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Magnetic resonance imaging studies of gastrointestinal, metabolic and appetitive responses to isoenergetic breakfasts of different plant grain origin

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

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Publications and awards arising during the PhD

Papers and reviews:

- **Alyami J**, Spiller RC, Marciani L. Magnetic resonance imaging to evaluate gastrointestinal function. Neurogastroenterology and Motility 2015; 27:1687-1692.
- **Alyami J**, Ladd N, Pritchard SE, et al. Glycaemic, gastrointestinal and appetite responses to breakfast porridges from ancient cereal grains: A MRI pilot study in healthy humans. Food Research International 2018: in press available online https://doi.org/10.1016/j.foodres.2017.11.071.
- **Alyami J**, Whitehouse E, et al. Assessment of motion of gastric contents in healthy subjects using MR tagged imaging (the manuscript is nearly ready for circulation)
- **Alyami J**, Whitehouse E, et al. Glycemic, Gastrointestinal , Hormonal and Appetitive Responses to Pearl Millet or Oats Porridge Breakfasts: a Randomized, Crossover Trial in Healthy Humans (the manuscript is under peer review at European Journal of Clinical Nutrition.

Conference abstracts:

- Midlands Academy of Medical Sciences in March 2017.
- 5th International Conference on Food Digestion that was held in Rennes, France from 4th to 6th April 2017
- Obesity week, Washington DC, USA (Oct, 2017).
- British chapter of ISMRM, London (March 2016).

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- The Nutrition Society Summer Conference 2018: Getting energy balance right held at the University of Leeds on 10 – 12 July 2018.

Awards:

- 2nd best PhD presentation prize at the 5th International Conference on Food Digestion that was held in Rennes, France from 4th to 6th April 2017.
- 2nd best PhD oral presentation at Sue Watson event, School of medicine, University of Nottingham, October 2017.
- Received 6 excellence awards from Saudi Arabian Cultural Bureau in London (one of the top 15 Saudi students in UK, September 2017).
- A finalist at 3MT® Competition, University of Nottingham (May 2018) and had been invited to present my 3MT® at a Research Celebration Evening taking place at the Djanogly Gallery, Lakeside Arts on Thursday 28th June, University of Nottingham
- The grant writing program award, school of Medicine, university of Nottingham (July 2018).

List of abbreviations

AACE	American Association of Clinical Endocrinologists
AMORE	Assessment of Millet, Oat and Rye Porridge Breakfasts Glucose and Gastric Emptying
ANOVA	Analysis of variance
AOAC	Association of Analytical Communities
AUC	Area under the curve
BMI	Body mass index
BRC	Biomedical Research Centre
bTFE	Balanced turbo field echo sequence
CCK	Cholecystokinin
CoV	Coefficient of variation
DF	Dietary fibre
EGIR	European Group for the Study of Insulin Resistance
EPI	Echo planar imaging
ELISA	Enzyme-linked immunosorbent assay
FA	Flip angle
FDA	Food and Drug Administration
FMP	Finger millet porridge
FOV	Field of view
GE	Gastric emptying
GI	Glycaemic index
GIP	Glucose-dependant insulinotropic polypeptide
GR	Glycaemic response
GIT	Gastrointestinal tract

GLP-1	Glucagon-like peptide 1
HDL	High Density Lipoprotein
HV	Healthy Volunteer
iAUC	Incremental area under the curve
IDF	International Diabetes Federation
IGT	impaired glucose tolerance
MMC	Migratory motor complex
MOM	Millet and Oats MRI
MR	Magnetic Resonance
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic Resonance Imaging
NCEP ATP III	National Cholesterol Education Programme Adult Treatment Panel III
NDDC	Nottingham Digestive Disease Centre
NGT	Normal glucose tolerance
NMR	Nuclear Magnetic Resonance
OGTT	Oral glucose tolerance test
PFC	Prospective food consumption
PhD	Doctor of Philosophy
PMP	Pearl millet porridge
PYY	Peptide YY
QMC	Queen’s Medical Centre
RARE	Rapid acquisition with relaxation enhancement
RF	Radiofrequency
ROI	Region of interest

RP	Rye porridge
SACN	Scientific Advisory Committee on Nutrition
SBWC	Small bowel water content
SCFA	Short chain fatty acids
SEM	Standard error of the mean
SOP	Scottish oats porridge
SPAMM	Spatial modulation of the magnetization
SPMIC	Sir Peter Mansfield Imaging Centre
TE	Echo time
TGs	Triglycerides
TR	Repetition time
T2DM	Type 2 diabetes mellitus
UoN	University of Nottingham
VAS	Visual analogue scale
WHO	World Health Organisation

