and does not involve the measurement of any metabolic pathways (Cappello et al., 2000). Limitations of the test include the need for subjects to remain still during measurements, competent operation and the issue that the placement area of the scan is open to subjectivity.

2.9.5 Paracetamol Absorption Test

The paracetamol absorption technique is a simple, non-invasive and economical method of assessing GE. Similar to the $^{13}$C OBT, the test is based on the principal that as paracetamol is rapidly absorbed upon reaching the small intestine, appearance of the drug in the blood acts as an indirect model for GE (Heading et al., 1973). A review conducted by Willems et al. (2001) identified 13 studies that found a good correlation ($r > 0.6$) between the paracetamol absorption technique and scintigraphy. Medhus et al. (1999) illustrated that the paracetamol absorption test is a sensitive test for assessing delayed and accelerated gastric emptying of a high caloric liquid meal when an algorithm that controls for individual pharmokinetics is employed. Weaknesses of the paracetamol test include that it cannot be used to measure solid GE. Furthermore, drug interactions such as oral contraceptive usage may enhance paracetamol clearance by almost 50% (Medhus et al., 1999).
2.9.6 $^{13}$C Octanoic Acid Breath Test

In 1993, Ghoos and colleagues proposed a safe, reliable and non-invasive alternative to scintigraphy for measurement of solid GE called the $^{13}$C octanoic acid breath test (OBT). The equivalent compound for measuring liquid GE is known as the $^{13}$C sodium acetate. The OBT uses a medium chain fatty acid called octanoic acid labelled with $^{13}$C as a marker of GE of the test meal. The test is based on the principle that upon reaching the duodenum in the small intestine, the labelled octanoate, is rapidly absorbed and is oxidised in the liver and excreted in breath as $^{13}$CO$_2$ (see Figure 2.5). The rate of pulmonary excretion of $^{13}$CO$_2$ acts as an indirect measurement of GE (Sanaka et al., 2004), with the latter acting as the rate-limiting step of this process (Pukkinen et al., 2006).
Figure 2.5 Sequential metabolic steps of Orally Administered $^{13}$C-labelled egg yolk. The excretion of $^{13}$CO$_2$ in breath is limited by the gastric emptying of the yolk (Adapted from: Sanaka et al., 2004; Perri et al., 2005).

A limitation to the OBT is that it does not allow for imaging or ‘regional assessment of gastric function’ (Maurer et al., 2002, p171). Results obtained using the OBT are similar to scintigraphy results based on the assumption that absorption, oxidation and excretion are comparable in each ‘normal individual’ (Ghoos et al., 1993; Choi et al., 1998). The OBT may overestimate GE parameters e.g. half-time and lag phase, compared to scintigraphy. It has been hypothesised that the greater duration of these GE parameters is a result of the time it takes for absorption and oxidation of octanoic acid subsequent to its emptying from the stomach (Ghoos et al., 1993). Furthermore, the breath test can
only be used to assess GE in populations with normal function of the small intestine, liver and lungs (Kondo, 2000). Despite its limitations, the non-invasive, non-radioactive nature of the $^{13}$C OBT and its lack of dependency on a skilled operator make the test suitable for repeated measurements. Thus, the OBT was chosen as the measurement technique for GE in the body of work presented in this thesis. In acknowledgement that intra-individual variability is a limitation in the measurement of GE, it was decided to use repeated-measures designed studies.

### 2.9.6.1 Validity and Reproducibility of the $^{13}$C Octanoic Acid Breath Test

Numerous validation studies have established that the $^{13}$C OBT is strongly correlated with scintigraphy for GE of liquid, semi-solid and solid meals in normal subjects and dyspeptic patients (Ghoos et al., 1993; Braden et al., 1995; Bromer et al., 2002). Furthermore, the OBT has been compared to ultrasonography (Capello et al., 2000; Punkkinen et al., 2006).

When compared to scintigraphy, GE half-time using 6-h breath collection from $^{13}$C OBT was shown to correlate positively with equivalent scintigraphy measures for solid ($r = 0.66, p = 0.05$) but not liquid GE ($r = 0.13, p = 0.74$) in 10 healthy adults (Bromer et al., 2002). It should be noted that the OBT overestimated GE half-time in comparison to scintigraphy. In another arm of this study, 23 patients with dyspeptic symptoms underwent simultaneous GE and OBT measurements and positive correlations were found for both solid and liquid half-emptying times (ibid). Similar findings were observed when Capello et al. (2000) compared solid-liquid meal GE measured with the OBT to ultrasonography in 14 healthy volunteers. Although the authors found a significant positive correlation between the two measures, the OBT over-reported GE parameters.

In contrast to the previous study, a more recent study by Punkkinen et al. (2006) only reported a weak correlation between the OBT and scintigraphy in a dyspeptic patient population. Criticisms of this study include the fact that measurement techniques were not conducted simultaneously and that different test meals were used. Given that GE is already a variable outcome measure with inter- and intra-individual variability (Brophy et al., 1986); results of this poorly controlled study should be interpreted with caution.

The reproducibility of the $^{13}$C OBT to measuring GE of solids in comparison to scintigraphy was examined by Choi et al. (1998) and results showed that within- and between-subject variability in GE measurements with the OBT agreed significantly with the variability observed in scintigraphy.
2.9.6.2 Complex theory of $^{13}$C kinetics

The simple sequential processes of the $^{13}$C OBT as originally described by Ghoos et al. (1993) are previously outlined in Section 2.6.6. The original theory of the OBT overlooks the more complex theory of the kinetics of the $^{13}$C label (Sanaka et al., 2008). A more detailed explanation of the theory of handling of $^{13}$C octanoate in the human body is outlined in Figure 2.6. The $^{13}$C labelled octanoate is absorbed after its departure from the stomach. The $^{13}$C labelled substance is then transferred to the liver via the portal venous system. It is preferentially oxidised in the liver to $^{13}$CO$_2$ (Perri et al., 1998). A small fraction of $^{13}$C drifts to another metabolic pathway; here $^{13}$C is temporarily fixed. The temporal fixation is known as the first-pass effect during absorption. After appearance in systemic circulation, $^{13}$C distributes over the bicarbonate pool in conjunction with its excretion through the respiratory and non-respiratory systems. Distribution is the ‘reversible movement’ of H$^{13}$CO$_3$ between the blood and the tissues; it takes place at ‘various rates and to various extents.’ H$^{13}$CO$_3$ infiltrates numerous organs throughout the body i.e. the blood bicarbonate pool. The well-perfused tissues e.g. heart, kidneys and the brain act in response to alterations in circulating CO$_2$ quicker than low-perfused tissues e.g. bone and skeletal muscle with equilibrium between the blood and low-perfused tissues (Sanaka et al., 2004). Equilibrium between the blood and the less-perfused tissues can take several hours to equilibrate. Thus the process of H$^{13}$CO$_3$ returning to the blood from the low-perfused tissues can be considered ‘irreversible non-respiratory elimination’ because of the duration of the conventional OBT (4 – 6-h) (Sanaka et al., 2004). The irreversible loss of $^{13}$CO$_2$ from the blood is known as elimination (Sanaka et al., 2004); $^{13}$C atoms get cleaved off of from octanoate by $\beta$-oxidation and enter the TCA cycle as acetyl CoA to form energy (Sherwood, 2004, p36).
Figure 2.6 Potential sites of Loss and Retention of Orally Administered $^{13}$C Octanoic Acid for Determination of Gastric Emptying Rate (Adapted from: Sanaka et al., 2004; Perri et al., 2005).
2.9.6.3 Methods of Analysis of the $^{13}$C Octanoic Acid Breath Test

Mathematical Model of Ghoos et al. (1993)

Ghoos and colleagues devised a mathematical model for the derivation of GE parameters from the $^{13}$C OBT. The formula came from the percentage of the cumulative pulmonary excretion of the $^{13}$C dose. The model was formed on the basis that ‘the breath test curve representing the cumulative dose in function of time is inversely analogue to the scintigraphic curve of gastric emptying’ (Ghoos et al., 1993). The technique involves fitting the raw data from the OBT to a modelled curve using a solver function in Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA). The modelled curve is established based on the following formula:

$$y = m (1 - e^{-kt}) \beta,$$

Where: $y$ is the percentage of cumulative $^{13}$C excretion in breath (PDR)

- $t$ is time
- $m$, $k$ and $\beta$ are constants with $m$ being the cumulative $^{13}$C recovery at infinite time

This formula allows for the calculation of values for the constants which are subsequently used to calculate the following GE parameters (see Figure 2.7) proposed by Ghoos et al. (1993):

1. Lag phase = $[\ln \beta/k]$

   - Lag phase describes the time taken to reach maximal excretion of $^{13}$CO$_2$ (Jackson et al., 2004). The parameter relates to the ‘time of the inflection point of the cumulative curve’ (Schommartz et al., 1998)

2. Half-time = $[-1/k] \times \ln [1 - 2^{-1/\beta}]$

   - This parameter describes the ‘time taken for 50 % of the $^{13}$C dose to be excreted’ in breath (Jackson et al., 2004).

Schommartz et al. (1998)

In 1998, Schommartz and colleagues proposed two more GE parameters:

1. Latency time = $1/k \times [\ln (\beta) + 1/\beta -1]$
• This describes the ‘initial delay of the cumulative exhalation curve’ and is the point of intersection of the tangent at the inflection point of the cumulative curve and the x-axis (Schommartz et al., 1998).

Ascension time = -1/k x [ln (1-2^{-1/β}) + ln (β) + 1/β -1]

• This parameter characterises ‘the time interval from latency phase to half-emptying time’ and represents the period of time where $^{13}$C excretion rates are high (Schommartz et al., 1998).

Figure 2.7 Schematic representation of gastric emptying parameters (adapted from: Schommartz et al., 1998). The solid purple line represents the total cumulative $^{13}$CO$_2$ excretion curve. GE half-time was calculated using the formula of Ghoos et al. (1993). Latency time is calculated as the intersection of the tangent (black dashed line) at the inflection point and the x-axis (Adapted from: Schommartz et al., 1998).
2.9.6.4 Other Methods of Measuring GE Parameters

Since the development of the non-linear regression method of Ghoos et al. (1993), a number of other models have been created for measurement of GE parameters from the $^{13}$COBT including the generalised linear regression (Viramontes et al., 2001), the Wagner-Nelson methods (Sanaka et al., 2006) and the Bayesian hierarchical modelling method (Bluck et al., 2011).

Odunsi and colleagues (2009) technically evaluated mathematical models to estimate solid GE half-time of a LF egg meal by $^{13}$C OBT with half-time from simultaneously measured scintigraphy. Total cumulative breath $^{13}$CO$_2$ excretion and four mathematical methods previously employed in the literature were used: (1) Ghoos method (Ghoos et al., 1993), (2) generalised linear regression (Viramontes et al., 2001), (3) linear regression (Szarka et al., 2008), and (4) the Wagner-Nelson method (Sanaka et al., 2006). A minimum of 12 breath samples were collected over ≥ 4-h postprandial period. The authors used the concordance correlation coefficient (CCC) for half-time calculated from each method as compared to half-time obtained from scintigraphy. The researchers found that linear regression (Viramontes; CCC = 0.93) and generalised linear regression (Szarka; CCC = 0.96) models provided the most accurate analyses of breath $^{13}$CO$_2$; the former methods are cost-effective due to the requirement of a low number of breath samples for the estimation of GE (approximately 5 samples). This evaluation used a large sample size and a wide spectrum of GE rates; thus making the results of the analyses representative of the whole population. It should also be noted that Odunsi and colleagues (2009) found that cumulative excretion of breath $^{13}$CO$_2$ method (CCC = 0.77) displayed a greater concordance correlation coefficient than the Ghoos method (CCC = 0.43) (Ghoos et al., 1993).

2.10 Measurement of Mouth to Caecum Transit Time

The H$_2$ breath test is a non-invasive, inexpensive test which can be used for measurement of mouth to caecum transit time (MCTT) or oocaecal transit time (Di Stefano et al., 2003). The measurement is based on the principle that the substrate ingested (e.g. lactulose or inulin) is indigestible in the small intestine i.e. H$_2$ production is negligible but upon reaching the caecum in the large intestine is rapidly metabolised by colonic bacteria (Bond & Levitt, 1975). Time between ingestion of the unabsorbable carbohydrate or fibre and rise in breath H$_2$ in end-exhalation is representative of MCTT; defined as an increase in breath H$_2$ over three consecutive readings of a minimum of a cumulative 10 parts per million (ppm) (Bond & Levitt, 1975).
Bond and Levitt (1975) found that transit time of lactulose measured by H₂ excretion was positively related to time for polyethylene glycol (PEG) to reach the distal ileum simultaneously \((r = 0.97)\), with the appearance of PEG occurring on average 7.6 minutes prior to H₂ excretion. A strong association between intestinal transit measured by the lactulose H₂ breath test and scintigraphy was examined in patients with ileoanal pouches (Ternent et al., 2001).

In 2003, Geboes et al. stated that there was a necessity for a more appropriate substrate for measurement of MCTT. Weaknesses of the lactulose as a substrate for the H₂ breath test included factors such as poor reproducibility and that the results of the MCTT are dose dependent. Bond and Levitt (1975) reported an inverse relationship between transit time and dose of lactulose (5, 10 or 20 g) in 9 subjects suggesting that as the quantity of the substrate increased, MCTT is accelerated. Geboes et al. (2003) showed a significant positive association between ileocaecal transit time using inulin and lactose \(^{13}\)C ureide \((r = 0.85; p < 0.001)\). In comparison to lactulose, 5 – 10 g inulin had no influence on transit time. The authors showed that neither substrate influenced GE despite previous findings that suggested that lactulose slowed GE (Miller et al., 1997). A recent study by Clegg and Shafat (2010) compared MCTT and GE of a LF pancake test meal using 12 g (i) liquid lactulose (L-L), (ii) solid lactulose (L-S) and (iii) solid inulin (IN-S) as the substrates. Employing L-L as the breath test substrate resulted in the shortest MCTT (85 min ± 43) compared with L-S (162 min ± 63) and IN-S (292 min ± 67; \(p = 0.007)\). In agreement with Miller et al. (1997), the authors found that L-L led to the slowest GE. Findings of another aspect of the experiment illustrated that IN-S reduced GE lag and latency phase (i.e. onset of stomach emptying) compared to a test meal containing no substrate, but had no affect on GE half-time. Based on these findings, Clegg and Shafat (2010) concluded that inulin is the most appropriate substrate for the H₂ breath test.

### 2.11 Change in Gastric Emptying in Obesity

A considerable body of research has compared GE in obese and lean cohorts (for detailed review see Clegg & Shafat, 2009). It was originally postulated by Hunt et al. (1975) that an enhanced gastric rate may reduce the satiating effect of food, thus leading to overeating and the onset of obesity. However, as can be seen in Table 2.2, finding of studies investigating whether GE is accelerated in the obese state are contradictory. Several studies have reported accelerated or shorter GE of solid (Wright et al., 1983; Zahorska-Markiewicz et al., 1986; Tosetti et al., 1996; Gryback et al., 1996; Näslund et al., 1998; Valera-Mora et al., 2005; Cardoso-Junior et al., 2007) and liquid test meals
(Vasquez-Roque et al., 2006) in obese individuals. Greater efficiency of antral grinding as well as the capacity to secrete more gastric fluids have been proposed as mechanisms in obese individuals that allow for increased emulsification of solid foods compared to their lean counterparts (Wright et al., 1983). On the contrary, further evidence suggests that GE is delayed or longer in the obese individuals for emptying of solid (Horowitz et al., 1983; Horowitz et al., 1986; Maddox et al., 1989; Jackson et al., 2004) and liquid meals (Horowitz et al., 1986; Maddox et al., 1989). Recently it was suggested that the slower GE observed in the obese may be a consequence of reduced fundal tone, a change in the sensitivity of stretch receptors or an alteration in antral area and mixing (Jackson et al., 2004). This research group have also hypothesized that a delayed GE rate may lead to the development of obesity instead of being an outcome of the obese state. Furthermore, numerous studies have documented no differences in solid (French et al., 1993; Glassbrenner et al., 1993; Hutson & Wald, 1993; Verdich et al., 2000) and liquid (Wright et al., 1983; Horowitz et al., 1983; Wisen & Johansson, 1992; Glassbrenner et al., 1993; Hutson & Wald, 1993; Cardoso-Junior et al., 2007) GE time between lean and obese individuals. The inconsistencies observed in findings may be a consequence of variations in methodologies and techniques employed to measure GE (see Section 2.9).

In Table 2.2, 15 studies are presented in which:

- Seven studies suggest that GE of solid food is accelerated in the obese state, while four studies suggest that obesity has no impact on GE and four studies suggest that obesity leads to delayed GE of solids.
- One study suggests that GE of liquids is accelerated in obesity, while six studies suggest that obesity does not impact on GE and two studies found that obesity led to delayed GE of liquid meals.
<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects</th>
<th>Method</th>
<th>Test Meal</th>
<th>GE in Obese</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright et al. (1983)</td>
<td>46 obese; 31 non-obese</td>
<td>Scint 212/81 (HF)</td>
<td>↑ ↔</td>
<td>Mixed (matched)</td>
<td></td>
</tr>
<tr>
<td>Horowitz et al. (1983)</td>
<td>15 obese; 11 lean</td>
<td>Scint 272/0</td>
<td>↓ ↔</td>
<td>Mixed</td>
<td></td>
</tr>
<tr>
<td>Horowitz et al. (1986)</td>
<td>7 obese; 11 lean</td>
<td>Scint 270/0 (HF) or 153</td>
<td>↓ ↓</td>
<td>Mixed</td>
<td></td>
</tr>
<tr>
<td>Zahorska-Markiewicz et al. (1986)</td>
<td>31 obese; 21 lean</td>
<td>Scint 410-440 (HF) ↑</td>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maddox et al. (1989)</td>
<td>31 obese; 31 lean</td>
<td>Scint 272/61 ↓ ↓</td>
<td>Mixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wisen &amp; Johansson (1992)</td>
<td>7 obese; 9 lean</td>
<td>Multiple marker dilution method 432 (HF) − ↔</td>
<td>Mixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>French et al. (1993)</td>
<td>8 obese; 7 lean</td>
<td>Scint 72 (LF) ↔ −</td>
<td>Mixed (matched)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glassbrenner et al. (1993)</td>
<td>24 obese; 8 lean</td>
<td>Scint 317 (HF) ↔ −</td>
<td>Mixed (matched)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hutson &amp; Wald (1993)</td>
<td>30 obese; 23 non-obese</td>
<td>Scint 379 ↔ ↔</td>
<td>Mixed (matched)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tosetti et al. (1996)</td>
<td>20 obese; 20 lean</td>
<td>Scint 638 ↑ −</td>
<td>Mixed (matched)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gryback et al. (1996)</td>
<td>9 obese; 21 lean</td>
<td>Scint 360 ↑ −</td>
<td>Mixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Näslund et al. (1998)</td>
<td>9 obese; 9 lean</td>
<td>US 280 ↑ −</td>
<td>Mixed (matched)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verdich et al.</td>
<td>19 obese;</td>
<td>Scint 597 ↔ −</td>
<td>Males</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Inconsistencies in Gastric Emptying Findings

Inconsistencies in the literature with regards to GE rates in obesity can be attributed to numerous factors including measurement techniques employed and differences between study designs such as variation in test meals, failure to use gender-, age- and height-matched pairs and control for the menstrual cycle.

Variation in test meals may play a fundamental role in the contradictory findings. Properties of a test meal such as meal composition, caloric density, weight or volume may all influence GE (Jackson et al., 2004). Factors such as a HF content (Hunt & Knox, 1968; Cecil et al., 1999; Clegg et al., 2010) or high-energy content of a meal (Jackson et al., 2004) are all potent delayers of GE. It is evident from Table 2.3 that test meals varied in caloric and dietary fat content. Meal size could explain some of the intra-study variability in findings. Using a small test meal (212 kcal), Wright et al. (1983) demonstrated accelerated GE in obese individuals compared to their lean counterparts. On the other hand, an omelette test meal (597 kcal) provided by Verdich et
al. (2000) caused no change in GE. The 385 kcal difference between the test meals makes it difficult to draw comparisons between these two studies.

Not all studies factor the menstrual cycle into consideration when testing female volunteers; previous studies in relation to GE in obesity have failed to test their female cohort during the same phase of the menstrual cycle (Wright et al., 1983; Horowitz et al., 1983; Horowitz et al., 1986; Zahorska-Markiewicz, 1986; Maddox et al., 1989; Glasbrenner, 1993; Tosetti et al., 1996). Neuromuscular function of the stomach may be affected by hormones related to the menstrual cycle including oestrogen and progesterone (Gill et al., 1987). These hormones may lead to feelings of nausea and gastric dysrhythmias in healthy females (ibid). Oestrogen is a smooth muscle relaxant and lower serum concentrations of oestrogen are associated with accelerated intestinal transit (Lewis et al., 1997). Recently, GE of glucose was shown to be slower in nine healthy, lean females during the follicular phase (days 6-12) compared to the luteal phase (days 18-24) of the menstrual cycle (Brennan et al., 2009). Gill and colleagues showed that emptying of a liquid phase marker was not significantly different between phases of the menstrual cycle but found that GE of a solid phase marker was slower during the luteal phase compared to the follicular phase in seven normally menstruating females (Gill et al., 1987). Monés et al. (1993) suggested that the menstrual cycle does not influence the solid gastric emptying rate; they found similar GE half-time between post-menopausal women and pre-menopausal women measured during the luteal and follicular phase of their cycle. This was in concordance with previous findings that suggested that differences in GE between gender is not related to female sex hormones (Wright et al., 1983). Thus, the issue of the menstrual cycle remains unresolved. Failure to control for the menstrual cycle could be partly responsible for inconsistency in findings regarding the effect of GE on obesity. Furthermore, many of the obese studies failed to use gender-matched controls (Horowitz et al., 1986; Wisen & Johansson, 1992; Glasbrenner, 1993; Hutson & Wald, 1993; Jackson et al., 2004). Until a clear conclusion has been reached regarding the effects of menstrual cycle and gender, it is important to control for these issues in future GE studies.

The aforementioned studies highlight the importance of interpreting and generalising results cautiously, especially when factors such as type of test meal, menstrual cycle, gender- and age-matched controls have not been taken into account.
2.13 The Role of Fat in Gastrointestinal Transit

2.13.1 Acute Exposure to or Infusion of Dietary Fat

Compared to other macronutrients, dietary fat has been shown to delay GE (Woodward, 1957). Acute ingestion of a HF soup delayed GE half-time by approximately 16 minutes, compared to a LF soup of equal energy content, mass and volume (Cecil et al., 1999). However, oral administration of the HF soup was shown to suppress hunger, increase fullness and decrease subsequent food intake more than its LF counterpart. The authors reported that differences were observed when nutrients were infused directly into the gut. In another arm of the study, Cecil and co-workers introduced the same soups intragastrically; similar rates of GE, sensations of appetite and subsequent food intake 2-h postprandially were reported. Building on the findings of Cecil et al. (1999), the effect of ingestion of fat at breakfast on the GE of a subsequent meal has also been explored by Clegg and Shafat (2010). The authors examined the effect of a (i) HF breakfast (771 kcal; 60 g fat; 222 g), (ii) a LF isoenergetic to HF breakfast (LFE) or (iii) LF breakfast of equal mass to HF (LFM) on the GE of a lunch soup given 3-h postprandially in nine healthy male volunteers. The HF breakfast was sufficient to delay GE half-time of the lunch compared to the LFM (GE half-time: HF 102 ± 11 min; LFM 95 ± 13 min, p < 0.01). Energy, fat and protein intake at an ad libitum buffet meal given 4-h after lunch was also significantly greater after ingestion of a HF breakfast. These data suggest that mass and energy content of food are responsible for regulation of subsequent food intake. Findings also highlight the hyperphagic effect of an acute HF meal (Clegg & Shafat, 2010). Maillot et al. (2008) have also shown that fat ingested at a test breakfast (25 g linoleic acid) was partly retained in the gut and metabolised after a subsequent lunch meal (44 g oleic acid) given 4.5-h later. The authors illustrated that an increased plasma and chylomicron TG concentration observed after lunch was associated with incomplete GE of fat from the test breakfast. It was speculated that feedback of fat into circulation led to TG retention within the gut as a result of delayed GE from ingestion of fat at breakfast (Maillot et al., 2008).

Early infusion studies have shown that nutrient interaction in the small intestine delay GE in humans. Miller et al. (1981) used an occluding balloon to separate the jejunoileal segment of the small intestine from the more proximal gut. Mixed meals (300 and 600 kcal) were administered while varying the jejunal perfusate. The study found that jejunileal chyme delayed GE, with its lipid (oleic acid), protein (casein hydrolysate) and carbohydrate (maltose) components all acting as inhibitors. Furthermore, Read and colleagues (1984) illustrated that GE of 100 ml of infused lactulose solution following a
previous infusion of fat emulsion, protein, hydrolysate, glucose, or saline into the jejunum, ileum or colon. Ileal infusion of fat emulsion or protein significantly delayed GE compared to the saline infusion. GE rate was unaffected by infusion of nutrients into the jejunum or colon. In another element of this study, Read et al. (1984) demonstrated that ileal infusion of fat emulsion significantly delayed GE of a solid test meal through the stomach and small intestine. The authors suggested that there appears to be a mechanism whereby unabsorbed food in the ileum may increase absorption by inhibiting the transit of food through the small gut (Read et al., 1984). When compared to saline, an ileal infusion of lipid emulsion (50% corn oil) was shown to significantly delay GE half-time (GE half-time: Lipid 203 ± 48 min, Saline 68 ± 12 min). A further study conducted by the same research group illustrated that infusion of lipid into the jejunum affected appetite in different ways than infusion into the ileum. Further research by Heddle et al. (1989) showed that intra-duodenal infusion of a triglyceride emulsion for 45 min after 25% of a solid meal had emptied from the stomach resulted in a significant delay in GE. It is clear from the aforementioned literature that acute oral exposure to and intra-duodenal exposure to fat is a potent inhibitor of GE.

**2.13.1.1 Physiochemical properties of fatty acids**

The impact of fatty acids on GI function is dependent on their physiochemical properties. Important features of fatty acid structure include their chain length and degree of saturation (Lawton et al., 2000). It is apparent from previous literature illustrating that ≥ 12 carbon atoms are necessary to induce a delay in GE, release GI hormones and suppress EI (Hunt & Knox, 1968; McLaughlin et al., 1999; Feltrin et al., 2004; Feltrin et al., 2006; Feltrin et al., 2008). The relationship between fatty acid chain length and GE was explored in healthy volunteers using an infused test meal containing salts of saturated fatty acids (C2 - C18). GE was measured by aspiration of gastric contents (Hunt & Knox, 1968). The authors found that there was only a minute increase in the delay of GE as the chain length of the fatty acid was increased from 2 - 10 carbon atoms. Conversely, salts of the fatty acids with 12-18 carbons were approximately four times more effective than short chain fatty acids in slowing GE. The role of carbon chain length was found to be an important factor in CCK secretion as well as gastric motility in healthy adults (McLaughlin et al., 1999). A similar and consistent elevation in plasma CCK was found after infusion of fatty acids with a carbon chain length ≥ 12. The authors concluded in comparison to C10 fatty acids, C12 fatty acids significantly reduced antral contractile amplitude and proximal gastric tone (McLaughlin et al., 1999).
The degree of saturation of fatty acids as well as the position of the end-most double bond can also influence GI function. GE half-time was significantly faster after a acute consumption of an n-3 PUFA meal (155 ± 6 min) compared to n-6 PUFA (237 ± 22 min; \( p \leq 0.001 \)), MUFA (219 ± 17 min; \( p \leq 0.01 \)), SFA meals (221 ± 15 min; \( p \leq 0.05 \)) in healthy adults (Robertson et al., 2002). Delayed CCK and reduced GLP-1 concentrations were suggested as the mechanisms responsible for enhanced GE of n-3 PUFA meal (Robertson et al., 2002). Compared to a saline control, ileal infusions of 6 g of C18:1 or C18:2, (but not C18:0), increased CCK secretion but not PYY secretion (Maljaars et al., 2009).

2.13.2 Chronic Exposure to Dietary Fat

In contrast to acute exposure to dietary fat, it has been documented that HF feeding may result in the adaptation of gastrointestinal function and led to an acceleration of GE, especially of high-fat meals (Castiglione et al., 2002). The time frame of HF intervention studies conducted to date is outlined in Figure 2.8).

Cunningham et al. (1991) were the first to demonstrate that a two week high-fat diet (HFD) reduced the delay in GE half time of a fatty test meal compared to 14 days on a low fat diet (LFD), (GE half-time: LFD 147 (88 - 206 min) (mean (range), HFD 98 (80 – 116 min). Additionally, MCTT was significantly accelerated after a 14-day HFD (MCTT: LFD 360 (130 - 350 min) (mean (range), LFD 240 (200 – 520 min). The authors found that exposure to a HFD resulted in a desensitisation of the nutrient responsive mechanisms that control GE. Furthermore, Castiglione et al. (2002) established that the consumption of a HFD for 14 days in healthy cohort accelerated linear emptying rate of GE of a HF (pre HFD: 0.36 ± 0.05 %·min⁻¹, post HFF 0.47 ± 0.03 %·min⁻¹ (mean ± SE)) but not a carbohydrate (LF) meal. These finding suggest that adaptation to previous dietary habits is nutrient specific. The effect of duodenal lipid infusion on antropyloroduodenal pressure, following a 14-day HF or LF diet has also been examined as a mechanism (Boyd et al., 2003). It is probable that the lower amplitude and tone of isolated pyloric waves as well as the increased number of antropyloroduodenal pressure sequences recorded after the HFD contribute to the acceleration in GE. The authors found that the HFD did not affect CCK or GLP-1 responses as well as food intake (Boyd et al., 2003).
A 21-day HFD raised fasting plasma CCK but did not affect upper gut motility, PYY or ghrelin concentrations during a CCK-8 infusion in lean, healthy males (Little et al., 2008). A pilot study conducted on six healthy males by Robertson et al. (2004) illustrated GE half-time was significantly accelerated after a mixed HF supplementation (88g fat) for a period of 14 and 21 days compared to baseline. Park and colleagues (2007) examined the effect of 14-day supplementation of different macronutrients in excess on gastric sensory and motor functions in normal-weight, overweight, and obese humans. HF supplementation for a period of 14 days (500 kcal of fat in excess of required calories) did not influence GE half-time of a solid egg meal (296 kcal; 35% of energy from fat), compared to the other diets, irrespective of BMI. A 3-day HF supplemented dietary intervention study in healthy males was shown to accelerate GE latency time (Figure 2.9)
and MCTT compared to the pre-intervention control diet or the control diet supplemented with a LF supplement (Clegg et al., 2011). Cunningham et al. (1991) previously showed that although a 4-day HFD was not sufficient to accelerate GE half-time compared to a LFD, it was an adequate time period to significantly reduce the quantity of food left in the stomach at 100 minutes postprandially.

Figure 2.9 Gastric emptying latency time (min) from the $^{13}$C octanoic acid breath test following each 3-day intervention periods: control, LF yogurt supplement and HF yogurt supplement in healthy males ($n = 11$). Data are mean ± SD, † $p = < 0.05$ vs. control * $p = < 0.05$ LF vs. HF supplement (Adapted from: Clegg et al., 2011).

Until recently, GI transit adaptation to a HFD for durations of greater than 14 days has not been evaluated in humans. Clegg and Shafat (2011) showed that a 7 day HFD accelerated GE latency time (pre 7 day HFD: 45 ± 8 min, post 7 day HFD 41 ± 10 min) and MCTT (pre 7 day HFD: 308 ± 43 min, post 7 day HFD 248 ± 83 min) of a HF meal (771 kcal; 60 g fat) compared to baseline ($n = 10$). However, no changes were reported in GI transit after 14, 21 or 28 days of a HF intervention ($n = 7$). These findings were similar to findings reported by Shafat and Rumsey (1998); rats fed a HFD for 27 days showed accelerated GE after 9 and 18 days but by day 27 pre-diet GI transit time was observed. Clegg and Shafat (2011) hypothesised that adaptation to a HFD would
gradually reduce the delay in GI transit over a 28 day as previously observed in a rat model of HF feeding (Brown et al., 1994). In rats, chronic intermittent ileal infusion of palm oil given 3 days·wk⁻¹ gradually attenuated the lipid-induced slowing of stomach to caecum transit time (SCTT), until 28 days when the delay was no longer than that induced by the infusion of a control emulsifier (Brown et al., 1994; see Figure 2.10).

**Figure 2.10** Histograms showing effects of an acute (weeks 1 - 8) palm oil infusion into the distal small intestine during chronic intermittent palm oil infusion into the distal small intestine (weeks 1 – 4, n = 10) and after the chronic palm oil infusion had ended (weeks 5 – 8, n = 5), on SCTT of the head of the meal (From: Brown et al., 1994).

The time course of deadaptation to a HF diet has not been previously explored in humans. Brown and colleagues illustrated that the lipid-induced delay in SCTT did not return during the 28 days after cessation of the chronic intermittent infusion of palm oil. The acute response to an ileal lipid infusion and SCTT of the head of the meal were also unaffected during this time period (Figure 2.10).
2.14 Effect of Chronic Exposure to Dietary Fat on Appetite and Energy Intake

It has been proposed that nutrient receptor mechanisms controlling eating behaviour may also be down-regulated in response to chronic exposure to dietary fat ultimately leading to increased EI (French et al., 1995). In rats, *ad libitum* intake of a (2.3 kcal∙ml⁻¹) HF (60 % energy from fat) liquid diet for 16 days led to a greater mean daily EI (~9 kcal∙day⁻¹) compared to an iso-caloric LFD (76 % energy from carbohydrate) (Warwick et al., 2002). In rats, high fat feeding has led to short term over-consumption of a high energy, high fat diet compared to low-fat feed cohorts, which is related to a reduced sensitivity to CCK (Savastano & Covasa, 2005).

Adaptation to a HF diet may also lead to changes in appetite responses in humans. As previously mentioned (Section 2.3.3) manipulation of dietary fat content of the diet for a 14-day period led to a 15.4 % increase in daily EI during the consumption of a HFD (45 – 50 % energy from fat) compared to a medium-fat diet (30 – 35 % energy from fat) and was related to weight gain (Lissner et al., 1987). French and colleagues illustrated that a 14-day HFD significantly increased average energy consumption compared with pre-diet levels by 160 kcal (660 kJ) per day. Additionally, a 14-day HFD resulted in greater sensations of hunger after an oral fat tolerance test but failed to increase EI in healthy adults (Boyd et al., 2003). Similarly, Little et al. (2008) demonstrated that a 21-day HFD did not influence EI during a CCK-8 infusion (Little et al., 2008). HF supplementation for a 14-day period had no effect on *ad libitum* caloric or macronutrient intake but enhanced maximum tolerated volume of the stomach, independent of BMI (Park et al., 2007). Park and co-workers postulated that toleration for a greater volume in the stomach with chronic exposure to dietary fat could lead to delayed time to satiation and possible over-consumption of food in an effort to reach a feeling of fullness. Exposure to a mixed HF supplemented diet for 7 days was sufficient to increase sensations of appetite but not *ad libitum* food intake (Clegg & Shafat, 2011). However, it appears that a 3-day HFD was too short a time course to influence appetite responses (Clegg et al., 2011).

2.14.1 Alterations in Control Mechanisms after High-Fat Feeding

A comprehensive review by Covasa (2010) illustrated that adaptation to a HFD results in alterations in gut morphology, regulatory peptides and neuronal responses, hence changing
gut functions (including GI transit) and ultimately leading to changes in appetite and metabolic regulation (Figure 2.11).

**Figure 2.11** Flowchart depicting the effects of a high-fat (HF) diet on physiological, neuronal and metabolic functions on appetite responses. Adaptation to an HF-diet causes direct changes in gut morphology, regulatory peptide secretion, and neuronal responses. These changes led to alterations in physiological and digestive gut functions such as increased production of fat-digesting enzymes, increased absorptive capacity of lipids, and decreased sensitivity to lipids and inhibitory gut peptides. They are associated with increased peptide secretion and subsequent changes in gastrointestinal responses such as gastric emptying, intestinal motility, and transit. Collectively, these morphological, physiological, and neuronal changes result in profound alterations in appetitive responses, such as increased hunger, decreased fat-induced satiation, and increased food consumption. This affects whole body metabolism, allowing for increased energy metabolism, fat storage, and energy harvest from the diet via gut microbial population changes. BBS; bombesin, NPY; neuropeptide Y, Apo A-IV; apolipoprotein A-IV (From: Covasa, 2010).
2.14.1.1 Cholecystokinin

It is evident that secretion of and sensitivity to CCK is modulated by dietary fat (Little et al., 2007). Spannagel et al. (1996) found that after exposure to a 14-day HFD, rats had a heightened CCK response to a lipid infusion. Covasa and Ritter (2000) illustrated that exposure to a HFD for 14-days reduced sensitivity to the satiating effects of an intraperitoneal injection of CCK-8. Rat pups fed a HFD had reduced sensitivity to CCK compared to rats fed an iso-caloric LF diet; the authors found that reversal of reduced sensitivity was possible with dietary changes but reduced sensitivity is quicker than its extinction (Swartz et al., 2010). Lo et al. (2010) showed that after 10-wk exposure to a HFD (20 % butter) CCK-knockout mice, were shown to have reduced weight gain and fat mass and enlarged adipocytes compared to their wild-type counterparts. This effect was likely to be caused by reduced fat absorption and increased energy expenditure in CCK-knockout mice. CCK has an important role to play in control of body weight in HF feeding.

After consumption of a 14-day HFD in humans, French et al. (1995) observed a decrease in the production of CCK, or a lowered sensitivity to released CCK following meal consumption, may be related to the reduction in the delay of GE and increases in EI observed (French et al., 1995). Fasting plasma CCK was raised after a 21-day HFD in lean males; it appears that in the absence of weight gain the sensitivity to exogenous CCK is not modified following a HFD (Little et al., 2008). Similarly, CCK response to a duodenal lipid infusion was not affected by a 14-day HFD (Boyd et al., 2003). Little et al. (2007) suggested that accelerated GE could be the mechanism responsible for increased CCK response in humans since postprandial CCK responses induced following intraduodenal lipid infusion in Boyd et al. (2003) would have bypassed the process of GE.

2.14.1.2 Glucagon-like Peptide-1, Peptide YY and Ghrelin responses

GLP-1 responses during a duodenal lipid infusion were not attenuated following a 14-day HFD (Boyd et al., 2003). Little et al. (2008) found no change in fasting PYY concentrations after a 21-day period of HF feeding. Robertson and colleagues (2004) established that after 14 days of dietary HF supplementation, plasma ghrelin concentrations were suppressed by 18 % compared to baseline, even though there was only a 3 % increase in body mass during this time.
2.15 Fatty Acid Sensing Mechanism in the Gastrointestinal Tract

At a cellular and molecular level, mechanisms by which fatty acids are detected in the mouth, small intestine and colon are involved in regulation of GI function and EI are not fully understood (Little & Feinle-Bisset, 2010). In recent years, potential G-protein coupled receptors (GPCRs) have been identified (Table 2.3); it appears that GPR40 and GPR120 are activated by medium- and long-chain free fatty acids (FFA) whereas GPR43 and GPR41 are most potently activated by short-chain FFAs (Miyauchi et al., 2009). As well as other sites including the pancreatic β-cell and adipose tissue, mounting research is suggesting that expression of these GPCRs occurs in the GI tract (Itoh et al., 2003; Hirasawa et al., 2005; Briscoe et al., 2003; Edfalk et al., 2008; Miyauchi et al., 2009).

Table 2.3 Characteristics of G-protein Coupled Receptors

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>GPR40</th>
<th>GPR43</th>
<th>GPR41</th>
<th>GPR120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist (FFA)</td>
<td>Medium-long C3<del>C4</del>C2</td>
<td>Short-chain C3&gt;C4&gt;&gt;C2</td>
<td>Short-chain C3&gt;C4&gt;&gt;C2</td>
<td>Medium-long</td>
</tr>
<tr>
<td>Expression without intestine</td>
<td>Pancreatic β-cell tissue</td>
<td>Adipose tissue</td>
<td>Adipose tissue</td>
<td>Adipose tissue</td>
</tr>
<tr>
<td>-Insulin secretion</td>
<td>GLP-1 secretion</td>
<td>-PYY production</td>
<td>-PYY secretion</td>
<td>-GLP-1 secretion</td>
</tr>
<tr>
<td>Physiological role</td>
<td>-GLP-1 secretion</td>
<td>-CCK secretion</td>
<td>-CCK secretion</td>
<td>-CCK secretion</td>
</tr>
</tbody>
</table>

(Adapted from: Miyauchi et al., 2009)

Edfalk and colleagues (2008) have shown that GPR40, which is expressed in GI tract endocrine cells, mediates FFA-stimulation of incretin hormones GLP-1 and GIP; this suggests that GPR40 can influence β-secretion both directly through the pancreatic cells and indirectly through FFA-stimulated insulin secretion in the GI tract (Miyauchi et al., 2009). GPR40-null mice had impaired GLP-1 and GIP secretion after administration of an acute oral fat load and this was associated with reduced insulin secretion and clearance of glucose (Edfalk et al., 2008). GPR120 is highly expressed in human and mouse intestinal L-cells and in mouse enteroendocrine STC-1 cells (Miyauchi et al., 2009). Saturated FFAs with a chain length of C14–C18 and unsaturated FFAs (C16–
C22) appear to stimulate GPR120. In mice, acute administration of linoleic acid (long-chain FFAs) was shown to increase secretion of GLP-1 and insulin (Hirasawa et al., 2005). Furthermore, using mouse enteroendocrine STC-1 cells, it was demonstrated by Tanaka and colleagues (2008) that long-chain FFA-induced CCK secretion was mediated by GPR120-coupled Ca\(^{2+}\) signalling, but not GPR40.

Although the physiological and pharmacological properties of FFA receptors require further investigation, it appears that G-protein coupled receptors could have a role to play in novel therapies for metabolic diseases (Miyauchi et al., 2009). Future research is also necessary to elucidate the role and underlying mechanisms of these fat sensing receptors in the control of appetite and energy intake in humans. Additionally it remains to be determined whether or not alterations in expression or sensitivity to these receptors have a role to play in the pathogenesis of obesity after chronic exposure to a HFD (Little & Feinle-Bisset et al., 2010).

### 2.16 Conclusion

In this review, the role of increased consumption of HF, energy-dense food in the pathogenesis of obesity has been outlined. The implication of acute and chronic exposure to dietary fat in the gastrointestinal tract has been the primary focus of this review. Although findings are inconsistent, it appears that GE is accelerated in the obese state.

This thesis will employ dietary fat as a model of obesity in an attempt to address some of the gaps in the literature including the effect of pharmacological (10 mg domperidone) and nutritional (3 g cinnamon) models on acute intake of dietary fat. Subsequently, the duration of adaptation and deadaptation to a HF supplemented diet will be explored in relation to gastrointestinal transit, appetite and substrate utilisation.
Chapter 3: Does Domperidone, a D$_2$-Antagonist, Accelerate Gastric Emptying Rates and Appetite in Healthy Adults?

3.1 Abstract

**Background/ Objective:** Accelerated gastric emptying (GE) may lead to reduced satiation, increased food intake and is associated with obesity and type 2 diabetes. Domperidone is a dopamine 2 (D$_2$) receptor antagonist with claims of gastrointestinal tract pro-kinetic activity. In humans, domperidone is used as an anti-emetic and treatment for gastrointestinal bloating and discomfort. The aim of this study was to determine the effect of acute domperidone administration on GE rate and appetite sensations in healthy adults.

**Methods:** A single-blind block randomised placebo-controlled crossover study assessed 13 healthy adults (5 males and 8 females, age 23.8 ± 1.5 yrs, weight 69.0 ± 11.1 kg, height 1.73 ± 0.11 m and BMI 23.3 ± 2.4 kg·m$^{-2}$). Volunteers ingested 10 mg domperidone or a matched placebo 30 min before a high-fat (HF) test meal. GE rate was determined using the $^{13}$C octanoic acid breath test. Breath samples and subjective appetite ratings were collected in the fasted state and during the 360 min postprandial period.

**Results:** GE half-time was similar following placebo (254 ± 54 min) and 10 mg domperidone ingestion (236 ± 65 min). Domperidone did not change appetite sensations during the 360 min postprandial period ($p > 0.05$).

**Conclusion:** In healthy adults, acute administration of 10 mg domperidone did not change GE or appetite sensations following a HF test meal.

**Keywords:** Domperidone, gastric emptying, fat, appetite
3.2 Introduction

Obesity has reached epidemic proportions worldwide and is closely related to the development of type 2 diabetes (T2DM) and cardiovascular disease (CVD) (Lawrence & Kopelman, 2004). Availability of highly palatable high-fat (HF) foods is a fundamental risk factor in the aetiology of obesity (Vucetic & Veyes, 2010). Changes in GE rate and the consequential reduction in satiation may be one mechanism linking HF intake to obesity. It is well recognised that a HF meal delays GE rate and can result in the acute suppression of appetite and energy intake (Little et al., 2007; Cecil et al., 1999; Clegg & Shafat, 2010). Chronically, exposure to a HF diet leads to desensitisation to the effects of fat and reduced delay in GE of a HF meal (Clegg et al., 2011; Cunningham et al., 1991; Castiglione et al., 2002). These observations suggest that GE rate is a dominant regulator of appetite and that an increase in GE rate can contribute to the aetiology of obesity (Clegg & Shafat, 2009; Covasa, 2010; Janssen et al., 2011). Levin and colleagues (1999) demonstrated that dopamine had the ability to delay GE and moderately prolong orocaecal transit time associated with a LF pancake meal in healthy adults. GE is susceptible to other external factors including drugs (Maes et al., 1994). Because of the potential role of accelerated GE in weight gain, pro-kinetic drugs that promote GE may increase the risk of weight gain.

Domperidone is a dopamine 2 (D2) receptor antagonist with claims of GI tract pro-kinetic activity that is used to relieve symptoms of gastrointestinal discomfort in diabetic and healthy populations (Xu et al., 2008). Acting peripherally, the drug does not cross the blood brain barrier and thus has minimal adverse extra-pyramidal effects on the central nervous system (Reddymasu et al., 2007). Domperidone is commonly used to alleviate symptoms of GI discomfort including postprandial nausea, abdominal bloating and early satiety in diabetic individuals with dyspepsia, gastropathy and to treat these drug therapy side-effects of in patients with Parkinson’s Disease (Patterson et al., 1999; Ahmad et al., 2006; Lertxundi et al., 2008). Even though domperidone only has a low degree of central nervous system activity, it has anti-emetic properties owing to its influence on the chemoreceptor trigger zone in the area of the postrema of the medulla (Patterson et al., 1999).

In Ireland, domperidone is readily available as an over-the-counter drug known as Motilium™. Given that the drug is advertised as a reliever of stomach discomfort after consumption of rich foods (McNeil Healthcare (Ireland) Limited, 2011); it is surprising that there is a paucity of research examining the effect of acute administration on domperidone on GE of a HF solid meal and appetite sensations. The efficacy of
domperidone to accelerate GE of low-nutrient meals has been established (Broekart, 1979), especially when GE is delayed (Brogden et al., 1982). In healthy adults, acceleration of GE has been documented after acute ingestion of domperidone (10 - 20 mg) using saline water and low-fat, low-energy density test drinks (Bateman et al., 1982; Valenzuela & Liu, 1982; Tatsuta et al., 1989). In contrast, one pilot study in healthy, elderly volunteers has shown that ingestion of 40 mg domperidone dissolved in water, given 60 minutes before a HF yoghurt preload (400 ml; 500 kcal) had no effect on antral area (GE) compared to a matched control (Parker, 2008). Although the exact mechanism explaining the effectiveness of domperidone is ill-defined (Parker, 2008), it is believed to be related to the capability of domperidone to enhance the amplitude of oesophageal motor function, increase antral-duodenal contractions and improve the coordination of peristalsis across the pylorus with ensuing acceleration of GE (Weihrauch et al., 1979; Friedman, 1983). Domperidone may accelerate gastric emptying by removing the ‘inhibitory drive into the stomach’ (Sanger & Alpers, 2008) which could be controlled via the sympathetic nervous system (Morgadinho et al., 1999). Domperidone has not been reported to alter intestinal motility and transit beyond the duodenum (Reddymasu et al., 2007).

The anti-emetic activity of D₂ receptor antagonists has also been proposed as a secondary mechanism which could increase gastric emptying by reducing postprandial symptoms such as nausea, early satiety and abdominal bloating (Sanger & Andrews, 2006). Dopamine D₂ receptor antagonists have also been shown to increase appetite and result in marked weight gain in rodents (Kaur & Kulkarni, 2002). Two mechanisms have been proposed to explain the association between GE and appetite; either stretch in the gastric wall is signalled through the vagus to reduce appetite (Näslund et al., 2001), or nutrient interaction with receptors on the intestinal mucosa trigger a hormonal response, or a combination of the two (Covasa, 2010).

GE may be the mechanism responsible for reduced satiety after domperidone ingestion. A gap in the literature exists regarding how acute domperidone ingestion affects GE rate of a HF solid meal. The current study was designed to examine the effects of acute administration of domperidone on the GE of a HF meal because this meal composition is more nutritionally relevant to the obesogenic environment (Vucetic & Veyes, 2010) than low-fat liquid meals (Valenzuela & Liu, 1982; Tatsuta et al., 1989).

It is acknowledged that the measurement of GE is associated with inter- and intra-variability (Brophy et al., 1986). GE is dependent on quantity and type of fat in the test meal (Cecil et al., 1999; Clegg et al., 2010; Robertson et al., 2004) and HF feeding has
been shown to accelerate GE of a HF (Cunningham et al., 1991; Clegg et al., 2011) but not a LF test meal (Castiglione et al., 2002). There is a paucity of research examining the relationship between habitual diet and GE of a HF test meal. It was decided to explore habitual dietary intake, including fatty acid intake, as a potential avenue which could explain some of the variability associated with GE.

3.2.1 Aims and Hypotheses

Using domperidone as a model of reduced dopaminergic activity, this chapter aimed to investigate if acute or oral administration of 10 mg of domperidone was sufficient to accelerate GE of a HF solid meal and reduce satiety responses in a healthy population, not exposed to HF feeding.

The following hypothesis will be addressed in this chapter (stated as the null hypothesis):

H₀₁: Acute administration of 10 mg domperidone will not change (i) GE of a HF test meal or (ii) change sensations of appetite compared to a matched placebo.

H₀₂: Habitual dietary intake, including fatty acid intake will not be associated with alterations in GE and adjustments for these covariates will not affect the primary end point.

3.3 Methods

3.3.1 Volunteers

Thirteen non-smoking, apparently healthy individuals (5 males and 8 females, age 23.8 ± 1.5 yrs, weight 69.0 ± 11.1 kg, height 1.73 ± 0.11 m and BMI 23.3 ± 2.4 kg∙m⁻²) consented to participate in this study. Prior to study entry, all potential volunteers completed a pre-test questionnaire. None of the volunteers had been previously diagnosed with a gastrointestinal-related medical condition or suffered from a gastrointestinal disturbance before or during the study. Female volunteers were pre-menopausal and were not pregnant or breastfeeding. None had taken domperidone within 2 months of study entry. Female volunteers were studied during the follicular phase of their menstrual cycle (Abell et al., 2007; Brennan et al., 2009). Neuromuscular function of the stomach may be affected by hormones related to the menstrual cycle; hormones including oestrogen and progesterone. These hormones may lead to feelings of nausea and gastric dysrhythmias in healthy females (Gill et al., 1987). Oestrogen is also a smooth muscle relaxant; lower serum concentrations of oestrogen are associated with accelerated intestinal transit (Lewis et al., 1997). In line with previous procedure
(Abell et al., 2007), it was decided that limiting testing to the first ten days of the menstrual cycle, when oestrogen levels were higher, would allow for standardised interpretation of study data. Eligible non-diabetic, non-smoking apparently healthy volunteers gave their informed consent to participate in the study, which was approved by the University of Limerick Ethics Committee (Study No: ULREC 08/03; Clinical Trial Registration No: ClinicalTrials.Gov NCT01347814).

3.3.2 Experimental Design

The experiment was a single-blind, placebo-controlled, randomised crossover study. Each volunteer underwent two x 1-day test trials (Figure 3.1) in random order where a high-fat breakfast meal was preceded by the ingestion of a (i) placebo or (ii) 10 mg Motilium pill. The trials were identical apart from the pills that were ingested. Test trials occurred 28-30 days apart to account for the female menstrual cycle (see Section 3.3.1).

Volunteers recorded their habitual diet using a weighed food diary for the three days (3-day WFD) prior to each test trial. On the day preceding each trial, volunteers were asked to minimise their consumption of foods naturally high in $^{13}$C abundance (Morrison et al., 2000) and refrain from alcohol consumption and vigorous exercise. WFDs were assessed using food composition tables (Morrison et al., 2000) to ensure that volunteers had not consumed foods rich in $^{13}$C prior to testing. It was important that foods eaten before testing were low in $^{13}$C abundance so that metabolism of dietary carbons from prior meals would not increase fasting $^{13}$CO$_2$ enrichment (Slater, 2004). It was important to restrict volunteers from exercising vigorously because increased energy expenditure may result in modifications in the relative proportion of fat and carbohydrate stores. Changes in the fat to carbohydrate oxidation ratio can affect the isotope ratio of $^{13}$C:$^{12}$C in breath CO$_2$ (Schoeller et al., 1977; Slater, 2004). Compared to carbohydrate stores, fat stores are less rich in $^{13}$C and strenuous activity prior to a $^{13}$C OBT would thus result in decreased fasting $^{13}$CO$_2$ breath enrichment (Schoeller et al., 1977; Morrison et al., 2000).
3.3.3 Experimental Procedure

Volunteers arrived at the metabolic suite between 0700 and 0900 after a 12-h overnight fast from food and fluids, except water. Volunteers were allowed one 200 ml glass of water on the morning of the study. Upon arrival, body mass of volunteers was calculated to the nearest 0.1 kg using digital scales (Seca delta, Seca, Germany) and stature was measured to the nearest mm using a freestanding stadiometer (Holtain Limited, Great Britain). Body mass index (BMI) was calculated as weight (kg) divided by height squared.
The test protocol is graphically portrayed in Figure 3.1. Following 15 min of seated rest, a baseline breath sample was collected for measurement of $^{13}$C abundance and a visual analogue scale (VAS) questionnaire was completed. Immediately after baseline measurement collection, the stopwatch was reset to zero ($t = -30$ min), 10 mg Domperidone (Motilium; Janssen-Cilag Ltd., U.K.) or placebo (Homeopathic medicine; Silicea, 6c, Weleda, Derbyshire, U.K.) was ingested orally with 100 ml of water. Thirty minutes were chosen because it is the time required to reach peak plasma concentration (McCallum, 1985). Subsequently, volunteers consumed a standardised test meal (see below) within a 15 min period. Upon completion of the meal the stopwatch was reset to zero and expired breath samples were collected at $t = 0$ min, every 5 min for the first half hour and thereafter in 15 min intervals until 360 min had elapsed for the detection of $^{13}$CO$_2$ (see measurements). VAS questionnaires were filled out every 30 min from $t = 30$ to $t = 360$ min. Room temperature was maintained at 23°C on all test days. At the end of postprandial assessments, volunteers were free to leave the laboratory.

3.3.4 Test Meal

The HF test meal comprised of three pancakes served with 20 g butter (Kerrygold Irish Creamery, Ireland) and 25g raspberry jam (Dunnes Stores, Ireland), along with 200 ml water (Ballygowan Ltd., Ireland). Pancakes were chosen as the test meal because it is a well accepted palatable solid meal and is easy to label with $^{13}$C octanoic acid (Maes et al., 1995). The test meal was put together using ingredients low in $^{13}$C abundance so that it did not influence breath $^{13}$CO$_2$ enrichment (Morrison et al., 2000; Slater, 2004). The pancake batter consisted of 50 g flour (Odlums, Shamrock Foods Ltd., Ireland), 50 g egg (large free-range; Dunnes Stores, Ireland), 150 ml whole milk (Dawn milk, Limerick, Ireland), 35 ml olive oil (Roma Foods, Ireland). Before cooking, 100 mg [1-$^{13}$C] octanoic acid (99 atom %; Eurisotop, St. Aubin, France) was solubilised in the egg yolk. After homogenising the yolk, the egg white was added and beaten. The labelled egg was subsequently mixed with the other ingredients to ensure uniform distribution of the label throughout the pancake batch (Clegg, 2007). The nutritional content of the meal was based on manufacturer’s information (Table 3.3). The fatty acid composition of the meal was estimated to be 37% SFAs, 52% MUFAs and 11% PUFAs. No other eating or drinking and minimal movement was permitted during test trials.
Table 3.1 Nutritional content of test meal

<table>
<thead>
<tr>
<th></th>
<th>Mass (g)</th>
<th>Energy content (kcal/kJ)</th>
<th>Protein (g)</th>
<th>CHO Energy content (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal</td>
<td>240</td>
<td>866/3623</td>
<td>16.9</td>
<td>62.2</td>
<td>62.5</td>
</tr>
</tbody>
</table>

3.3.5 Gastric Emptying

GE rate of the test meal was determined by using the $^{13}$C octanoic acid breath test (Ghoos et al., 1993). Duplicate breath samples were collected into 10 ml tubes (Exetainers; Labco, Buckinghamshire, UK) filled through a drinking straw. The analysis of the $^{13}$CO$_2$/^12CO$_2$ enrichment in breath samples was performed using an ABCA (Europa Scientific, Crewe, UK) isotope ratio mass spectrometer with a continuous flow inlet and auto-sampler. Calibration of the mass spectrometer was carried out using a reference gas of known isotopic composition and results were expressed as delta over baseline relative to the standard Vienna Pee Dee Belemnite (VPDB). Carbon dioxide ($^1$CO$_2$) production rate was assumed to be 300 mmol·h$^{-1}$·m$^{-2}$ body surface area (Shreeve et al., 1970). Body surface area (BSA) was calculated using the weight-height formula of Haycock et al. (1978). The isotope data were expressed as a percentage of $^{13}$C dose recovered per hour (PDR) and as a cumulative percentage of $^{13}$C dose recovered over the 6 h postprandial period (cPDR). These data were fitted to the GE model developed by Ghoos et al. (1993). For all data, the $r^2$ coefficient between the modelled and raw data was calculated and $r^2 > 0.90$. From this model, measurements of GE half-time and lag phase were ascertained using the formulae of Ghoos et al. (1993). Latency and ascension times were also calculated (Schommartz et al., 1998; see section 2.4.6 for definitions).

3.3.6 Appetite Sensations

Sensations of hunger, desire to eat, fullness, thirst, tiredness and coldness were assessed throughout the test using a VAS questionnaire (Blundell et al., 2010). The VAS questionnaire was assembled as an A4 booklet; each page represented a different time point and had an identical set of questions. Each page of the questionnaire was folded out of view after each rating. The questions presented to volunteers at each time point were: ‘How hungry are you right now?’, ‘How thirsty are you right now?’, ‘How much
would you like to eat right now?', ‘How full are you right now?', ‘How tired are you right
now? and ‘How cold are you right now? The variables thirst, tiredness and cold were
used to distract volunteers from their satiety status of which analysis was of importance
and were not subsequently analysed.’ A 150 mm horizontal line was printed under each
question with the most positive response positioned to the right (very/ a lot) and the most
negative response positioned to the left of the line (not at all). Volunteers were instructed
to mark their response to each question across the line corresponding to their feelings at
a given time point. The rating of the response was quantified by measuring the distance
(mm) from the left starting point of the line to the pen mark made by the volunteer.

3.3.7 Dietary Analysis and Assessment of Underreporting

Food diaries were analysed for energy, macronutrient and fatty acid intake using
CompEat P ro V ersion 6 (Nutrition Systems, G rantham, UK). This software uses

Basal m etabolic rate ( BMR) w as es timat ed by us ing the S chofield ( 1985) pr ediction
equation adapt ed by t he FA O/WHO/UNU r eport ( 2004). The r atio o f E I/BMR was
calculated for each volunteer. An EI/BMR <1.14 was the classification for energy
underreporters based o n t he c ut-off l imits dev ised by G oldberg et al . ( 1991) a nd
employed i n t he methods of Y annakoulia et al . ( 2007). Normal energy reporters were
volunteers with 1.14≤EI/BMR≤2.4 whereas overreporters as having an EI/BMR >2.4
(ibid).

3.3.8 Statistical Analyses

All results were analysed using SPSS (version 15.0, SPSS Inc., Chicago, Ill., USA). All
data were tested for normal distribution using the Shapiro-Wilk test. Mean habitual 3-day
and 1-day dietary intake and GE parameters were compared by using two-tailed, paired
Student’s t-tests. The total area under response versus time curve (AUC) was calculated
using the trapezoidal rule and was used as a summary measures for postprandial
appetite. Appetite sensation data were transformed by natural log to account for non-
equal variance and analyzed using a two-way repeated-measures analysis of variance
(RM ANOVA) with one within-subject factor (time) and one between-subject factor
(treatment). Pearson’s Correlations were used to examine the relationship between 3-
day and 1 -day macronutrient and fatty acid intake, as well as demographic variables,
and GE parameters. Covariate adjustment was measured using one-way analysis of
covariance (ANCOVA). Data were expressed as the mean ± standard deviation of the
mean (SD). A value of $p < 0.05$ was considered statistically significant. Coefficient of variation (CV) was quantified as follows: $(SD/mean) \times 100$.

A retrospective power analysis was also conducted. The power calculation was conducted for the primary endpoint evaluation. To detect a 16.6% change in GE rate (Levein et al., 1999) with a two-sided significance level of 5% and a power of 80%, a sample size of 12 volunteers was necessary; anticipating for the possibility of drop-out, 14 volunteers were recruited.

3.4 Results

All aspects of the test trials were well tolerated by volunteers and they reported the meals to be palatable.

3.4.1 Habitual food intake

Dietary intake may have a significant effect on GE rates and it is important to ensure similar diet in the days prior to repeated measures of GE (Clegg et al., 2011). Assessment of dietary intake (food diary derived measurements were collected from $n = 11$ volunteers) demonstrated that there was no significant difference in mean energy, macronutrient or fibre intake over the three day period prior to each treatment (Table 3.2). Analysis of 1-day data indicated that volunteers consumed more protein (20.1 ± 23.7 g) before the placebo condition and protein as percentage of energy intake in the day preceding the placebo treatment (Table 3.3) compared to domperidone. The mean value of EI/BMR for the cohort was 1.40 ± 0.39 (mean 3-day food intake for $n = 11$). Low-energy reporters represented 27% of the cohort. None of the volunteers were classified as energy overreporters.
Table 3.2 3-day Energy, Macronutrient and Fibre Intake before Placebo and Domperidone Treatments

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 11)</th>
<th>Domperidone (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI (kcal)</td>
<td>2187 ± 783</td>
<td>2124 ± 704</td>
<td>0.64</td>
</tr>
<tr>
<td>EI (kJ)</td>
<td>9150 ± 3275</td>
<td>8885 ± 2944</td>
<td>0.64</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>86.6 ± 28.3</td>
<td>79.1 ± 27.0</td>
<td>0.28</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>83.6 ± 33.9</td>
<td>81.1 ± 38.5</td>
<td>0.73</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>291.0 ± 119.4</td>
<td>288.2 ± 78.2</td>
<td>0.93</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>17.0 ± 5.9</td>
<td>16.6 ± 6.0</td>
<td>0.80</td>
</tr>
<tr>
<td>Protein (% of EI)</td>
<td>16.2 ± 2.8</td>
<td>15.5 ± 3.5</td>
<td>0.52</td>
</tr>
<tr>
<td>Fat (% of EI)</td>
<td>33.8 ± 7.0</td>
<td>32.8 ± 4.9</td>
<td>0.66</td>
</tr>
<tr>
<td>CHO (% of EI)</td>
<td>50.0 ± 7.0</td>
<td>51.1 ± 5.8</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between treatments using Student’s t-test, *p > 0.05.

Table 3.3 1-day Energy, Macronutrient and Fibre Intake before Placebo and Domperidone Treatments

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 11)</th>
<th>Domperidone (n = 11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI (kcal)</td>
<td>2166 ± 806</td>
<td>2122 ± 734</td>
<td>0.77</td>
</tr>
<tr>
<td>EI (kJ)</td>
<td>9064 ± 3372</td>
<td>8879 ± 3071</td>
<td>0.77</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>95.4 ± 31.7</td>
<td>75.3 ± 28.4</td>
<td>0.02*</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>77.9 ± 37.9</td>
<td>83.0 ± 37.4</td>
<td>0.46</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>288.6 ± 115.1</td>
<td>285.7 ± 99.1</td>
<td>0.92</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>18.2 ± 9.3</td>
<td>15.5 ± 6.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Protein (% of EI)</td>
<td>18.3 ± 4.3</td>
<td>15.0 ± 3.7</td>
<td>0.01**</td>
</tr>
<tr>
<td>Fat (% of EI)</td>
<td>31.6 ± 7.9</td>
<td>34.6 ± 6.4</td>
<td>0.27</td>
</tr>
<tr>
<td>CHO (% of EI)</td>
<td>50.1 ± 7.02</td>
<td>44.0 ± 12.1</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. Significant difference between treatments using Student’s t-test: *p < 0.05; **p < 0.01.
3.4.2 Gastric Emptying

There were no significant differences between placebo and domperidone treatments in any GE parameters (Table 3.4). GE half-time was accelerated by domperidone in ten out of thirteen volunteers who participated in this study (Figure 3.2 (a)). Domperidone accelerated lag phase in seven out of thirteen volunteers (Figure 3.2 (b)).

Table 3.4 Gastric Emptying Parameters under Placebo and Domperidone Treatments

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>Placebo (n = 13)</th>
<th>Domperidone (n = 13)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-time (min)</td>
<td>254 ± 54</td>
<td>236 ± 65</td>
<td>0.33</td>
</tr>
<tr>
<td>Lag phase (min)</td>
<td>140 ± 31</td>
<td>132 ± 34</td>
<td>0.30</td>
</tr>
<tr>
<td>Latency time (min)</td>
<td>38 ± 12</td>
<td>38 ± 12</td>
<td>0.74</td>
</tr>
<tr>
<td>Ascension time (min)</td>
<td>216 ± 48</td>
<td>198 ± 59</td>
<td>0.35</td>
</tr>
<tr>
<td>cPDR (%)</td>
<td>38.2 ± 5.1</td>
<td>39 ± 4</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between treatments, \( p > 0.05 \).

Figure 3.2 (a) Individual changes in gastric emptying half-time (GE half-time) between placebo and domperidone (drug) treatments. Solid lines indicate a decrease (i.e. an acceleration) and dashed lines indicate an increase (i.e. a delay) in GE half-time. Purple solid bold line indicates group mean GE half-time.
Figure 3.2 (b) Individual changes in gastric emptying lag phase (GE lag phase) between placebo and dom peridone (drug) treatments. Solid lines indicate a decrease (i.e. an acceleration) and dashed lines indicate an increase (i.e. a delay) in GE lag phase. Solid bold line indicates group mean GE half-time.

3.4.3 Appetite Sensations

Sensations of hunger, desire to eat and fullness under placebo and dom peridone conditions are illustrated in Figure 3.3 (a), (b) and (c), respectively. Volunteers reported moderate sensations of hunger and desire to eat and low levels of fullness in the fasted state; these sensations did not differ between placebo and dom peridone condition ($p > 0.05$). Immediately after consuming the test meal, all volunteers reported reduced hunger and desire to eat and increased fullness. As expected, these sensations were gradually reversed during the 360 min postprandial period. Domperidone did not affect the way sensations changed over time (no time x treatment interaction, $p > 0.05$). There were no differences detected in postprandial time-averaged AUC appetite sensation profiles between conditions ($p > 0.05$; see Table 3.5).
Figure 3.3 (a), (b) and (c) Visual Analogue Scale (VAS) Sensations of (a) hunger (b) desire to eat, and (c) fullness in healthy subjects before (-30 min) and after ingestion of a high-fat breakfast under placebo (○) and domperidone (●) treatments. Values are Mean ± SD (error bars; n = 13). No significant differences were found between treatments, $p > 0.05$ by two-way ANOVA.

Table 3.5 Postprandial time-averaged areas under response versus time curve (AUCs) for hunger, desire to eat (DTE) and fullness under Placebo and Drug (Domperidone) Treatments (n = 13)

<table>
<thead>
<tr>
<th>VAS Scores</th>
<th>Treatment</th>
<th>AUC (mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>Placebo</td>
<td>60.1 ± 26.5</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>63.0 ± 27.9</td>
<td></td>
</tr>
<tr>
<td>DTE</td>
<td>Placebo</td>
<td>65.0 ± 26.8</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>66.7 ± 30.0</td>
<td></td>
</tr>
<tr>
<td>Fullness</td>
<td>Placebo</td>
<td>72.7 ± 28.9</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>68.0 ± 26.6</td>
<td></td>
</tr>
</tbody>
</table>
AUC values expressed as Mean ± SD. Time-averaged postprandial area under the curve was calculated as the mean response during the 6-h postprandial period. No significant differences were found between treatments, $p > 0.05$.

3.4.4 Further Analyses

3.4.4.1 Association between Anthropometric Data, Habitual Energy, Macronutrient and Fibre Intake and Gastric Emptying Half-Time

There was no significant relationship between age, body mass, height, BMI and GE half-time under placebo or domperidone treatments ($p > 0.05$; data not shown). The relationship between mean 3-day energy, macronutrient and fibre intake from the 3-day WFD and GE half-time under placebo and domperidone treatments were also assessed (Table 3.6 (a)).

Table 3.6 (a) Relationships between Mean 3-Day Energy, Macronutrient and Fibre Intake from Weighed Food Diaries (3-day WFD) and GE Half-time under Placebo and Drug (Domperidone) Treatments

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 11)</th>
<th>Domperidone (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>EI (kcal·day$^{-1}$)</td>
<td>-0.51 0.11</td>
<td>-0.38 0.26</td>
</tr>
<tr>
<td>Protein (g·day$^{-1}$)</td>
<td>-0.50 0.12</td>
<td>-0.26 0.44</td>
</tr>
<tr>
<td>Fat (g·day$^{-1}$)</td>
<td>-0.55 0.08</td>
<td>-0.39 0.23</td>
</tr>
<tr>
<td>CHO (g·day$^{-1}$)</td>
<td>-0.37 0.26</td>
<td>-0.34 0.31</td>
</tr>
<tr>
<td>Fibre (g·day$^{-1}$)</td>
<td>-0.10 0.77</td>
<td>-0.31 0.35</td>
</tr>
<tr>
<td>Protein (% of EI·day$^{-1}$)</td>
<td>0.01 0.98</td>
<td>0.33 0.32</td>
</tr>
<tr>
<td>Fat (% of EI·day$^{-1}$)</td>
<td>-0.31 0.36</td>
<td>-0.34 0.31</td>
</tr>
<tr>
<td>CHO (% of EI·day$^{-1}$)</td>
<td>0.34 0.30</td>
<td>0.07 0.85</td>
</tr>
</tbody>
</table>

Mean values were not significantly associated with GE half-time by Pearson’s Correlation: $p > 0.05$. 
Additionally, these relationships were examined using the 1-day WFD i.e. food intake data from the day preceding the test trial (Table 3.6 (b)). There were no significant correlations between 3-day or 1-day intake of intake of energy, macronutrients or fibre and GE half-time.

Table 3.6 (b) Relationships between 1-Day Energy, Macronutrient and Fibre Intake from Weighed Food Diaries (1-day WFD) and GE Half-time under Placebo and Drug (Domperidone) Treatments

<table>
<thead>
<tr>
<th></th>
<th>1-day WFD v GE half-time</th>
<th>Placebo (n = 11)</th>
<th>Domperidone (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>EI (kcal∙day⁻¹)</td>
<td>-0.24</td>
<td>0.47</td>
<td>-0.26</td>
</tr>
<tr>
<td>Protein (g∙day⁻¹)</td>
<td>-0.23</td>
<td>0.49</td>
<td>-0.36</td>
</tr>
<tr>
<td>Fat (g∙day⁻¹)</td>
<td>-0.18</td>
<td>0.59</td>
<td>-0.30</td>
</tr>
<tr>
<td>CHO (g∙day⁻¹)</td>
<td>-0.24</td>
<td>0.47</td>
<td>-0.13</td>
</tr>
<tr>
<td>Fibre (g∙day⁻¹)</td>
<td>0.06</td>
<td>0.87</td>
<td>-0.35</td>
</tr>
<tr>
<td>Protein (% of EI∙day⁻¹)</td>
<td>0.08</td>
<td>0.81</td>
<td>-0.16</td>
</tr>
<tr>
<td>Fat (% of EI∙day⁻¹)</td>
<td>-0.09</td>
<td>0.79</td>
<td>-0.26</td>
</tr>
<tr>
<td>CHO (% of EI∙day⁻¹)</td>
<td>0.05</td>
<td>0.88</td>
<td>-0.33</td>
</tr>
</tbody>
</table>

Mean values were not significantly associated with GE half-time by Pearson’s Correlation: \( p > 0.05 \).

3.4.4.2 Association between Habitual Fatty Acid Intake and Gastric Emptying Half-Time

The relationship between mean 3-day fatty acid intake from the 3-day WFD and GE half-time under placebo and domperidone treatments were also assessed. A significant inverse correlation was found between mean 3-day intake of linolenic acid (C18:3; 0.17 ± 0.15 g) and GE half-time under the placebo treatment \( r = -0.60, p = 0.05 \); see Figure 3.4). No other significant correlations were found between specific fatty acid intake in habitual diet and GE half-time under placebo or domperidone treatments (data not
shown). Additionally, the relationship between 1-day intake of specific fatty acids and GE half-time was explored. No significant relationships were found between specific fatty acid intake in habitual diet and GE half-time under placebo or domperidone treatments.

![Graph showing the relationship between mean 3-day intake of linoleic acid (C18:3) and gastric emptying (GE) half-time of a high-fat breakfast under placebo treatment (n = 11).](image)

**Figure 3.4** Relationship between mean 3-day intake of linoleic acid (C18:3) and gastric emptying (GE) half-time of a high-fat breakfast under placebo treatment (n = 11).

3.4.4.3 Covariate-Adjusted Analysis

Adjustment for 1- or 3-day intake of C18:3 (n = 11) did not affect GE parameters ($p > 0.05$).

3.5 Discussion

The present study was designed to examine the effect of an acute oral administration of 10 mg of domperidone on GE rate of a solid high-fat meal using the $^{13}$C OBT and associated subjective appetite sensations in healthy, young adults.

No acceleration in GE was found after an acute administration of 10 mg domperidone. Mean GE half-time was long in duration (254 ± 54 min under placebo treatment) but was similar to previous studies that have used a HF test meal (Clegg et al., 2011). Large intra-subject variability made it difficult to observe alterations in GE rates as a result of
domperidone ingestion (GE half-time under respective placebo and dom peridone treatments: CV = 50.4 and 41.2% ; Lag phase: CV = 27.6 and 25.2%). Individual analysis of GE data illustrated that domperidone delayed GE half-time in three volunteers whereas lag phase was delayed in six out of thirteen cases. Domperidone has previously been shown to accelerate GE of liquid meals (Bateman et al., 1982; Valenzuela & Liu, 1982; Tatsuta et al., 1989). The lack of effect of domperidone on GE rate of a HF test meal was surprising. However, these findings extend the results of a previous study that failed to show an effect of 40 mg domperidone on GE rate of a high nutrient liquid pre-load (30% fat) in healthy, elderly volunteers (Parker, 2005). Regulation of GE is predominantly influenced by the interaction of macronutrients with the small intestine (Lin et al., 1990). Thus, the degree of intestinal feedback to the stomach is associated with the length and region of the small intestine exposed to nutrient (ibid). Fat is the most potent generator of feedback because it has higher energy density and compared to other macronutrients, it has a slower rate of absorption (Lin et al., 1996). Infusion of lipid emulsion directly into the ileum delayed GE in humans, but was not affected by 20 mg domperidone (Welch et al., 1987). The authors concluded that the ileal brake is not mediated by dopaminergic mechanisms, although it may be that when fat is sensed in the ileum, the delay in GE is strong enough to overwhelm any dopaminergic acceleration. In agreement with Parker et al. (2005), current data suggest that domperidone does not have a pro-kinetic effect on energy-dense high-fat content meals. It is probable that domperidone may only be effective as a gastrointestinal pro-kinetic drug when the degree of intestinal feedback is low, such as in low-fat, low-energy test meals (Broekart, 1979; Valenzuela & Liu, 1982; Tatsuta et al., 1989). Additionally, it has previously been documented that domperidone is more effective as a pro-kinetic drug when GE is already delayed, such as dyspeptic patients (Brogden et al., 1982).

The anti-emetic activity of D₂ receptor antagonists has also been proposed as a secondary mechanism which could increase gastric emptying by reducing postprandial symptoms such as nausea, early satiety and abdominal bloating (Sanger & Andrews, 2006). Dopamine D₂ receptor antagonists have also been shown to increase appetite and result in marked weight gain in rodents (Kuar & Kulkarni, 2002; Lee & Clifton, 2002). In the current study domperidone did not influence appetite sensations. Parker (2005) also demonstrated no effect on appetite sensations by acute administration of 40 mg domperidone at any time point in a test meal compared to a matched placebo. A weakness of Parker’s study was that volunteers were provided with a buffet meal before the pre-load would have been fully emptied from the stomach. It is possible that volunteers were still fully satiated at 100 min when they were presented with the
buffet meal. To overcome this limitation, measurements of GE rate and appetite sensations in the present study were made up until six hours postprandially. The lack of appetite effect may be understood as evidence against any central effects of domperidone (Reddymasu et al., 2007), or, alternatively, the dominance of GE as a regulator of appetite sensation (Little et al., 2007). Thus, the similar GE rates observed here is the most probable explanation for no difference in satiety between domperidone and placebo treatments (Xu et al., 2008). It is well documented that GE has an influential role to play in appetite regulation and EI (Little et al., 2007). One mechanism that may link GE and appetite is gastric distension. Gastric distension triggers stretch receptors which, through vagal activity, reduce satiety and satiation (Näslund et al., 2001). Jones et al. (1997) have shown a direct relation between feelings of fullness and antral (gastric) distension. Nutrient interaction with the intestinal submucosa may also stimulate the release of gut peptides such as CCK (Lissner et al., 1987; Welch et al., 1985; Moran & Schwartz, 1994). However, as domperidone did not induce acceleration in GE, it is not possible to deduce whether or not the ability of domperidone to alleviate GI symptoms is mediated by accelerated GE. Therefore, the effect of GE on postprandial appetite sensations after administration of domperidone should be interpreted cautiously.

Habitual dietary intake of volunteers was comparable with past national data from the North South Food Consumption Survey for males (n = 662, 2639 kcal, 34.8 % fat) and females (n = 717, 1800 kcal, 35.6 %; Harrington et al., 2001). No significant difference was found between domperidone and placebo conditions for energy and macronutrient intake, except for volunteers consuming more protein in their diet in the day preceding the placebo treatment. It should be noted that this did not have an effect on total energy intake and protein intake was not associated with GE half-time. It was decided based on these findings to provide more rigorous guidelines for the recording dietary intake in the days preceding test trials. In order to control for the possible effect of background diet, volunteers were asked to record their habitual dietary intake using a WFD for the three days prior to the first trial and would then repeat this diet before the second trial in future GI studies.

Associations between intake of energy, macronutrients and fatty acids and GE half-time under placebo and domperidone treatments were also assessed. A higher intake of linolenic acid (C18:3) was significantly associated with a decreased GE half-time under the placebo treatment. This relationship was not evident between diet in the day preceding testing and GE half-time. A covariate-adjusted analysis for C18:3 was employed to achieve an unbiased estimate of the effect of 10 mg domperidone on GE.
However, there were no significant differences between conditions when adjustments were made.

Interestingly background intake of fatty acids including C16:0 and C18:1, which were high in the test meal were not strongly associated with GE of the meal. This suggests that GE of a HF test meal is not just specifically affected by background intake of that specific fatty acid. Recently, G protein-coupled receptors (GPR) have been shown to have an affinity to different long chain (e.g. GPR40 or GPR120) fatty acids (Miyauchi et al., 2010; Moore et al., 2009). These receptors have a role to play in secretion of gut peptides such as CCK, GLP-1 and PYY (Miyauchi et al., 2010). Background intake of specific fatty acids may lead to down-regulation of receptor expression and subsequently result in desensitisation to fat intake (Itoh et al., 2003).

There are several potential limitations of this study. The lack of effect of domperidone on GE rate of the high-fat test meal could be as a result of the dosage of domperidone being too low to counteract the energy content of the meal. As suggested by Parker (2005), perhaps a larger dose of domperidone would have been more effective in accelerating the GE rate of the meal employed in this study. However, 10 mg is the recommended dose for healthy adults. Furthermore, domperidone is reported to reach maximum serum concentration after half an hour and the drug effects last for 4-7 hours (Nagarsenker et al., 2000). Although the study did not confirm this, it was expected that administering domperidone 30 minutes before a meal would maximise small intestinal exposure and accelerate GE (Ma et al., 2010).

Dopamine helps to regulate energy balance by altering food intake through rewarding satiety and energy expenditure circuits of the brain (Meguid et al., 2000). We explored the effect of domperidone, a dopamine 2 (D2) receptor antagonist, on GE and appetite sensations of a HF test meal in an acute setting. Chronic high-fat feeding may lead to acceleration of GE (Clegg et al., 2011; Cunningham et al., 1991; Castiglione et al., 2002). It has been suggested that modulation of gastrointestinal function, as a result of exposure to a HFD, may lead to increased energy intake and adiposity (Little et al., 2007). A chronic intake of dopamine D2 receptor antagonists may increase appetite and result in marked weight gain in rodents (Kuar & Kulkarni, 2002; Lee & Clifton, 2002). In an obese cohort, individuals with the greatest BMI had the lowest density of D2 receptors (Wang et al., 2002). Mice that were chronically exposed to fat also had a lower density of D2 receptors (Huang et al., 2006) suggesting that dopamine may have a fat sensor mechanism. It appears unlikely that adaptation in GI function through chronic exposure
to fat is the mechanism responsible for previously reported findings (Kuar & Kulkarni, 2002; Huang et al., 2006; Lee & Clifton, 2002) since we found no alteration in GE.

In summary, acute administration of 10 mg domperidone failed to accelerate GE of a HF solid meal. Domperidone also had no detectable effect on appetite sensations in a healthy population. Potential confounding variables including domperidone dose and energy or fat content of the test meal need to be addressed prior to future work in this area.
Chapter 4: Effect of Cinnamon on Gastric Emptying, Postprandial Lipaemia and Glycaemia, Appetite Responses to an Acute High-Fat Meal

4.1 Abstract

Background/ Objectives: Cinnamon has been shown to delay GE of a high-carbohydrate meal and reduce postprandial glycaemia in healthy adults. However, it is dietary fat which is implicated in the etiology of obesity and is associated with T2DM and CVD. The current study aimed to determine the effect of 3 g cinnamon (*Cinnamomum zeylanicum*) on GE, postprandial lipaemic and glycaemic responses, as well as appetite sensations and subsequent food intake following a HF meal.