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Abstract

Introduction: Cereal grain based porridges are commonly consumed throughout the world. Cereal types that are popular in the Western world, such as oats and rye, have been characterised with respect to health related variables such as glycaemic response and suppression of hunger. However, other '*ancient* grains' consumed in the Asia and in Africa, such as millets, have been studied minimally although they are believed to offer health benefits. Consuming these grains may be advantageous in the control of obesity and type 2 diabetes, the incidence of which are increasing globally. They are also a sustainable food, due to their tolerance of extreme weather and harsh growing conditions. It is thus timely to consider whether they should be exploited to a greater extent as a human food, both from a health and environmental perspective. Concurrently the development of novel magnetic resonance imaging (MRI) techniques enable a deeper understanding to be gained of the impact on health of the interaction between the GI tract and specific food. The aim of this project was thus to investigate the glycaemic, gastrointestinal, hormonal and appetitive response ratings to breakfast porridge made from different grains in healthy subjects (HVs) whilst exploiting and developing MRI.

Methods: Firstly**,** a cross-over pilot study (AMORE) was undertaken in sixteen healthy participants [ten female and six male, 20.9 (SD 0.9) years old, BMI 22.1 (SD 2.9) kg/m^2] to produce data on postprandial glucose levels (using finger prick), gastric emptying, small bowel water contents (using MRI) and satiety (using VAS scales and food diaries) following

consumption of isoenergetic breakfast porridges made from oats, rye (both in flakes form), and finger (ragi) millet and pearl (bajri) millet grains (in flour form).

Secondly, a cross-over study (MOM) was undertaken in twenty six healthy subjects (17 females and 9 males, aged 28.5 (SD 9.6) years old, and with a BMI of 23.4 (SD 3.2) kg/m^2) who consumed two iso-energetic/iso-volumetric PMP or SOP breakfast meals, provided with a drink of water. At baseline and for two hours postprandially, MRI measurements were taken of gastric volume. Blood samples were collected to measure glucose, insulin, GLP-1, GIP and PYY. An *ad libitum* test meal was offered at lunch. Self-reported appetite ratings were collected over the visit and food intake records were kept for the remainder of the day.

Lastly, a MRI developmental study (Tagging study) was undertaken to investigate the feasibility of using a continuously tagged magnetic resonance imaging sequence to monitor and assess gastric handling (such as flow and mixing) of breakfast porridge meals in HVs.

Key results: Seven participants completed the entire protocol in the pilot study and were included in the final analysis. A subgroup analysis with the n =10 paired comparison between the same individuals that completed the oats, rye and pearl millet was also considered. The study showed that the gastric volume AUC was higher for pearl millet than oats and rye (n =10, $P < 0.001$). The incremental area under the curve (iAUC) for blood glucose was not significantly different between the meals although this showed a trend to be lower for pearl millet. Hunger VAS

was lower for pearl millet compared to oats and rye (n= 10, *P* = 0.01). There was a significant correlation between total gastric volume AUC and average appetite AUC (r =−0.47, P < 0.010).

The MOM study showed iAUC2h blood glucose was not significantly different between the porridges (*P* > 0.05). The iAUC2h gastric volume was larger for PMP compared with SOP (*P* = 0.045). The iAUC2h GIP concentration was significantly lower for PMP compared with SOP (*P* = 0.001). The other hormones and appetite responses were similar between meals.

The tagging study showed that the shorter delay time resulted in higher coefficient of variation (CoV) across the proximal and distal part of the stomach and whole stomach regions. Tagged cine-MRI can be used to assess dynamic intragastric handling of breakfast meals.

Conclusions: Pearl millet porridge elicited glycaemic and appetite responses comparable to oats, a known health-promoting grain. GIP is an incretin hormone that has been linked to triacylglycerol absorption in adipose tissue, therefore the lower GIP response for PMP, may be an added health benefit. PMP could represent a sustainable, alternative breakfast intervention.

The tagging method developed is relatively easy to implement and could provide an additional parameter to add to the scan card for future studies.

1. Introduction

1.1 Obesity

Obesity is defined as an excessive or abnormal accumulation of body fat, also known as adipose tissue, that may impair health[1]. It is considered to be a disease of positive energy balance (greater energy consumption than expenditure) which over time results in fat accumulation as an energy store[1, 2].

Obesity has been found to have a devastating impact on health-related quality of life[3]. Obesity and overweight are associated with decreased overall life expectancy by increases in early mortality[4]. Obese individuals are at a greater risk of developing type 2 diabetes (T2DM)[5], stroke[6] and, coronary artery disease[7, 8] as a consequence of associated metabolic conditions including insulin resistance, hyperlipidaemia, hypertension and non- alcoholic fatty liver disease. In addition obesity is associated with gallbladder disease[9, 10], other liver disease[11] and several cancers[12-14].

The Body Mass Index (BMI) (originally known as the Quetelet index) is the most widely used method to categorise body fat. It is calculated as weight in kilograms, divided by height squared in meters and expressed with the unit of kg/m^2. The desirable range for BMI values for adults is between 18 and 24 kg/m^2. The World Health Organisation (WHO) indicates that a BMI of 25 to 30 kg/m^2 is considered overweight and greater than 30 kg/m^2 is considered obese[15, 16].