Methods: A single-blind randomised crossover study assessed nine healthy, young subjects. GE rate of a HF meal supplemented with 3 g cinnamon or placebo was determined using the $^{13}$C OBT. Breath, blood samples and subjective appetite ratings were collected in a fasted state and during the 360 min postprandial period, followed by an *ad libitum* buffet meal. GE and 1- and 3-day fatty acid intake relationships were also examined.

Results: Cinnamon did not significantly delay GE parameters, postprandial TG, glucose concentrations or appetite responses ($p < 0.05$). Strong relationships were evident between GE half-time and habitual 1- and 3-day intake of palmitoleic acid (C16:1), eicosenoic acid (C20:1), and total omega-3 ($n$-3) intake.

Conclusion: The ingestion of 3 g cinnamon had no effect on the GE of a HF meal and did not alter the postprandial appetite and metabolic responses to a HF test meal. This study found no evidence to support the use of 3 g cinnamon supplementation for the prevention or treatment of metabolic disease. Dietary fatty acid intake requires consideration in future gastrointestinal studies.

Keywords: Cinnamon, fat, gastric emptying, appetite, metabolic variables
4.2 Introduction

Free-living individuals are in the postprandial hyper-triglyceridemic state for the majority of a 24-h period (Williams, 1997). It has been established that non-fasting triglyceride (TG) levels are independently associated with cardiovascular events (Bansal et al., 2007). Elevated postprandial TG concentrations are associated with greater risk of developing atherosclerosis than elevated fasting concentrations (Patsch et al., 2002; Karpe et al., 1994). Similarly, blood glucose response 2-h postprandially is a more accurate predictor of mortality from CVD than fasting blood glucose (DECODE, 2001).

GE is a rate-limiting step in blood glucose homeostasis in healthy individuals; it has been shown to be inversely related to postprandial glycaemia (Horowitz et al., 1993). Acute exposure to fat in the small intestine leads to a delay in GE and has the potential to alter metabolic variables in the postprandial state. The addition of 50 g fat to a meal was shown to reduce postprandial glycaemic and insulin responses observed in healthy adults (Cunningham & Read, 1989). On the contrary, accelerated GE, through HF feeding may lead to heightened postprandial glycaemia and lipaemia (Darwiche et al., 2001) and thus result in increased risk for development of atherosclerosis, coronary artery disease and T2DM (Castiglione et al., 2002).

Dietary supplementations, such as traditional spices, that can limit lipaemia and glycaemia in the fed state, have important implications for prevention and management of metabolic diseases. Two decades ago, cinnamon (Cinnamomum zeylanicum) was proposed as a treatment for T2DM when it was shown to display insulin-mimetic properties (Khan et al., 1990). Of 49 herbs, spices and medicinal plant extracts tested for insulin-dependent utilisation of glucose in vitro, cinnamon was shown to have the highest degree of bioactivity, enhancing insulin activity > 20-fold higher than any other extract tested (Broadhurst et al., 2000).
Figure 4.1 Potential Anti-hyperglycaemic Actions of Cinnamon  (Adapted from: Kirkham et al., 2009)

Cinnamon has been proposed to act on numerous mechanisms relating to glucose and insulin function (Figure 4.1). These actions include improved cellular uptake of glucose through stimulation of insulin receptor PI-3-kinase activity and inhibiting tyrosine phosphates (Imparl-Radosevich et al., 1998). An bioactive element in cinnamon, methyl hydroxyl chalcone polymer (MHCP), was an effective insulin mimetic; 0.1 mg/ml of this polymer had an effect on glucose uptake and glycogen synthesis that was comparable to that of insulin (Jarvill-Taylor et al., 2001). Cinnamon may also promote insulin receptor activity by increasing phosphorylated intracellular protein IRS-1 concentrations and increasing the binding to PI3-kinase, which results in enhanced cellular glucose uptake in fructose-induced insulin resistant rats (Qin et al., 2004). Furthermore, polyphenol type-
A polymer, a water soluble component found in cinnamon may reduce inflammation through antioxidant effects (Anderson et al., 2004).

Cinnamon intake may also decrease insulin resistance through reducing hyperglycaemia and hyperlipidaemia. A placebo controlled clinical trial indicated that chronic supplementation of cinnamon lowered fasting serum glucose, triacylglycerol, and total and LDL-cholesterol concentrations in T2DM patients (30 men and 30 women aged 52.2 ± 6.3 yrs) receiving oral blood glucose lowering medication (Khan et al., 2003). Cinnamon or placebo (wheat flour) capsules were incorporated into the normal diets of patients for 40 days in doses of 1, 3, or 6 g. The three concentrations of cinnamon had comparable lowering effects on mean fasting serum glucose (18 - 20 %), triglyceride (23 - 30 %), LDL cholesterol (7 - 27 %) and total cholesterol (12 - 26 %) concentrations. The authors concluded that there was no dose response to cinnamon and that 1 g cinnamon daily may be effective for the control of blood glucose and lipid concentrations in the diabetic population. Recently, a double-blind placebo controlled study illustrated that the intake of 2 g of cinnamon for 3 months significantly reduced glycated haemoglobin (HbA1c) in poorly controlled T2DM patients compared to a matched placebo. The authors also reported a significant reduction in fasting blood glucose (FBG) at week 12 compared to baseline in the cinnamon group. However, these alterations were not significant compared to the placebo group. Mang et al. (2006) reported a reduction in FBG when T2DM patients were given 3 g of cinnamon daily for 120 days. Conversely, Vanschoonbeek et al. (2006) demonstrated no effect of cinnamon ingestion (1.5 g daily for 42 days) on whole body insulin insensitivity, oral glucose tolerance or lipid profiles in postmenopausal T2DM patients. Baker et al. (2008) conducted a meta-analysis of five prospective randomised control trials and indicated that cinnamon did not significantly alter A1c, fasting blood glucose or lipid parameters in patients with type 1 or 2 diabetes (Baker et al., 2008). The low numbers of eligible trials as well as discrepancies between the five trials (dose of cinnamon, level of glycaemic control, ethnicity, diet, lack of confirmation of double blinding) were limitations of this meta-analysis.

Beneficial effects of cinnamon on hyperglycaemia and insulin sensitivity have also been demonstrated in healthy populations. Acutely, a 5 g cinnamon bolus improved glycaemic responses and insulin sensitivity when given 12 hours prior to, or with, an oral glucose tolerance test (OGTT) in seven healthy sedentary males (Solomon & Blannin, 2007). This study provides evidence that acute 5 g cinnamon bolus intake can improve in vivo glycaemic control and insulin sensitivity both immediately and 12-h post-ingestion. Further research carried out by Solomon and Blannin (2009) examined the effect of daily consumption of 3 g cinnamon for 14 days on glucose tolerance and insulin sensitivity in
eight sedentary healthy males. In concordance with their previous study, the authors illustrated that glucose and insulin responses to OGTT as well as insulin sensitivity were improved following cinnamon ingestion. However, the team failed to demonstrate any long-term effects of prolonged cinnamon supplementation on insulin sensitivity after supplement ingestion had been discontinued. The 3 g bolus ingestion of cinnamon induced comparable enhancements in glucose AUC (-10 v.s. -13 %) to the 5 g bolus employed in their previous work (Solomon et al., 2007) suggesting that the former dose is adequate.

The majority of literature has examined cinnamon from the perspective of glucose disappearance from circulation using high concentrations of extract in vitro (Jarvill-Taylor et al., 2001; Anderson et al., 2004; Qin et al., 2004). Concentrations employed in vitro are not directly applicable to doses suitable for consumption in free-living individuals. In addition to the rate of glucose removal from circulation, plasma glucose concentration is also determined by the rate of glucose entering the circulation (Schenk et al., 2003). Circulating glucose comes from three sites; intestinal absorption during the fed state, glycogenolysis and gluconeogenesis (Aronoff et al., 2004). GE is an important determinant of the rate of glucose appearance and blood glucose homeostasis in healthy and diabetic populations (Horowitz et al., 1993; Rayner et al., 2001). It has been suggested that GE is responsible for 40 – 50 % of the variation in postprandial glycaemia in healthy adults (Horowitz et al., 1993). Delayed GE could be part of the mechanism by which cinnamon improves glucose tolerance. Hlebowicz and colleagues were the first to examine the effect of cinnamon on GE rate. When combined with a semi-solid, low-fat rice pudding test meal (330 kcal; 16 g carbohydrate; 58 % of energy from carbohydrate), 6 g cinnamon reduced postprandial glycaemia and delayed GE rate, without affecting appetite sensations in fourteen healthy subjects (Hlebowicz et al., 2007). Although it is recognised that the rate of stomach emptying acts as a mechanism for controlling blood glucose homeostasis in healthy individuals (Horowitz et al., 1993; Blair et al., 1991), this study found that the decrease of postprandial blood glucose concentrations was more apparent than the effect of cinnamon on GE rate. The authors concluded that the change in GE rate could not be the sole explanation for the lower blood glucose following a meal containing cinnamon. More recently, the same research group investigated the effect of the addition of a more realistic amount of cinnamon to rice pudding (3 g or 1 g) using a similar methodology to their previous work (Hlebowicz et al., 2009). Ingestion of a cinnamon (3 g) supplemented meal reduced postprandial serum insulin and increased GLP-1 concentrations but had no effect on GE rate, satiety, blood glucose, GIP or ghrelin concentrations in the postprandial period.
Hlebowicz et al. (2009) remarked that a relationship may exist between the quantity of cinnamon consumed and the delay in GE and blood glucose concentration in a healthy population. There may also be a correlation between the amount of cinnamon consumed and the reduction in insulin concentration.

4.2.1 Aims and Hypotheses

The effect of cinnamon on GE of a HF test meal has not previously been evaluated. This study aimed to determine if acute supplementation of 3 g cinnamon would reduce appetite responses or postprandial glycaemic and lipaemic responses to a high-fat (HF) meal through a delay in GE. Additionally, the relationship between habitual dietary and fatty acid intake in the three days prior to testing and GE of the HF test meal was examined.

The following hypotheses will be addressed in this chapter (stated as the null hypotheses):

H₀₁: Acute ingestion of 3 g cinnamon will not (i) delay GE of a HF test meal nor will it reduce (ii) appetite, food intake or (iii) metabolic variables in the postprandial period compared to a matched placebo in healthy volunteers.

H₀₂: Habitual dietary intake, including fatty acid intake will not be associated with alterations in GE and adjustments for these covariates will not affect the primary end point.

4.3 Methods

This study was completed in collaboration with Dr Conor McClean and Dr Gareth Davison from the University of Ulster, Jordanstown, Northern Ireland (see Appendix 5 for provisional manuscript). The recruitment, design and randomisation for the study were undertaken by the author of the thesis. The collaborators collected data in conjunction with the author of this thesis to examine the effect of a cinnamon-supplemented high fat meal on vascular function, blood pressure and oxidative stress. None of these data are presented in this thesis.

4.3.1 Volunteers

Nine apparently healthy subjects (3 male, 6 female; age 26.2 ± 3 yrs; body mass 66.5 ± 11.3 kg; BMI 22.4 ± 2.5 kg·m⁻², percentage body fat (% BF) 22.2 ± 6.5 %) consented to participate in the study, which was approved by the University of Limerick Research
Ethics Committee (ULREC: 08/61; Clinical Trial Registration No: ClinicalTrials.gov Identifier: NCT01350284). The study commenced on 18th June 2009 and ended on 17th July 2009. Volunteers had no history of GI-related complaints, CVD or diabetes and were not currently taking an antioxidant or lipid-lowering medication. Volunteers were non-smokers and were recreationally trained (participated in at least 120 min·week−1 individual or team sport) as determined by administration of a 3 month exercise questionnaire (McClean et al., 2007; Martins et al., 2007). Fasting blood lipid and glucose parameters were all within the normal limits (Table 4.1). Volunteers were required to rate at least 50% of the food items that would be presented in the buffet meal as 5 or higher in a food preference questionnaire for study inclusion. Two volunteers were identified as restrained eaters [scoring > 12 on the eating restraint section (factor 1) of the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985; Martins et al., 2008)].

| Table 4.1 Fasting Plasma Glucose and Lipid Parameters before Placebo and Cinnamon Conditions |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Placebo (n = 8) | Cinnamon (n = 8) | p-value |
| Glucose (mmol·l⁻¹)             | 4.62 ± 0.31     | 5.02 ± 0.39     | 0.06    |
| TG (mmol·l⁻¹)                  | 0.71 ± 0.13     | 0.80 ± 0.29     | 0.35    |
| Total Chol (mmol·l⁻¹)          | 3.65 ± 0.82     | 4.03 ± 0.91     | 0.10    |
| HDL (mmol·l⁻¹)                 | 1.48 ± 0.34     | 1.68 ± 0.34     | 0.15    |
| LDL (mmol·l⁻¹)                 | 1.85 ± 0.62     | 1.98 ± 0.76     | 0.26    |

Data are mean ± S.D. No significant differences were found between conditions using paired, two-tailed Student’s t-test, p > 0.05.

4.3.2 Anthropometric Measures

Anthropometric measures were collected 7 days prior to the first test day. Participants drank 500 ml of water one hour prior to anthropometric measurements and were asked not to consume any food for three hours preceding their appointment. Participants were measured in light clothing. Stature was measured to the nearest millimetre using a freestanding stadiometer (Holtain Limited, Great Britain). A MC-180MA multi-frequency body composition analyser (TANITA UK Ltd., UK) 50 kHz leg-to-leg bio-impedance...
analysis (BIA) system was used to determine body fat (% BF) and body mass to the nearest 10\textsuperscript{th} of a kilogram. The BIA system employs a tetra-polar footpad-style electrode arrangement. Participants were instructed to stand on the metal electrode plates of the analyser in their bare feet. Percentage body fat was calculated using the manufacturer’s software.

4.3.3 Experimental Design

Volunteers were blinded to the real nature of the study and were informed that the study aimed to investigate the effect of food flavourings on GE of a high-fat test meal. The experiment was a single-blind, placebo-controlled, randomised crossover study. Each subject underwent two separate 1-day test trials in random order: (i) wheat flour (placebo) and (ii) cinnamon supplemented meal (Figure 4.2). The test days were identical apart from the content of the capsules. In order to control for confounding effects of the menstrual cycle, test trials were separated by an interval of 28 days (Burton et al., 2009).

During the 3 days before the first trial, subjects recorded all of the food and drink that they consumed and repeated this diet before the second trial. Volunteers were provided with written instructions as well as a set of scales and a diary for record keeping. They were instructed to include a detailed description of all food items, including the ingredients of mixed meals, and brand names of whole foods. A physical activity diary was also logged during this time period. Volunteers gave details of the duration and intensity of their daily activity levels for the three days prior to each test trial. Volunteers were instructed to minimise their consumption of foods naturally high in $^{13}$C abundance for 24-h prior to each trial and were also asked to refrain from alcohol consumption and vigorous exercise during the test day. In order to control for confounding effects of the menstrual cycle, test trials were separated by an interval of 28 days (Burton et al., 2009).

4.3.4 Experimental Procedure

Volunteers reported to the laboratory after a 12-h overnight standard fast. Upon waking, the consumption of 200 ml of water was permitted. Volunteers were scheduled to arrive at the lab at the same time for each session. Volunteers remained seated throughout the sessions when supine blood measurements were not being taken. Following of 15 min interval of supine rest, a fasting blood sample was collected via venepuncture ($t = -25$ min). Volunteers returned to the seated position and after a 10 min interval, baseline breath samples were taken and visual analogue scale (VAS) questionnaires were completed ($t = -15$ and -10 min). Once baseline measurements were taken, subjects...
consumed the test meal within 15 m in (Table 4.2). Upon completion of the meal, the stopwatch was reset ($t = 0 \text{ min}$) and sequential postprandial measurements of GE, appetite sensations and plasma glucose and lipids responses were taken. Exhaled breath samples were collected at $t = 0 \text{ min}$, every 5 min for the first half hour after meal consumption and thereafter in 15-min intervals from $t = 30$ until 360 min for the detection of $^{13}$CO$_2$ (see measurements). VAS questionnaires were administered after the consumption of breakfast ($t = 0 \text{ min}$) and every 30 min until $t = 360 \text{ min}$. Blood samples were collected hourly from $t = 60 \text{ to } t = 240 \text{ min}$. At the end of the postprandial assessments ($t = 360 \text{ min}$), volunteers were presented with an assortment of cold lunch-type buffet foods (Table 4.3; see section 4.3.9). Volunteers were given 30 min (i.e. $t = 360 - 390 \text{ min}$) to eat ad libitum until ‘comfortably full’. After the ingestion of the buffet meal, volunteers completed another VAS questionnaire and were then free to leave the laboratory. Additional VAS questionnaires were administered after consumption of the test meal and buffet meal ($t = 0$ and 390 min) to evaluate meal palatability as well as sensations of nausea and well-being. They recorded their subsequent food intake for the remainder of the test day.

### 4.3.5 Test Meal

The high-fat test breakfast (Table 4.2) was rich in poly-unsaturated fat and was based on sunflower oil. The fat composition of the meal was 19.5 % SFA, 27 % MUFA and 53 % PUFA. The test meal was put together using ingredients low in $^{13}$C abundance so that it did not influence breath $^{13}$CO$_2$ enrichment (Morrison et al., 2000; Slater, 2004). The pancakes were prepared under standardised conditions and consisted of 36 g flour (Odlums, Shamrock Foods Ltd., Ireland), 44 g egg (medium free-range; Dunnes Stores, Ireland), 70 g whole milk (Dunnes Stores, Ireland), 30 g sunflower oil (Flora, Ireland). The test meal consisted of three pancakes served with 20 g of chocolate spread (Nutella, FerreroUK Ltd, England) and 300 ml of water (Dunnes Stores, Ireland). 135 mg (150µl) [1-$^{13}$C] octanoic acid (99 atom %; Cambridge Isotope Laboratories, Andover, MA, USA) was solubilised in the egg yolk prior to cooking as previously described (Section 3.3.4). Based on the literature that was available at the time of designing the study, 3 g was deemed as an appropriate supplemental dose of cinnamon (Kahn et al., 2003). Volunteers were instructed to ingest 8 gelatin capsules containing (i) 3 g Cinnamon powdered spice (Cinnamomum zeylanicum; Schwartz, UK) or (ii) 3 g placebo (wheat flour; Odlums, Shamrock Foods Ltd., Ireland). Volunteers were asked to consume four capsules directly before and after their meal. No other eating or drinking was permitted.
during the sessions. Volunteers sat in individual booths and the kitchen door remained closed during cooking to minimise the effect of factory stimulation (sight or smell of food) on subsequent gastrointestinal responses (Arosio et al., 2004).

Figure 4.2 Timeline outlining the test day protocol
Table 4.2 Nutritional content of test meal

<table>
<thead>
<tr>
<th>Meal</th>
<th>Mass (g)</th>
<th>Energy content (kcal/kJ)</th>
<th>Protein (g)</th>
<th>Carbohydrate (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>178</td>
<td>632/2644</td>
<td>13.1</td>
<td>42.1</td>
<td>45.7</td>
</tr>
</tbody>
</table>

4.3.6 Gastric Emptying
GE was measured following the methodology described in Section 3.3.5.

4.3.7 Appetite Sensations
Sensations of appetite were measured using a 150 mm VAS questionnaire as previously described in Section 3.3.6. Volunteers also rated the palatability of the test meal and buffet at $t = 0$ and 390 min respectively (Flint et al., 2000; Appendix 2B).

4.3.8 Blood sampling and analyses
Blood samples (~11 ml) were obtained, with minimal stasis by extracting blood from a prominent forearm antecubital vein, while volunteers rested in the supine position. Immediately after blood collection, antithrombotic vacutainers containing potassium ethylenediaminetetraacetic acid (K₃ EDTA) and sodium fluoride were mixed by inversion and placed on ice. All samples were separated by centrifugation at 4560 rpm (4000g) for 15 minutes at 4°C within 30 minutes of collection and stored at -70°C until analysis.

Further analysis of blood samples was conducted by Tom Trinick and Ellie Duly in Ulster Hospital, Dundonald, Belfast, Northern Ireland. Plasma glucose was determined by an immobilised enzyme membrane method in conjunction with a Clark electrode on a YSI 2300 analyser (Yellow Springs, USA). Total cholesterol, TAGs and HDL were measured by enzyme assay kits, using an automated analyser (Aeroset™, Abbott Labs, USA; See McClean et al., 2007). LDL cholesterol was calculated using the Friedewald equation (Friedewald et al., 1972). All samples for each volunteer were analysed in a single analyser run. CVs were < 7.7 % for glucose and < 10.0 % for all blood lipids.

4.3.9 Ad Libitum Buffet Meal
After the final postprandial samples were collected ($t = 360$ min), volunteers were presented with an assortment of lunch-type buffet foods in excess of expected
consumption (Table 4.3). Volunteers were provided with a selection of foods as opposed to a homogenous meal which allowed for the measurement of macronutrient-specific appetite in conjunction with total energy intake (Martins et al., 2007). Volunteers were not told that their food intake was to be measured and foods were presented in an unpackaged manner so that no nutritional information was available. This ensured that volunteers were unaware of the energy and macronutrient composition of the foods they were consuming. Volunteers were given thirty minutes to eat ad libitum until 'comfortably full'. A maximum of 19 buffet items were offered to volunteers. The items included in the buffet were ones that subjects had previously ranked as > 5 on a scale of 1 - 10 on a food preference sheet. The quantity of food consumed was weighed to the nearest 0.1 g before serving and the amount of leftover food was subtracted from the initial weight to provide a measure of energy and macronutrient intake. Time taken to complete the buffet meal completion (in min) was also recorded. Energy and macronutrient composition were calculated using CompEat Pro Nutritional Analysis software (Version 6; Nutrition Systems, Grantham, UK). This nutritional software employed McCance and Widdowson’s 6th Edition food composition tables (Food Standards Agency, 2002). Subjects were asked to record subsequent food intake for the remainder of the day using a weighed food diary.

4.3.10 Dietary Analysis and Assessment of Underreporting

Dietary analysis and assessment of underreporting were analysed as described previously (Section 3.3.7).
Table 4.3 Composition of cold ad libitum buffet meal. The energy and macronutrient content of foods are given per actual weight of food provided. Nutrient information on each food is based on manufacturer's data.

<table>
<thead>
<tr>
<th>Food items</th>
<th>Amount served (g)</th>
<th>Energy content (kcal)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ham, sliced&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115</td>
<td>125</td>
<td>18.3</td>
<td>2.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Chicken, sliced&lt;sup&gt;b&lt;/sup&gt;</td>
<td>115</td>
<td>142</td>
<td>30.3</td>
<td>0.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Cheese, grated&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100</td>
<td>472</td>
<td>29.3</td>
<td>2.9</td>
<td>38.5</td>
</tr>
<tr>
<td>Tomatoes, sliced&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125</td>
<td>16</td>
<td>1.3</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Lettuce&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60</td>
<td>8</td>
<td>0.7</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Cucumber, sliced&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80</td>
<td>8</td>
<td>0.6</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Sweetcorn, drained&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90</td>
<td>70</td>
<td>2.4</td>
<td>14.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Coleslaw&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100</td>
<td>117</td>
<td>2.1</td>
<td>7.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Brown bread, 6 slices&lt;sup&gt;e&lt;/sup&gt;</td>
<td>235</td>
<td>487</td>
<td>21.6</td>
<td>96.1</td>
<td>3.5</td>
</tr>
<tr>
<td>White bread, 6 slices&lt;sup&gt;e&lt;/sup&gt;</td>
<td>235</td>
<td>515</td>
<td>20.5</td>
<td>101.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Butter&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30</td>
<td>162</td>
<td>0.2</td>
<td>0.4</td>
<td>17.7</td>
</tr>
<tr>
<td>Mayonnaise&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30</td>
<td>363</td>
<td>0.8</td>
<td>0.9</td>
<td>40.0</td>
</tr>
<tr>
<td>Relish&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30</td>
<td>87</td>
<td>0.8</td>
<td>20.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Yoghurt&lt;sup&gt;c&lt;/sup&gt;</td>
<td>415</td>
<td>191</td>
<td>15.8</td>
<td>30.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Cookies, 6 biscuits&lt;sup&gt;g&lt;/sup&gt;</td>
<td>75</td>
<td>383</td>
<td>4.7</td>
<td>51.0</td>
<td>17.9</td>
</tr>
<tr>
<td>Chocolate swiss roll&lt;sup&gt;h&lt;/sup&gt;</td>
<td>120</td>
<td>488</td>
<td>4.4</td>
<td>66.8</td>
<td>22.7</td>
</tr>
<tr>
<td>Crisps&lt;sup&gt;i&lt;/sup&gt;</td>
<td>60</td>
<td>310</td>
<td>4.7</td>
<td>32.2</td>
<td>18.0</td>
</tr>
<tr>
<td>Water&lt;sup&gt;c&lt;/sup&gt;</td>
<td>300</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2315</strong></td>
<td><strong>3942.6</strong></td>
<td><strong>158.3</strong></td>
<td><strong>430.7</strong></td>
<td><strong>179.9</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup>Aldi Stores Ltd., Ireland; <sup>b</sup>Glen Valley Foods, Ireland; <sup>c</sup>Dunnes Stores, Ireland; <sup>d</sup>Spring Fresh, Ireland; <sup>e</sup>Joseph Brennan Bakeries Ltd., Ireland; <sup>f</sup>Ballymaloe Country Relish, Hyde Ltd., Ireland; <sup>g</sup>Burton's Food Ltd., UK; <sup>h</sup>Gateaux, Premier Foods, Ireland; <sup>i</sup>Hunky Dory Crisps, Ireland. Abbreviations: g, grams; CHO, carbohydrates.

4.3.11 Statistical Analyses

Appetite sensations were transformed by natural log to account for non-equal variance. Fasting measurements of plasma lipid and glucose concentrations were
calculated as the mean of the values collected at baseline ($t = -30$ to $-20$ min). Baseline GE and appetite sensation scores were calculated as the mean of values collected at $t = -15$ and $-10$ min. The total area under response versus time curve (AUC) was calculated using the trapezoidal rule and was used as a summary measure for postprandial appetite. Blood biomarkers and appetite sensations were analysed using a two-way (time x supplement) RM ANOVA. Paired-sample t-tests were used to compare GE parameters, habitual diet, physical activity intensity and duration as well as food intake at the buffet meal (quantity, energy consumed and macronutrient distribution).

Relationships between background dietary intake as well as demographic characteristics (i.e. potential confounders) and GE half-time were compared using Pearson's correlations. Covariate adjustment was measured using one-way analysis of covariance (ANCOVA). Statistical significance was established at the $p < 0.05$ level and the mean values ± SD are reported. All statistical analyses were carried out using SPSS-version 16.0 (SPSS, Inc., Chicago, IL, USA).

A prospective power calculation was conducted for GE half-time, the primary endpoint evaluation. A sample size of 9 volunteers was necessary to detect a 15.8 % change in GE rate (Hlebowicz et al., 2007) in a two-sided paired-sample t test with alpha set at 5 % and a power of 80 %.

### 4.4 Results

Test days were well tolerated by all volunteers. There were no adverse reactions to the test meals or cinnamon capsules.

#### 4.4.1 Habitual food intake and physical activity

Volunteers successfully repeated their food diaries as indicated by no significant differences in mean energy, macronutrient or fibre intake ($p > 0.05$) for 3 days or 1 day prior to each condition (Table 4.4 (a) and (b)). Compared to the cinnamon trial, there was a trend towards increased protein intake ($15.1 ± 20.3$ g) for the day preceding the placebo condition. Volunteers also successfully repeated their 3-day physical activity diaries and there were no differences in total physical activity and intensity level of activities (Table 4.5). The mean value of EI/BMR for the cohort was $1.26 ± 0.17$. Low-energy reporters represented 11 % of the cohort. None of the volunteers were classified as energy over reporters.
### Table 4.4 (a) 3-day Energy, Macronutrient and Fibre Intake before Placebo and Cinnamon Conditions

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Intake (kcal)</td>
<td>1987 ± 318</td>
<td>1904 ± 385</td>
<td>0.40</td>
</tr>
<tr>
<td>Energy Intake (kJ)</td>
<td>8347 ± 1277</td>
<td>7964 ± 1609</td>
<td>0.40</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>83.8 ± 28.1</td>
<td>74.8 ± 25.9</td>
<td>0.12</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>70.5 ± 15.1</td>
<td>66.2 ± 18.2</td>
<td>0.30</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>264.3 ± 44.6</td>
<td>255.2 ± 63.5</td>
<td>0.68</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>16.9 ± 4.3</td>
<td>17.4 ± 3.7</td>
<td>0.72</td>
</tr>
<tr>
<td>Protein (% of energy intake)</td>
<td>19.4 ± 6.0</td>
<td>15.6 ± 3.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Fat (% of energy intake)</td>
<td>31.5 ± 3.6</td>
<td>31.9 ± 6.2</td>
<td>0.79</td>
</tr>
<tr>
<td>CHO (% of energy intake)</td>
<td>49.2 ± 4.7</td>
<td>52.4 ± 5.5</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. Carbohydrate; CHO. No significant differences were found between conditions using Student’s t-test, \( p > 0.05 \).

### Table 4.4 (b) 1-day Energy, Macronutrient and Fibre Intake before Placebo and Cinnamon Conditions

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Intake (kcal)</td>
<td>2002 ± 276</td>
<td>1880 ± 326</td>
<td>0.32</td>
</tr>
<tr>
<td>Energy Intake (kJ)</td>
<td>8376 ± 1155</td>
<td>7864 ± 1362</td>
<td>0.32</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>91.1 ± 26.8</td>
<td>76.0 ± 27.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>65.9 ± 16.0</td>
<td>70.5 ± 23.3</td>
<td>0.59</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>252.8 ± 79.9</td>
<td>232.2 ± 150.8</td>
<td>0.58</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>17.7 ± 4.9</td>
<td>16.4 ± 6.0</td>
<td>0.44</td>
</tr>
<tr>
<td>Protein (% of energy intake)</td>
<td>22.8 ± 14.6</td>
<td>15.9 ± 5.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Fat (% of energy intake)</td>
<td>29.3 ± 4.8</td>
<td>33.5 ± 8.0</td>
<td>0.10</td>
</tr>
<tr>
<td>CHO (% of energy intake)</td>
<td>47.7 ± 12.5</td>
<td>50.7 ± 6.8</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. Carbohydrate; CHO. No significant differences were found between conditions using Student’s t-test, \( p > 0.05 \).
Table 4.5 Mean 3-day Duration of light, moderate, vigorous and total duration of Physical Activity (min) before Placebo and Cinnamon Conditions (n = 9)

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (min)</td>
<td>60.0 ± 71.6</td>
<td>58.3 ± 73.2</td>
<td>0.92</td>
</tr>
<tr>
<td>Moderate (min)</td>
<td>84.4 ± 81.6</td>
<td>78.9 ± 66.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Vigorous (min)</td>
<td>50.8 ± 60.4</td>
<td>57.2 ± 83.7</td>
<td>0.79</td>
</tr>
<tr>
<td>Total (min)</td>
<td>195.2 ± 89.6</td>
<td>194.4 ± 115.5</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions using Student’s t-test, p > 0.05.

4.4.2 Gastric Emptying

No significant effect of cinnamon supplementation was observed on GE parameters (Table 4.6). GE half-time was delayed by cinnamon in five out of nine volunteers who participated in this study (Figure 4.3 (a)). Cinnamon delayed lag phase accelerated in six out of nine volunteers (Figure 4.3 (b)).

Table 4.6 Gastric Emptying (GE) Parameters after the Ingestion of High-Fat Meal Supplemented with 3 g Wheat Flour (placebo) or Cinnamon.

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-time (min)</td>
<td>237 ± 32</td>
<td>245 ± 49</td>
<td>0.42</td>
</tr>
<tr>
<td>Lag phase (min)</td>
<td>136 ± 12</td>
<td>143 ± 22</td>
<td>0.21</td>
</tr>
<tr>
<td>Latency time (min)</td>
<td>40 ± 6</td>
<td>43 ± 9</td>
<td>0.19</td>
</tr>
<tr>
<td>Ascension time (min)</td>
<td>197 ± 35</td>
<td>202 ± 48</td>
<td>0.60</td>
</tr>
<tr>
<td>cPDR (%)</td>
<td>55.1 ± 10.0</td>
<td>56.3 ± 4.9</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions, p > 0.05.
Figure 4.3 (a) Individual changes in gastric emptying half-time (GE half-time) between placebo and cinnamon conditions. Solid lines indicate a decrease (i.e. an acceleration) and dashed lines indicate an increase (i.e. a delay) in GE half-time. Purple solid bold line indicates group mean GE half-time.

Figure 4.3 (b) Individual changes in gastric emptying lag phase (GE lag phase) between placebo and cinnamon conditions. Solid lines indicate a decrease (i.e. an acceleration) and dashed lines indicate an increase (i.e. a delay) in GE lag phase. Purple solid bold line indicates group mean GE half-time.
4.4.3 Blood biomarkers

4.4.3.1 Plasma glucose
Baseline plasma glucose concentrations tended to be higher before cinnamon supplementation compared to placebo ($p = 0.06$); ($n = 8$ for all blood derived measurements). There was no significant interaction between time and supplement ($p > 0.05$) when plasma glucose concentrations were corrected for baseline.

![Figure 4.4](image)

**Figure 4.4** Mean plasma glucose concentrations corrected for baseline (fasting) glucose levels in healthy subjects after ingestion of a high-fat test meal supplemented with 3 g placebo (○) or cinnamon (●). Values are Mean ± SD (error bars; $n = 8$). No significant differences were found between conditions, $p > 0.05$ by ANCOVA.

4.4.3.2 Plasma lipids
Baseline plasma T G, total c holesterol, H DL and LD L concentrations did not differ significantly between trials. There were no changes in TAG between trials (time x condition interaction, $p > 0.05$; Table 4.7) but there was a main effect for time (pooled placebo and cinnamon data, $p < 0.05$). There were no changes in plasma total cholesterol, HDL, or LDL levels either within or between the trials (time x supplement interaction; $p > 0.05$).
Table 4.7 Mean plasma lipid values after the ingestion of high-fat meal supplemented with 3 g wheat flour (placebo) or cinnamon

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>Placebo</td>
<td>0.84 ± 0.27</td>
<td>0.93 ± 0.36</td>
<td>1.01 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>0.80 ± 0.32</td>
<td>0.88 ± 0.32</td>
<td>0.95 ± 0.37</td>
</tr>
<tr>
<td>Total Chol</td>
<td>Placebo</td>
<td>3.80 ± 0.59</td>
<td>3.79 ± 0.55</td>
<td>3.85 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>3.93 ± 0.43</td>
<td>3.77 ± 0.68</td>
<td>3.78 ± 0.69</td>
</tr>
<tr>
<td>HDL</td>
<td>Placebo</td>
<td>1.45 ± 0.31</td>
<td>1.39 ± 0.27</td>
<td>1.45 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>1.49 ± 0.41</td>
<td>1.46 ± 0.38</td>
<td>1.45 ± 0.36</td>
</tr>
<tr>
<td>LDL</td>
<td>Placebo</td>
<td>1.97 ± 0.57</td>
<td>1.98 ± 0.54</td>
<td>1.92 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>2.07 ± 0.30</td>
<td>1.91 ± 0.48</td>
<td>1.90 ± 0.41</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions, $p > 0.05$.

4.4.4 Appetite Sensations

Sensations of hunger, desire to eat and fullness under placebo and cinnamon conditions are illustrated in Figure 4.5 (a), (b) and (c), respectively. Fasting sensations of appetite did not differ significantly between conditions ($p > 0.05$); volunteers reported moderate sensations of hunger and low levels of fullness before consuming the test breakfast. Immediately after consuming the test meal, all volunteers reported reduced hunger and desire to eat as well as increased fullness. Changes to appetite sensation scores were evident over time after ingestion of test meals ($p < 0.05$). No significant effect of condition was observed on sensations of hunger. Volunteers reported significantly lower fullness under cinnamon supplementation at $t = 120$ min ($p = 0.03$). DTE scores were greater after cinnamon supplementation, and this increase became significant at $t = 330$ min ($p = 0.03$) compared to the respective time point under the control condition.

No differences between sensations of palatability, pleasantness, nausea and stomach pain were recorded when conditions were compared after the test breakfast ($t = 0$ min) and buffet-style lunch meal ($t = 390$ min). There were no differences between total AUC in appetite sensation profiles between conditions ($p > 0.05$; Table 4.7). Thirst, tiredness
and coldness (i.e. the distracter variables) did not vary significantly with supplementation or over time (data not shown).

(a)

(b)
**Figure 4.5 (a), (b) and (c) Visual Analogue Scale (VAS) Sensations of (a) hunger (b) desire to eat, and (c) fullness in healthy volunteers before (-15 min) and after ingestion of a high-fat test meal supplemented with 3 g placebo (○) or cinnamon (●). Values are Mean ± SD (error bars; n = 9). Significant differences were found between conditions, \( p < 0.05 \) by RM ANOVA.**

Summary measures of appetite responses were evaluated as postprandial time averaged AUC (Table 4.8). Volunteers reported significantly lower sensations of hunger and DTE under the placebo condition.
### Table 4.8

Time-averaged postprandial areas under response versus time curve (AUCs) for hunger, desire to eat (DTE) and fullness under Placebo and Cinnamon Conditions (n = 9).

<table>
<thead>
<tr>
<th>VAS Scores</th>
<th>Condition</th>
<th>AUC (mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>Placebo</td>
<td>59.3 ± 28.0</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>66.5 ± 20.9</td>
<td></td>
</tr>
<tr>
<td>DTE</td>
<td>Placebo</td>
<td>58.2 ± 25.6</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>68.1 ± 22.8</td>
<td></td>
</tr>
<tr>
<td>Fullness</td>
<td>Placebo</td>
<td>78.1 ± 21.4</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Cinnamon</td>
<td>71.2 ± 22.3</td>
<td></td>
</tr>
</tbody>
</table>

AUC values expressed as Mean ± SD. Time-averaged postprandial area under the curve was calculated as the mean response during the 6-h postprandial period. No significant differences were found between treatments, \( p > 0.05 \).

#### 4.4.5 Subsequent Food Intake

Analysis of the buffet meal indicated that there were no differences between the conditions for quantity of food consumed as well as total EI and fat, carbohydrate and protein intake (Table 4.9). There was no significant difference in evening food diaries which volunteers were asked to complete for the remainder of the test day subsequent to leaving the lab (data not shown).
Table 4.9 Mean Energy & Macronutrient Intake in the *ad libitum* Buffet Meal after Placebo and Cinnamon Conditions

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI (kcal)</td>
<td>730 ± 270</td>
<td>794 ± 362</td>
<td>0.28</td>
</tr>
<tr>
<td>EI (kJ)</td>
<td>3054 ± 1132</td>
<td>3324 ± 1513</td>
<td>0.28</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>39.1 ± 17.3</td>
<td>40.6 ± 21.0</td>
<td>0.58</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>32.5 ± 14.5</td>
<td>33.6 ± 17.2</td>
<td>0.75</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>73.2 ± 35.4</td>
<td>85.4 ± 44.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Protein (% of EI)</td>
<td>21.3 ± 9.7</td>
<td>21.9 ± 9.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Fat (% of EI)</td>
<td>40.0 ± 8.7</td>
<td>35.6 ± 9.7</td>
<td>0.12</td>
</tr>
<tr>
<td>CHO (% of EI)</td>
<td>40.0 ± 8.4</td>
<td>43.4 ± 12.2</td>
<td>0.17</td>
</tr>
<tr>
<td>Quantity eaten (g)</td>
<td>420.4 ± 120.0</td>
<td>466.9 ± 150.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Time (min)</td>
<td>15 ± 3</td>
<td>15 ± 5</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. EI; Energy intake, CHO; Carbohydrate. No significant differences were found between conditions, *p* > 0.05.

4.4.6 Further Analyses

4.4.6.1 Association between Anthropometric Data, Habitual Energy, Macronutrient and Fibre Intake and Gastric Emptying Half-Time

There was a significant relationship between GE half-time and body mass (*r* = -0.72, *p* = 0.03) and height (*r* = -0.73, *p* = 0.03) under the placebo condition. Similar relationships were evident under the cinnamon condition (weight: *r* = -0.80, *p* = 0.01; height: *r* = -0.71, *p* = 0.03). No relationship was evident between GE half and age or BMI under placebo and cinnamon conditions. The relationship between mean 3-day energy, macronutrient and fibre intake from the 3-day WFD and GE half-time under placebo and cinnamon conditions were also assessed (Table 4.10 (a)). Additionally, these relationships were examined using the 1-day WFD i.e. food intake data from the day preceding the test trial (Table 4.10 (b)). Strong inverse relationships were evident between GE half-time and absolute 3-day and 1-day protein intake.
Table 4.10 (a) Relationships between Mean 3-Day Energy, Macronutrient and Fibre Intake from Weighed Food Diaries (3-day WFD) and GE Half-time under Placebo and Cinnamon Conditions

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>EI (kcal·day⁻¹)</td>
<td>-0.46</td>
<td>0.22</td>
</tr>
<tr>
<td>Protein (g·day⁻¹)</td>
<td>-0.81</td>
<td>0.01**</td>
</tr>
<tr>
<td>Fat (g·day⁻¹)</td>
<td>-0.33</td>
<td>0.38</td>
</tr>
<tr>
<td>CHO (g·day⁻¹)</td>
<td>0.01</td>
<td>0.97</td>
</tr>
<tr>
<td>Fibre (g·day⁻¹)</td>
<td>-0.48</td>
<td>0.19</td>
</tr>
<tr>
<td>Protein (% of EI·day⁻¹)</td>
<td>-0.50</td>
<td>0.17</td>
</tr>
<tr>
<td>Fat (% of EI·day⁻¹)</td>
<td>0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>CHO (% of EI·day⁻¹)</td>
<td>0.64</td>
<td>0.07</td>
</tr>
</tbody>
</table>

EI; energy intake, CHO; carbohydrate. Mean values were significantly associated with GE half-time by Pearson’s Correlation: * p < 0.05; ** p < 0.01.

Table 4.10 (b) Relationships between 1-Day Energy, Macronutrient and Fibre Intake from Weighed Food Diaries (1-day WFD) and GE Half-time under Placebo and Cinnamon Conditions

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 9)</th>
<th>Cinnamon (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>EI (kcal·day⁻¹)</td>
<td>-0.06</td>
<td>0.89</td>
</tr>
<tr>
<td>Protein (g·day⁻¹)</td>
<td>-0.78</td>
<td>0.01*</td>
</tr>
<tr>
<td>Fat (g·day⁻¹)</td>
<td>0.10</td>
<td>0.80</td>
</tr>
<tr>
<td>CHO (g·day⁻¹)</td>
<td>0.26</td>
<td>0.49</td>
</tr>
<tr>
<td>Fibre (g·day⁻¹)</td>
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<td>0.79</td>
</tr>
<tr>
<td>Protein (% of EI·day⁻¹)</td>
<td>0.14</td>
<td>0.72</td>
</tr>
<tr>
<td>Fat (% of EI·day⁻¹)</td>
<td>0.15</td>
<td>0.71</td>
</tr>
<tr>
<td>CHO (% of EI·day⁻¹)</td>
<td>-0.19</td>
<td>0.63</td>
</tr>
</tbody>
</table>

EI; energy intake, Carbohydrate; CHO. Mean values were significantly associated with GE half-time by Pearson’s Correlation: * p < 0.05.
4.4.6.2 Association between Habitual Fatty Acid Intake and Gastric Emptying Half-Time

The relationship between mean 3-day fatty acid intake from the 3-day WFD and GE half-time under placebo and cinnamon conditions were also assessed. No significant differences were observed between 3-day and 1-day background fatty acid intake before cinnamon and placebo trials. Mean 3-day intake of palmitoleic acid (C16:1, 0.28 ± 0.17 g; $r = -0.76, p < 0.05$; Figure 4.6 (a)), eicosenoic acid (C20:1, 0.10 ± 0.13 g; $r = -0.70, p < 0.05$; Figure 4.5 (b)) and total n-3 intake respectively (0.50 ± 0.36 g; $r = -0.80, p < 0.01$; Figure 4.5 (c)) were inversely related to GE half-time of the placebo-supplemented HF meal. Mean 3-day intake of C16:1, C20:1 and total n-3 fatty acids accounted for 58, 49 and 64% of the variance in GE half-time respectively. Additionally, the relationship between 1-day intake of specific fatty acids and GE half-time was explored. 1-day intake of palmitoleic acid (C16:1, 0.29 ± 0.17 g; $r = -0.81, p < 0.01$; Figure 4.5 (a)), eicosenoic acid (C20:1, 0.11 ± 0.12 g; $r = -0.77, p < 0.05$; Figure 4.5 (b)) and total n-3 intake respectively (0.48 ± 0.37 g; $r = -0.79, p < 0.01$; Figure 4.5 (c)) were inversely related to GE half-time of the placebo-supplemented HF meal. 1-day intake of C16:1, C20:1 and total n-3 fatty acids accounted for 66, 59 and 62% of the variance in GE half-time respectively. No other significant correlations were found between mean 3-day and 1-day specific fatty acid intake in habitual diet and GE half-time under placebo or cinnamon conditions (data not shown).
Figure 4.6 (a) Relationship between Mean 3-day and 1-day Intake of Palmitoleic Acid (C16:1) and Gastric Emptying (GE) half-time of a high-fat breakfast under placebo condition (n = 9).

Figure 4.6 (b) Relationship between Mean 3-day and 1-day Intake of Eicosenoic Acid (C20:1) and Gastric Emptying (GE) half-time of a high-fat breakfast under placebo condition (n = 9).
4.4.6.3 Covariate-Adjusted Analyses

Adjusting for single covariates namely, body mass, BMI or 1-day or 3-day background intake of total n-3, C16:1, C20:1 or total fat intake, no significant differences were detected in GE parameters.

4.5 Discussion

The current study tested the primary hypothesis that supplementing a single HF breakfast with 3 g of cinnamon would change GE of a high-fat solid meal utilising the $^{13}$C OBT, and consequently reduce postprandial metabolic variables and appetite responses.

This study showed no significant change in GE parameters using 3 g of cinnamon. Under the placebo condition, mean GE half-time was $237 \pm 32$ min, similar to that seen in other studies that have employed a similar HF test meal ($234 \pm 54$ min; energy content: 599 kcal; fat 40 g; Clegg & Shafat, 2008). Individual analysis of GE data illustrated that cinnamon accelerated GE half-time in four volunteers whereas lag phase was accelerated in three out of nine cases. Large inter-subject variability in GE
parameters was apparent; there was a difference of 90 and 113 minutes in the slowest and fastest half-emptying time recorded in volunteers under placebo and cinnamon conditions respectively (CV = 57.5 and 49.4%). Lag phase variations of 34 and 73 minutes were also evident under the aforementioned conditions (CV = 31.3 and 30.6%). This confirms previous findings which suggest that there is large intra-variability in GE (Brophy et al., 1986). The test meal (65% of energy from fat) was mainly from sunflower oil, which contains approximately 70% linoleic acid [C18:2n-6, a PUFA] (Sobrino et al., 2003). Long-chain fatty acids have a potent inhibitory effect on GE rate (Cecil et al., 1999; Hunt & Knox, 1968) and have also been shown to increase CCK and GLP-1 concentrations (Feltrin et al., 2004). It is suggested that cinnamon does not delay GE over and above the effects of the fat content of the meal.

Similar postprandial glycaemic and lipaemic responses were observed under both conditions. However, it should be noted that we were unable to measure a hyperglycemic or hyperlipidaemic state due to the frequency of sampling. Studies (Solomon & Blannin, 2007; Hlebowicz et al., 2007) which employed the largest doses of cinnamon relative to CHO in the test meal (CHO to cinnamon ratio of 15:1 or lower) appear to have had the most potent effects on reducing postprandial glycaemia (Mettler et al., 2009). In spite of our high ratio of 14:1, we did not achieve a significant blood glucose-lowering effect. This is possibly due to glucose absorption from the small intestine being affected by the fat content of a meal (Normand et al., 2001).

Recent data indicates that the addition of 3 g cinnamon to a low-fat rice pudding test meal had no significant effect on GE rate or postprandial glycaemia in healthy individuals (Hlebowicz et al., 2009). However, cinnamon did significantly lower serum insulin levels and increase GLP-1 concentrations, a GI peptide which has been shown to increase glucose-dependent secretion of insulin, delay GE and reduce glucose absorption and postprandial glycaemia (Deane et al., 2010; Näslund et al., 1999). When added to the same test meal, 6 g cinnamon significantly delayed GE and reduced postprandial glycaemia but the decrease in blood glucose concentration 64.1 mmol·min·l⁻¹ was greater than the 2.5% lower rate of GE suggesting that GE cannot be the sole mechanism explaining lower blood glucose responses following cinnamon ingestion (Hlebowicz et al., 2007). In agreement with the findings of others, (Hlebowicz et al., 2007; Mettler et al., 2009; Hlebowicz et al., 2009) the current study found that volunteers had similar sensations of appetite and subsequent ad libitum food intake under placebo and cinnamon conditions. Cinnamon is unlikely to be relevant in affecting the postprandial response to HF meals. This is a novel finding that was never considered before in the extant literature.
A significant inverse association between body mass, but not BMI, and GE half-time was observed under both placebo and cinnamon conditions suggesting that as body mass increased, GE was accelerated (i.e. shorter). This finding is in agreement with a study which showed a relationship between faster GI handling of food and a high body weight in heavier weight, non-obese adults (Johansson & Ekelund, 1976). On the contrary, Lavigne et al. (1978) showed a linear relation between GE of solid food and body size in normal-weight individuals. The observation in the current study is consistent with the concept that GE is accelerated in the obese state (Wright et al., 1983; Tosetti et al., 1996; Cardoso-Junior et al., 2007; Clegg et al., 2009; for full review see Section 2.4.5). Adjusting for body mass or BMI did not affect the way GE responded to cinnamon.

The habitual dietary intake of volunteers was comparable to past national data (Harrington et al., 2001). No significant difference was found between mean 3-day and 1-day intake of energy, macronutrient and fibre intake prior to placebo and cinnamon conditions. Strong inverse correlations were observed between GE half-time and absolute 3-day and 1-day protein intake. Assessment of previous day dietary intake indicated that a higher intake of C16:1, C20:1 and total n-3 was associated with a shorter GE half-time of the HF meal supplemented with the wheat flour placebo. However, when adjustments were made for these potential confounders, we did not detect a delay in GE parameters after cinnamon ingestion. A single meal, supplemented with n-3 PUFAs, was less capable of triggering GLP-1 and CCK compared to other fats, resulting in a more rapid GE of a HF breakfast (Robertson et al., 1999) while others (Riber et al., 1996) showed that n-3 PUFA fish oil reduced CCK release and gallbladder contraction without affecting GE. Both GLP-1 and CCK are putative mediators of the ileal brake, a feedback mechanism responsible for delaying transit and facilitating digestion, in response to lipids in the distal GI tract (Dobson et al., 2000). Our current findings extend these observations to illustrate that even short-term intake of n-3 fatty acids is associated with faster GE rates, in a population who were not eating a HF diet but adjustment for this fatty acid did not lead to a significant effect of the intervention. Because the subjects in the current study ate an identical test-meal, mechanisms apart from acute release of GLP-1 and CCK, must mediate the effects of specific fatty acids on GI transit. Recently, a 3-day HF yoghurt supplementation, rich in C18:2n-6 accelerated the GE rate of a test meal rich in the same fatty acid (Clegg et al., 2011). It is interesting to note that background intake of C18:2n-6, which was high in the test meal, did not show a strong association with GE of the meal. It is tempting to speculate about the potential mechanisms for fat sensing and adaptation following the recently sequenced GPR120 protein, expressed on intestinal cells, and demonstrated to be differentially sensitive to
different fatty acids (Moore et al., 2009). These observations suggest that GE half-time of a HF meal is not just specifically affected by a background intake of that specific fatty acid and that the process of adaptation to a HF diet may involve mechanisms other than desensitisation to a specific fatty acid. It is likely that different adaptations are continuously taking place in the gut, in response to the balance of fatty acids in the diet.

It is likely the study was underpowered and therefore a small effect below the detection threshold of the study cannot be ruled out. Three grams of cinnamon were used because it was shown to have a similar chronic effect on fasting serum glucose and lipid profiles as 6 g (Khan et al., 2003). Similarly Mang et al., 2006 and Solomon and Blannan (2009) have conveyed that 3 g improved blood glucose responses in diabetic and healthy populations respectively. However, recent evidence suggests a dose-dependent relationship for cinnamon (1 – 3 g) consumed and the delay in GE (Hlebowicz et al., 2009).

In conclusion, data presented in this study found no evidence for delayed GE rate in response to a HF meal supplemented with 3 g cinnamon compared to a matched placebo. Furthermore, cinnamon did not modulate postprandial glycaemic and lipaemic responses, or reduce appetite sensations or subsequent food intake in an ad libitum buffet meal. Given the association between 1- and 3-day fatty acid intake and GE half-time, controlling for background fatty acid composition requires consideration in future gastrointestinal studies.
Chapter 5: Review of the Carbon Dioxide Production Rate Assumption in the $^{13}$C Octanoic Acid Breath Test

5.1 Abstract

**Background/ Objective:** The $^{13}$C octanoic acid breath test (OBT) is a safe and repeatable non-invasive method for measuring GE. However, true cumulative percentage of $^{13}$C recovered in breath (cPDR) is dependent on accurate measurement of $\dot{V}$CO$_2$ production rate. The current study aimed to quantify differences in the $^{13}$C OBT results obtained using directly measured $\dot{V}$CO$_2$ gathered throughout the $^{13}$C OBT with a constant directly measured and a predicted resting $\dot{V}$CO$_2$ production rate.

**Methods:** The GE rate of a HF test meal was assessed in twenty seven healthy normal weight volunteers (age 24.2 ± 3.2 yrs, weight 68.82 ± 9.8 kg; BMI 22.3 ± 1.8 kg·m$^2$). Breath samples, and exhaled air samples for measurement of $\dot{V}$CO$_2$ production rates, were gathered during the fasted state and at regular intervals throughout the 6-h postprandial period. A constant predicted value 300 mmol CO$_2$·min$^{-1}$·m$^{-2}$· normalised for BSA was employed (Shreeve et al., 1970).

**Results:** Directly measured and predicted resting $\dot{V}$CO$_2$ production rates underestimated cPDR by 4.8 and 2.7% compared to directly measured $\dot{V}$CO$_2$ gathered throughout the $^{13}$C OBT. GE parameters were positively misinterpreted using resting and estimated values of $\dot{V}$CO$_2$, with GE half-time appeared 12 and 9 minutes longer respectively compared to directly measured $\dot{V}$CO$_2$.

**Conclusion:** The findings highlight the importance of directly measuring $\dot{V}$CO$_2$ production rates throughout the $^{13}$C OBT and could partly explain the inconsistencies in the literature regarding the directionality of GE rates in obesity.

**Keywords:** $^{13}$C OBT, gastric emptying, $\dot{V}$CO$_2$ production rates.
5.2 Introduction

The $^{13}$C OBT was originally proposed as a valid alternative to scintigraphy, the ‘reference method’ for measuring GE by Ghoos and colleagues (Ghoos et al., 1993). Justification for a new technique included the need for a more cost-effective tool that required less expertise and was appropriate for repeated within-subject applications (ibid). The tool is suitable for use in vulnerable paediatric, pregnant and geriatric populations because it is non-invasive and does not pose a radiation risk to patients (Modak et al., 2007). The OBT is based on the principle that the labelled substrate, $^{13}$C octanoic acid, is firmly retained in the stomach but upon reaching the duodenum is rapidly absorbed. The $^{13}$C octanoate is subsequently oxidised in the liver and excreted in breath as $^{13}$CO$_2$ (see section 2.6.6.2 for full review of the process). Excretion of $^{13}$C in breath acts as an indirect representation of GE, the rate-limiting step in the process (Perri et al., 2005).

In $^{13}$C breath tests, it is vital to have an accurate measurement of $\dot{V}CO_2$ production rate for quantification of the true percentage of $^{13}$C recovered (PDR) in breath at a given time point and cumulatively, cPDR, over the 6 h postprandial period. The cPDR model is based on the ‘empirical fact that the breath test curve representing the cumulative dose in function of time is inversely analogue to the scintigraphic curve of gastric emptying’ (Ghoos et al., 1993). In research, it is common practice to estimate PDR based on a predicted resting $\dot{V}CO_2$ production rate of 5 mmol·min$^{-1}$·m$^{-2}$ body surface area (Shreeve et al., 1970). Body surface area (BSA) is calculated using the weight-height equation of Haycock et al. (1978). A limitation of this assumption is that it does not take into account that increases in $\dot{V}CO_2$ production rate will cause an underestimation of the tracer labelled CO$_2$ due to dilution with unlabelled CO$_2$ generated endogenously from metabolism (Slater et al., 2006). Although movement is often minimised during the OBT (Schommartz et al., 1997; Robertson et al., 2002; Clegg & Shafat, 2010), consumption of a test meal can increase $\dot{V}CO_2$ production rate and thus lead to an underestimation of true cPDR. Increased $\dot{V}CO_2$ production rate during the postprandial period is known as diet-induced thermogenesis (DIT) or the thermic effect of the food consumed (Westerterp, 2004). DIT is the summation of energy requirements for digestion, intestinal absorption and clearance of ingested nutrients (Crovetti et al., 1997). Although DIT is dependent on factors such as the nutrient composition of the diet, it is acknowledged that DIT represents approximately 10% of daily energy expenditure (Westerterp, 2004). There is also the possibility that using a constant predicted resting value of $\dot{V}CO_2$ might underestimate early increases in $\dot{V}CO_2$ production rates as a result of DIT and...
overestimate values of $\dot{V}CO_2$ in the latter measurement period of the OBT when the thermic effect of food would be reduced (Westerterp, 2004). This could lead to positive misinterpretations of GE parameters such as GE half-time and lag phase (Ghoos et al., 1993).

As well as its potential influence on cumulative PDR over the duration of the test day, using a constant predicted resting value of $\dot{V}CO_2$ may also influence calculation of GE parameters such as GE half-time. There is a possibility that the assumption of $\dot{V}CO_2$ causes a bias by ignoring DIT after test meal consumption, therefore leading to an overestimation of early $\dot{V}CO_2$ production rate and an overestimation of $\dot{V}CO_2$ production rate at the later stages of the breath test, leading to higher GE half-time compared to scintigraphy. Ghoos and colleagues (1993) reported that GE determined by the OBT overestimated half-time by 66 minutes compared to the scintigraphy value. Thus the authors subtracted this value of time from half-time obtained using scintigraphy to give a corrected value of half-time measured by the OBT. It is feasible that some of the 66 minute bias as is as a result of estimating $\dot{V}CO_2$ production rate and this highlights the importance of examining breath test GE parameters using estimated and directly measured values of $\dot{V}CO_2$ production rate.

5.2.1 Aims and Hypotheses

The objective of this study was to quantify differences in $^{13}$C OBT results obtained using directly measured $\dot{V}CO_2$ production rates and predicted $\dot{V}CO_2$ production rates (Shreeve et al., 1970).

The following hypothesis is addressed in this chapter (stated as the null hypothesis):

$H_{01}$: Using directly measured $\dot{V}CO_2$ production rate throughout the $^{13}$C OBT will not result in (i) a constant value of directly measured resting $\dot{V}CO_2$ production rate (RMR) (ii) a constant predicted resting value of $\dot{V}CO_2$ normalised for BSA (Shreeve et al., 1970; Haycock et al., 1978).

5.3 Methods

5.3.1 Volunteer Characteristics

Twenty seven healthy adults (age $24.2 \pm 3.2$ yrs, weight $68.82 \pm 9.8$ kg; BMI $22.3 \pm 1.8$ kg·m$^2$) consented to partake in this study following ethical approval from the Education
and Health Sciences Research Ethics Committee (EHSREC No: 09/ 74). Prior to study participation, volunteers filled out a pre-test questionnaire to ensure that they had no medical conditions that would compromise study endpoints. None had any history of gastrointestinal disorders or disturbances within 3 months of study entry. Written informed consent was obtained from each volunteer prior to study participation. Data for this chapter came from normal weight volunteers who were recruited for, and completed, the first (control) test trial in study three (see Chapter 6). It should be noted that five out of twenty seven of these volunteers did not complete the HF intervention in Chapter 6 (see Figure 6 for explanation of drop-outs) and hence there is a discrepancy in numbers between the two chapters.

5.3.2 Experimental Design

This study was a subsection of a larger intervention study (For details see Section 6.3.). Volunteers refrained from vigorous physical activity and alcohol for 24 hours prior to testing (Clegg et al., 2011). To minimise $^{13}$C basal abundance in breath, volunteers were asked to avoid spicy foods, pineapple and corn products (Morrison et al., 2000). To control for the effect of the menstrual cycle on gastrointestinal transit and appetite, all testing was conducted on female volunteers during the follicular phase of their cycle (Brennan et al., 2009).

5.3.3 Test Procedure

Volunteers arrived at the metabolic suite after a 12-h overnight fast. Measurements of body mass and stature were collected as previously outlined (Section 3.3.3). Following 15 minutes of supine rest on an examination couch and a 5-min familiarisation period with the Douglas bag technique, a 15-min resting metabolic rate (RMR) exhaled air sample was collected ($t = -45$ min) whilst the volunteer remained supine (see below). Volunteers returned to a seated position and after a 10-min interval baseline abundance of $^{13}$C breath samples were collected ($t = -20$ and -15 min). Once baseline measurements were taken, volunteers consumed the test meal within 10 min (see below). Upon completion of the meal, the stopwatch was reset to zero ($t = 0$ min), and sequential postprandial measurements of breath $^{13}$CO$_2$ were collected at $t = 0$ min, every 5 min for the first 30 min after meal consumption and thereafter in 15-min intervals from $t = 30$ until 360 min for the detection of $^{13}$CO$_2$. Collection of 12-min expired air samples was carried out in 60-min intervals starting at $t = 45$ min in the seated position.
5.3.4 Test Meal

The HF test meal (Table 5.1) was rich in monounsaturated fat and was based on olive oil. The test meal was put together using ingredients low in $^{13}$C abundance so that it did not influence breath $^{13}$CO$_2$ enrichment (Morrison et al., 2000; Slater, 2004). The pancakes were prepared under standardised conditions using 40 g flour (Odlums, Shamrock Foods Ltd., Ireland), 44 g egg (medium free-range; Dunnes Stores, Ireland), 70 g semi-skimmed milk (Dunnes Stores, Ireland) 40 g olive oil (Tesco Stores Ltd., Cheshunt, UK). Three pancakes were served with 20 g chocolate spread (Nutella, Ferrero UK Ltd, England) and 200 ml of water. 12 g inulin (Raftiline HP, Orafti, Belgium) was added to the flour. The pancake was enriched with 150 μl (135mg) $^{13}$C octanoic acid (Cambridge Isotope Laboratories, Andover, MA, USA), which was solubilised in the egg yolk prior to cooking as previously described (Section 3.3.4). After homogenising the yolk, it was mixed with the other ingredients to ensure uniform distribution of the label throughout the pancake batch. No other eating or drinking was permitted during the sessions. The kitchen door remained closed during cooking to minimise the effect of olfactory stimulation (sight or smell of food) on subsequent gastrointestinal responses (Arosio et al., 2004).

<table>
<thead>
<tr>
<th>Table 5.1 Nutritional content of test meal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mass</strong></td>
</tr>
<tr>
<td>(g)</td>
</tr>
<tr>
<td>Meal</td>
</tr>
</tbody>
</table>

5.3.5 Gastric Emptying

GE was measured using isotope ratio mass spectrometry (DeltaV, ThermoFisher Scientific, Hemel Hempstead, UK) as previously described (Section 3.3.5).

5.3.6 Predicting resting $\dot{V}$CO$_2$ production rate

$\dot{V}$CO$_2$ production rate was predicted from body surface area. Resting $\dot{V}$CO$_2$ was assumed to remain constant at 5 mmol CO$_2$·min$^{-1}$·m$^{-2}$ body surface area (Shreeve et al., 1970), which equates to 300 mmol CO$_2$·min$^{-1}$·m$^{-2}$. Body surface area (BSA) was calculated from height and weight using the Haycock et al. (1978) equation:

$$\text{BSA (m}^2\text{)} = 0.024265 \times (\text{height (cm)}^{0.3964} \times (\text{weight (cm)}^{0.5378})$$
Predicted $\dot{\text{CO}_2}$ (mmol·h$^{-1}$) = 300 x BSA

5.3.7 Measuring $\dot{\text{CO}_2}$ production rate

Resting metabolic rate (RMR) was collected following a 12 h fast and a 24 h abstention period from vigorous physical activity and alcohol. In order to minimise increased energy expenditure, volunteers were asked to drive to the laboratory. The measurement was carried out in a darkened room and a thermoneutral environment (21 – 24 °C) with minimal external stimuli to ensure quiet surroundings. The Douglas Bag technique was employed to collect expired air samples (Consolazio et al., 1963). Measurement of the fraction of expired O$_2$ and CO$_2$ (FEO$_2$ and FECO$_2$) was made for a 15 min duration and subsequently, non-protein respiratory exchange ratio (RER), rate of energy expenditure and substrate utilisation were calculated using indirect calorimetry equations adapted from Consolazio et al. (1963):

$$\text{RER} = \frac{\dot{\text{CO}_2}}{\dot{\text{O}_2}}$$

Rate of fat oxidation (g·min$^{-1}$) = $(1.689 \times \dot{\text{O}_2}) - (1.689 \times \dot{\text{CO}_2})$

Rate of carbohydrate oxidation (g·min$^{-1}$) = $(4.12 \times \dot{\text{CO}_2}) - (2.91 \times \dot{\text{O}_2})$

Rate of energy expenditure (kJ·min$^{-1}$) = $(15.88 \times \dot{\text{O}_2}) - (4.87 \times \dot{\text{CO}_2})$

5.3.8 Expired Air Collection and Analysis

As previously mentioned, the Douglas Bag technique was employed to collect expired air samples (Consolazio et al., 1963). Prior to the collection of samples, the Servomex gas analyser was calibrated against known reference gases (100% N$_2$ balance and 15% O$_2$, 5% CO$_2$; BOC Gases Ireland, BOC Ltd, Limerick, Ireland) and barometric pressure was measured. All gas measurements were corrected to standard room temperature and pressure (STPD) for a dry gas. The fraction of oxygen [FIO$_2$] and carbon dioxide [FICO$_2$] in the inspired air were assumed to be constant (20.8 % and 0.05 % respectively).

Analysis of expired air samples was conducted in two separate steps:

(i) 0.5 L of air was extracted through a sampling valve of the Douglas bag at a constant flow rate. This air passed into a pre-calibrated gas analyser (Servomex 1440 Series, Servomex Group Ltd, East Sussex, UK) for measurement of the fraction of O$_2$ and CO$_2$ (FEO$_2$ and FECO$_2$).
(ii) For the measurement of sample volume, the remaining expired air was extracted from the Douglas bag at a constant flow rate by an air fan (~ 30 l·min⁻¹) using a dry gas meter (Harvard Apparatus Ltd, Kent, UK).

Values of O₂ and C O₂ from expired air (\( \dot{V} \text{E} \)) were calculated using the Haldane Transformation. Analysis of expired air was calculated using an automated spreadsheet (Egan, 1999) using the following equations:

(1) Calculation of \( \dot{V} \text{E} \) at ambient temperature, pressure and saturated with water vapour (ATPS)

\[
\dot{V} \text{E} \text{ (ATPS)} = (V/t) \times 60
\]

Where: \( \dot{V} \text{E} \) ~ indicates expired flow rate (l·min⁻¹)

\( V \) ~ indicates volume (L)

\( t \) ~ indicates duration of sample (s)

(2) Calculation of \( \dot{V} \text{E} \) at body temperature, pressure and saturated with water vapour (BTPS)

\[
\dot{V} \text{E} \text{ (BTPS)} = \dot{V} \text{E} \text{ (ATPS)} \times (273 + 37)/(273 + T) \times (P_B - P_{H2O})/(P_B - 47.08)
\]

Where: \( P_B \) ~ indicates barometric pressure (mmHg)

\( T \) ~ temperature of sample gas in volume measuring device (°C)

\( P_{H2O} \) ~ partial pressure of water at \( T \) (mmHg)

-Body temperature was assumed to be 37°C, and \( P_{H2O} \) at body temperature was assumed to be 47.08 mmHg.

(3) Calculation of \( \dot{V} \text{E} \) at standard temperature, pressure and pressure, dry (STPD) (Wasserman et al., 1994)

\[
\dot{V} \text{E} \text{ (STPD)} = \dot{V} \text{E} \text{ (BTPS)} \times (273)/(273 + 37) \times (P_B - 47.08)/(760)
\]

(4) Estimation of \( \dot{V} \text{I} \) (Adapted from Wilmore and Costill, 1973)

\[
\dot{V} \text{I} = \dot{V} \text{E} \times (1 - (FEO_2 + FECO_2)/(1 - (FIO_2 + FICO_2))
\]

Where: \( \dot{V} \text{I} \) ~ indicates inspired flow rate (l·min⁻¹)

\( FEO_2 \) ~ indicates fraction of O₂ in expired air

\( FECO_2 \) ~ indicates fraction of CO₂ in expired air
FIO₂ ~ indicates fraction of O₂ in inspired air

FICO₂ ~ indicates fraction of CO₂ in inspired air

(5) Calculation of \( \dot{\text{V}} \text{O}_2 \) and \( \dot{\text{V}} \text{CO}_2 \) (Consolazio et al., 1963)

\[
\dot{\text{V}} \text{O}_2 \ (\text{l} \cdot \text{min}^{-1}) = (\dot{\text{V}}_i \times \text{FIO}_2) - (\dot{\text{V}}_e \times \text{FEO}_2)
\]

\[
\dot{\text{V}} \text{CO}_2 \ (\text{l} \cdot \text{min}^{-1}) = (\dot{\text{V}}_i \times \text{FICO}_2) - (\dot{\text{V}}_e \times \text{FECO}_2)
\]

(6) \( \dot{\text{V}} \text{CO}_2 \ (\text{l} \cdot \text{min}^{-1}) \) measurements were converted to mmol.min\(^{-1}\) using the calculation previously employed by Amarri et al. (1998):

\[
\dot{\text{V}} \text{CO}_2 \text{(mmol·min}^{-1}) = \dot{\text{V}} \text{CO}_2 \text{(mmol·min}^{-1})/(R \times (273.15 + \ ^\circ\text{C})
\]

Where: \( R \) (gas constant) ~ indicates 0.08206 litres/ atom per \(^\circ\text{C} \) per mol

-Room temperature was assumed to be 23\(^\circ\text{C}\)

5.3.9 Calculation of percentage dose recovered (PDR) in breath

\(^{13}\text{C}\) enrichment in breath over baseline was determined by subtracting \(^{13}\text{C}\) abundance in baseline breath samples (mean of two samples) from that of the post-dose samples. PDR in each post-dose breath sample was calculated using the following formula: The cumulative PDR (cPDR) was determined by adding individual PDR values that were averaged over the time interval between samples using the trapezoidal rule (Amarri et al., 1998; Slater et al., 2006).

\[
PDR \ h^{-1} = \left( (\text{At}\% \ t_0 - \text{At}\% \ t_b) + (\text{At}\% \ t_{0+1} - \text{At}\% \ t_b)/2 \right) \times (t_{0+1} - t_0) \times R_{\text{PDB}} \times 10^{-3} \times \dot{\text{V}} \text{CO}_2 \times 100\% \text{ Tracer dose}
\]

Where: \text{At}\% \(^{13}\text{C}\) (Atom Percent) = \( (^{13}\text{C}/ \ ^{12}\text{C} + ^{13}\text{C}) \)

\[
R_{\text{PDB}} = ^{13}\text{C}/^{12}\text{C} \text{ in PDB (international standard Pee Dee Belemnite)} = 0.0112372
\]

\text{At}\% \ t_0, \text{At}\% \ t_{0+1}, \text{At}\% \ t_b \text{ are enrichments at time points } t_0, t_{0+1} \text{ (post-dose) and } t_b \text{ (baseline) respectively}

\text{Tracer dose (mmol) = weight substrate (i.e. 135 mg) / molecular weight (i.e. 145.2 g·mol}^{-1})

\[
\dot{\text{V}} \text{CO}_2 \text{(mmol·h}^{-1}) = \dot{\text{V}} \text{CO}_2 \text{(mmol·min}^{-1})/(R \times (273.15 + \ ^\circ\text{C}) \times 60
\]
The cPDR was calculated by adding the PDR values for each time point and represented the area under the curve for the duration of the OBT. cPDR and GE parameters were calculated and using the following values of CO₂ production rates (\(\dot{V}CO₂\)):

1. A constant predicted value of resting \(\dot{V}CO₂\) production rate normalised for body surface area (Section 5.2.6)
2. A constant value of directly measured resting \(\dot{V}CO₂\) production rate at rest (RMR) by indirect calorimetry (Section 5.2.7)
3. Sequential values of directly measured \(\dot{V}CO₂\) production rate at rest (RMR) and postprandially indirect calorimetry (Section 5.2.7).

5.3.10 Statistical Analyses

The relationship between GE half-time by directly measured \(\dot{V}CO₂\) production throughout the test day and (i) a constant predicted value of resting \(\dot{V}CO₂\) production or (ii) a constant value of directly measured resting \(\dot{V}CO₂\) production were examined using Pearson's Correlations, and analysed in PASW-version 18.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was established at the \(p < 0.05\) level and the mean values ± standard deviation are reported. Bias and limits of agreement of PDR and GE parameters were calculated using the Bland & Altman (1986) method. The data were analysed in Microsoft Excel 2007 using Analyse-it Method Evaluation software (Microsoft Corporation, Redmond, WA). The most accurate method was taken to have no bias i.e. the mean difference between directly measured and predicted \(\dot{V}CO₂\) production rates for PDR and GE calculations equalled zero.

5.4 Results

Measurements of \(\dot{V}CO₂\) production rates from twenty-seven normal weight volunteers were evaluated and used to calculate cumulative \(^{13}\)C percentage dose recovered (cPDR) and GE parameters at 6h postprandially. A comparison of sequential values of directly measured \(\dot{V}CO₂\) values from throughout the test day was made with (i) a constant value of directly measured resting \(\dot{V}CO₂\) production rate (RMR) and (ii) a constant predicted resting value of \(\dot{V}CO₂\) normalised for BSA (Shreeve et al., 1970; Haycock et al., 1978) and are illustrated in Table 5.2.
Table 5.2 Comparison of cumulative $^{13}$C percentage dose recovered (cPDR) and gastric emptying (GE) parameters obtained using directly measured $\dot{V}$CO$_2$ values throughout the test day, a constant value of directly measured resting $\dot{V}$CO$_2$ value and a constant predicted resting $\dot{V}$CO$_2$ value normalised to body surface area (Shreeve et al., 1970; Haycock et al., 1978). Values are mean ± standard deviation (SD), n = 27. Correlations were significantly different from the measured parameter: **p < 0.01.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measured</th>
<th>Measured resting</th>
<th>r</th>
<th>Predicted</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>cPDR (%)</td>
<td>34.6 ± 7.5</td>
<td>29.8 ± 7.2</td>
<td>0.77**</td>
<td>32.0 ± 5.6</td>
<td>0.65**</td>
</tr>
<tr>
<td>Half-time (min)</td>
<td>202 ± 35</td>
<td>215 ± 36</td>
<td>0.93**</td>
<td>212 ± 35</td>
<td>0.86**</td>
</tr>
<tr>
<td>Lag phase (min)</td>
<td>133 ± 22</td>
<td>140 ± 23</td>
<td>0.96**</td>
<td>138 ± 23</td>
<td>0.86**</td>
</tr>
<tr>
<td>Latency time (min)</td>
<td>48 ± 9</td>
<td>50 ± 10</td>
<td>0.93**</td>
<td>49 ± 9</td>
<td>0.82**</td>
</tr>
<tr>
<td>Ascension time (min)</td>
<td>155 ± 31</td>
<td>165 ± 31</td>
<td>0.90**</td>
<td>163 ± 31</td>
<td>0.87**</td>
</tr>
</tbody>
</table>

Table 5.3 Bias and Limits of Agreement between cumulative $^{13}$C percentage dose recovered (cPDR) and gastric emptying (GE) parameters obtained using directly measured $\dot{V}$CO$_2$ values throughout the test day and a constant value of directly measured resting $\dot{V}$CO$_2$ value, n = 27.

<table>
<thead>
<tr>
<th></th>
<th>cPDR (%)</th>
<th>Half-time (min)</th>
<th>Lag phase (min)</th>
<th>Latency time (min)</th>
<th>Ascension time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias</td>
<td>-4.83</td>
<td>12.45</td>
<td>7.26</td>
<td>2.06</td>
<td>10.39</td>
</tr>
<tr>
<td>Limits of Agreement</td>
<td>-14.60, 4.92</td>
<td>-13.81, 38.70</td>
<td>-5.91, 20.43</td>
<td>-5.03, 9.14</td>
<td>-16.63, 37.41</td>
</tr>
</tbody>
</table>
Table 5.4 Bias and limits of agreement between cumulative $^{13}$C percentage dose recovered (cPDR) and gastric emptying (GE) parameters obtained using directly measured $\dot{V}CO_{2}$ values throughout the test day and a constant predicted value of resting $\dot{V}CO_{2}$ value normalised to body surface area (Shreeve et al., 1970; Haycock et al., 1978), $n=27$.

<table>
<thead>
<tr>
<th></th>
<th>cPDR (%)</th>
<th>Half-time (min)</th>
<th>Lag phase (min)</th>
<th>Latency time (min)</th>
<th>Ascension time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias</td>
<td>-2.66</td>
<td>9.54</td>
<td>5.17</td>
<td>1.16</td>
<td>8.38</td>
</tr>
<tr>
<td>Limits of Agreement</td>
<td>-13.91, 8.59</td>
<td>-26.23, 45.31</td>
<td>-17.85, 28.18</td>
<td>-9.82, 12.14</td>
<td>-22.18, 38.95</td>
</tr>
</tbody>
</table>

5.4.1 Cumulative $^{13}$C Percentage Dose Recovered

5.4.1.1 Comparison of Cumulative 13C Percentage Dose Recovered (cPDR) calculated using directly measured $\dot{V}CO_{2}$ throughout the test day and constant value of directly measured $\dot{V}CO_{2}$ at rest

The relationship between cPDR calculated using directly measured $\dot{V}CO_{2}$ values from throughout the test day and a constant value of directly measured $\dot{V}CO_{2}$ provided an $R^2$ of 0.59, $p < 0.01$ (see Figure 5.1).
Figure 5.1 The relationship between cumulative $^{13}$C percentage dose recovered (cPDR) calculated using directly measured $\dot{VCO}_2$ throughout the test day and constant value of directly measured $\dot{VCO}_2$ at rest.

Figure 5.2 shows a Bland-Altman residual plot of cPDR obtained from directly measured $\dot{VCO}_2$ at rest and throughout 6 h postprandial period and a constant prediction from directly measured $\dot{VCO}_2$ at rest. There was a bias of -4.83 % (95% CI -6.80 to -2.86) between these two methods of analysis and the limits of agreement range (mean difference ± 2 SD) from -14.60 to 4.92 %.
Figure 5.2 Bland-Altman residual plot of cumulative $^{13}$C per centage dose recovered (cPDR) calculated using directly measured $\dot{V}CO_2$ throughout the test day and constant value of directly measured $\dot{V}CO_2$ at rest. The arrows indicate the mean difference plus two standard deviations (Mean + 2SD), the mean difference and the mean minus two standard deviations (Mean-2SD).

5.4.1.2 Comparison of Cumulative $^{13}$C Percentage Dose Recovered (cPDR) calculated using directly measured $\dot{V}CO_2$ throughout the test day and a constant value of predicted $\dot{V}CO_2$ at rest, normalised to body surface area

Figure 5.3 illustrates the relationship between cPDR calculated using directly measured $\dot{V}CO_2$ values gathered throughout the test day and a constant value of predicted $\dot{V}CO_2$. This provided an $R^2$ of 0.43, $p < 0.01$. 
Figure 5.3 The relationship between cumulative $^{13}$C percentage dose recovered (cPDR) calculated using directly measured $\dot{V}$CO$_2$ throughout the test day and constant value of predicted $\dot{V}$CO$_2$ at rest.

The Bland-Altman residual plot (Figure 5.4) highlights the bias between the two methods i.e. using directly measured $\dot{V}$CO$_2$ during rest and throughout the 6-h postprandial period and a constant value of predicted $\dot{V}$CO$_2$ at rest to measure cPDR. The plot shows that 2.66% of the data (95% CI -4.93 to -0.39) lies outside the limits of agreement range (mean difference ± 2 SD) from -13.91 to 8.59 %.
Figure 5.4  Bland-Altman residual plot of cumulative $^{13}$C percentage dose recovered (cPDR) calculated using directly measured $\dot{V}$CO$_2$ throughout the test day and a constant value of predicted $\dot{V}$CO$_2$ at rest. The arrows indicate the mean difference plus two standard deviations (Mean + 2SD), the mean difference and the mean minus two standard deviations (Mean-2SD).

5.4.2.1 Comparison of Gastric emptying (GE) half-time calculated using directly measured $\dot{V}$CO$_2$ throughout the test day and a constant value of directly measured $\dot{V}$CO$_2$ at rest

The relationship between GE half-time calculated using directly measured $\dot{V}$CO$_2$ values gathered throughout the test day and a constant value of directly measured $\dot{V}$CO$_2$ provided an $R^2$ of 0.86, $p < 0.01$ (see Figure 5.5).
Figure 5.5 The relationship between gastric emptying (GE) half-time calculated using directly measured $\dot{V}$CO$_2$ throughout the test day and a constant value of directly measured $\dot{V}$CO$_2$ at rest.

Figure 5.6 highlights the Bland-Altman residual plot of GE half-time obtained from directly measured $\dot{V}$CO$_2$ throughout the test day and a constant value of directly measured $\dot{V}$CO$_2$ at rest. There was a bias of 12.45 min (95% CI 7.15 to 17.75) between these two methods of analysis and the limits of agreement range (mean difference ± 2 SD) from -13.81 to 38.70 min.
Figure 5.6 Bland-Altman residual plot of gastric emptying (GE) half-time calculated using directly measured \( \dot{V}_{\text{CO}_2} \) throughout the test day and a constant value of directly measured \( \dot{V}_{\text{CO}_2} \) at rest. The arrows indicate the mean difference plus two standard deviations (Mean + 2SD), the mean difference and the mean minus two standard deviations (Mean-2SD).

5.4.2.2 Comparison of Gastric emptying (GE) half-time calculated using directly measured \( \dot{V}_{\text{CO}_2} \) throughout the test day and a constant value of predicted \( \dot{V}_{\text{CO}_2} \) at rest, normalised to body surface area

Figure 5.7 illustrates the relationship between GE halftime calculated using directly measured \( \dot{V}_{\text{CO}_2} \) values from throughout the test day and a constant value of predicted \( \dot{V}_{\text{CO}_2} \). This provided an \( R^2 \) of 0.75, \( p < 0.01 \).
Figure 5.7 The relationship between gastric emptying half-time calculated using directly measured $\dot{V}CO_2$ throughout the test day and a constant value of predicted $\dot{V}CO_2$ at rest.

The Bland-Altman residual plot (Figure 5.7) highlights the bias between the two methods i.e. using directly measured $\dot{V}CO_2$ during rest and throughout the 6-h postprandial period and constant prediction from directly measured $\dot{V}CO_2$ at rest to measure gastric emptying half-time. The plot shows that 9.54 min of the data (95% CI 2.32 to 16.76) lies outside the limits of agreement range (mean difference ± 2 SD) from -26.23 to 45.31 min.
Figure 5.8 Bland-Altman residual plot of gastric emptying (GE) half-time calculated using directly measured $\dot{V}_{CO_2}$ throughout the test day and a constant value of predicted $\dot{V}_{CO_2}$ at rest. The arrows indicate the mean difference plus two standard deviations (Mean + 2SD), the mean difference and the mean minus two standard deviations (Mean -2SD).

5.5 Discussion

The isotope $^{13}$C OBT shows promise as an alternative to the ‘reference GE measurement technique,’ scintigraphy (Sanaka et al., 2010). In contrast to scintigraphy, the OBT is non-invasive, does not pose a radiation risk to patients and is not dependent on a highly skilled operator (Ghoos et al., 1993). Numerous validation studies have established that the OBT is strongly correlated with scintigraphy for GE of liquid, semi-solid and solid meals in normal subjects and dyspeptic patients (Ghoos et al., 1993; Braden et al., 1995; Brome et al., 2002). However, the $^{13}$C OBT is not without its limitations. Excretion of $^{13}$C in breath acts as an indirect measurement of GE (Perri et al., 2005). Factors such as delayed $^{13}$C excretion (retention) and loss can lead to incomplete $^{13}$CO$_2$ recovery, independent of GE rate (Sanaka et al., 2010). However, these authors have previously argued that pulmonary excretion of $^{13}$CO$_2$ represents GE because ‘post-gastric processes’ have lower inter- and intra-subject variability (Sanaka et al., 2008). Methodological issues may also lead to a failure in accurately quantifying $^{13}$C PDR in breath. Quantification of $^{13}$C PDR requires an exact measurement of $\dot{V}_{CO_2}$...
production rate, and many researchers use a predicted resting value for $\dot{V}CO_2$ based on the formula of Shreeve et al. (1970); which assumes a constant $\dot{V}CO_2$ production rate for the duration of the breath test. This study aimed to assess differences in $^{13}C$ OBT outcome measures obtained using directly measured $\dot{V}CO_2$ production rates and predicted $\dot{V}CO_2$ production rates (Shreeve et al., 1970).

Findings of this study illustrated that both directly measured and predicted resting $\dot{V}CO_2$ production rates underestimated CPDR compared to using directly measured $\dot{V}CO_2$ values from throughout the test day. There was a wide range in variability in both positive and negative directions. However cPDR by directly measured resting $\dot{V}CO_2$ was 1.96 % more biased than the assumption based prediction of PDR. Factors such as movement and consumption of a test meal, i.e. DIT during the OBT, are known to increase $\dot{V}CO_2$ production rates. Relying upon an assumption will not account for factors that can increase $\dot{V}CO_2$ production rate and can result in lower $^{13}CO_2$ enrichment in the breath compared to direct measurement of $\dot{V}CO_2$ (Slater et al., 2006; Amari et al., 1998).

It has recently been hypothesised that GE parameters i.e. half-time, lag phase, latency time and ascension time would be less sensitive to changes in $CO_2$ production rates (Clegg & Shafat, 2010). On the contrary, data presented in this chapter highlighted that all GE parameters were positively misrepresented by using resting measured and estimated values of $\dot{V}CO_2$ (Table 5.3; Table 5.4). GE half-time, which is the time it takes for 50 % of the total dose of $^{13}C$ to be excreted in breath, appeared 12 and 9 min longer with measured resting and predicted $\dot{V}CO_2$ respectively than directly measured $\dot{V}CO_2$ values from throughout the test day.

When normalised for BSA, the $\dot{V}CO_2$ production rate assumption of Shreeve et al. (1978) would suggest that obese individuals have a higher resting $\dot{V}CO_2$ than leaner individuals in the fasted state creating a bias towards overestimation of GE parameters due to lower $^{13}C$ enrichment in the breath. However, the assumption of Shreeve and colleagues does not take into account that although obese individuals have a greater BSA, this does not infer greater $\dot{V}CO_2$ production rate since adipose tissue is less metabolically active than skeletal muscle. Another argument is that obese individuals have significantly reduced sensitivity to the thermic effect of food, when compared to their normal weight counterparts (De Jonge & Bray, 1997). Compared to lean individuals, this would lead to
of a reduced $\dot{V}CO_2$ production rate in the early postprandial period in an obese cohort. One could speculate that use of a constant predicted resting value of $\dot{V}CO_2$ in an obese individual might overestimate $\dot{V}CO_2$ production rate, leading to a greater bias towards delayed GE in obese compared to lean individuals. The combination of the aforementioned factors could lead to a greater bias towards over-estimation of GE parameters in obese compared to lean individuals. However, future work needs to quantify the bias in GE parameters using the estimated, as opposed to directly measured $\dot{V}CO_2$ production rate in obese and lean individuals.

The difference in GE rates of obese individuals compared to their lean counterparts remains inconclusive in the literature (for full review see section 2.4.5). Two of these studies used the OBT to measure GE and found conflicting results. Jackson et al. (2004) reported delayed GE half-time and lag phase of a solid HF test meal (2 MJ (477 kcal)-40% energy from fat) in obese individuals compared to lean individuals whereas the opposite was shown by Cardoso-Junior et al. (2007) using a standard test meal (1 MJ (250 kcal)-fat content not specified). It is difficult to make direct meaningful comparisons between these studies since the test meals varied in energy and fat content. However, the conflicting results may be partly attributed to using different assumptions to estimate $\dot{V}CO_2$ production rate as opposed to using direct measurements of $\dot{V}CO_2$ throughout the OBT. The former used the Schofield’s value for basal metabolic rate (BMR) multiplied by 1.3 (Schofield, 1985) which assumes a respiratory quotient of 0.85, while Cardoso-Junior et al. (2007) used the assumption of Shreeve et al. (1970) normalised for body surface area (Haycock et al., 1978). The findings of this thesis suggest that employing an estimated or measured resting value of $\dot{V}CO_2$ leads a delay in the appearance of GE half-time by 9 - 12 minutes in a lean cohort compared to using a direct measurement of $\dot{V}CO_2$. If obese individuals do have reduced DIT (De Jonge & Bray, 1997) using the Shreeve et al. (1970) assumption of $\dot{V}CO_2$ for this cohort would create a bias towards delayed GE compared to directly measuring $\dot{V}CO_2$.

A recognised limitation to this study was the use of the Douglas Bag technique for measurement of $\dot{V}CO_2$ production rate, as a lack of familiarity with the mouthpiece apparatus can cause hyperventilation and thus lead to a measurement that inaccurately reflects $\dot{V}CO_2$ (Rizza, 1997, p105). Future research should use a ventilated canopy system for measurement of $\dot{V}CO_2$ production rate.
In conclusion, the findings of this study suggest that the predicted value and directly measured \( \dot{V}\text{CO}_2 \) production rate at rest overestimated cumulative \(^{13}\text{C}\) percentage dose recovered and underestimated GE parameters compared to directly measured \( \dot{V}\text{CO}_2 \) production rates from throughout the postprandial period. If direct measurements of \( \dot{V}\text{CO}_2 \) production rate are not feasible, a predicted value of \( \dot{V}\text{CO}_2 \) normalised for body surface area should be used cautiously in healthy normal weight adults.
Chapter 6: Effect of a 5-day Adaptation and Deadaptation Period to a High-fat Diet Supplemented with Specific Fatty Acids on Gastrointestinal Transit, Appetite and Substrate Utilisation

6.1 Abstract

Background/ Objectives: High-fat diets (HFDs) of as little as three days have been shown to accelerate GI transit. This study aimed to assess if 5-day HF supplemented diet varying in fatty acid composition were sufficient to accelerate GI transit, appetite responses and substrate utilisation and whether a 5-day deadaptation period to a HF diet would reverse the responses induced by adaptation to a HFD.

Methods: Twenty four healthy adults (weight 72.2 ± 4.4 kg; BMI 23.4 ± 3.9 kg/m²) completed three 5-day interventions. Volunteers were randomly divided into 3 HF groups: olive oil (OO), olive oil and fish powder blend (OF) or olive oil and macadamia oil group (OM). In the first intervention period, normal diet was supplemented with LF milkshakes. The second and third interventions involved repeating previous diet along with HF or LF milkshakes respectively. Each 5-day intervention period was followed by measurement of GE, MCTT, appetite responses and substrate utilisation.

Results: When adjusted for background dietary fat intake, GE was accelerated after the HF intervention but there was no significant trial x group interaction. Satiety was reduced after a 5-day HF intervention and had not returned to baseline levels after a 5-day deadaptation period. During the HF intervention, the OF group had reduced energy and fat intake compared to the OO and OM group.

Conclusions: This study illustrated that a 5-day is a sufficient period to accelerate GI transit and appetite and that satiety is not returned to baseline levels following a 5-day deadaptation period. n-3 PUFAs appear to have an appetite suppressant effect.

Keywords: gastric emptying, high-fat diet, n-3 PUFAs, MCTT, appetite
6.2 Introduction

The World Health Organization envisages that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese (WHO, 2011) with overconsumption of HF, energy dense foods cited as a major contributing factor to the epidemic (Rolls, 2000). There is a direct relation between intake of dietary fat and adverse weight profiles (Rolls, 1995) and in countries where obesity is escalating rapidly, consumption of fat may contribute to 45% of total daily energy intake (Golay & Bobbioni, 1997).

Dietary fat has potent stimulatory effects within the small intestine (Heddle et al., 1988) and compared to a LF meal, acute oral ingestion of a HF meal causes a delay in GE (Cecil et al., 1999) to the degree that it can influence the GE rate of a subsequent meal (Clegg et al., 2010). On the contrary, chronic exposure to dietary fat can lead to physiological alterations of the GI tract (Covasa et al., 2010; Little et al., 2007; French et al., 1995). A recently published study has illustrated that exposure to a HF supplemented diet for as little as 3 days was sufficient to accelerate GE latency phase (control: 50 ± 13 min; HF intervention: 40 ± 7 min) and MCTT (control: 280 ± 60 min; HF intervention: 226 ± 84 min) (Clegg et al., 2011). Furthermore, it was illustrated by the same research group that a 7-day HFD accelerated GE latency phase (control: 45 ± 8 min; HF intervention 41 ± 10 min) and MCTT (control: 308 ± 43 min, HF intervention 248 ± 83 min) in 10 healthy males. However, no changes were reported in GI transit after 14, 21 or 28 days of a HFD intervention in a subset of 7 of the same cohort (Clegg & Shafat, 2011). Cunningham and colleagues (1991) have examined the effect of HF feeding on GE and MCTT after a period of 4 and 14 days in healthy adults. A 14-day HFD reduced the delay in GE half-time by a mean of 49 min and MCTT by 120 min compared to a LFD. The same study found no difference in GE half-time or MCTT after 4 days of HF feeding, although significantly less food was left in the stomach at 100 min postprandially (Cunningham et al., 1991). Additionally, it was substantiated that consumption of a HFD for 14 days reduced linear GE rate of a HF (pre HFD: 0.36 ± 0.05 ·min⁻¹, post HFF 0.47 ± 0.03 ·min⁻¹ (mean ± S E)) but not a LF meal (Castiglione et al., 2002). One mechanism which has been proposed to explain the acceleration in GE in response to HF feeding was demonstrated by Boyd and colleagues (2003); a 14-day HFD attenuated pyloric tonic and phasic pressure and increased antropyloroduodenal pressure-wave sequences compared to a LFD without affecting CCK or GLP-1 responses. Conversely, a more recent study illustrated that a 21 day HFD raised fasting plasma CCK but did not affect upper gut motility or polypeptide-YY and ghrelin concentrations during a CCK-8 infusion in lean, healthy males (Little et al., 2008). M CTT at 7 - and 21 -days after...
commencement of a HF supplemented diet, but not 14-days, was slower compared to baseline (Henderson et al., 1998).

Adaptation to a HF diet may also lead to changes in appetite responses in humans. Short term exposure to a HF supplemented diet for 7 days has been shown to reduce satiety but not food intake in an *ad libitum* buffet (Clegg & Shafat, 2011), whereas a 3-day HFD had no affect on appetite responses (Clegg et al., 2011). Exposure to a 14-day HFD resulted in a significant increase in average energy consumption compared with pre-diet levels (Energy Intake: Post HFD: 10.3 (SE 0.5) MJ·day⁻¹, Pre HFD: 9.6 (SE 0.6) MJ·day⁻¹) (French et al., 1995). Furthermore, a 14-day HFD resulted in greater sensations of hunger after an oral fat tolerance test but failed to increase EI in healthy adults (Boyd et al., 2003). A more recent study by Park et al. (2007) concluded that HF supplementation resulted in increased maximum tolerated volume of the stomach, independent of BMI. It was suggested by the authors that chronic exposure to dietary fat could lead to delayed time to satiation, thus leading to overeating in an attempt to reach a sensation of fullness.

The timeframe of the extant literature examining the effect of HF dietary intervention on GI transit is illustrated in Figure 2.8. It is evident that there is a dearth of research examining the effect of short-term HF feeding on GE and MCTT. In addition, the time course of deadaptation to a HF diet has not previously been explored in humans. In rats, 28 days was not a sufficient time period for the lipid-induced delay to return to baseline stomach to caecum transit time after a 28 day period of chronic intermittent ileal infusion of palm oil (Brown et al., 1994). Increasing amounts of research are showing that the quality of dietary fat may be equally pertinent as absolute quantity of fat with regards to development of lifestyle disease (MacIntosh et al., 2003; Khor, 2004). The influence of fatty acids on GI function is fatty acid chain length dependent, with previous literature illustrating that ≥ 12 carbon atoms are necessary to induce a delay in GE, release GI hormones and suppress EI (Hunt & Knox, 1968; Matzinger et al., 2000; Mc Laughlin et al., 1999; Feltrin et al., 2004). Additionally, Robertson and colleagues (2002) established that GE half-time was significantly faster after acute consumption of an *n*-3 PUFA meal (155 ± 6 min) compared to *n*-6 PUFA (237 ± 22 min; *p* ≤ 0.001), MUFA (219 ± 17 min; *p* ≤ 0.01) and SFA meals (221 ± 15 min; *p* ≤0.05). Ingestion of the *n*-3 PUFA meal resulted in delayed CCK secretion and reduced GLP-1 concentrations compared to the SFA and MUFA meal conditions respectively; this was suggested as the mechanism responsible for enhanced GE (Robertson et al., 2002). A study by Maljaars and colleagues (2009)
demonstrated that compared to a saline control, ileal infusions of 6 g of C18:1 or C18:2, 
(but not C18:0), increased CCK secretion, while study fatty acid chain length exerted no 
influence on PYY secretion.

The degree of fatty acid saturation may also affect appetite, eating behaviour as well as 
postprandial thermogenesis and substrate oxidation (Lawton et al., 2000; Casas-
Agustench et al., 2008; Maljaars et al., 2009). Maljaars and colleagues showed that 
acute infusion of unsaturated fatty acids increased satiety without affecting food intake. 
Compared to a SFA meal, Casas-Agustench and co-workers (2008) showed that 5-h 
postprandial thermogenesis was 28% and 23% higher after acute intake of meals rich 
in PUFA and MUFA respectively; the authors did not find that fat quality affected 
substrate oxidation or satiety. GE was proposed as a potential mechanism responsible 
for differences in substrate utilisation and thermogenesis after ingestion of fatty meals 
varying in fat quality (Casas-Agustench et al., 2008). Chronic ingestion of a diet rich in 
oleic acid for 28 days resulted in greater fat oxidation than a diet enriched in palmitoleic 
acid (Kien et al., 2005).

The findings of Clegg et al. (2011) suggested that 3 days was a sufficient time period to 
adapt to a narrow range of PUFA, predominantly sunflower oil and accelerate GI transit 
of a HF meal rich in the same oil. Evidence from acute studies (Robertson et al., 2002; 
Maljaars et al., 2009) suggests that fat quality of a meal leads to different responses in 
the GI tract. Furthermore, observational evidence from Chapter 4 indicated that 
background intake of palmitoleic acid (C16:1), eicosenoic acid (C20:1) and total n-3 
intake were inversely related to GE half-time of a HF meal rich in linoleic acid. This 
finding suggests that GE half-time of a HF meal is not just specifically affected by a 
background intake of that specific fatty acid and that the process of adaptation to a HF 
diet may involve mechanisms other than desensitization to a specific fatty acid.

6.2.1 Aims and Hypotheses

A gap remains in the literature regarding how 5-day supplemented diets rich in various 
fatty acids affect gastrointestinal transit, appetite and substrate utilisation. This study 
examined the effect of a 5-day HFD supplemented with specific fatty acids on GE, 
MCTT, appetite, food intake and substrate utilisation. Another novel aspect of this 
chapter was examining whether a subsequent 5-day period is sufficient to reverse the 
effects of HF feeding on GE and other aforementioned parameters.
The following hypotheses will be addressed in this chapter (stated as the null hypotheses):

**H\textsubscript{o}1:** Exposure to a 5-day HF supplemented diet will not change GE or MCTT nor will it change appetite and substrate utilisation compared to baseline.

**H\textsubscript{o}2:** Following a 5-day deadaptation period, a HF supplemented diet will not reverse the change in GE or MCTT induced by adaptation to a HF diet nor will it change sensations of appetite, food intake or substrate utilisation.

**H\textsubscript{o}3:** Exposure to different fatty acids in a HF supplemented diet will not result in different rates of GE and MCTT nor will it alter sensations of appetite, food intake or substrate utilisation.

**H\textsubscript{o}4:** Habitual dietary intake, including fatty acid intake will not be associated with alterations in GE and adjustments for these covariates will not affect the primary endpoint.

### 6.3 Methods

#### 6.3.1 Volunteer Characteristics

This study was conducted on 24 apparently healthy adults (age 24.9 ± 4.4 yrs; height 1.75 ± 0.09 m; weight 72.2 ± 4.4 kg; BMI 23.4 ± 3.9 kg·m\(^{-2}\)). Prior to study participation, volunteers filled out a pre-test questionnaire to ensure that they had no medical conditions that would compromise study endpoints. Volunteers were screened and deemed eligible for participation having met the following criteria on the pre-test questionnaire: (i) aged 18-40 years (ii) no history of diabetes or cardiovascular disease (iii) no gastrointestinal disorders or gastrointestinal disturbances within 3 months of study entry (iv) no allergies to wheat or dairy products. Females were required to have a regular menstrual cycle. Average dietary restraint scores were 7.3 ± 3.3. Two volunteers were identified as restrained eaters [scoring > 12 on the eating restraint section (factor 1) of the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985; Martins et al., 2008)]. This study was ethically approved by the Education and Health Sciences Research Ethics Committee (EHSREC No: 09/74). Written informed consent was obtained from each volunteer prior to study participation.
6.3.2 Recruitment Process

Volunteers were recruited from the Limerick area using a variety of media. Figure 6.1 illustrates the number of volunteers engaged in the study, including cases of exclusion and attrition.

Figure 6.1 Study flowchart illustrating volunteer recruitment, randomisation and drop-out at each stage of intervention for olive oil and fish powder (OF), olive oil (OO), olive oil and macadamia oil (OM) groups respectively.

6.3.3 Experimental Design

Volunteers participated in a randomised, single-blind parallel study. The timeline of the intervention is highlighted in Figure 6.2. Volunteers were randomised to one of three fatty
acid intervention arms using the 'randbetween' function in Excel 2007 (Microsoft Corp. Redmond, WA, USA). Volunteers whose entry into the spreadsheet generated a '-1', '0' and '1' were assigned to the olive oil and fish powder (OF), olive oil (OO), olive oil and macadamia oil (OM) groups respectively. Each volunteer completed three test trials. The first and second test trials were separated by 28 days. The third trial was on the 6th day after the second trial (5 days apart). All testing for females volunteers was conducted during the follicular phase of their menstrual cycle to control for the effect of the menstrual cycle on GI transit and appetite (Brennan et al., 2009).

6.3.4 Dietary Standardisation

Volunteers completed a 5-day weighed-food diary (WFD) preceding the first trial (Control; CON). They were provided with a LF chocolate milkshake supplement daily which they were required to drink alongside their normal diet. Prior to the second and third trial, volunteers were instructed to repeat this WFD as accurately as possible with the addition of a HF or LF milkshake supplement respectively. The supplement could be consumed at any stage throughout the day except in the 12 hour prior to test trials. There was a high level of compliance with the study regimen which is partly attributable to frequent contact with the volunteers. Volunteers refrained from vigorous physical activity and alcohol for 24 hours prior to testing (Clegg et al., 2011). To minimise $^{13}$C basal abundance in breath, volunteers were asked to avoid spicy foods and corn products (Morrison et al., 2000). Prior to testing volunteers were blinded to the nature of the supplement and they were informed that the study was examining the effect of macronutrients in the diet on digestive and cardiovascular health (Appendix 1 B).
6.3.5 Dietary Supplementation

It was decided to supplement volunteers' habitual diet with HF supplements. This methodology has previously been employed by Henderson et al. (1998), Robertson et al. (2004), Clegg et al. (2011) and Clegg & Shafat (2010). In comparison to the usual procedure of presenting subjects with foods that they might not be accustomed to in artificial LF and HF diets (i.e. confounding factor), dietary manipulation of habitual food...
intake to produce a HF diet may reduce variability within the study (Henderson et al., 1998).

The control supplement consisted of 275 g semi-skimmed milk (Dunnes Stores, Ireland), 25 g chocolate milkshake mix (Nesquik; Nestlé, York, UK), 15 g dried skimmed milk (Marvel; Chivers Ireland Ltd., Dublin, Ireland) and 1 g xanthan gum (Doves Farm Foods, Berkshire, UK) combined to form a milkshake. This milkshake was brought up to a volume of 580 ml with still water. The CON, LF and HF milkshake supplement were identical apart from the addition of fatty acids to the latter (see Table 6.1). Volunteers were randomly assigned to one of three HF supplements varying in fatty acid composition: (i) OO: 90 g olive oil (Tesco Stores Ltd., Cheshunt, UK) (ii) OF: 86.67 g olive oil and 3.3 g of n-3 EPA/DHA fish powder (MEG-3; Ocean Nutrition, Dartmouth, Nova Scotia, Canada) and (iii) OM: 74.82 g olive oil and 15.18 g macadamia nut oil (Swanson Health Products, Fargo, ND, US).

Table 6.1 Nutritional content of milkshake supplement with and without fat

<table>
<thead>
<tr>
<th></th>
<th>Energy content (kcal/ kJ)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON/ LF Milkshake</td>
<td>272/ 1138</td>
<td>16.4</td>
<td>40.8</td>
<td>6.2</td>
</tr>
<tr>
<td>HF milkshake</td>
<td>1082/ 4527</td>
<td>16.4</td>
<td>40.8</td>
<td>96.2</td>
</tr>
</tbody>
</table>

Based on the manufacturers’ information, 3.3 g of fish powder was given to provide 500 mg EPA/DHA to the supplement. This quantity was based on recommendations for EPA/DHA intake in healthy adults (International Society for the Study of Fatty Acids and Lipids). The quantity of macadamia oil was matched to give an equal amount of C16:1 to EPA. The fatty acid composition of the fat blends was quantified retrospectively by gas chromatography (Table 6.2).
Table 6.2 Fatty acid composition of test oil blends*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>OO</th>
<th>OF</th>
<th>OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>-</td>
<td>0.29</td>
<td>-</td>
</tr>
<tr>
<td>C16:0</td>
<td>11.48</td>
<td>11.34</td>
<td>10.71</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.58</td>
<td>1.24</td>
<td>2.59</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.54</td>
<td>0.61</td>
<td>0.47</td>
</tr>
<tr>
<td>C18:1</td>
<td>80.65</td>
<td>78.51</td>
<td>80.82</td>
</tr>
<tr>
<td>C18:2</td>
<td>-</td>
<td>0.08</td>
<td>-</td>
</tr>
<tr>
<td>C18:3</td>
<td>-</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>C20:1</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>C20:2</td>
<td>-</td>
<td>0.09</td>
<td>-</td>
</tr>
<tr>
<td>C20:4</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>C20:5</td>
<td>-</td>
<td>0.29</td>
<td>-</td>
</tr>
<tr>
<td>C22:5</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>C22:6</td>
<td>-</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>6.75</td>
<td>7.39</td>
<td>5.42</td>
</tr>
</tbody>
</table>

* Fatty acid composition of the test supplements were analysed by Deirdre Ní hEidhín in the Department of Life Sciences, University of Limerick. Values are the mean of three replicates (expressed as % of total fatty acids in experimental fat blends).

6.3.6 Test Procedure

Volunteers arrived at the metabolic suite after a 12-h overnight fast. See test day outline in Figure 6.2. Measurements of body mass and stature were collected as previously outlined (Chapter 3). Following 15 minutes of supine rest on an examination couch and a 5-min familiarisation period with the douglas bag technique, a 15-min resting metabolic rate (RMR) expired air sample was collected ($t = -45$ min) whilst the volunteer remained supine (see below). Volunteers returned to a seated position and after a 10-min interval baseline abundance of $^{13}$C and $H_2$ breath samples were collected and visual analogue scale (VAS) questionnaires were completed ($t = -20$ and $-15$ min). Once baseline measurements were taken, volunteers consumed the test meal within 10 min (see below). Upon completion of the meal, the stopwatch was reset to zero ($t = 0$ min), and sequential postprandial measurements of breath $^{13}$CO$_2$, breath-$H_2$ and appetite sensations were taken from this point onwards. $^{13}$CO$_2$ breath samples were collected at $t$
= 0 min, every 5 min for the first 30 min after meal consumption and thereafter in 15-min intervals from \( t = 30 \) until 360 min for the detection of \(^{13}\text{CO}_2\). VAS questionnaires were administered after consumption of breakfast \((t = 0 \text{ min})\) and every 30 min until \( t = 360 \) min. \( \text{H}_2 \) breath samples were taken at \( t = 0, 10, 20, 30, 40, 45, 60 \text{ min} \) throughout the postprandial period. Collection of 12-min expired air samples was carried out in 60-min intervals starting at \( t = 45 \text{ min} \). At the end of the postprandial assessments \((t = 360 \text{ min})\), volunteers were presented with an assortment of cold lunch-type buffet foods. After ingestion of the buffet meal, volunteers completed another VAS questionnaire and were then free to leave the laboratory. Additional VAS questionnaires were administered after consumption of the test meal and buffet meal \((t = 0 \text{ and } 390 \text{ min})\) to evaluate meal palatability as well as sensations of nausea and well-being. Volunteers recorded their subsequent \textit{ad libitum} food intake for the remainder of the test day.

### 6.3.7 Test meal

The HF test meal (Table 6.3) was rich in monounsaturated fat and was based on olive oil. The test meal was put together using ingredients low in \(^{13}\text{C} \) abundance so that it did not influence breath \(^{13}\text{CO}_2\) enrichment (Morrison et al., 2000; S later, 2004). The pancakes were prepared under standardised conditions using 40 g flour (Odlums, Shamrock Foods Ltd., Ireland), 44 g egg (medium free-range; Dunnes Stores, Ireland), 70 g semi-skimmed milk (Dunnes Stores, Ireland) and 40 g olive oil (Tesco Stores Ltd., Cheshunt, UK). Three pancakes were served with 20 g chocolate spread (Nutella, Ferrero UK Ltd, England) and 200 ml of water. 12 g inulin (Raftiline HP, Orafti, Belgium) was added to the flour. The test meal was enriched with 150 μl (135mg) \(^{13}\text{C} \) octanoic acid (Cambridge Isotope Laboratories, Andover, MA, USA), which was solubilised in the egg yolk prior to cooking as previously described (Section 3.3.4). After homogenising the yolk, it was mixed with the other ingredients to ensure uniform distribution of the label throughout the pancake batch.

<table>
<thead>
<tr>
<th>Table 6.3 Nutritional content of test meal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meal</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Meal</td>
</tr>
</tbody>
</table>
6.3.8 *Ad Libitum* Buffet Meal

At the end of the postprandial period, volunteers were provided with a buffet meal and were given 30 min (i.e. $t = 360 - 390$ min) to eat *ad libitum* until 'comfortably full' (See Section 4.3.9). The selection presented was in excess of anticipated consumption and consisted of a variety of food items varying in macronutrient content (Table 6.3). Volunteers consumed the buffet meal in isolation to minimise the effect of experimenter presence on feeding behaviour (Herman & Polivy, 2005). Volunteers were blinded to the purpose of the buffet meal (i.e. measurement of energy and macronutrient intake) and were instead informed that the buffet meal aimed to explore changes in food palatability after consuming the milkshake supplements.
Table 6.4 Energy and Macronutrient Content of 21 Food Items based on Manufacturer’s Information

<table>
<thead>
<tr>
<th>Food items</th>
<th>Amt served (g)</th>
<th>Energy content (kcal)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ham, sliced</td>
<td>115</td>
<td>125</td>
<td>18.3</td>
<td>2.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Chicken, sliced</td>
<td>115</td>
<td>153</td>
<td>32.2</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Cheese, grated</td>
<td>100</td>
<td>410</td>
<td>25.5</td>
<td>2.5</td>
<td>33.5</td>
</tr>
<tr>
<td>Tomatoes, sliced</td>
<td>60</td>
<td>11</td>
<td>0.5</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Lettuce</td>
<td>50</td>
<td>7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Cucumber, sliced</td>
<td>80</td>
<td>8</td>
<td>0.6</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Sweetcorn, drained</td>
<td>90</td>
<td>70</td>
<td>2.4</td>
<td>14.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Coleslaw</td>
<td>100</td>
<td>117</td>
<td>2.1</td>
<td>7.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Brown bread, 6 slices</td>
<td>235</td>
<td>486</td>
<td>21.6</td>
<td>96.1</td>
<td>3.5</td>
</tr>
<tr>
<td>White bread, 6 slices</td>
<td>235</td>
<td>515</td>
<td>20.4</td>
<td>101.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Butter</td>
<td>50</td>
<td>269</td>
<td>0.2</td>
<td>0.3</td>
<td>29.5</td>
</tr>
<tr>
<td>Mayo</td>
<td>50</td>
<td>363</td>
<td>0.8</td>
<td>0.9</td>
<td>40</td>
</tr>
<tr>
<td>Relish</td>
<td>50</td>
<td>87</td>
<td>0.8</td>
<td>20.7</td>
<td>0</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>250</td>
<td>243</td>
<td>10.5</td>
<td>29.0</td>
<td>9.3</td>
</tr>
<tr>
<td>Chocolate</td>
<td>100</td>
<td>505</td>
<td>8.0</td>
<td>61.8</td>
<td>25.0</td>
</tr>
<tr>
<td>Swiss roll</td>
<td>100</td>
<td>450</td>
<td>4.9</td>
<td>55.7</td>
<td>22.7</td>
</tr>
<tr>
<td>Crisps</td>
<td>60</td>
<td>310</td>
<td>4.7</td>
<td>32.2</td>
<td>18</td>
</tr>
<tr>
<td>Peaches</td>
<td>220</td>
<td>106</td>
<td>0.9</td>
<td>25.7</td>
<td>0</td>
</tr>
<tr>
<td>Orange juice</td>
<td>500</td>
<td>170</td>
<td>30.0</td>
<td>45.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cola</td>
<td>500</td>
<td>210</td>
<td>0</td>
<td>53.0</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>700</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3760</td>
<td>4128</td>
<td>154.1</td>
<td>428.0</td>
<td>201.8</td>
</tr>
</tbody>
</table>

a Dunnes Stores, Ireland; b O’Kanes, Ireland; c Sunny South, Ireland; d Joseph Brennan Bakeries Ltd., Ireland; e Ballymaloe Country Relish, Hyde Ltd., Ireland; f Glenisk, Ireland; g Mars Ireland, Ireland; h Cadbury Ireland Ltd., Ireland; i Hunky Dory Crisps, Ireland; j Squeez, Ireland; k Coca Cola Ltd, Ireland. Abbreviations: g, grams; CHO, carbohydrates.

6.3.9 Resting Metabolic Rate

See Section 5.2.7 detailed review.
6.3.10 Expired Air Collection and Analysis

See Section 5.2.8

6.3.11 Gastric Emptying

GE was measured as previously described (Section 3.3.5).

6.3.12 Mouth to Caecum Transit Time

Measurements of breath H₂ were collected using a H₂ meter device (Micromedical, Chantham, UK) for analysis of mouth to caecum transit time (MCTT). Upon reaching the caecum, inulin (a non-digestible substrate added to the test meal) is metabolised by colonic bacteria which releases H₂ gas in the breath. Volunteers were asked to exhale slowly and gently into the H₂ meter through a mouthpiece until maximal exhalation was reached whilst wearing a nose clips. MCTT was defined as an increase in breath H₂ over three consecutive readings amounting to a minimum cumulative increase of 10 ppm (Bond et al., 1975).

6.3.13 Appetite Sensations

A 150-mm VAS questionnaire was used to monitor hunger, satiety, fullness, and prospective food consumption (PFC). Desire to eat (DTE) something fatty, salty, sweet or savoury before the breakfast test meal and throughout the postprandial period was also assessed (Appendix 2A). DTE something salty, sweet or savoury were used as distracter variables and were not subsequently analysed. Directly after the test meal and ad libitum buffet meal consumption, volunteers rated the meal palatability (visual appeal, smell, taste, aftertaste and pleasantness) as well as feelings of general wellbeing and nausea (Flint et al., 2000; Appendix 2B). Measurement of questionnaire responses was conducted as previously described (Section 3.3.6).

6.3.14 Dietary Analysis and Assessment of Underreporting

See section 3.3.7.

6.3.15 Statistical Analyses

Statistical analysis was conducted using SPSS (PASW Statistics Version 18; Surrey, UK). Prior to statistical analysis, data were tested for normality using the Shapiro-Wilk normality test. Skewed data were logarithmically transformed. GE, appetite, substrate utilisation, energy intake and food intake were explored using t wo-way repeated measures analysis of variance (RM ANOVA) with one within- (trial) and between- subject
(group) factors. The total area under response versus time curve (AUC) was calculated using the trapezoidal rule and was used as a summary measures for postprandial appetite, energy expenditure and substrate utilisation responses. Post hoc Fisher’s least significant difference (LSD) tests and 95% confidence intervals (CI) were used to identify where differences lay. One-way RM ANOVA was used to analyse pooled GE data for adaptation and deadaptation. Pearson’s correlations were used to examine the relationship between habitual dietary intake (1- and 5-day), anthropometric data and GE parameters. Covariate adjustment was measured using two-way analysis of covariance (ANCOVA). All data are expressed as mean ± SD and significance was accepted at the $p < 0.05$ level.

6.4 Results

Test days were well tolerated by all volunteers. Data from two volunteers was disregarded because they did not have a complete data set. Additionally, data of one volunteer was omitted from analysis following notification of a diagnosis of a GI disorder.

6.4.1 Habitual Food Intake

6.4.1.1 5-day Energy and Macronutrient Intake

Mean energy and macronutrient intake for 5-days prior to CON, HF and LF conditions are illustrated in Table 6.5. There was no significant main effect of trial for energy intake ($p = 0.21$). However, there was a significant interaction ($p = 0.03$). Output from significant RM ANOVA analyses only is shown in Table 6.6. The OF group had a lower energy intake than the OO group. Interpretation of 95% CI revealed that the OF group had a lower EI than the OO group before the HF and LF trials. The OF group also had a lower intake of energy before the HF trial than the OM group. There was no significant main effect of trial for fat intake ($p = 0.36$) but there was a significant trial x group interaction ($p = 0.03$). Post hoc analysis revealed that the OF group consumed less fat than the OO group ($p = 0.04$). 95% CI showed that the OF group consumed less fat before the HF and LF test. There were no significant main or interaction effects for protein, fat or fibre intake. There was a main effect of trial for calcium intake (Table 6.5). There were no significant group by trial interaction. Post hoc analysis revealed that calcium intake was lower before the HF trial than before the CON trial ($p = 0.03$). There were no significant main effects or group x trial interaction effects for the proportion of energy intake derived from protein, fat or CHO. The mean value of EI/BMR for the cohort was 1.23 ± 0.19
(CON trial; mean 5-day EI, n = 24). Low-energy reporters represented 29% of the cohort. None of the volunteers were classified as energy over reporters.

Table 6.5 5-day Energy, Macronutrient, Fibre and Calcium Intake before Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 24)

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>**EI <em>(kcal·day⁻¹)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>2030 ± 414</td>
<td>2076 ± 618</td>
<td>2206 ± 1025</td>
</tr>
<tr>
<td>OF</td>
<td>2010 ± 306</td>
<td>1504 ± 293 † ‡</td>
<td>1721 ± 292 †</td>
</tr>
<tr>
<td>OM</td>
<td>1978 ± 232</td>
<td>2187 ± 554</td>
<td>2039 ± 772</td>
</tr>
<tr>
<td>**EI <em>(kJ·day⁻¹)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>8492 ± 1734</td>
<td>8684 ± 2586</td>
<td>9231 ± 4286</td>
</tr>
<tr>
<td>OF</td>
<td>8410 ± 1282</td>
<td>6292 ± 1224 † ‡</td>
<td>7199 ± 1220 †</td>
</tr>
<tr>
<td>OM</td>
<td>8875 ± 971</td>
<td>9150 ± 2319</td>
<td>8529 ± 3229</td>
</tr>
<tr>
<td>**Protein <em>(g·day⁻¹)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>82.0 ± 19.1</td>
<td>83.5 ± 21.9</td>
<td>91.6 ± 33.6</td>
</tr>
<tr>
<td>OF</td>
<td>90.0 ± 20.9</td>
<td>71.0 ± 16.0</td>
<td>77.2 ± 17.6</td>
</tr>
<tr>
<td>OM</td>
<td>83.0 ± 17.7</td>
<td>88.1 ± 27.4</td>
<td>86.3 ± 25.2</td>
</tr>
<tr>
<td>**Fat <em>(g·day⁻¹)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>82.5 ± 14.8</td>
<td>86.5 ± 24.4</td>
<td>84.3 ± 33.1</td>
</tr>
<tr>
<td>OF</td>
<td>77.7 ± 11.6</td>
<td>57.8 ± 10.2 †</td>
<td>65.3 ± 13.1 †</td>
</tr>
<tr>
<td>OM</td>
<td>74.9 ± 15.7</td>
<td>89.8 ± 30.4</td>
<td>72.3 ± 24.2</td>
</tr>
<tr>
<td>**CHO <em>(g·day⁻¹)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>254.6 ± 70.4</td>
<td>256.1 ± 99.7</td>
<td>285.6 ± 161.3</td>
</tr>
<tr>
<td>OF</td>
<td>252.7 ± 52.4</td>
<td>197.7 ± 48.9</td>
<td>214.6 ± 48.6</td>
</tr>
<tr>
<td>OM</td>
<td>253.2 ± 36.8</td>
<td>268.5 ± 74.6</td>
<td>277.1 ± 129.2</td>
</tr>
<tr>
<td><strong>Fibre (g·day⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>13.1 ± 4.4</td>
<td>12.1 ± 5.5</td>
<td>16.8 ± 13.4</td>
</tr>
<tr>
<td>OF</td>
<td>18.1 ± 6.5</td>
<td>14.1 ± 8.5</td>
<td>15.3 ± 8.5</td>
</tr>
<tr>
<td>OM</td>
<td>16.0 ± 7.8</td>
<td>14.2 ± 6.0</td>
<td>16.5 ± 10.4</td>
</tr>
<tr>
<td>**Calcium <em>(mg·day⁻¹)</em> a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>1011 ± 378</td>
<td>981 ± 348</td>
<td>1040 ± 440</td>
</tr>
<tr>
<td>OF</td>
<td>1016 ± 232</td>
<td>781 ± 253</td>
<td>861 ± 306</td>
</tr>
<tr>
<td>OM</td>
<td>1110 ± 267</td>
<td>1049 ± 445</td>
<td>1022 ± 396</td>
</tr>
<tr>
<td>**Protein <em>(% of EI·day⁻¹)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

142
Data are given as the mean ± SD. EI; energy intake, CHO; carbohydrate, CON; control trial, HF; high-fat trial, LF; LF trial. * Statistical analysis performed on logarithmically transformed data. Differences were found between and within conditions using RM ANOVA, p < 0.05: a Main effect of trial for HF compared to CON trial by LSD. † Trial x group interaction compared to O group by LSD and 95% CI ‡ Trial x group interaction compared to M/O group by LSD and 95% CI.

Table 6.6 Significant RM ANOVA Output Analyses for 5-day Energy and Macronutrient Intake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main Effect Trial</th>
<th>Group x Trial Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>EI</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat Intake</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calcium Intake</td>
<td>2,42</td>
<td>3.371</td>
</tr>
</tbody>
</table>

Df; degrees of freedom, F; F-ratio, EI; energy intake

6.4.1.2 1-day Energy and Macronutrient Intake

Mean energy and macronutrient intake for 1-day prior to CON, HF and LF conditions are illustrated in Table 6.7. Volunteers successfully repeated their food diaries as indicated by no significant differences in mean energy, macronutrient, fibre or calcium intake (p > 0.05) for 1 day prior to each condition.
Table 6.7 1-day Energy, Macronutrient, Fibre and Calcium Intake before Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 24)

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI (kcal·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>1907 ± 456</td>
<td>2122 ± 912</td>
<td>2198 ± 640</td>
</tr>
<tr>
<td>OF</td>
<td>2052 ± 634</td>
<td>1419 ± 555</td>
<td>1685 ± 415</td>
</tr>
<tr>
<td>OM</td>
<td>1919 ± 251</td>
<td>2266 ± 1302</td>
<td>2195 ± 1305</td>
</tr>
<tr>
<td>EI (kJ·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>7753 ± 1853</td>
<td>8876 ± 3815</td>
<td>8359 ± 2680</td>
</tr>
<tr>
<td>OF</td>
<td>8587 ± 2653</td>
<td>5938 ± 2323</td>
<td>7049 ± 1738</td>
</tr>
<tr>
<td>OM</td>
<td>8028 ± 1049</td>
<td>9482 ± 5449</td>
<td>9184 ± 5461</td>
</tr>
<tr>
<td>Protein (g·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>82.9 ± 26.4</td>
<td>95.1 ± 36.6</td>
<td>86.5 ± 36.8</td>
</tr>
<tr>
<td>OF</td>
<td>88.2 ± 28.8</td>
<td>60.1 ± 34.4</td>
<td>74.5 ± 24.3</td>
</tr>
<tr>
<td>OM</td>
<td>98.3 ± 27.5</td>
<td>101.1 ± 40.9</td>
<td>92.9 ± 43.4</td>
</tr>
<tr>
<td>Fat* (g·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>81.2 ± 26.6</td>
<td>90.6 ± 49.8</td>
<td>86.9 ± 38.4</td>
</tr>
<tr>
<td>OF</td>
<td>76.8 ± 24.3</td>
<td>55.0 ± 27.5</td>
<td>65.5 ± 25.4</td>
</tr>
<tr>
<td>OM</td>
<td>67.0 ± 20.2</td>
<td>82.8 ± 45.1</td>
<td>77.0 ± 47.2</td>
</tr>
<tr>
<td>CHO* (g·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>211.0 ± 83.1</td>
<td>245.6 ± 113.0</td>
<td>231.4 ± 71.3</td>
</tr>
<tr>
<td>OF</td>
<td>268.3 ± 117.6</td>
<td>182.6 ± 56.7</td>
<td>196.4 ± 74.0</td>
</tr>
<tr>
<td>OM</td>
<td>232.0 ± 65.5</td>
<td>284.0 ± 219.3</td>
<td>300.3 ± 207.8</td>
</tr>
<tr>
<td>Fibre* (g·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>12.8 ± 5.7</td>
<td>14.6 ± 8.8</td>
<td>12.5 ± 4.3</td>
</tr>
<tr>
<td>OF</td>
<td>20.6 ± 9.2</td>
<td>13.4 ± 8.4</td>
<td>16.4 ± 9.4</td>
</tr>
<tr>
<td>OM</td>
<td>12.1 ± 4.8</td>
<td>21.3 ± 25.0</td>
<td>20.3 ± 25.5</td>
</tr>
<tr>
<td>Calcium * (mg·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>1203.5 ± 846</td>
<td>1152.7 ± 493</td>
<td>823.1 ± 168</td>
</tr>
<tr>
<td>OF</td>
<td>1075.3 ± 3776</td>
<td>668.0 ± 451</td>
<td>851.0 ± 345</td>
</tr>
<tr>
<td>OM</td>
<td>986.2 ± 2966</td>
<td>894.8 ± 453</td>
<td>1020.3 ± 359</td>
</tr>
<tr>
<td>Protein (% of EI·day⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>17.9 ± 3.6</td>
<td>19.1 ± 7.7</td>
<td>17.0 ± 4.4</td>
</tr>
<tr>
<td>OF</td>
<td>17.6 ± 4.3</td>
<td>16.2 ± 4.2</td>
<td>21.5 ± 7.7</td>
</tr>
<tr>
<td>OM</td>
<td>24.2 ± 13.4</td>
<td>19.3 ± 5.6</td>
<td>21.9 ± 12.4</td>
</tr>
</tbody>
</table>
### Fat (% of EI·day⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>39.5 ± 10.0</td>
<td>38.1 ± 7.6</td>
<td>37.8 ± 9.3</td>
</tr>
<tr>
<td>OF</td>
<td>34.1 ± 7.3</td>
<td>34.1 ± 6.6</td>
<td>38.6 ± 8.4</td>
</tr>
<tr>
<td>OM</td>
<td>31.1 ± 6.8</td>
<td>33.7 ± 6.5</td>
<td>31.4 ± 6.2</td>
</tr>
</tbody>
</table>

### CHO * (% of EI·day⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>42.6 ± 11.8</td>
<td>43.9 ± 7.9</td>
<td>45.0 ± 11.5</td>
</tr>
<tr>
<td>OF</td>
<td>48.2 ± 6.9</td>
<td>49.7 ± 6.2</td>
<td>196.4 ± 74.0</td>
</tr>
<tr>
<td>OM</td>
<td>49.7 ± 6.2</td>
<td>46.7 ± 10.2</td>
<td>46.5 ± 13.1</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. EI; energy intake, CHO; carbohydrate, CON; control trial, HF; high-fat trial, LF; LF trial. * Statistical analysis performed on logarithmically transformed data. No differences were found within or between conditions using RM ANOVA, p < 0.05.

### 6.4.2 Body Mass

There was no significant effect of trial (p = 0.16) or trial x group interaction (p > 0.05) (Table 6.8).

**Table 6.8** Body Mass after the Ingestion of High-Fat Meal Supplemented Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 24)

<table>
<thead>
<tr>
<th>Body Mass * (kg)</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>76.4 ± 13.8</td>
<td>76.9 ± 14.5</td>
<td>76.9 ± 14.5</td>
</tr>
<tr>
<td>OF</td>
<td>65.6 ± 10.7</td>
<td>65.6 ± 10.6</td>
<td>65.9 ± 10.8</td>
</tr>
<tr>
<td>OM</td>
<td>74.7 ± 23.3</td>
<td>75.4 ± 23.4</td>
<td>75.5 ± 23.3</td>
</tr>
</tbody>
</table>

Data are given as the mean ± S.D. * Statistical analysis performed on logarithmically transformed data.

### 6.4.3 Gastric Emptying

#### 6.4.3.1 Gastric Emptying using directly measured \( \dot{V}CO_2 \) data

GE parameters calculated using directly measured \( \dot{V}CO_2 \) data after the 5-day CON, HF and LF interventions are highlighted in Table 6.9. There was no main effect of trial, (p = 0.24), or trial x group interaction effect for GE half-time. There was no main effect of trial
for lag phase, \((p = 0.28)\), or interaction effect. Similar GE latency time, ascension time and cPDR were observed within and between CON, HF and LF trials.

**Table 6.9** Gastric Emptying (GE) Parameters after the Ingestion of High-Fat Meal Supplemented Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests using directly measured \(\dot{V}_{CO_2}\) data (n = 24)

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half-time (min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>195 ± 36</td>
<td>200 ± 45</td>
<td>199 ± 43</td>
</tr>
<tr>
<td>OF</td>
<td>195 ± 31</td>
<td>187 ± 28</td>
<td>184 ± 25</td>
</tr>
<tr>
<td>OM</td>
<td>219 ± 39</td>
<td>195 ± 26</td>
<td>201 ± 32</td>
</tr>
<tr>
<td><strong>Lag phase (\hat{.}) (min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>130 ± 22</td>
<td>138 ± 30</td>
<td>130 ± 27</td>
</tr>
<tr>
<td>OF</td>
<td>131 ± 16</td>
<td>124 ± 14</td>
<td>122 ± 17</td>
</tr>
<tr>
<td>OM</td>
<td>139 ± 29</td>
<td>131 ± 17</td>
<td>130 ± 32</td>
</tr>
<tr>
<td><strong>Latency time (min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>47 ± 8</td>
<td>53 ± 12</td>
<td>46 ± 10</td>
</tr>
<tr>
<td>OF</td>
<td>49 ± 5</td>
<td>45 ± 3</td>
<td>44 ± 7</td>
</tr>
<tr>
<td>OM</td>
<td>49 ± 18</td>
<td>49 ± 8</td>
<td>45 ± 8</td>
</tr>
<tr>
<td><strong>Ascension time (\hat{.}) (min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>149 ± 30</td>
<td>146 ± 36</td>
<td>153 ± 36</td>
</tr>
<tr>
<td>OF</td>
<td>146 ± 30</td>
<td>142 ± 27</td>
<td>140 ± 21</td>
</tr>
<tr>
<td>OM</td>
<td>170 ± 38</td>
<td>146 ± 22</td>
<td>156 ± 27</td>
</tr>
<tr>
<td><strong>cPDR (\hat{.}) (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>37.1 ± 9.9</td>
<td>37.6 ± 3.9</td>
<td>36.8 ± 6.0</td>
</tr>
<tr>
<td>OF</td>
<td>35.0 ± 7.1</td>
<td>35.3 ± 11.4</td>
<td>39.6 ± 6.5</td>
</tr>
<tr>
<td>OM</td>
<td>30.9 ± 6.0</td>
<td>37.9 ± 4.6</td>
<td>38.2 ± 3.4</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions using RM ANOVA, \(p > 0.05\). * Statistical analysis performed on logarithmically transformed data.
Figure 6.3 Gastric emptying half-time (GE half-time) in Control (CON), 5-day HFD (HF), 5-day deadaptation to HFD (LF) trials across olive oil (OO), olive oil and fish powder (OF) and olive oil and macadamia oil blend supplements (n = 8 per group). Pooled data (n = 24). Data are given as the mean ± SD. Significantly different to CON trial: * p < 0.05 when adjustments were made for 1-day total dietary fat intake or C14:0 intake, ** p < 0.01 when adjustments were made for 1-day MUFA or SFA intake.

When adjustments were made for 1-day intake of total dietary fat GE half-time was significantly faster at the HF compared to the LF trial, (p = 0.03) (Significant RM ANOVA output shown in Table 6.10). No significant group x trial interaction was evident. Similar main effects of trial on half-time were reported when single adjustments were made for 1-day intake of SFA (p = 0.001), MUFA (p = 0.01) and C14:0 intake (p = 0.03). Adjustments for covariates did not result a significant main effect of trial for lag phase or latency time.

There was a significant main effect of trial for ascension time when adjustments were made for 1-day intake of MUFA, (p = 0.03) or SFA, (p = 0.001), indicating that ascension time was accelerated at HF compared to CON trial. There was also a significant main effect of trial when single adjustments were made for body mass (p = 0.02) or BMI (p = 0.03).
Table 6.10 Significant RM ANOVA Output Analyses for gastric emptying parameters after adjustment for 1-day background diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-time</td>
<td>2,40</td>
<td>5.446</td>
<td>0.05</td>
</tr>
<tr>
<td>(1-day total fat intake adjustment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascension time</td>
<td>2,40</td>
<td>4.423</td>
<td>0.03</td>
</tr>
<tr>
<td>(1-day total MUFA intake adjustment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascension time</td>
<td>2,40</td>
<td>8.542</td>
<td>0.001</td>
</tr>
<tr>
<td>(1-day total SFA intake adjustment)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Df; degrees of freedom, F; F-ratio, MUFA; monounsaturated fatty acids, SFA; saturated fatty acids.

6.4.3.2 Gastric Emptying using directly measured and estimated resting \( \dot{V} \text{CO}_2 \) data

GE parameters calculated using directly measured resting and estimated \( \dot{V} \text{CO}_2 \) data after the 5-day CON, HF and LF interventions are highlighted in Appendix 3 A and B. Similar GE parameters were observed within and between CON, HF and LF trials without adjusting for covariates.

6.4.3 Mouth to Caecum Transit Time

One volunteer from the OM group was classed as a ‘non-responder’ to the inulin \( H_2 \) breath test (i.e. did not show an increase in breath \( H_2 \) after the consumption of the test meal). This volunteer was subsequently removed from MCTT data. There was no significant main effect of trial, \( p = 0.22 \) or significant trial x group interaction \( p > 0.05 \) prior to covariate adjustment (Table 6.10). When adjustments were made for 5-day intake of total dietary fat or total n-3 intake, there was a strong trend towards accelerated MCTT at the HF trial compared to CON trial \( p = 0.06 \) and \( p = 0.05 \) respectively).
Table 6.10 Mouth to Caecum Transit Time after the Ingestion of High-Fat Meal Supplemented Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 23)

<table>
<thead>
<tr>
<th>MCTT (min)</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>OO</td>
<td>223 ± 67</td>
<td>239 ± 72</td>
<td>256 ± 53</td>
</tr>
<tr>
<td>OF</td>
<td>256 ± 81</td>
<td>246 ± 50</td>
<td>266 ± 50</td>
</tr>
<tr>
<td>OM</td>
<td>281 ± 71</td>
<td>248 ± 65</td>
<td>276 ± 78</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions using RM ANOVA, p > 0.05.

Figure 6.4 Representation of a typical (volunteer 3) breath hydrogen (H2) curve over the time course of the test day for each of the three test conditions: control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF). Mouth to caecum transit times for CON, HF and LF are 240, 225 and 225 min respectively (indicated by red arrow), ppm; part per million.
6.4.4 Appetite Sensations

There was no main effect or interaction effect for mean fasting (baseline) sensations of appetite. Immediately after consumption of the breakfast test meal, volunteers reported lower hunger, PFC, DTE fatty food and greater satiety and fullness. Changes in appetite sensations were evident in the 6-h postprandial period ($p < 0.05$).

There was a main effect of hunger, ($p = 0.02$), but no group x trial interaction effect (See Table 6.11 for all significant RM ANOVA appetite analyses output). Post hoc analysis revealed that volunteers were less hungry after HF ($p = 0.01$) and LF trials ($p = 0.03$) compared to CON at 60 minutes postprandially.

For PFC, there was a significant group x trial interaction immediately after the breakfast test meal (0 minutes), ($p = 0.01$). Post hoc analysis (95 % CI) revealed that during the LF trial, the OF group had lower ratings of PFC than the OO group. There was a significant main effect of trial at 60 minutes, ($p = 0.02$), 90 minutes, ($p = 0.05$), 120 minutes, ($p = 0.01$) and 300 minutes postprandially, ($p = 0.05$). At 60 and 90 minutes postprandially, ratings of PFC were greater during the LF than CON trial ($p = 0.01$ and $p = 0.03$, respectively). At 120 minutes postprandially, greater ratings of PFC were observed at HF ($p = 0.004$) and LF trials ($p = 0.02$) compared to CON. At 300 minutes, higher PFC ratings were evident at HF compared to CON trial ($p = 0.02$).

There was a main effect for DTE fatty foods at 330, ($p = 0.02$), and 360 minutes postprandially, ($p = 0.002$). Post hoc analysis revealed that volunteers had less DTE fatty foods at LF ($p = 0.02$) compared to CON trial at 330 minutes postprandially. There was also less DTE fatty foods at LF compared to CON ($p = 0.004$) and HF trials ($p = 0.025$) at 360 minutes postprandially.

There was a main effect of satiety at 60 minutes postprandially, ($p = 0.01$). Post hoc analysis revealed that volunteers were less satisfied at the LF ($p = 0.003$) compared to CON trial. Main effects of trial, but no interaction effects were observed at 180 minutes, ($p = 0.04$), and 210 minutes after meal ingestion, ($p = 0.05$). Volunteers were less satisfied after HF compared to CON ($p = 0.03$) and LF trials ($p = 0.04$) at 180 minutes postprandially. Similarly, at 210 minutes postprandially, volunteers were less satisfied after HF compared to CON ($p = 0.02$) and LF trials ($p = 0.02$). Immediately after the ad libitum buffet meal, there was no main effect of trial for satiety but a main group x trial interaction was evident, ($p = 0.05$). Post hoc analysis (95 % CI) revealed that at the HF trial, the OF group were more satisfied compared to OO and OM groups.
For fullness (see Figure 6.5), there was a significant main effect of trial at 120 minutes postprandially, ($p = 0.01$). Post hoc analysis revealed that volunteers reported lower sensations of fullness at HF ($p = 0.01$) and LF trials ($p = 0.03$) compared to CON. There was a main effect for fullness at 210 minutes, ($p = 0.01$) with lower hunger sensations reported at HF compared to CON trials ($p = 0.02$). At 240 minutes, there was a main effect of hunger, ($p = 0.01$), with volunteers reporting lower sensations of fullness at HF ($p = 0.01$) and LF trials ($p = 0.03$) compared to CON. At 270 minutes, a main effect of hunger was also observed, ($p = 0.02$), with volunteers feeling less full after HF compared to CON trials ($p = 0.02$). There was also a main effect for trial at 300 minutes postprandially, ($p = 0.004$), with volunteers reporting lower sensations of fullness at HF ($p = 0.006$) and LF trials ($p = 0.02$) compared to CON.

(a)
Figure 6.5 (a), (b) and (c) Visual Analogue Scale (VAS) Sensations of fullness in healthy volunteers before (-15 min) and after ingestion of a high-fat test meal under Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) trials across (a) olive oil (OO), (b) olive oil and fish powder blend (OF) and (c) olive oil and macadamia oil blend (OM) supplements \((n = 8)\). Data are given as the mean ± SD. Statistical analysis
performed on logarithmically transformed data. Differences were found between trials using RM ANOVA, \( p < 0.05 \): \(^a\) Main effect of trial for HF compared to CON trial by LSD. \(^c\) Main effect of trial for LF compared to HF trial by LSD. Standard error bars for the LF values were similar in magnitude to other data on the same figure and are omitted to improve clarity.

**Table 6.11 Significant RM ANOVA Output Analyses for Appetite Sensations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main Effect Trial</th>
<th>Group x Trial Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Df</td>
<td>F</td>
</tr>
<tr>
<td><strong>Hunger</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>2,42</td>
<td>4.497</td>
</tr>
<tr>
<td><strong>PFC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60 min</td>
<td>2,42</td>
<td>4.654</td>
</tr>
<tr>
<td>90 min</td>
<td>2,42</td>
<td>3.332</td>
</tr>
<tr>
<td>120 min</td>
<td>2,42</td>
<td>5.916</td>
</tr>
<tr>
<td>300 min</td>
<td>2,42</td>
<td>4.017</td>
</tr>
<tr>
<td><strong>DTE fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330 min</td>
<td>2,42</td>
<td>4.610</td>
</tr>
<tr>
<td>360 min</td>
<td>2,42</td>
<td>7.298</td>
</tr>
<tr>
<td><strong>Satiety</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>2,42</td>
<td>5.631</td>
</tr>
<tr>
<td>180 min</td>
<td>2,42</td>
<td>4.136</td>
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<tr>
<td>210 min</td>
<td>2,42</td>
<td>3.374</td>
</tr>
<tr>
<td>390 min</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Fullness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>2,42</td>
<td>4.486</td>
</tr>
<tr>
<td>210 min</td>
<td>2,42</td>
<td>4.891</td>
</tr>
<tr>
<td>240 min</td>
<td>2,42</td>
<td>5.677</td>
</tr>
<tr>
<td>270 min</td>
<td>2,42</td>
<td>4.592</td>
</tr>
<tr>
<td>300 min</td>
<td>2,42</td>
<td>6.227</td>
</tr>
</tbody>
</table>

Df; degrees of freedom, \( F \); \( F \)-ratio, PFC; prospective food consumption, DTE; desire to eat.
Summary measures of appetite responses were evaluated as postprandial time averaged AUC (Table 6.12). There was no significant main effect or interaction effect for hunger, satiety PFC or DTE fatty food ($p > 0.05$). There was a significant main effect of trial for fullness, ($p = 0.02$), but no interaction effect was evident (see Table 6.13 for significant RM ANOVA output). Post hoc analysis revealed that volunteers were less full after the HF trial than after the control ($p = 0.02$). There was no difference between ratings of fullness between the CON and LF trials ($p = 0.14$) or HF and LF trials ($p = 0.06$).

Table 6.12 Time-averaged postprandial area under the curve (AUCs) for hunger, satiety, fullness, prospective food consumption (PFC) and desire to eat (DTE) fatty food at Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests ($n = 24$)

<table>
<thead>
<tr>
<th>VAS Scores</th>
<th>Condition</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total AUC (mm)</td>
<td>Total AUC (mm)</td>
<td>Total AUC (mm)</td>
</tr>
<tr>
<td>Hunger</td>
<td>OO</td>
<td>67.2 ± 17.9</td>
<td>71.5 ± 20.0</td>
<td>68.9 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>OF</td>
<td>65.6 ± 23.7</td>
<td>68.3 ± 21.5</td>
<td>62.1 ± 22.3</td>
</tr>
<tr>
<td></td>
<td>OM</td>
<td>73.2 ± 22.9</td>
<td>79.1 ± 34.9</td>
<td>78.5 ± 30.8</td>
</tr>
<tr>
<td></td>
<td>OO</td>
<td>97.9 ± 13.8</td>
<td>89.2 ± 15.3</td>
<td>90.0 ± 13.8</td>
</tr>
<tr>
<td>Satiety</td>
<td>OF</td>
<td>99.6 ± 20.2</td>
<td>93.2 ± 18.9</td>
<td>99.9 ± 19.5</td>
</tr>
<tr>
<td></td>
<td>OM</td>
<td>87.4 ± 17.9</td>
<td>76.9 ± 35.6</td>
<td>80.5 ± 33.0</td>
</tr>
<tr>
<td>Fullness a</td>
<td>OO</td>
<td>101.5 ± 12.2</td>
<td>90.1 ± 17.9</td>
<td>91.8 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>OF</td>
<td>101.0 ± 19.8</td>
<td>94.7 ± 20.9</td>
<td>102.6 ± 18.1</td>
</tr>
<tr>
<td></td>
<td>OM</td>
<td>88.2 ± 22.2</td>
<td>78.0 ± 36.0</td>
<td>82.3 ± 32.4</td>
</tr>
<tr>
<td>PFC</td>
<td>OO</td>
<td>51.9 ± 25.9</td>
<td>53.5 ± 19.2</td>
<td>55.6 ± 14.5</td>
</tr>
<tr>
<td></td>
<td>OF</td>
<td>39.4 ± 18.1</td>
<td>50.0 ± 19.7</td>
<td>45.5 ± 19.3</td>
</tr>
<tr>
<td></td>
<td>OM</td>
<td>64.3 ± 28.5</td>
<td>74.6 ± 41.2</td>
<td>70.6 ± 32.5</td>
</tr>
<tr>
<td>DTE Fat</td>
<td>OO</td>
<td>64.4 ± 31.5</td>
<td>56.3 ± 41.4</td>
<td>56.3 ± 39.8</td>
</tr>
<tr>
<td></td>
<td>OF</td>
<td>52.4 ± 46.9</td>
<td>50.6 ± 46.3</td>
<td>55.0 ± 49.8</td>
</tr>
<tr>
<td></td>
<td>OM</td>
<td>42.4 ± 31.3</td>
<td>34.6 ± 33.9</td>
<td>29.0 ± 32.9</td>
</tr>
</tbody>
</table>

AUC values expressed as Mean ± SD. Time-averaged postprandial area under the curve was calculated as the mean appetite response during the 6-h postprandial period. PFC; prospective food consumption, DTE; desire to eat. * Statistical analysis performed on
logarithmically transformed data. Differences were found between and within conditions using RM ANOVA, $p < 0.05$: a Main effect of trial for HF compared to CON trial by LSD.

**Table 6.13** Significant RM ANOVA Output Analysis for postprandial time-averaged hunger

<table>
<thead>
<tr>
<th>Parameter</th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>2,42</td>
<td>4.649</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Df; degrees of freedom, $F$; F-ratio

### 6.4.5 Palatability of Breakfast Test Meal

There were no significant main effects or interaction effects for ratings of pleasantness or aftertaste of the breakfast test meal (data not shown). There was a significant main effect for smell of the breakfast test meal, ($p = 0.04$), but no group x trial interaction effect (Figure 6.6 (a); see Table 6.13 for ANOVA output). Post hoc analysis revealed that volunteers rated the breakfast test meal as smelling better at HF ($p = 0.04$) and LF ($p = 0.05$) compared CON trial. There was a significant main effect for taste of the breakfast test meal, $F(2,42) = 6.630$, $p = 0.01$, but no trial x group interaction effect (Figure 6.6 (b)). Post hoc analysis revealed that volunteers rated the breakfast test meal as tasting better at HF ($p = 0.01$) and LF ($p = 0.02$) compared CON trial.
Figure 6.6 (a) Ratings of smell of breakfast test meal in Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) trials across olive oil (OO), olive oil and fish powder blend (OF) and olive oil and macadamia oil blend (OM) supplements (n = 8). Data are given as the mean ± SD. * Statistical analysis performed on logarithmically transformed data. Differences were found between trials using RM ANOVA, p < 0.05: a Main effect of trial for HF compared to CON trial by LSD. b Main effect of trial for LF compared to CON trial by LSD.

Figure 6.6 (b) Ratings of taste of breakfast test meal in Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) trials across olive oil (OO), olive oil and fish powder blend (OF) and olive oil and macadamia oil blend (OM) supplements (n = 8). Data are
given as the mean ± SD. * Statistical analysis performed on logarithmically transformed data. Differences were found between trials using RM ANOVA, p < 0.05:  

- Main effect of trial for HF compared to CON trial by LSD.
- Main effect of trial for LF compared to CON trial by LSD.

There was no main effect or interaction effect of feelings of general wellbeing after the breakfast meal. There was a significant main effect of nausea, (p = 0.04), but no trial x group interaction effect (Figure 6.6 (c)). Post hoc analysis revealed that volunteers felt more nauseous at HF (p = 0.02) and LF trials (p = 0.04) compared to CON.

Figure 6.6 (c) Ratings of nausea in Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) trials across olive oil (OO), olive oil and fish powder blend (OF) and olive oil and macadamia oil blend (OM) supplements (n = 8). Data are given as the mean ± SD. * Statistical analysis performed on logarithmically transformed data. Differences were found between trials using RM ANOVA, p < 0.05:  

- Main effect of trial for HF compared to CON trial by LSD.
- Main effect of trial for LF compared to CON trial by LSD.
Table 6.14 Significant RM ANOVA Output Analysis for palatability of breakfast test meal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>df</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smell</td>
<td>2,42</td>
<td>4.151</td>
<td>0.04</td>
</tr>
<tr>
<td>Taste</td>
<td>2,42</td>
<td>6.630</td>
<td>0.01</td>
</tr>
<tr>
<td>Nausea</td>
<td>2,42</td>
<td>4.151</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Df; degrees of freedom, F; F-ratio

6.4.6 Palatability of Ad Libitum Buffet Meal

There were no significant main effects or interaction effects for palatability of the ad libitum buffet meal or feelings of general wellbeing or nausea (data not shown).

6.4.7 Subsequent Food Intake

There was no significant main effect of trial, \( p = 0.25 \) or group x trial interaction for EI in the buffet (Table 6.15). Similarly, there was no significant effect of trial or interaction between group and trial for protein, carbohydrate or fat intake \( (p > 0.05) \). There was no significant main effect of trial or trial x group interaction for quantity eaten in the buffet meal \( (p > 0.05) \). For time taken to eat the buffet meal, there was no significant trial x group interaction \( (p > 0.05) \). However, there was a main effect of trial for time taken to eat the buffet meal \( (g, \ p = 0.05; \text{See Table 6.16}) \). Post hoc analysis by LSD revealed that compared to baseline, volunteers spent a shorter amount of time eating the buffet at LF \( (p = 0.03) \) but not at HF \( (p = 0.20) \) trials. There was no main effect or interaction effect for eating rate \( (p > 0.05) \).
<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EI (kcal)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>1367 ± 359</td>
<td>1225 ± 342</td>
<td>1240 ± 427</td>
</tr>
<tr>
<td>OF</td>
<td>1314 ± 538</td>
<td>1337 ± 530</td>
<td>1349 ± 517</td>
</tr>
<tr>
<td>OM</td>
<td>1323 ± 554</td>
<td>1359 ± 532</td>
<td>1571 ± 689</td>
</tr>
<tr>
<td><strong>EI (kJ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>5728 ± 1500</td>
<td>5137 ± 1430</td>
<td>5201 ± 1782</td>
</tr>
<tr>
<td>OF</td>
<td>5512 ± 2256</td>
<td>5610 ± 2223</td>
<td>5658 ± 2168</td>
</tr>
<tr>
<td>OM</td>
<td>5542 ± 2322</td>
<td>5693 ± 2227</td>
<td>6588 ± 2885</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>59.8 ± 22.3</td>
<td>49.2 ± 18.3</td>
<td>51.7 ± 18.3</td>
</tr>
<tr>
<td>OF</td>
<td>67.4 ± 34.0</td>
<td>70.2 ± 31.4</td>
<td>68.0 ± 33.0</td>
</tr>
<tr>
<td>OM</td>
<td>64.1 ± 24.6</td>
<td>67.7 ± 25.5</td>
<td>72.3 ± 27.7</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>59.2 ± 24.3</td>
<td>48.5 ± 20.2</td>
<td>49.3 ± 23.0</td>
</tr>
<tr>
<td>OF</td>
<td>49.7 ± 25.7</td>
<td>52.2 ± 25.9</td>
<td>51.8 ± 21.2</td>
</tr>
<tr>
<td>OM</td>
<td>64.1 ± 24.6</td>
<td>55.5 ± 27.2</td>
<td>61.9 ± 32.5</td>
</tr>
<tr>
<td><strong>CHO (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>162.1 ± 56.4</td>
<td>156.5 ± 37.2</td>
<td>156.2 ± 47.0</td>
</tr>
<tr>
<td>OF</td>
<td>163.8 ± 62.5</td>
<td>162.1 ± 65.4</td>
<td>166.5 ± 62.2</td>
</tr>
<tr>
<td>OM</td>
<td>157.6 ± 65.9</td>
<td>160.0 ± 59.2</td>
<td>195.8 ± 83.9</td>
</tr>
<tr>
<td><strong>Protein % (of EI)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>17.6 ± 5.1</td>
<td>16.2 ± 4.7</td>
<td>17.7 ± 6.6</td>
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<tr>
<td>OF</td>
<td>19.9 ± 3.3</td>
<td>20.6 ± 3.6</td>
<td>19.5 ± 3.7</td>
</tr>
<tr>
<td>OM</td>
<td>20.4 ± 7.0</td>
<td>20.1 ± 5.0</td>
<td>18.9 ± 4.0</td>
</tr>
<tr>
<td><strong>Fat % (of EI)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>37.7 ± 8.0</td>
<td>34.4 ± 7.7</td>
<td>49.3 ± 23.0</td>
</tr>
<tr>
<td>OF</td>
<td>32.9 ± 6.6</td>
<td>34.5 ± 6.9</td>
<td>34.3 ± 3.6</td>
</tr>
<tr>
<td>OM</td>
<td>34.9 ± 7.6</td>
<td>35.7 ± 5.3</td>
<td>34.4 ± 6.8</td>
</tr>
<tr>
<td><strong>CHO % (of EI)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>47.0 ± 8.3</td>
<td>51.9 ± 6.4</td>
<td>51.0 ± 5.4</td>
</tr>
<tr>
<td>OF</td>
<td>50.7 ± 7.3</td>
<td>48.6 ± 7.9</td>
<td>49.7 ± 6.7</td>
</tr>
<tr>
<td>OM</td>
<td>48.0 ± 5.2</td>
<td>47.5 ± 4.6</td>
<td>50.3 ± 7.2</td>
</tr>
<tr>
<td><strong>Quantity eaten (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.15 Mean Energy and Macronutrient Intake in the *ad libitum* Buffet Meal
Data are given as the mean ± S.D. * Statistical analysis performed on logarithmically transformed data. Differences were found between conditions using RM ANOVA, $p < 0.05$: $^b$ Main effect of trial for LF compared to CON trial by LSD.

Table 6.16 Significant RM ANOVA Output Analysis for time taken to consume the ad libitum buffet meal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>df</th>
<th>$F$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>2.42</td>
<td>3.306</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Df; degrees of freedom, $F$; $F$-ratio

6.4.8 Energy and Substrate Utilisation
There was no main effect or interaction effect for mean fasting (baseline) energy expenditure, fat or carbohydrate oxidation (Table 6.17).

Table 6.17 Fasting values for energy expenditure, fat oxidation and carbohydrate oxidation at Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 24)
<table>
<thead>
<tr>
<th>Condition</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy Expenditure</strong>&lt;sup&gt;1&lt;/sup&gt; (kJ·h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>307.9 ± 64.9</td>
<td>324.9 ± 34.7</td>
<td>286.0 ± 42.1</td>
</tr>
<tr>
<td>OF</td>
<td>293.2 ± 90.1</td>
<td>300.0 ± 81.6</td>
<td>298.0 ± 99.6</td>
</tr>
<tr>
<td>OM</td>
<td>314.9 ± 86.4</td>
<td>314.8 ± 83.2</td>
<td>324.6 ± 108.1</td>
</tr>
<tr>
<td><strong>Fat Oxidation</strong>&lt;sup&gt;2&lt;/sup&gt; (g·h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>3.47 ± 3.22</td>
<td>3.90 ± 2.04</td>
<td>4.05 ± 1.81</td>
</tr>
<tr>
<td>OF</td>
<td>3.78 ± 1.38</td>
<td>4.10 ± 1.19</td>
<td>4.62 ± 1.63</td>
</tr>
<tr>
<td>OM</td>
<td>4.55 ± 2.60</td>
<td>4.23 ± 1.33</td>
<td>3.69 ± 0.99</td>
</tr>
<tr>
<td><strong>CHO Oxidation</strong>&lt;sup&gt;2&lt;/sup&gt; (g·h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>10.07 ± 7.80</td>
<td>10.10 ± 3.61</td>
<td>7.48 ± 2.77</td>
</tr>
<tr>
<td>OF</td>
<td>8.51 ± 7.32</td>
<td>8.18 ± 3.28</td>
<td>6.89 ± 2.68</td>
</tr>
<tr>
<td>OM</td>
<td>8.03 ± 5.51</td>
<td>8.75 ± 3.93</td>
<td>11.28 ± 7.17</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. CHO; Carbohydrate. * Statistical analysis performed on logarithmically transformed data. No differences were found within or between conditions using RM ANOVA, p > 0.05.

Summary measures of energy expenditure and substrate utilisation as time-averaged postprandial AUC were also evaluated (Table 6.18). There was no significant main effect or interaction effect for energy expenditure, fat or carbohydrate oxidation (p > 0.05).
Table 6.18 Time-averaged postprandial areas under response versus time curve (AUCs) for energy expenditure, fat oxidation and carbohydrate oxidation at Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 24)

<table>
<thead>
<tr>
<th>Condition</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Expenditure ('kJ∙h⁻¹')</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>354.8 ± 46.9</td>
<td>366.1 ± 53.2</td>
<td>345.1 ± 45.7</td>
</tr>
<tr>
<td>OF</td>
<td>346.9 ± 84.7</td>
<td>342.1 ± 58.9</td>
<td>351.1 ± 71.2</td>
</tr>
<tr>
<td>OM</td>
<td>370.6 ± 88.9</td>
<td>359.1 ± 83.2</td>
<td>367.1 ± 76.0</td>
</tr>
<tr>
<td>Fat Oxidation (g∙h⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>5.11 ± 2.92</td>
<td>4.64 ± 2.77</td>
<td>4.23 ± 2.30</td>
</tr>
<tr>
<td>OF</td>
<td>4.38 ± 1.87</td>
<td>5.04 ± 1.29</td>
<td>5.47 ± 1.29</td>
</tr>
<tr>
<td>OM</td>
<td>6.02 ± 2.54</td>
<td>5.8 ± 1.64</td>
<td>5.95 ± 1.74</td>
</tr>
<tr>
<td>CHO Oxidation ('g∙h⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>9.26 ± 4.73</td>
<td>10.81 ± 5.92</td>
<td>9.19 ± 4.84</td>
</tr>
<tr>
<td>OF</td>
<td>10.28 ± 6.66</td>
<td>8.50 ± 2.46</td>
<td>8.24 ± 2.77</td>
</tr>
<tr>
<td>OM</td>
<td>7.93 ± 4.78</td>
<td>7.76 ± 2.59</td>
<td>7.65 ± 1.47</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. Time-averaged postprandial area under the curve was calculated as the mean response during the 6-h postprandial period. CHO; Carbohydrate. * Statistical analysis performed on logarithmically transformed data.

6.4.9 Further Analyses

6.4.9.1 Pooled Gastric Emptying Data

Data from OO, OF and OM groups (n = 24) to examine the effect of a HF intervention on GE parameters is presented below (Table 6.19). There were no significant trial effects for GE half-time (p = 0.23), lag phase (p = 0.27), latency time (p = 0.14) and ascension time (p = 0.11).
Table 6.19 Pooled gastric emptying parameters after the ingestion of High-Fat Meal Supplemented Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests (n = 24).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-time <em>(min)</em></td>
<td>203 ± 36</td>
<td>194 ± 33</td>
<td>195 ± 34</td>
</tr>
<tr>
<td>Lag phase <em>(min)</em></td>
<td>133 ± 22</td>
<td>131 ± 21</td>
<td>127 ± 21</td>
</tr>
<tr>
<td>Latency time <em>(min)</em></td>
<td>48 ± 11</td>
<td>49 ± 9</td>
<td>45 ± 8</td>
</tr>
<tr>
<td>Ascension time (min)</td>
<td>155 ± 33</td>
<td>145 ± 28</td>
<td>150 ± 28</td>
</tr>
<tr>
<td>cPDR (%)</td>
<td>34.3 ± 7.9</td>
<td>36.9 ± 7.2</td>
<td>38.2 ± 5.4</td>
</tr>
</tbody>
</table>

Data are given as the mean ± S.D. * Statistical analysis performed on logarithmically transformed data. No significant differences were found between conditions using RM ANOVA, p > 0.05.

6.4.9.2 Association between Anthropometric Data, Habitual Energy, Macronutrient and Fibre Intake and Gastric Emptying Half-Time

When data as pooled from the CON trial (n = 24), there were no significant relationships between GE half-time calculated using directly measured tCO₂ data and age (r = -0.15, p = 0.47), body mass (r = -0.21, p = 0.32), height (r = -0.275, p = 0.19) and BMI (r = -0.11, p = 0.61). Similar relationships were evident for lag phase (weight: r = -0.80, p = 0.01; height: r = -0.71, p = 0.03). No relationship was evident between GE lag phase and age, body mass, height and BMI (data not shown).

6.4.9.3 Association between Anthropometric Data, Habitual Energy, Macronutrient and Fibre Intake and Gastric Emptying Half-Time

The relationship between mean 5- and 1-day energy, macronutrient and fibre intake from the WFD and pooled GE half-time data (n = 24) from the CON trial were also assessed (Table 6.20). No significant relationships were evident between 5-day food intake and GE half-time. An inverse relationship was evident between GE half-time and 1-day total fat intake and calcium intake, such that shorter GE half-time was associated with an increased intake of total fat and calcium intake. Total fat and calcium intake explained 20 and 16 % of variance in GE half-time respectively.
Table 6.20 Relationships between Mean 5-Day Energy, Macronutrient and Fibre Intake from 5-day and 1-day Weighed Food Diaries (WFD) and GE Half-time at the Control Trial (n = 24)

<table>
<thead>
<tr>
<th></th>
<th>5-day WFD v GE half-time</th>
<th>1-day WFD v GE half-time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>EI (kcal·day⁻¹)</td>
<td>-0.21</td>
<td>0.32</td>
</tr>
<tr>
<td>Protein (g·day⁻¹)</td>
<td>-0.31</td>
<td>0.15</td>
</tr>
<tr>
<td>Fat (g·day⁻¹)</td>
<td>-0.27</td>
<td>0.20</td>
</tr>
<tr>
<td>CHO (g·day⁻¹)</td>
<td>-0.03</td>
<td>0.91</td>
</tr>
<tr>
<td>Fibre (g·day⁻¹)</td>
<td>-0.29</td>
<td>0.17</td>
</tr>
<tr>
<td>Protein (% of EI)</td>
<td>-0.21</td>
<td>0.33</td>
</tr>
<tr>
<td>Fat (% of EI)</td>
<td>0.21</td>
<td>0.33</td>
</tr>
<tr>
<td>CHO (% of EI)</td>
<td>-0.03</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Mean values were significantly associated with GE half-time by Pearson’s Correlation: * p < 0.05.

6.4.9.4 Association between Habitual Fatty Acid Intake and Gastric Emptying Half-Time

The relationship between 5-day and 1-day fatty acid intake from WFDs and GE half-time were also examined. No significant correlations were apparent between 5-day habitual intake and GE half-time. A significant inverse correlation was found between the intake of MUFA and GE half-time (MUFA intake: 24.9 ± 9.7 g·day⁻¹; r = -0.41, p = 0.05). Significant inverse correlations were also evident between GE half-time and the intake of butyric acid (C4:0 0.27 ± 0.25 g·day⁻¹; r = -0.47, p = 0.021), caproic acid (C6:0 0.27 ± 0.25 g·day⁻¹, r = -0.48, p = 0.02), caprylic acid (C8:0 0.16 ± 0.14 g·day⁻¹, r = -0.48, p = 0.02), capric acid (C10:0 0.35 ± 0.31 g·day⁻¹, r = -0.45, p = 0.03), lauric acid (C12:0 0.50 ± 0.39 g·day⁻¹, r = -0.45, p = 0.03) and myristic acid (C14:0 1.50 ± 1.15 g·day⁻¹, r = -0.48, p = 0.02) but not total intake of SFAs (SFAs: 29.51 ± 11.91 g·day⁻¹, r = -0.04, p = 0.11). No other significant correlations were found between mean 5-day and 1-day specific fatty acid intake in habitual diet and GE half-time (data not shown).
6.5 Discussion

The effect of a 5-day HF supplemented diet varying in fatty acid composition on GI transit, appetite and metabolic responses have not previously been evaluated. There is also a paucity of research in humans examining whether or not a 5-day period is sufficient to reverse GI adaptations to a HF supplemented diet.

To our knowledge, this study was the first to explore the effect of HF interventions varying in fatty acid composition on GI transit. The results of this study illustrated that GE half-time and ascension time (calculated using directly measured \( \text{CO}_2 \) data) were accelerated at the HF compared to the CON trial when adjustments were made for background 1-day intake of dietary fat, specific fatty acids, or anthropometric data. GE half-time represents the time taken for 50% of the \( ^{13}\text{C} \) dose to be excreted in breath whereas, as cension time represents the time period between latency phase and half-time where \(^{13}\text{C} \) excretion rates are high (Jackson et al, 2004; Schommartz et al, 1994). The current findings of the significance of background dietary intake on GE rate are in agreement with observational findings in Chapter Four which suggested that there was a strong inverse relation between habitual intake of specific fatty acids (total n-3 and C16:1) in 1- and 3-day habitual diet and GE half-time. Robertson et al. (2002) showed that GE half-time was faster after an acute n-3 PUFA meal compared to meals rich in SFAs, PUFAs or MUFAs. The authors also showed that after an n-3 PUFA meal there was a lower release of CCK, reduced GLP-1 response postprandially and an accelerated appearance of chylomicron TG in plasma without influencing PPL. However, volunteers responded similarly to the other three test meals, namely SFA, PUFA and MUFAs; this confirmed previous results suggesting that a carbon chain length of greater than 12 has similar potency for the release of CCK and delaying GE (Hunt & Knox, 1968; McLaughlin et al., 1999). In the current study mean 5-day daily EI (including CON supplement) at baseline increased from 9.7 MJ (32% fat) to 12.3 MJ (52% fat) during the HF supplementation period. The type of fatty acid supplement (i.e. OO, OF or OM) did not affect how volunteers adapted to the HF intervention; it appears that the properties of n-3 and palmitoleic acid in the OF and OM supplements respectively could have been overridden by the potency of oleic acid which was present in a much larger quantity. Expression G protein-coupled receptors such as GPR40, GPR120 or CD36 in the GI tract are responsible for the secretion of gut peptides which inhibit GE, including CCK, GLP-1 and PYY (Mirauchi et al, 2009). It is possible that modulation of fatty acid receptor expression could have taken place in the GI tract in response to HF feeding as well as background intake of specific fatty acids (Feinle-Bisset et al., 2010).
Previously, a 3-day yoghurt supplemented diet rich in sunflower oil (90 g) reduced the GE latency phase of a HF sunflower oil meal (40 g) (Clegg et al., 2011). The authors argued that the GI tract only had to become accustomed to a narrow range of fatty acids, predominantly linoleic acid; this may have facilitated faster adaptation in the GI tract compared to studies using artificial mixed HFDs (Cunningham et al., 1991). On the contrary, after 7-day period, a mixed HF supplemented diet was shown to accelerate GE half-time of an oral fat tolerance test (Robertson et al., 2004) and latency phase of a HF sunflower oil meal (Clegg & Shafat, 2011). The findings of Clegg and Shafat (2011) suggest that a short-term HF diet may be a sufficient time period to adapt to a larger range of fatty acids present in HF supplements (chocolate mousse and yoghurts containing sunflower oil and peanuts which are high in oleic acid). However, on closer inspection of the dietary supplementation employed in this study, it appears that on five out of seven days, including the day prior to testing, volunteers were given supplements high in sunflower oil. Since the HF test meal was high in sunflower oil, it appears that this study also concludes that volunteers had adapted to a narrow range of fatty acids. In the current study, analysis of the GE parameters (including the examination of pooled data; n = 24), without adjustments for background intake or weight profiles masked the effect of the HF intervention rich in olive oil (oleic acid) on GE of an olive oil test meal. At baseline, volunteers had a high mean habitual intake (5-day) of oleic (9.1 ± 4.4 g) suggesting that they might have been more accustomed to the this fatty acid and that volunteers may have been less responsive to this fatty acid in the GI tract. Recent data found a strong inverse association between sensitivity to oleic acid (C18:1) in the GI tract and dietary fat intake (Stewart et al., 2011). It is acknowledged that missing fatty acid data made it difficult to truly quantify background intake of oleic acid. However, using fatty acid composition of plasma TG as a biomarker of recent dietary history (n = 232), Hodson and colleagues (2008) illustrated that the most abundant fatty acid was C18:1 n-9 (33.7 mol %). Considering that failure to adjust for background dietary intake of fatty acids masked the effect of the intervention of GE parameters, it appears that that the GI tract is responsive to background intake of fatty acids as well as adaptation to a narrow range of fatty acids in a HF supplement which are similar to the composition of a HF test meal.

In agreement with 3-day and 4-day HFDs (Clegg et al., 2011; Cunningham et al., 1991), but in contrast to 7-day HFDs (Henderson et al., 1998; Clegg & Shafat, 2011) findings in healthy adults, the current study found that MCTT of the HF meal was similar between trials. Clegg and colleagues (2011) suggested that a shorter time for the head of the meal to reach the caecum i.e. MCTT was in concordance with the finding of accelerated
GE of the first portion of the HF test meal i.e. latency phase. In support of this statement, it is not surprising that no significance in MCTT was established considering that latency time was similar between trials.

The time course of deadaptation to a HF diet has not been previously examined in humans. In the current study, covariate-adjusted data illustrated at the LF trial, GE parameters were not significantly different to the CON trial suggesting that parameters were similar to those observed prior to HF feeding. Brown et al. (1994) illustrated that 28 days was not an adequate period of time to reverse the lipid-induced delay in stomach to caecum transit time caused by a 28-day intermittent ileal infusion of palm oil (2.7 ml·wk⁻¹) in rodents. This finding suggests that regulatory mechanisms within the small intestine remained desensitised to the effect of chronic ileal infusion of fat, even after a 28 day period where normal habitual diet was restored. The contrast in findings could be related to the shorter-term, less intensive nature of our HF intervention. Brown and colleagues (1994) speculated that, in contrast to humans there might be greater attenuation in gastrointestinal patterns after a period of HF infusions in rats because they would be accustomed to a diet that is lower in fat.

Mean 5-day, but not 1-day EI and fat intake were lower in the OF group at HF and LF trials compared to the OO group. Fat intake was also lower for the OF group compared to the OM group during the 5-day HF intervention, suggesting that n-3 fatty acids may have an appetite suppressing effect. This is in agreement with previous research that has suggested that overweight and obese subjects reported lower sensations of hunger and greater fullness to a test dinner after an 8-wk energy-restricted diet supplemented with 1300 mg·day⁻¹ of long chain n-3 fatty acids (Parra et al., 2008). Research by Wang et al. (2002) indicated that n-3 fatty acid supplementation may reduce appetite due to its effect on leptin concentrations.

Following the HF intervention, volunteers had reduced satiety and fullness responses in the postprandial period and wanted to consume more food prospectively (PFC) compared to the CON trial. Similarly, exposure to HFDs leads to reduced satiation in both humans (Clegg & Shafat, 2011; Boyd et al., 2003) and rats (Warwick & Weingarten, 1995). Conversely, Castiglione et al. (2002) showed similar ratings of hunger and satiety after HF and LF diets. The authors criticised visual analogues scales as a subjective measurement of appetite sensations, stating that the method might be too ‘crude’ for any changes in satiety to be observed. Interestingly, the current study found that following a 5-day deadaptation period to the HFD i.e. LF intervention, reduced satiation responses to a HF test meal were still evident. From this novel result, it could be speculated that
volunteers were still responsive to the effect of a HFD on appetite down-regulatory responses after a 5-day adaptation period. However, at the LF trial, it should be noted that volunteers had less desire to eat fatty foods compared to CON trials. This could suggest that volunteers were starting to experience reduced preference for fatty foods. Although volunteers did not consume a LFD after the HF intervention, it is interesting to note that recent evidence shows that cravings for HF foods were averted in obese adults randomly assigned to a LFD compared to a low-carbohydrate diet (Martin et al., 2011). Lower ratings of PFC were observed in the LF trial for OF compared to the OO group which further suggests that supplementing a HF olive oil diet with n-3 may lead to a less deleterious effect on appetite responses. Mattes (1993) examined the effect of a 12-wk LFD on fat perception and dietary induced hedonic shifts and showed that volunteers were better at perceiving differences in the fat content of custards after LF feeding. Conversely, a shorter-term intervention established that a 28-day HFD was not sufficient to induce changes in hedonic ratings of regular fat and LF custards compared to LFDs (Stewart & Keast, 2011). However, scores for the hedonic task did decline over the HF intervention suggesting that significant adaptations in hedonic ratings may have been if a longer intervention was employed. The authors have speculated that HF feeding can lead to hyposensitivity to fatty acids and thus, greater quantities of fat are needed in the oral cavity before a fatty acid taste is detected.

Ratings of smell and taste following the HF and LF interventions were significantly high compared to the CON trial. Stewart and Keast (2011) evaluated the effects of HF and LFDs on taste sensitivity to oleic acid (C18:1) in lean and overweight/obese volunteers (OW/OB). Consumption of an LFD for 28 days increased taste sensitivity to oleic acid in both cohorts whereas HF feeding for 28 days resulted in lean volunteers only. The failure of the HFD to reduce sensitivity to oleic acid in OW/OB group was attributed to these individuals having adapted taste thresholds as a result of greater habitual fat intake. It is tempting to speculate that the findings of our study are related to G protein-coupled receptors mediating taste signals in the oral cavity in response to the HF diet (Geraedts et al., 2010). It has been elucidated that LCFAs receptors, namely GPR40, GPR120 and CD36 are expressed in the taste cells of the oral cavity (Matsumura et al., 2007; Mattes, 2009b). Given that the receptors implicated in oral and small intestinal fatty acid detection are homologous, it is possible that over-exposure to fatty acids following a HFD could result in a reduction in expression or sensitivity of these receptors in both the mouth and small intestine (Little & Feinle-Bisset, 2010). This in turn would lead to reduced response to the HF trial and increased fat amount, and increased intake.
In agreement with previous findings (Clegg & Shafat, 2011; Boyd et al., 2003), we found no change in ad libitum food intake. Conversely, rats fed a HFD over-consumed at a 3-h ad libitum high-energy, HF meal compared to their counterparts on a LFD (Savastano and Covasa, 2005). In humans, Lissner and colleagues (1987) revealed that a 14-day HFD (45 – 50 % energy from fat) increased EI by 15.4 % compared to a medium HFD (30 – 35 % energy from fat). Similarly, it was documented that a 14-day HFD resulted in a 160 kcal∙day⁻¹ surfeit in EI compared to intake prior to HF feeding (French et al., 1995). Clegg and Shafat (2010) demonstrated that short-term hyperphagia was possible after a single HF meal; a HF breakfast was shown to be sufficient to significantly increase energy and fat intake at an ad libitum buffet meal 7-h postprandially. It was demonstrated in the current study that at the LF trial volunteers spent a mean time consuming food of 5 minutes less than the CON trial; hence a similar quantity of calories within a shorter time period was consumed. Cunningham et al. (1991) showed that in response to 14-day HF feeding, subjects had a slower rate of eating compared to a LFD, but this was not associated with the amount of the test meal consumed. The authors were surprised by this finding, expecting to find that desensitisation of fat receptors might have increased the capacity for a meal and enhanced the rate of eating. Together, these data suggest that fat desensitisation can occur in the small intestine, stomach, or oral cavity. In some studies, but not all, this is associated with increased appetite sensations, an enhanced rate of eating and increases in EI. All these point to potential mechanisms mediating the link between the development of obesity and HFDs.

In addition to the effect of GE, it is possible that HF supplementation could have had an influential effect on central appetite control (Park et al., 2007). It is also plausible that changes may have taken place in the absorptive capacity of enterocytes in the gut which could result in reduced sensitivity to gut peptides such as CCK and GLP-1 and appetite dys-regulation (Covasa, 2010). Absorptive mucosal cell renewal within the intestinal epithelium is highly regulated; the process from cell birth to death takes only 3 - 5 days (Näthke & McCarthy, 2009, p108). Therefore, the GI tract can adapt efficiently to exposure to a HFD, leading to alterations in brush-border morphology and increased digestive and absorptive capacity for lipids (Balint et al., 1980; Singh et al., 1972; Sagher et al., 1991; Spannagel et al., 1996; Shafat & Rumsey, 1997). Rats fed a regular chow diet supplemented with 20 % lard had greater absorptive capacity for oleic acid in the jejunum than controls (Singh et al., 1972). Balint and colleagues (1980) demonstrated enhanced uptake of oleic acid, after a 28-day period of HF feeding in rats (45 % vegetable oil), was associated with ileal mucosal hypertrophy. A diet high in olive oil (45 % of total energy) increased villus height to crypt depth ratio in the ileum and jejunum of
the rat (Sagher et al., 1991). Furthermore, feeding a 14-day HF load (20% of energy from fat) compared to a LFD (5% of energy) enhanced the release of CCK and pancreatic secretory response to intraduodenal infusion of oleic acid; the authors suggested that increased CCK was due to greater efficiency in digestion of triglycerides (Spannagel et al., 1996). In humans, HF feeding is also associated with increased CCK concentration (French et al., 1995). These data illustrate that adaptations to mechanisms other than accelerated GI transit can take place in the gut in response to HF feeding.

The relationship between habitual dietary intake for 5-days and 1-day before the CON trial and GE half-time using directly measured \[^{14}\text{CO}_2\] data was also examined. No significant relationships were found between 5-day habitual EI, macronutrient and fatty acid intake and GE half-time. We did find significant inverse associations between the 1-day intake of total dietary fat, MUFAs, medium-chain SFAs (C6:0 - C12:0) and long-chain SFAs (C12:0 - C14:0) and GE half-time. Considering the direct relationship between dietary fat intake and adverse weight profiles (Rolls, 1995), our finding of an inverse correlation between GE half-time and total fat intake seems fitting with the hypothesis that GE is accelerated in obesity (Wright et al., 1983; Zahorska-Markiewicz et al., 1986; Tosetti et al., 1996; Gryback et al., 1996; Näslund et al., 1998; Valera-Mora et al., 2005; Cardoso-Junior et al., 2007). In comparison to long-chain TGs which are transported as chylomicrons in the lymphatic system, when medium-chain TGs are converted to fatty acids, they can bypass this step and are transported directly into the venous portal vein (Bloom et al., 1951); this means that MCFAs have shorter GI transit compared to their LC FA counterparts (Clegg, 2010). This finding also links well with research that has established that a carbon chain length of ≥ C12 is necessary to delay GE (Hunt & Knox, 1968). These correlations further the findings from Chapter Four which suggest that GE half-time of a test meal is affected by background intake of a variety of fatty acids. This chapter also failed to show a significant correlation between background intake of oleic acid, which was high in the test meal, and GE half-time.

Accurate assessment of dietary fatty acid intake is necessary to examine the association between habitual dietary intake and GI transit. Limitations of our dietary assessment methodology include: (1) Respondent-dependent issues. Under-reporting of dietary consumption is a common limitation of volunteers recording their food intake, although this has been shown to be of greater concern in obese compared to lean respondents (Macdiarmid & Blundell, 1998). It has also been shown that volunteers may alter their habitual diet during a period of dietary monitoring (Hill et al., 2001). In the current study,
volunteers were asked to repeat their 5-day WFD from CON trial at HF and LF trials. This placed a large time burden on the respondents and accurate recording of food intake was dependent on a high level of motivation (Biro et al., 2002; Hodson et al., 2008). (2) Database-related issues. Food composition tables are out of date and reference data for all food items is unavailable (Biro et al., 2002). Furthermore, food composition tables fail to offer data sets for the complete range of fatty acids (Dobson et al., 2008). In the current study, for the 10 main foods that contributed to total dietary fat in a 5-day habitual diet in the CON trial, data was available on 96, 96 and 94% of total MUFA, PUFA and SFA content of foods respectively. However, analysis of specific fatty acid data in habitual data is confounded by lack of reference data. For example, for content of C18:1, C18:2 and C18:0, data was only available on 46, 43 and 53% of foods. As a result of the limitations associated with the dietary assessment methodology employed in the current study, it is recommended that fatty acid composition of blood and adipose tissue are also measured as biological biomarkers of fatty acid intake in future gastrointestinal studies (as outlined in Dobson et al., 2008). It is acknowledged that lacking data could have led to misinterpretation of the association between GE parameters and habitual intake of dietary fat as a result of a type I error. However, the fact that strong associations were evident between MUFA and SFA parameters, where the majority of reference data is available, strengthens our findings.

The current study found similar EE, fat and carbohydrate oxidation values at baseline and for time-averaged measurements of the postprandial period. Previously, Casas-Agustench et al. (2008) reported that acute HF test meals varying in fatty acid chain length did not affect substrate oxidation. Exposure to a HFD rich in oleic acid for 28 days resulted in greater fat oxidation compared to a diet enriched in palmitic acid (Kien et al., 2005). Schrauwen et al. (1997) illustrated that fat oxidation only equalled fat intake by day seven after switching from a LF to HF diet. It appears that volunteers in the current study may not have increased their fat oxidation fully in response to the HFD. Similar findings with regards to fat oxidation in the current trial could be explained by the duration and intensity of the HF intervention employed. However, it is acknowledged that examination of specific time points in conjunction with postprandial time-averaged response may have elucidated some alterations in metabolic responses.

It is appreciated that the supplements provided in the current study are both dense in energy and fat content and thus it is not possible to say if the fat content of the diet per se was responsible for study outcomes. However, it is the overconsumption of HF energy dense foods which are implicated in the aetiology of obesity (Drewnowski, 2003; Lissner et al., 1987) and the majority of HF intervention studies have employed diets that
are high in energy and fat content (Cunningham et al., 1991; Castiglione et al., 2002; Boyd et al., 2003; Robertson et al., 2004; Clegg et al., 2011; Clegg & Shafat, 2011). The use of an *ad libitum* buffet meal provided an objective quantification of energy and macronutrient intake at the end of the test day. However, buffet meals as a measurement of food intake are not without their limitations; consuming a meal under laboratory conditions is an artificial environment and may influence a subject’s eating behaviours (Herman & Polivy, 2005). In the current study, measurement of food intake at the buffet meal occurred 6 hours subsequent to ingestion of the test meal. Although this has been employed in previous studies (Clegg & Shafat, 2011), and was shorter than the 9-h gap between meals in Cunningham et al. (1991), it is not a realistic gauge of eating behaviour in free-living situations. However it is important to consider that the primary objective of this study was to examine the effect of the intervention on GE of a HF test meal and it has been confirmed that for meals with a long half-emptying time, it is more accurate to measure GE for 6-h postprandially (Clegg & Shafat, 2010).

It is acknowledged that the low sample size in the current study, and hence the possibility of type II errors, could have made it difficult to detect differences within-trials and between-groups. The use of Fisher’s least significant difference (LSD) may have resulted in type I errors. It is acknowledged that if adjustments had been made for multiple comparisons using the Bonferroni correction significant differences may not have been found. Assuming that these trends are repeatable in future trials, for a gastric emptying half-time difference between CON and HF trials in (based on pooled group data), a sample size of 40 subjects would have 85% power to detect a difference in means of 9.1 assuming a SD of differences of 18.4, using a two-sided paired t-test with a 0.05 two-sided significance level. For a difference between CON and LF trials, a sample size of 48 subjects would have 85% power to detect a difference in means of 18.70 assuming a SD of differences of 8.40, using a two-sided paired t-test with a 0.05 two-sided significance level.

Since we observed reduced satiety in response to HF supplementation, it can be speculated that HF supplementation for an extra two days had a more deleterious effect on health than the findings observed relating to a 3-day HF supplemented diet (Clegg et al., 2011). Clegg and colleagues illustrated that GE latency time and MCTT were accelerated after 3-day HF supplementation but this effect was not translated to changes in postprandial lipaemia or subjective appetite, which are significant factors in the aetiology of obesity and metabolic syndrome (Rolls, 1995; Darwiche et al., 2002; Castiglione et al., 2002). It is feasible that lack of covariate-adjusted analyses in the aforementioned paper could have masked changes, or failed to show the full effect of the
intervention, independent of confounding variables including dietary fatty acid intake. In
the current study, volunteers consumed a greater quantity of fat (OO and OM group) and
energy (OO) during the 5-day HF supplementation period, when compared to the OF
group. All supplement groups had reduced satiety in response to HF test meal, even
after 5-day deadaptation period.

The current study illustrated that a 5-day HF supplemented diet altered GE half-time and
ascension time when adjustments were made for habitual dietary intake or weight profile.
These findings highlight that the complex interaction between factors which lead to
adaptation in GI transit, including the HF supplementation period, composition of the test
meal and background diet. Adaptation in the GI tract is not just a case of adaptation to a narrow range of fatty acids as previously hypothesised (Clegg et al.,
2011). MCTT or time-averaged postprandial energy expenditure and substrate utilisation
were not significantly altered in response to a HF intervention. Interestingly, we did show
that a 5-day HF intervention was sufficient to reduce satiety sensations associated with a
HF meal with affecting ad libitum food intake. Furthermore, it was illustrated that 5-days
was not a sufficient time period to fully reverse the effect of HF feeding on subjective
sensations of satiety. HF supplementation with n-3 fatty acids appeared to have an
appetite-suppressing; during the 5-day HF intervention period volunteers had a decrease
in mean habitual EI and/or intake compared to the OO and OM groups. This finding
provides some evidence for the importance of fat quality in the aetiology of obesity and
T2DM (MacIntosh et al., 2003; Khor, 2004).
Chapter 7: Conclusion

7.1 Overview

Obesity is one of the most significant health burdens of the 21st century (WHO, 2008). A high intake of dietary fat is a salient environmental factor in the aetiology of obesity. Chronic exposure to fat may lead to down-regulation of the appetite control system through mechanisms including alterations to the GI tract (French et al., 1995; Little et al., 2007; Covasa, 2010). In order to design effective preventative strategies and valuable treatments for obesity, it is necessary to gain a greater understanding of how GI dysfunction regulates appetite and EI.

In this thesis a number of models were used to gain a greater insight into the regulation of GE and appetite in healthy adults. The $^{13}$C octanoic acid breath test (OBT) was used to assess GE in all studies conducted as part of this thesis; breath samples were collected during the fasted state (at baseline) and for 6-h postprandially for determination of GE. Firstly, acute pharmacological and nutritional models (Chapter 3 and 4), which explored the effects of 10 mg domperidone and 3 g cinnamon respectively, on GE and appetite responses after ingestion of HF meals are presented. Habitual dietary intake data, ascertained by WFD, revealed that 1-day and 3-day background intake of specific fatty acids was strongly correlated with the rate of GE (Chapter 4). Also detailed in this thesis, was a methodology for improving the accuracy of the measurement of GE parameters in the $^{13}$C OBT, through direct measurement of $\dot{V}CO_2$ production rate (Chapter 5). The findings of Chapters 3 - 5 culminated in the decision to examine the effect of a 5-day HF dietary intervention supplemented with specific fatty acids on adaptation and deadaptation of GI transit using directly measured $\dot{V}CO_2$ data (Chapter 6). While the results of the aforementioned studies were discussed in preceding chapters, this section aims to draw conclusions based on the overall findings of this body of work. Limitations of this body of work will also be highlighted and recommendations for future research shall be proposed.

In Chapter 3, an acute pharmacological model, 3 g domperidone administered or ally before a HF test meal, was shown to have no effect on GE rate or appetite sensations. From emerging literature, it appears that receptor antagonists have a fat receptor mechanism; a lower density of D2 receptor antagonists were present after a period of HF feeding in mice (Huang et al, 2006) and was associated with greater adiposity in an obese human cohort (Wang et al, 2002). The findings presented in Chapter 3 suggest
that adaptation in GI function is through chronic exposure to fat and is not the mechanism responsible for the findings of Huang et al. (2006) and Wang et al. (2002). Chapter 4 demonstrated that 3 g cinnamon supplementation had no effect on GE of a HF test meal and appetite responses in the postprandial period. Levels of glycaemia and lipaemia were also comparable to those observed under the placebo condition. Oral administration of domperidone (10 mg) and ingestion of cinnamon (3 g) were unable to induce an effect on GE over and above the effect of fat on rate of emptying. Collectively, these studies confirm that LCFAs are potent inhibitors of GE (Hunt & Knox, 1968; Cecil et al, 1999; Clegg et al, 2009). LCFAs stimulate the secretion of CCK and GLP-1, gut peptides which are known inhibitors of GE, through G-protein coupled fatty acid receptors such as GPR120-coupled Ca^{2+} signalling (Tanaka et al, 2008; Hirasawa et al, 2005).

The relationship between habitual dietary intake and GE half-time was explored in Chapter 3 and 4. Of particular significance, it was shown in Chapter 4 that there was a strong inverse relation between habitual intake (1 – 3 days) of palmitoleic (C16:1), eicosenoic (C20:1) and n-3 fatty acids and GE half-time of a test meal rich in linoleic acid (C18:2n-6). The association between GE of a HF meal and ingestion of specific fatty acids in habitual diet, which were not high in the test meal may suggest that the process of adaptation to a HFD is affected by mechanisms other than adaptation to background intake of that specific fatty acid as previously speculated (Clegg et al., 2011). Fatty acid receptors such as G protein-coupled receptors, GPR40, GPR120 as well as CD36 have been shown to have an affinity to specific LCFAs and appear to aid in the secretion of gut peptides including CCK, GLP-1 and PYY (Feinle-Bisset et al., 2010; Geraedts et al., 2010). Background intake of specific fatty acids may led to a down-regulation of receptors in the GI tract and oral cavity and led to reduced sensitivity to fat ingestion (Itoh et al., 2003; Geraedts et al., 2010; Mattes, 2009b). These observational findings culminated in the decision to explore the effect of a short-term HF supplemented diet varying in fatty acid composition of GI transit (Chapter 6).

The agreement between GE parameters obtained using directly measured $\dot{V}CO_2$ production rate and (1) estimated $\dot{V}CO_2$ production rate using widely used assumptions (Shreeve et al., 1970; Haycock et al., 1978) (2) directly measured $\dot{V}CO_2$ production rate at rest only was examined using Bland-Altman plots (Chapter 5). It was illustrated that, in comparison to GE parameters calculated using directly measured $\dot{V}CO_2$, that the other two methods overestimated GE parameters to a similar degree. GE half-time and lag phase appeared 12 and 9 m in longer with measured resting and predicted $\dot{V}CO_2$
respectively than directly measured $\dot{V}CO_2$ values gathered throughout the 6-h postprandial period. As a consequence of this finding, the analysis of GE parameters (Chapter 6) was calculated based on directly measured $\dot{V}CO_2$ production rate.

The over-consumption of dietary fat has a fundamental role to play in the aetiology of obesity. There is a direct relation between intake of dietary fat and adverse weight profiles (Rolls, 1995). Hunt and colleagues (1975) hypothesised that a shorter rate of GE may reduce the satiating effect of nutrients and led to over-consumption and obesity. However, there is controversy over the directionality of GE in obese patients compared to lean individuals. Some have shown delayed GE (solid meals: Wright et al., 1983; Zahorska-Markiewicz et al., 1986; Tosetti et al., 1996; Gryback et al., 1996; Näslund et al., 1998; Valera-Mora et al., 2005; Cardoso-Junior et al., 2007; liquid meal: Vasquez-Roque et al., 2006) whereas others have shown an acceleration of nutrients from the stomach into the small intestine (solid meals: Horowitz et al., 1983; Horowitz et al., 1986; Maddox et al., 1989; Jackson et al., 2004; liquid meals: Horowitz et al., 1986; Maddox et al., 1989). In Chapter 6, a 5-day HF feeding intervention in healthy adults was used as a short-term model of development of obesity. It was shown that a 5-day HF supplemented diet accelerated GE half-time and as cession time, when adjustments were made for background dietary fat intake. Thus, a combination of reduction of gastric distension, through its influence on gastric stretch and tension receptors (Grundy, 2002) could have been partly responsible for lower subjective satiety responses after this short-term HF intervention. The presence of nutrients within the small intestinal lumen also contributes to satiety (Geliebter et al., 1988; Read et al., 1994). Gut peptides including CCK, GLP-1 and PYY are released in response to the presence of nutrients in the small intestine and act to reduce appetite (Horner et al., 2011). The time-frame of deadaptation to a HF diet has not been previously explored in humans. A rat model created by Brown and colleagues (1994) showed that a 28-day period was not a sufficient time span to reverse the HFD-induced delay in GE. In the current study, using a less intensive shorter-term HF supplemented diet, GE rates at the LF trial were not significantly different to CON; this suggests that GE was returning to baseline levels. However, satiety responses were still reduced at the LF trial. Therefore, it is proposed that mechanisms other than GI transit, including alterations in central appetite signaling and brush-border morphology were responsible for altered satiety responses to a HFD (Covasa, 2010). Even though gut peptide responses were not measured in the current thesis, it is feasible that there was a reduced sensitivity to CCK after HF feeding as documented by others (French et al., 1995; Covasa & Ritter, 2000). Furthermore, fasting GLP-1 concentrations were significantly altered in mice chronically exposed to fat (Anini & Brubaker, 2002). CCK and
GLP-1 act through G-protein coupled receptors such as GPR120 and GPR40 (Feinle-Bisset et al., 2010; Geraedts et al., 2010). Covasa (2010) suggested that HF feeding and hence, ‘chronic agonism’ of these receptors, may result in desensitisation and down-regulation. The findings of this study illustrate that a myriad of factors have a role to play in the integrative process of regulating appetite.

7.2 Limitations

- Future HF intervention studies should employ a control arm to ensure that differences recorded between trials are as a result of the intervention itself and not as a result of external confounding factors or bias.
- In this thesis, a standard amount of $^{13}$C octanoate (100 – 150 mg) was used in the $^{13}$OBT as opposed to an amount relative to the body weight of the volunteer (e.g. 1 -1.5 mg∙kg$^{-1}$). A criticism of this method is that $^{13}$C enrichment could be lower in the breath of heavier individuals. However, the precision of the isotope ratio mass spectrometer employed in Chapter 6 was 0.06 δ and the Δ in δ from baseline was approximately -25 to -17 (i.e. 8 δ units). Therefore, it is probable that lower $^{13}$C enrichment would have a significant impact on results.
- Calculation of GE parameters from the $^{13}$CO$_2$ excretion curve relies on the extrapolation of data using the Ghoos model (Ghoos et al., 1993). It is acknowledged that this model was devised based on a LF test meal of ~ 250 kcal. The fat and caloric content of the test meals employed in this thesis were high (fat content of test meals: 45.7 – 62.5 g; energy content: 632 – 866 kcal) and hence, there was a slow rise in $^{13}$CO$_2$/$^{12}$CO$_2$ ratio. In order to validate whether or not a more accurate calculation of GE parameters could be obtained by conducting a longer OBT i.e. less need for extrapolation of data, one volunteer from Chapter 6 provided breath samples for a duration of 12 -h postprandially (Appendix 4 A and B). GE parameters were calculated using 6-h and 12-h datasets. GE parameters were obtained using a constant predicted resting CO$_2$ value because it was not feasible to directly measure CO$_2$ production rate when the volunteer had left the metabolic suite at 6-h postprandially. Upon leaving the metabolic suite, the volunteer was permitted to exercise and eat ad libitum. As is evident from Appendix 3 A, GE parameters ascertained through use of 6-h and 12-h data are similar suggesting that 6-h is a sufficient time-period for calculation of GE half-time, lag phase, latency and as cension time using a HF test meal. It should be noted that the volunteer exercised after the CON and HF trial; this would have resulted in enhanced recovery of $^{13}$C from the bicarbonate pool and
could be part of the explanation of why there are greater discrepancies in cPDR between 6-h and 12-h measurements at these trials compared to the LF trial.

7.3 Recommendations for Future Research

- An expert panel have recently made attempts to formulate a standardised oral fat tolerance test (FTT); they recommend that the FTT meal should consist of 75 g fat (containing mixtures of saturated and unsaturated fatty acids), 25 g carbohydrate and 10 g protein (Kolovou et al., 2011). In line with this idea of a standard FTT, efforts should be made to standardise the gastrointestinal response to fat loading. Measurement of GE using a test meal that is physiologically relevant to the aetiology of obesity-related chronic diseases, would help researchers draw firmer conclusions on whether or not GE to a fatty meal is delayed or accelerated in the obese state.

- An area worthy of investigation in future research is the evaluation of different mathematical models for the estimation of GE parameters after ingestion of a solid HF test meal. Odunsi et al. (2009) have recently evaluated mathematical models to estimate GE half-time of a LF test meal and found that the linear regression models of Virmontes and Szarka resulted in more accurate analyses of breath $^{13}$CO$_2$ than the Ghoos model. As well as being more accurate, the aforementioned regression models are also more cost-effective because they require less frequent collection of breath samples (Odunsi et al. 2009). Thus, these models would aid in the collection of normative data on GE rate of HF test meals and would also help lead to a conclusion on the directionality of the GE rate in the obese state.

- The quantity of fat used in the HF supplement was standardised in Chapter 6. It would be reasonable to consider adding fat to the supplement relative to body weight, especially when using a mixed cohort. However, it is appreciated that this would result in a non-standardised intervention.

- This thesis showed that GE parameters are over-estimated using a constant predicted value of $\dot{\nu}$CO$_2$. Thus, calculation of GE parameters using directly measured $\dot{\nu}$CO$_2$ production rate is recommended for future GI studies. Ideally, future studies should use the ventilated canopy technique for increased accuracy of $\dot{\nu}$CO$_2$ production rate.

- A liquid HF supplement was used for a 5-day HF intervention in Chapter 6 and changes in GI transit were quantified on test trials using a solid HF test meal. This study raises further questions: Firstly: how would a more physiologically
relevant HF solid supplement affect GI transit of a HF solid meal? Secondly: how would a liquid HF supplementation affect GI transit of a liquid HF test meal? Thirdly: how would adaptation to a specific fatty acid supplemented HFD affect GI transit of a test meal rich in another fatty acid?

- Findings from Chapter 6 illustrate that there is a complex inter-relationship between mechanisms responsible for appetite regulation. Thus, future studies which examine the time course of adaptation and deadaptation to a HF diet in humans should examine a multitude of factors involved in regulation of appetite instead of looking at an isolated mechanism (i.e. GI transit).

- The influence of habitual dietary intake on fatty acid receptors (e.g. GPR40, GPR120, CD36) in the gustatory system and the GI tract should be examined. Furthermore, it should be quantified how these receptors adapt and deadapt to a period of HF feeding.

- The findings of this thesis also open to further investigation of examining differences in oral fat tasting and preference change upon switching from a HF to LF diet in healthy and obese cohorts.

- Development of a dual stable isotope tracer technique for measurement of GE and MCTT simultaneously, would allow for a more accurate quantification of MCTT and would remove the issue of non-response to the inulin H2 breath test as was evident in Chapter 6. In a similar approach, two different substrates, namely 13C octanoate and 2H octanoate, for simultaneous measurement of GE using the OBT, were correlated with synchronised GE assessment by scintigraphy (Bluck et al., 2002). The authors found that appearance of deuterated octanoate in the body water pool was more closely associated with scintigraphy than the kinetics observed using octanoate as the substrate. When labelled with 13C, Lactose-ureide, a substrate which is metabolised by bacteria upon reaching the colon, was shown to be a valid alternative to scintigraphy for measurement of MCTT (Geypens et al., 1999). Based on the observations of Bluck et al. (2002) and Geypens et al. (1999), it is proposed that a dual label tracer methodology could employ deuterium 2H octanoate for measurement of GE and 13C-labelled lactose-ureide for calculation of MCTT.

- Assessment of habitual dietary intake is confounded by a lack of up-to-date, reference data for fatty acid composition of foods in dietary analysis software. Measurement of biological biomarkers of fatty acid intake, alongside self-reported dietary assessment would provide a more accurate insight into the association
between habitual intake of specific dietary fatty acids and GI transit. Dobson and colleagues (2008) noted that while fatty acid composition of adipose tissue is accepted as the gold standard method for characterising dietary fatty acid intake of stable weight adults, it has a slow turnover rate. Using stable isotope techniques, Strawford et al. (2004) illustrated that adipose tissues have a half-time of between 6 and 9 months. Fatty acid composition of plasma TGs could act as a biomarker of recent dietary intake (Dobson et al., 2008).

- Monitoring of habitual dietary intake by fatty acid profile in conjunction with recording of dietary intake e.g. WFD should take place for a minimum of one day before GI transit research in order to reduce inter-subject variability. If this is not feasible, a LF evening meal could be used on the evening prior to testing in an attempt to standardise dietary intake prior to testing (Robertson et al., 2004).

7.4 Key Findings

- Oral administration of 10 mg domperidone had no effect on GE of a HF test meal or appetite responses in healthy adults.
- Supplementing a HF meal with 3 g cinnamon did not alter GE rate, nor did it affect appetite or metabolic variables in the postprandial period.
- There appears to be a link between habitual dietary intake and GE half-time which is not specific to the fatty acid composition of the test meal. This important finding could explain some of the inter-subject variability in GI transit studies (Brophy et al., 1986).
- Quantification of GE parameters using an assumption of \( \dot{V}CO_2 \) production rate multiplied by body surface area (Shreeve et al., 1970; Haycock et al., 1978) overestimated GE half-time and lag phase by 12 and 9 minutes respectively compared to \( \dot{V}CO_2 \) measured directly and regularly through the OBT.
- When adjustments were made for habitual dietary intake of fat, a 5-day HF supplemented diet varying in fatty acid composition accelerated GE and reduced satiety without affecting MCTT, energy expenditure or substrate utilisation.
- Supplementing a HF diet with \( n-3 \) FAs has an appetite suppressing effect.
- Even after a 5-day deadaptation period to HF supplementation, volunteers had reduced satiety responses to a HF test meal, without affecting ad libitum food intake.
7.5 Conclusions

This thesis confirms previous findings (Cecil et al., 1999; Clegg & Shafat, 2009) that dietary fat is a potent regulator of GE and appetite, such that pharmacological and nutritional models were not able to induce a change in rate of entry of nutrients from the stomach into the small intestine. Furthermore, this thesis quantifiably justified the use of directly measured \( \Delta CO_2 \) production rate data for improved accuracy of GE parameters. A short-term HFD was shown to accelerate GE and appetite sensations without affecting GI transit. This thesis highlights the complexity of appetite regulation; over which control is based on an array of mechanisms including gastric distension, hormonal responses to gut peptides and fatty acid receptors in the GI tract and gustatory system. It appears that pathways other than the alteration of GI transit were also responsible for a change of appetite sensations after a 5-day HFD. This body of work exemplifies the significance of recent dietary patterns, in terms of both supplementation and background dietary intake, in the regulation of GI transit and appetite.
Bibliography


International Diabetes Federation (2005) The IDF consensus worldwide definition of the metabolic syndrome [article online].


Appendices

Appendix 1

Appendix 1 A Ethics Application Approval Email (Chapter 6)

From: Anne O'Brien  
Sent: 23 June 2010 11:00  
To: Oonagh Markey; Amir Shafat  
Subject: Amendments to EHSREC09-74

Dear Oonagh, Amir

Thank you for your Research Ethics application which was recently reviewed by the Education and Health Sciences Research Ethics Committee. The recommendation of the Committee is outlined below:

EHSREC09-74 Effect Of 5-Day Adaptation & Deadaptation Period To A Fatty Acid Supplemented Diet On Gastrointestinal Transit, Postprandial Lipaemia & Appetite

Principal Investigator: Amir Shafat  
Other Investigators: Oonagh Markey

Recommendation: Amendments to application EHSREC09-74 (details below) Approved

6(a) study aim: To investigate if 5-day high fat feeding and de-feeding of various fatty acid compositions modulates (iv) fat oxidation and energy expenditure  
6(b) Hypothesis added: No difference in fat oxidation or energy expenditure following HF test meal subsequent to a high-fat feeding or de-feeding period.  
Section 6(c) Plan of investigation  
- Before the commencing test trials, volunteers will record their dietary intake using a weighed food diary  
- Volunteers will repeat their dietary intake (based on 7 day weighed food diary)  
- Baseline expired gas analysis samples will be collected (SS009).  
- Gas analysis samples will be taken at regular intervals until 6h postprandially.

Section 6(d) Addition of research procedure SS009 Expired Gas Analysis  
Section 7(g) Expired gas sampling procedures presents minimal discomfort or hazard to volunteers.

Volunteer Information Sheet (Sentences added):  
Before the commencing test trials, you will be asked to record their dietary intake for 7 days using a weighed food diary using food diary sheets and a set of scales that will be provided by the investigator.  
You will be asked to repeat your 7 day weighed food diary for 5 days (for the 5 applicable days) before your first test day.  
You will give an initial gas analysis sample (this simply involves breathing into a mouthpiece).  
Gas analysis samples will be collected regularly for up to 6h after the first meal.  
Gas analysis procedure presents minimal risk.

Regards  
Anne O’Brien  
Administrator to Education and Health Sciences  
Research Ethics Committee
Appendix 1B Ethical Application Cover Sheet (Chapter 6)

Cover Sheet Questionnaire

Section 1. Ethical Issues

1. Does this application involve research with:
   a. People under the age of 18
   b. People under the age of 18 who are not in educational contexts and engaged in a normal range of teaching, learning and assessment activities
   c. People with diagnosed psychological impairments
   d. People with a diagnosed learning difficulty
   e. People dependent on the protection/under the control/influence of others (e.g. people in care, prisoners, students with whom the researcher has a supervisory relationship, etc.)
   f. Relatives of sick people (e.g. parents of sick children)
   g. People who may have only a basic knowledge of English
   h. Other populations that are potentially vulnerable
      Please describe:

2. Does this application deal with:
   a. Sensitive personal issues? (e.g. suicide, bereavement, gender identity, sexuality, fertility, abortion, gambling)?
   b. Illegal activities, illicit drug taking, substance abuse engaging in criminal behaviour?
   c. Any act that might diminish self-respect or cause shame, embarrassment or regret?
   d. Research into politically and/or racially/ethically and/or commercially sensitive areas?
   e. Issues which might otherwise give rise to a risk of loss of employment for the participant?
   f. Other issues that may be considered sensitive
      Please describe:

3. Does the proposed research procedures involve:
   a. Use of personal records without consent?
   b. Deception of participants or use of placebos?
   c. The offer of large inducements to participate?
   d. Audio or visual recording without consent?
   e. Invasive physical interventions or treatments?
   f. Research that might put researchers or participants at substantial risk?
   g. Storage of results or data for less than 7 years?
   h. Dealing with topics, using methodologies, or reporting of findings in a way that is likely to cause pain, discomfort, embarrassment, or changes to lifestyle for participants
   i. Other procedures that may be considered invasive
      Please describe: blood sampling
Section 2. Approved Procedures

If the answer to any of the questions in Section 1 is ‘yes’, then please complete this section before proceeding to Section 3 & 4.

Does the research follow any ULREG Approved Procedure in relation to this sensitivity or intrusion?
Yes ☐ No ☑

Procedure Name and Approval Number:

3(b) Deception of participants or use of placebos.

Participants will be blinded to the real nature of the study i.e. effect of a 5-day adaptation & deadaptation period to a fatty acid supplemented diet on gastrointestinal transit, postprandial lipaemia & appetite. They will be originally informed that the study is examining the effect of macronutrients in the diet on digestive and cardiovascular health. They will be informed that they will be receiving a macronutrient supplement in an attempt to prevent them from altering their dietary patterns. Participants will be asked to consume a low-fat supplement (e.g. muffin/milkshake) for 5-days for two out of three trials. The other trial will involve participants consuming the same supplement with the addition of up to 100g of dietary fat. Research suggests that free-living individuals can consume more than 150g fat in a single sitting and almost 200g in a full day (Blundell & MacDiarmid, 1997). Therefore the level of fat present in the supplement provided in this study is ecologically valid. Upon study completion, volunteers will be provided with an explanation of the nature of the study. It will be explained to volunteers that it was deemed necessary to take this course of action because failure to blind volunteers would have resulted in a weaker methodological design. Awareness of the real nature of the study could have resulted in a manipulation in dietary fat patterns in response to the fact that they were aware that they were being studied in relation to dietary fat intake (i.e. Hawthorne Effect). Furthermore, the "deception" in this case is mild and concerns a generalisation of the intervention - we do not purposefully deceive but instead we generalise. Although this compromises full informed consent, and we have taken the harsh interpretation that this is a form of deception, we do not think this is a deception in the full sense of the word. Specifically, subjects are informed and consent to all aspects of this study, with a single component (dietary fat) deliberately generalised (but not masked or misleading) to prevent any changes to eating behaviour.


3(i) SS023. Blood sampling by venepuncture.

Venous blood sampling will be carried out by a trained phlebotomist within a clean laboratory environment.
Appendix 2

Appendix 2 A Visual Analogue Scale (Chapter 6)

Visual Analogue Scale
Name ______________ Sub No. ____ Date _______ Test _______

**Time: 0 min (after breakfast)**

*How hungry do you feel?*

- I am not hungry at all
- I have never been more hungry

*How satisfied do you feel?*

- I am completely empty
- I cannot eat another bite

*How full do you feel?*

- Not at all full
- Totally full

*How much do you think you can eat?*

- Nothing at all
- A lot

*Would you like to eat something sweet?*

- Very Much
- Not at all

*Would you like to eat something salty?*

- Very Much
- Not at all

*Would you like to eat something savoury?*

- Very Much
- Not at all

*Would you like to eat something fatty?*

- Very Much
- Not at all
Appendix 2 B Visual Analogue Scale for Meal Palatability and Feelings of General Well-being after Test Breakfast Meal and Ad Libitum Buffet Meal (Chapter 6)
Appendix 3

Appendix 3  A Gastric Emptying (GE) Parameters after the Ingestion of High-Fat Meal under Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests using directly measured resting \( \dot{\mathrm{V}} \mathrm{CO}_2 \) data (n = 24)

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
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<tbody>
<tr>
<td>Half-time * (min)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>OO</td>
<td>206 ± 41</td>
<td>213 ± 57</td>
<td>196 ± 36</td>
</tr>
<tr>
<td>OF</td>
<td>213 ± 34</td>
<td>197 ± 23</td>
<td>211 ± 43</td>
</tr>
<tr>
<td>OM</td>
<td>228 ± 34</td>
<td>213 ± 28</td>
<td>216 ± 19</td>
</tr>
<tr>
<td>Lag phase * (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>137 ± 26</td>
<td>145 ± 35</td>
<td>130 ± 24</td>
</tr>
<tr>
<td>OF</td>
<td>140 ± 16</td>
<td>130 ± 13</td>
<td>136 ± 27</td>
</tr>
<tr>
<td>OM</td>
<td>145 ± 28</td>
<td>139 ± 18</td>
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<tr>
<td>Latency time (min)</td>
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<tr>
<td>OO</td>
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<td>47 ± 4</td>
<td>47 ± 10</td>
</tr>
<tr>
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<td>49 ± 8</td>
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</tr>
<tr>
<td>Ascension time (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>156 ± 35</td>
<td>158 ± 49</td>
<td>149 ± 28</td>
</tr>
<tr>
<td>OF</td>
<td>163 ± 32</td>
<td>150 ± 20</td>
<td>164 ± 36</td>
</tr>
<tr>
<td>OM</td>
<td>177 ± 34</td>
<td>163 ± 23</td>
<td>169 ± 18</td>
</tr>
<tr>
<td>cPDR * (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>31.5 ± 8.8</td>
<td>31.1 ± 8.8</td>
<td>31.4 ± 5.8</td>
</tr>
<tr>
<td>OF</td>
<td>30.0 ± 9.0</td>
<td>32.4 ± 5.8</td>
<td>31.3 ± 7.0</td>
</tr>
<tr>
<td>OM</td>
<td>28.2 ± 6.5</td>
<td>33.4 ± 4.4</td>
<td>35.4 ± 19.3</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions using R M A NOVA, \( p > 0.05 \). * Statistical analysis performed on logarithmically transformed data.
Appendix 3 B Gastric Emptying (GE) Parameters after the Ingestion of High-Fat Meal under Control (CON), 5-day HFD (HF), 5-day Deadaptation to HFD (LF) Tests using a constant predicted measurement of resting $\sqrt{\text{CO}_2}$ data ($n = 24$)

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>CON</th>
<th>HF</th>
<th>LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>**Half-time ** (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>208 ± 38</td>
<td>213 ± 57</td>
<td>196 ± 36</td>
</tr>
<tr>
<td>OF</td>
<td>210 ± 36</td>
<td>197 ± 23</td>
<td>211 ± 43</td>
</tr>
<tr>
<td>OM</td>
<td>228 ± 34</td>
<td>213 ± 28</td>
<td>216 ± 19</td>
</tr>
<tr>
<td>**Lag phase ** (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>138 ± 18</td>
<td>143 ± 36</td>
<td>130 ± 24</td>
</tr>
<tr>
<td>OF</td>
<td>138 ± 25</td>
<td>130 ± 13</td>
<td>136 ± 27</td>
</tr>
<tr>
<td>OM</td>
<td>145 ± 28</td>
<td>139 ± 18</td>
<td>138 ± 11</td>
</tr>
<tr>
<td>**Latency time ** (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>50 ± 11</td>
<td>53 ± 12</td>
<td>47 ± 10</td>
</tr>
<tr>
<td>OF</td>
<td>49 ± 4</td>
<td>47 ± 4</td>
<td>47 ± 10</td>
</tr>
<tr>
<td>OM</td>
<td>51 ± 18</td>
<td>49 ± 8</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>**Ascension time ** (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>158 ± 32</td>
<td>160 ± 46</td>
<td>149 ± 28</td>
</tr>
<tr>
<td>OF</td>
<td>161 ± 33</td>
<td>150 ± 20</td>
<td>164 ± 36</td>
</tr>
<tr>
<td>OM</td>
<td>177 ± 34</td>
<td>163 ± 23</td>
<td>169 ± 18</td>
</tr>
<tr>
<td>**cPDR ** (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OO</td>
<td>34.1 ± 4.7</td>
<td>31.5 ± 7.2</td>
<td>36.9 ± 3.1</td>
</tr>
<tr>
<td>OF</td>
<td>32.4 ± 3.6</td>
<td>33.7 ± 4.1</td>
<td>36.7 ± 5.1</td>
</tr>
<tr>
<td>OM</td>
<td>29.8 ± 7.1</td>
<td>35.3 ± 4.3</td>
<td>35.0 ± 2.7</td>
</tr>
</tbody>
</table>

Data are given as the mean ± SD. No significant differences were found between conditions using RM ANOVA, $p > 0.05$. * Statistical analysis performed on logarithmically transformed data.
Appendix 4

Appendix 4 A Comparison of cumulative 13C percentage dose recovered (cPDR) and gastric emptying (GE) parameters obtained using a constant predicted resting $\dot{V}CO_2$ value normalised to body surface area (Shreeve et al., 1970; Haycock et al., 1978) for 6-h and 12-h post prandially. Modelled on data from volunteer 28 (OF group; Chapter 6).

<table>
<thead>
<tr>
<th>GE Parameters</th>
<th>Predicted 6-h</th>
<th>Predicted 12-h</th>
<th>Difference (12-h – 6-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cPDR (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>41.1</td>
<td>46.4</td>
<td>5.3</td>
</tr>
<tr>
<td>HF</td>
<td>42.8</td>
<td>48.9</td>
<td>6.1</td>
</tr>
<tr>
<td>LF</td>
<td>49.0</td>
<td>51.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Half-time (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>182</td>
<td>183</td>
<td>1</td>
</tr>
<tr>
<td>HF</td>
<td>201</td>
<td>198</td>
<td>-3</td>
</tr>
<tr>
<td>LF</td>
<td>196</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td>Lag phase (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>109</td>
<td>109</td>
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</tr>
<tr>
<td>HF</td>
<td>119</td>
<td>118</td>
<td>-1</td>
</tr>
<tr>
<td>LF</td>
<td>108</td>
<td>107</td>
<td>-1</td>
</tr>
<tr>
<td>Latency time (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>33</td>
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<td>LF</td>
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<td>29</td>
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<tr>
<td>Ascension time (min)</td>
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<tr>
<td>HF</td>
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<td>163</td>
<td>-3</td>
</tr>
<tr>
<td>LF</td>
<td>167</td>
<td>167</td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix 4 B A representation of typical (volunteer 28) $^{13}$CO$_2$ excretion over the time course of the 6-h (360 min) test day (indicated by arrow) and extended 6-h time period (720 min) for each of the three test conditions: control (CON), 5-day HFD (HF), 5-day deadaptation to HFD (LF). Postprandial breath samples commenced after completion of test meal (0 min)
Effect of cinnamon on gastric emptying, arterial stiffness, postprandial lipemia, glycemia, and appetite responses to high-fat breakfast

Markey et al.

http://www.candiab.com/content/10/1/78 (7 September 2011)
Effect of cinnamon on gastric emptying, arterial stiffness, postprandial lipemia, glycemia, and appetite responses to high-fat breakfast

Oonagh Markey1, Conor M McLean2, Paul Medlow2, Gareth W Davison2, Tom R Trinick2, Ellie Duly3 and Amir Shafal1*

Abstract
Background: Cinnamon has been shown to delay gastric emptying of a high-carbohydrate meal and reduce postprandial glycaemia in healthy adults. However, it is dietary fat which is implicated in the etiology and is associated with obesity, type 2 diabetes and cardiovascular disease. We aimed to determine the effect of 3 g cinnamon (Cinnamomum zeylanicum) on GE, postprandial lipemic and glycemic responses, oxidative stress, arterial stiffness, as well as appetite sensations and subsequent food intake following a high-fat meal.

Methods: A single-blind randomized crossover study assessed nine healthy, young subjects. GE rate of a high-fat meal supplemented with 3 g cinnamon or placebo was determined using the 13C octanoic acid breath test. Breath, blood samples and subjective appetite ratings were collected in the fasted and during the 360 min postprandial period, followed by an ad libitum buffet meal. Gastric emptying and 1-day fatty acid intake relationships were also examined.

Results: Cinnamon did not change gastric emptying parameters, postprandial triacylglycerol or glucose concentrations, oxidative stress, arterial function or appetite (p < 0.05). Strong relationships were evident (p < 0.05) between GE T50 and 1-day palmitoleic acid (r = 0.78), eicosanoic acid (r = 0.84) and total omega-3 intake (r = -0.72). The ingestion of 3 g cinnamon had no effect on GE, arterial stiffness and oxidative stress following a HF meal.

Conclusions: 3 g cinnamon did not alter the postprandial response to a high-fat test meal. We find no evidence to support the use of 3 g cinnamon supplementation for the prevention or treatment of metabolic disease. Dietary fatty acid intake requires consideration in future gastrointestinal studies.

Trial registration: Trial registration number: at http://www.clinicaltrial.gov: NCT01350284

Keywords: gastrointestinal, antioxidant capacity, obesity, type 2 diabetes, polyphenols, fatty acids, omega-3 fatty acids

Background
Free-living individuals are in the postprandial hyper-triglyceremic state for the majority of a 24-h period [1]. Dietary supplementation, such as traditional spices, that can limit lipemia and glycemia in the fed state, have important implications for prevention and management of metabolic diseases. Two decades ago, cinnamon (Cinnamomum zeylanicum) was proposed as a treatment for type 2 diabetes (T2D) when it was shown to display insulin-mimetic properties [2]. Cinnamon has been proposed to act on numerous mechanisms relating to glucose and insulin function including improved cellular uptake of glucose through stimulation of insulin receptor kinase activity, increased insulin receptor phosphorylation and glycogen synthesis activity and reducing inflammation through antioxidant effects [3-7]. Chronic supplementation of 1 to 6 g cinnamon has been shown to have similar effects with regards to lowering of
fasting glucose and lipid levels in diabetic patients [8].

Acute, a 5 g cinnamon bolus improved glycemic responses and insulin sensitivity when given 12 hours prior to, or with, an oral glucose tolerance test (OGTT) in healthy adults [9].

In addition to rate of glucose removal from circulation, plasma glucose concentration is also determined by the rate of glucose entering the circulation [10]. Gastric emptying (GE) is an important determinant of rate of glucose appearance and blood glucose homeostasis in healthy and diabetic populations [11,12]. Delayed GE could be part of the mechanism by which cinnamon improves glucose tolerance. When combined with a semi-solid, low-fat meal, 6 g cinnamon reduced postprandial glyceria and delayed GE in healthy subjects [13].

Vascular dysfunction has emerged as a critical step in the development and progression of CVD, specifically atherosclerosis [14]. Vascular dysfunction refers to impairments in nitric oxide (NO)-mediated endothelium-dependent dilation, which is inversely related to an increase in vessel stiffness [15]. These impairments may stem from decreased NO synthesis and/or release, in combination with exaggerated consumption by reactive oxygen species (ROS) [16]. It has been postulated that postprandial lipemia may promote vascular dysfunction via an oxidative stress pathway [17-19].

The actions of dietary fatty acids on the gastrointestinal (GI) tract are still poorly understood. Acute ingestion of dietary fat can potently delay GE and reduce appetite [20]; these effects are partly mediated by the secretion of GI peptides including glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) [21]. Dietary fatty acid intake may be one factor that affects inter- and intra-subject variability in GE rates.

We aimed to determine if acute supplementation of 3 g cinnamon would reduce postprandial glycemic and lipemic responses to a high-fat (HF) meal through a delay in GE or an alternative mechanism. We also evaluated the effect of cinnamon ingestion on oxidative stress, vascular function, appetite sensations and subsequent food intake in healthy subjects. Additionally, we examined the relationship between previous day fatty acid intake and GE of the HF test meal.

Methods

Subjects

Nine apparently healthy subjects (3 male, 6 female; age 26.2 ± 3 years; mass 66.5 ± 11.3 kg; BMI 22.4 ± 2.5 kg/m²; body fat 22.2 ± 6.5%) consented to participate in the study, which was approved by the Local Research Ethics Committee. Subjects had no history of GI-related complaints, CVD or diabetes and were not currently taking antioxidant or lipid-lowering medication. Subjects were non-smokers and were recreationally active (performing < 60 min of moderate to intense exercise per day, on every day of the week) determined using a 3 month exercise questionnaire. Fasting blood lipid, glucose and blood pressure (BP) levels were all within the normal limits. Subjects were required to rate at least 50% of the food items that would be presented in the buffet meal as 5 or higher in a food preference questionnaire for study inclusion. Two subjects were identified as restrained eaters (scoring > 12 on the eating restraint section (factor 1) of the Three-Factor Eating Questionnaire [22]).

Experimental design

Each subject completed two separate 1-day trials separated by 28 days: cinnamon and placebo (wheat flour) supplementation. Trials were conducted in a single-blind, placebo controlled randomized fashion and were identical apart from the content of the capsules. During the 3 d before the first trial, subjects recorded all of the food and drink that they consumed and repeated this diet before the second trial. Energy, macronutrient and fatty acid intake were calculated using CompEat Pro Nutritional Analysis software (Version 5.8; Nutrition Systems, Grantham, UK). Physical activity was also logged during this time period. Subjects were instructed to minimize their consumption of foods with naturally high 13C abundance on the day preceding each trial and were asked to refrain from alcohol consumption and vigorous exercise. Apart from these requirements, subjects ate and exercised ad libitum between the two testing periods.

Study day protocol

Subjects reported to the laboratory after a 12 h overnight fast. Following 15 min interval of supine rest, measurements of arterial stiffness (see measurements) and BP were obtained and a baseline blood sample was collected. Subjects returned to the seated position and after a 10 min interval, baseline breath samples were taken and visual analogue scale (VAS) questionnaires were completed (t = -15 and -10 min). Once baseline measurements were taken, subjects consumed the test meal within 15 min (see below). Upon completion of the meal (t = 0 min), sequential postprandial measurements of GE, appetite sensations, arterial stiffness, BP and plasma glucose and lipids responses were taken. Expired breath samples were collected at t = 0 min, every 5 min for the first half hour after meal consumption and thereafter in 15-min intervals from t = 30 until 360 min for the detection of 13CO2 (see measurements). VAS questionnaires were administered after consumption of breakfast (t = 0 min) and every 30 min until t = 360 min. Arterial stiffness, BP measurements and blood
samples were collected hourly from t = 60 to t = 270 min (t = 240 min for blood samples). At the end of postprandial assessments (t = 360 min), subjects were presented with an assortment of cold lunch-type buffet foods. The meal comprised of six slices of wholegrain bread (235 g), six slices of white bread (235 g), 115 g sliced ham, 115 g sliced chicken, 100 g grated cheddar cheese, 60 g lettuce, 125 g cherry tomatoes, 80 g sliced cucumber, 90 g sweet corn, 100 g coleslaw, 30 g butter, 30 g mayonnaise, 30 g relish, 415 g strawberry yoghurt, 6 biscuits (75 g), 120 g chocolate Swiss roll, 60 g crisps and 300 ml water. The total energy content of the buffet meal was 16504 kJ. The selection presented was in excess of anticipated consumption. Subjects were given 30 min (i.e. t = 360 - 390 min) to eat ad libitum until ‘comfortably full’. After ingestion of the buffet meal, subjects completed another VAS questionnaire and were then free to leave the laboratory. Additional VAS questionnaires were administered after consumption of the test meal and buffet meal (t = 0 and 390 min) to evaluate meal palatability as well as sensations of nausea and well-being. They recorded their subsequent food intake for the remainder of the test day.

Test meal
The test meal consisted of three pancakes (36 g flour, 44 g egg, 70 g whole milk, 30 g sunflower oil) served with 20 g chocolate spread and 300 ml of water. The test meal was enriched with 150 μl 13C octanoic acid (Cambridge Isotope Laboratories, Andover, MA, USA), which was solubilized in the egg yolk prior to cooking. After homogenizing the yolk, it was mixed with the other ingredients to ensure uniform distribution of the label throughout the pancake batch. The test meal provided 2646 kJ of energy and consisted of 42 g carbohydrate, 46 g fat and 13 g protein. Subjects were instructed to ingest 8 gelatin capsules (four directly before and after meal) totaling 3 g cinnamon powdered spice (Cinnamomum zeylanicum; Schwartz, UK) or a wheat flour placebo (Odlums, Ireland).

Measurements
Gastric emptying
Gastric emptying was determined using the 13C octanoic acid breath test [23,24]. Breath samples were collected into 10 ml tubes (Exetainers; Labco, Bucks, UK). The analysis of the 13CO2/12CO2 enrichment in breath samples was performed using an ABCA (Europa Scientific, Crewe, UK) isotope ratio mass spectrometer. GE parameters, gastric half emptying time (T1/2), lag phase (Tlag), latency time (Tim), and ascension time (Tasc) were calculated using previously described methods [23,25]. Cumulative excretion of 13CO2 (as a percent of ingested dose) was also calculated.

Arterial stiffness
Arterial stiffness was measured using the PulseTrace PCA 2 device (Micro Medical, Kent, UK). The device measures the digital volume pulse (DVP) through the use of a photoplethysmographic transducer placed on the index finger of the right hand, transmitting infra-red light at 940 nm. The device permits the calculation of the stiffness index (SI; m/s) and the reflection index (RI; %) [26,27]. Heart rate (HR) in beats/min (bpm) was also measured by transmission of the DVP.

Blood pressure
Systemic arterial blood pressure (BP) was measured at the brachial artery using an Omron M5-I fully automatic BP monitor (Surrey, UK). Measurements were taken in triplicate directly after arterial stiffness measurements in the supine position and an average of these readings was recorded.

Blood analysis
Blood samples were collected into K-EDTA and NaF tubes and placed on ice. Serum separating clot activator tubes were allowed to clot at room temperature. All samples were separated within 30 min of collection and stored at -70°C until analysis. Plasma glucose was determined by an immobilized enzyme membrane method in conjunction with a Clark electrode on a YSI 2300 analyzer (Yellow Springs, USA). Total cholesterol, TAGs and HDL were measured by enzyme assay kits, using an automated analyzer (Aeroset™, Abbott Labs, USA [28]). LDL cholesterol was calculated using the Friedewald equation [29]. All samples for each subject were analyzed in a single analyzer run. CVs were < 7.7% for glucose and < 10.0% for all blood lipids.

Serum lipid hydroperoxides
Serum lipid hydroperoxides (LOOHs) (as measures of lipid peroxidation) were incubated with FOX-1 assay and quantified using a Shimadzu UV-VIS 1240 Spectrophotometer, (Mason Technology, Belfast, Northern Ireland [28]). CV was < 16% for LOOH.

Appetite sensations and subsequent energy intake
Sensations of hunger, desire to eat, fullness, thirst, tiredness and coldness were assessed using a VAS questionnaire [30]. Although it was important to examine general well being, the latter sensations were primarily included so that subjects were unaware that our primary outcome measures were sensations of appetite. Each VAS assessed a sensation on a 150 mm horizontal line anchored at the beginning and end by opposing statements.

The quantity of food consumed in the buffet meal was recorded to the nearest 0.1 g and the total energy (kJ) and macronutrient intakes (% of energy) were subsequently calculated. Time taken to complete the buffet meal completion (in min) was also recorded.

Statistics
Data were checked for normal distribution before statistical analysis was performed. Appetite sensation data
was transformed by natural log. Fasting measurements of arterial stiffness, blood pressure and plasma lipid and glucose concentrations were calculated as the mean of the values collected at baseline (−t = −30 to −20 min). Baseline GE and appetite sensation scores were calculated as the mean of values collected at t = −15 and −10 min. Blood biomarkers, appetite sensations and arterial stiffness data were analyzed using a two-way (time × supplement) repeated-measures ANOVA. Incremental area under the glycemic curve (IAUC) was calculated using the trapezoidal rule by subtracting baseline values from measured plasma glucose concentrations. Relationships between variables were compared using Pearson correlations. Paired-sample t tests were used to compare GE parameters, habitual diet, physical activity intensity and duration as well as food intake at the buffet meal (quantity, energy consumed and macronutrient distribution). Statistical significance was established at the p < 0.05 level and the mean values ± SD are reported. All statistical analyses were carried out using SPSS-version 16.0 (SPSS Inc., Chicago, IL, USA). Power calculation was conducted for gastric emptying T_{50%} as the primary endpoint evaluation. A sample size of 9 subjects was necessary to detect a 15.8% change in GE rate [13] in a two-sided paired Students’ t-test with alpha set at 5% and a power of 80%. Trial registration number: at http://www.clinicaltrial.gov: NCT013500284.

Results

Test days were well tolerated by all subjects. Subjects successfully repeated their food and physical activity diaries as indicated by no significant differences in energy or macronutrient intake (p > 0.05) and in total and exercise intensities respectively (p > 0.05) for 3 days prior to each treatment. Usual dietary intake was consistent with guidelines for healthy living (51% of energy from carbohydrate, 31% fat, and 18% protein).

Gastric Emptying
No significant effect of cinnamon supplementation was observed on gastric emptying parameters (Table 1).

Cardiovascular Measures

Arterial stiffness

There were no mean differences between or within the groups for SI (time × supplement interaction, p > 0.05) (Table 2). However, a main effect for time was observed whereby SI decreased postprandially in both supplements (pooled placebo and drug data, p < 0.05). There were no reported differences either between or within supplements for RI over time following the ingestion of the test meal (time × supplement interaction, p > 0.05) (Table 2).

Table 1 Mean values for gastric emptying parameters and food intake from the buffet meal after the ingestion of high-fat meal supplemented with 3 g wheat flour (placebo) or cinnamon

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Cinnamon</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE emptying</td>
<td>T_{50%} (min)</td>
<td>237 ± 32</td>
</tr>
<tr>
<td></td>
<td>T_{50%} (min)</td>
<td>136 ± 12</td>
</tr>
<tr>
<td></td>
<td>T_{50%} (min)</td>
<td>40 ± 6</td>
</tr>
<tr>
<td></td>
<td>T_{50%} (min)</td>
<td>197 ± 35</td>
</tr>
<tr>
<td></td>
<td>Cumulative excursion of^{15}CO_{2} (%)</td>
<td>55.1 ± 10.0</td>
</tr>
<tr>
<td>Food intake</td>
<td>Energy intake (kcal)</td>
<td>3056 ± 1130</td>
</tr>
<tr>
<td></td>
<td>Quantity consumed (g)</td>
<td>420 ± 20</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate (%)</td>
<td>40 ± 13</td>
</tr>
<tr>
<td></td>
<td>Fat (%)</td>
<td>40 ± 8</td>
</tr>
<tr>
<td></td>
<td>Protein (%)</td>
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<td></td>
<td>Time (min)</td>
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<tr>
<td>T_{50%}</td>
<td>Gastric half emptying time</td>
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</tr>
<tr>
<td>t_{50%}</td>
<td>Ascension time</td>
<td>31 ± 15</td>
</tr>
<tr>
<td>t_{50%}</td>
<td>Latency time</td>
<td>32 ± 15</td>
</tr>
</tbody>
</table>

Blood pressure and heart rate

There were no differences either between or within the groups for both systolic and diastolic BP over time following the ingestion of the test meal (time × supplement interaction, p > 0.05). There were no mean differences between or within the groups for HR (time × supplement interaction, p > 0.05). However, a main effect for time was observed whereby HR decreased over time across both trials (60 ± 10 bpm at baseline vs. 52 ± 10 bpm at 30 min).

Table 2 Mean stiffness and reflection index values after the ingestion of high-fat meal supplemented with 3 g wheat flour (placebo) or cinnamon

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Cinnamon</th>
</tr>
</thead>
<tbody>
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<td>Stiffness Index (mV/Vec)</td>
<td>Baseline</td>
<td>5.58 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>5.53 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>5.42 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>180 min</td>
<td>5.39 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>240 min</td>
<td>5.34 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>300 min*</td>
<td>5.47 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>Reflection Index (%)</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>61.26 ± 12.73</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>61.64 ± 8.79</td>
</tr>
<tr>
<td></td>
<td>180 min</td>
<td>60.74 ± 12.63</td>
</tr>
<tr>
<td></td>
<td>240 min</td>
<td>71.57 ± 15.84</td>
</tr>
<tr>
<td></td>
<td>300 min</td>
<td>62.78 ± 12.59</td>
</tr>
</tbody>
</table>

Data are means ± SD, n = 9. *Main effect for time for Stiffness Index, pooled placebo and drug data, p < 0.05.
9 bpm at 300 min post meal in placebo trial; 61 ± 12 bpm at baseline vs. 51 ± 9 bpm at 300 min post meal in cinnamon trial: pooled placebo and drug data, p < 0.05).

**Blood biomarkers**

**Plasma glucose**

Baseline plasma glucose concentrations tended to be higher after cinnamon supplementation compared to placebo (p > 0.05; n = 8 for all blood derived measurements). Plasma glucose concentrations changed across time in response to both supplementations (p < 0.0001) (Figure 1). There was no significant interaction between time and supplement (p > 0.05). Three hours after meal ingestion all plasma glucose concentrations were similar to baseline concentrations, regardless of supplement. IAUC following placebo (-22.2 ± 87.6 mmol/L/min) was similar to that following cinnamon ingestion (-14.2 ± 29.1 mmol/L/min, p = 0.819).

**Plasma lipids**

Baseline plasma TAG, total cholesterol, HDL and LDL concentrations did not differ significantly between trials (Table 3). There were no changes in TAG between trials (time × treatment interaction, p > 0.05) but there was a main effect for time (pooled placebo and cinnamon data, p < 0.05). There were no changes in plasma total cholesterol, HDL, or LDL levels either within or between the trials (time × supplement interaction; p > 0.05).

**Serum lipid hydroperoxides**

There was a main effect for time for LOOHs with levels increasing over time throughout the course of the trials (pooled placebo and cinnamon data, p < 0.05) but there were no changes in mean LOOHs between or within the groups (time × supplement interaction, p > 0.05) (Figure 2).

**Appetite sensations and subsequent energy intake**

The variables thirst, tiredness and coldness did not vary significantly with supplementation or over time (data not shown). Baseline appetite sensation VAS scores did not differ significantly between conditions. Changes to appetite sensation scores were evident over time after ingestion of test meals (p < 0.05). No significant effect of treatment was observed on sensations of hunger (Figure 3), desire to eat and fullness (data not shown). No

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Cinnamon</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG (mmol/L)</td>
<td>0.84 ± 0.27</td>
<td>0.80 ± 0.32</td>
</tr>
<tr>
<td>60 min</td>
<td>0.93 ± 0.36</td>
<td>0.88 ± 0.32</td>
</tr>
<tr>
<td>120 min</td>
<td>1.01 ± 0.36</td>
<td>0.95 ± 0.37</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>3.80 ± 0.59</td>
<td>3.93 ± 0.43</td>
</tr>
<tr>
<td>60 min</td>
<td>3.79 ± 0.55</td>
<td>3.77 ± 0.66</td>
</tr>
<tr>
<td>120 min</td>
<td>3.85 ± 0.52</td>
<td>3.78 ± 0.69</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.45 ± 0.31</td>
<td>1.49 ± 0.41</td>
</tr>
<tr>
<td>60 min</td>
<td>1.39 ± 0.27</td>
<td>1.46 ± 0.38</td>
</tr>
<tr>
<td>120 min</td>
<td>1.45 ± 0.32</td>
<td>1.45 ± 0.36</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>1.97 ± 0.57</td>
<td>2.07 ± 0.30</td>
</tr>
<tr>
<td>60 min</td>
<td>1.98 ± 0.54</td>
<td>1.91 ± 0.48</td>
</tr>
<tr>
<td>120 min</td>
<td>1.92 ± 0.46</td>
<td>1.90 ± 0.41</td>
</tr>
</tbody>
</table>

Data are means ± SD, n = 8. No significant differences were found between treatments, p > 0.05.

![Figure 1](image1.png)  
**Figure 1** Plasma glucose concentrations in healthy subjects after the ingestion of a high-fat test meal supplemented with 3 g cinnamon or placebo. Values are means, with standard deviations represented by vertical bars (n = 8).

![Figure 2](image2.png)  
**Figure 2** Lipid hydroperoxides (LOOHs) concentrations in healthy subjects before and after the ingestion of a high-fat test meal supplemented with 3 g cinnamon or placebo. Values are means, with SD represented by vertical bars (n = 8).
differences between sensations of palatability, pleasantness, nausea and stomach pain recorded when treatments were compared after the test breakfast (t = 0 min) and buffet-style lunch meal (t = 290 min). No significant effect of treatment was observed for quantity of food consumed, energy intake, percentage of energy provided by carbohydrate, fat or protein and time taken to consume the buffet meal (Table 1).

**Relationship between variables**

Significant positive correlations were observed between TAG levels and LOOHs (r = 0.52, p < 0.0001) and SI and LOOH (r = 0.24, p < 0.05). GE T_{half} was inversely related to energy intake in the buffet meal after cinnamon ingestion (r = -0.67, p < 0.05) but not in the placebo trial (r = -0.62, p < 0.05).

**Relationship between gastric emptying and 3-day background fatty acid intake**

No significant differences were observed between 1-day background fatty acid intake before cinnamon and placebo trials. 1-day intake of palmitoleic acid (C16:1, r = -0.78, p < 0.05), eicosapentaenoic acid (C20:1, r = -0.84, p < 0.01) and total n-3 intake respectively (r = -0.72, p < 0.05) were inversely related to GE T_{half} of the placebo-supplemented HF meal (Table 4). 1-day intake of C16:1, C20:1 and total n-3 fatty acids accounted for 61, 71 and 51% of the variance in GE T_{half} respectively. No significant relationships between GE T_{half} after cinnamon ingestion and 1-day of palmitoleic acid, eicosapentaenoic acid or total n-3 intake were observed (p > 0.05).

**Discussion**

The current study tested the hypothesis that supplementing a single HF breakfast with 3 g of cinnamon would delay GE of a high-fat solid meal utilizing the $^{13}$C octanoic acid breath test, and consequently reduce postprandial blood glucose and lipid concentrations.

**Gastric Emptying, Metabolic Variables and Appetite**

We were unable to induce significant changes in GE using 3 g of cinnamon. The test meal (65% of energy from fat) was mainly from sunflower oil, which contains approximately 70% linoleic acid (C18:2n-6, a PUFA [31]). Long-chain fatty acids have a potent inhibitory effect on GE rate [20,32] and have also been shown to increase CCK and GLP-1 concentrations [21]. We propose that cinnamon does not delay GE over and above the effects of the fat content of the meal. Furthermore, we found similar postprandial glycemic and lipemic responses under both conditions. However, it should be noted that we were unable to measure a hyperglycemic or hyperlipidemic state. Studies which employed the largest doses of cinnamon relative to carbohydrate in the test meal (carbohydrate/cinnamon ratio of 15 or lower [9,13]) appear to have had the most potent effects on reducing postprandial glycemia [33]. In spite of the current high ratio of 14, we did not achieve a significant blood glucose-lowering effect. This is possibly due to glucose absorption from the small intestine being affected by the fat content of a meal [34].

Recent data indicates that the addition of 3 g cinnamon to a low-fat rice pudding test meal had no significant effect on GE rate or postprandial glycemia in healthy individuals [35]. However, cinnamon did significantly lower serum insulin levels and increase GLP-1 concentrations, a GI peptide which has been shown to increase glucose-dependent secretion of insulin, delay GE and reduce glucose absorption and postprandial glycemia [36,37]. When added to the same test meal, 6 g cinnamon significantly delayed GE and reduced postprandial glycemia but the decrease in blood glucose concentration was more apparent than the delay in GE rate suggesting that GE cannot be the sole mechanism.
explaining lower blood glucose responses following cinnamon ingestion [13]. Agreeing with the findings of others [13,33,34], we found that cinnamon did not influence appetite sensations or subsequent food intake, probably as a result of similar GE rates between conditions [38]. Together with data presented in the current study, cinnamon is unlikely to be relevant in affecting the postprandial response to HF meals.

Diet and fatty acid intake

Assessment of previous day dietary intake indicated that a higher intake of C16:1, C20:1 and total n-3 was associated with a shorter GE $T_{\text{half}}$ of the HF meal supplemented with the wheat flour placebo. To our knowledge, this is the first observation of specific dietary fatty acids from preceding diet affecting GI transit in humans. A single meal, supplemented with n-3 PUFAs, was less capable of triggering GLP-1 and CCK compared to other fats, resulting in a more rapid GE of a HF breakfast [39] while others showed that n-3 PUFAs fish oil reduced CCK release and gallbladder contraction without affecting GE [40]. Both GLP-1 and CCK are putative mediators of the ileal brake, a feedback mechanism responsible for delaying transit and facilitating digestion, in response to lipids in the distal GI tract [41]. Our current findings extend these observations to illustrate that even short-term intake of n-3 fatty acids is associated with faster GE rates, in a population who were not eating a HF diet. This means that mechanisms apart from acute release of GLP-1 and CCK, must mediate the effects of specific fatty acids on GI transit. Recently, a 3-day HF yoghurt supplementation, rich in C18:2n-6 accelerated the GE rate of a test meal rich in the same fatty acid [42]. It is interesting to note that background intake of C18:2n-6, which was high in the test meal, did not show a strong association with GE of the meal. Our observations suggest that GE $T_{\text{half}}$ of a HF meal is not just specifically affected by a background intake of that specific fatty acid and that the process of adaptation to a HF diet may involve mechanisms other than desensitization to a specific fatty acid. It is likely that different adaptations are continuously taking place in the gut, in response to the balance of fatty acids in the diet. It is tempting to speculate about the potential mechanisms for fat sensing and adaptation following the recently sequenced GPR120 protein, expressed on intestinal cells, and demonstrated to be differentially sensitive to different fatty acids [43].

Vascular Function and antioxidant capacity

One of the principle findings of this study was that no changes were observed in relation to the measures of vascular function. Interestingly, there was a main effect for time indicating a decrease in SI which at face value appears paradoxical given the transient impairment in vessel function following the ingestion of a HF meal [44,45]. This apparent contradiction might be explained by the fact the test meal we used may not have been of sufficient energy and, in particular, fat content to evoke a change in vessel function. In most of the related literature the postprandial TAG concentration associated with vascular dysfunction (~2.0 mmol/l; [17,18,44]) is double than that presently observed. Moreover, the HF meal in the current study contained 297 - 1742 kJ less energy (and 14 - 34 g less fat) than other similarly designed investigations [17,45-47]. Such discrepancies in meal composition further highlight the need for a standardized, physiologically relevant HF meal [48] to be used in future corresponding studies, similar to the OGGT.

Due to its polyphenolic nature, cinnamon is thought to exhibit antioxidant properties [3,49] which may be anti-atherogenic. It is proposed that the impairments in blood vessel function following the ingestion of HF loads are perpetrated via an oxidative stress mechanism that can increase the unwanted consumption of NO and favor the formation of further ROS, such as peroxynitrite (ONOO-) [17,19,28,44]. In the present study cinnamon ingestion had no apparent effect on indices of oxidative stress as a main effect for time was observed for LOOH.

It has been documented that even high-normal fasting glucose levels can aggravate arterial stiffness [50] but when cinnamon was added to rats fed a high-fat high-fructose diet hepatic glycogen, hepatic insulin receptors and GLUT transporter in muscle tissue all increased [51]. Despite no reported change in arterial stiffness using our physiologically relevant high-fat meal, the possibility therefore exists that cinnamon could modulate stiffness by affecting (hepatic) glycemic control and thus this relationship merits further scrutiny.

Emerging research postulates the existence of a diurnal variation in endothelial function [52]. Fluctuations in the competitive balance between intrinsic local vasodilator function and sympathetic nervous system (SNS) α-adrenoreceptor-mediated vasoconstriction have recently been proposed as one potential mechanism to explain such findings [53]. Given the conceptual relationship between endothelial function and arterial stiffness, and the observed main effect for a decrease in HR (as an indirect measure of SNS activity), it is tempting to speculate that this explanation may in some way account for the SI data in the current investigation. Conversely, this was tempered by the fact that no changes in RI were recorded.

This study may have been underpowered and therefore a small effect below the detection threshold of the study cannot be ruled out. Three grams of cinnamon
was used as because it was shown to have a similar chronic effect on fasting serum glucose and lipid profiles as 6 g [8]. However, recent evidence suggests a dose-dependent relationship for cinnamon consumed and the delay in GE [13,35].

Conclusions

We found no evidence for delayed GE rate or reduced indices of oxidative stress in response to a HF meal supplemented with 3 g cinnamon compared to placebo. Cinnamon did not change postprandial glycemic and lipemic responses, arterial stiffness or appetite. Given the association between 1-day fatty acid intake and GE T-body, we suggest that controlling for background fatty acid composition requires consideration in future gastrointestinal studies.

List of abbreviations

GE: gastric emptying; T2D: type 2 diabetes; CVD: cardiovascular disease; HF: high-fat; GGT: oral glucose tolerance test; NO: nitric oxide; OX: reactive oxygen species; GI: gastrointestinal; GIP: 1-glucagon-like peptide-1; CCR: cholecystokinin; Kir: blood pressure; VAS: visual analog scale; TVG: gastric halffemptying time; T90: lag phase; TVG: latency time; TVA: ascension time; DVP: digital voleu pulse; IS: stiffness index; RI: refection index; LOOH: lipid hydroperoxides; SNS: sympathecic nervous system.

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Authors’ contributions

The author’s responsibilities were as follows - OM and AS contributed to all aspects of the study. PA, MA, JG, TW and EJ contributed to acquisition of data, or analysis and interpretation of data. All authors read, reviewed and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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