BMI values can provide useful information about increasing body fat allowing professionals to conduct comparisons of body frame status

between people. These comparisons can identify the individual and groups at risk of increased morbidity and premature mortality. However, BMI is not a perfect measure to diagnose obesity because it does not differentiate between excess body fat and excess muscle weight. Athletic men, for example, with an increased proportion of lean body mass, may have a BMI above 30 kg/m^2 [17] but not have detrimental stores of body fat. Combining the use of the waist circumference method which gives an indication of abdominal fat distribution, associated with liver fat deposition[11] and BMI can give a more clinically meaningful indication of risk to health associated with body fat and is also used for defining obesity[15, 16].

1.1.1 Causes of obesity

Obesity is a disease caused by a positive energy balance[18]. For instance, if a person consumes more energy than they expend by 5% per day, this would result approximately in 0.5 kg weight gain per year and over many years - this weight gain leads to obesity[19]. However, the aetiology of obesity is more complex; there are genetic and environmental factors that contribute to the development of obesity.

Genetic Factors

Numerous studies have shown that biological and genetic factors have strongly influenced individuals' susceptibility to obesity[20, 21]. Only few genes associated with obesity have been identified in people. Melanocortin-4 receptor gene is considered to be the most common gene

associated with obesity. This gene acts to suppress nutrient intake and the frequent deficiencies in this gene can lead to sever obesity[20].

Although single genes associated with obesity have been recognized, the majority of obesity present today is considered to be due to the interaction of polygenic influences with environmental factors[21, 22].

Environmental Factors

The dietary changes over recent decades are thought to play a role in the concurrent rise in obesity. Firstly, the increase in meal portion size is considered one of the major changes in the diet. Restaurants and food manufactures have in the recent past presented large meals as a sign of a good deal to customers although they may have prioritised quantity over quality[23]. Although the Food and Drug Administration (FDA) have developed recommended amounts for different items, most market portions range from 2 to 8 times the suggested standard portion[23]. Providing all other factors remain constant the increase in food consumption leads to a direct increase in energy intake, in the absence of an increase in energy expenditure this subsequently results in weight gain[21, 24].

The intake of sugar-sweetened beverages and high fructose corn syrup, which have increased, show a strong positive correlation with obesity. In a longitudinal cohort study of women's weight over a four year period the number of sugary drinks consumed was self-recorded. The weight gain of the participants who had reported consumed a greater number of sugary drinks was the highest, whereas the group who

reported drinking fewer numbers of those drinks had the least weight gain[25].

Another environmental factor is changes in physical activity. Nowadays, more individuals use cars to commute to and from work, have office jobs and fewer have labour-intensive jobs[21]. Furthermore, the increase in the time spent watching television plays an important role in the increase of childhood obesity. It is suggested that the time spent in front of the television was positively associated with BMI compared with that performing less sedentary activities. The Children who spend a longer time in front of television were both sedentary and targeted advertising via commercials may encourage children to continuously snack even if they are not hungry. Again, such behaviour will lead to an increase in energy intake without the necessary energy expenditure (energy imbalance) and results in obesity[21, 26]. The combined effect of being sedentary and overweight may also amplify the health risks[21, 26].

The rise in obesity and non-chronic no communicable diseases in low and middle income countries such as India and China has been also linked to a large shift from consumption of coarse grains such as millets to consumption of rice and wheat among the population[27].

1.1.2 Prevalence and health consequence of obesity

The global prevalence of obesity is increasing. The prevalence rates are continuing to rise rapidly particularly amongst younger people. Obesity rates have increased more than threefold between 1975 and 2016. The WHO estimated that there are 1.9 billion adults aged 18 years and older

who are overweight worldwide, 300 million of whom are obese[28]. The increase in overweight and obesity amongst children and adolescents has increased dramatically from just 4% in 1975 to just over 18% in 2016. Forty one million children under 5 years of age and over 340 million children and adolescents aged 5-19 were overweight or obese in 2016[28]. This is likely to result in even higher rates of adult obesity, as these already overweight and obese cohorts age, with increasing health complications, because of longer exposure to obesity. In other words, ageing people are going to have worse health as they age whilst being obese.

Obesity imposes a substantial economic burden on the individual, and on families and nations in both developed and less developed countries[29]. According to the WHO, obesity accounts for approximately 2-7% of total health care costs in a number of industrialized nations[2]. In a systematic review of the direct costs of obesity it has been reported that the medical cost of obese individuals were approximately 30% greater than that of normal weight individuals[14].

Obesity is considered as a major public health problem and it has become an epidemic in many developing and developed nations. In every region in the world, there are more obese people than those who are underweight except in parts of sub-Saharan Africa and Asia[28]. Obesity and overweight increase the risk of premature death and disease[28].

Metabolic syndrome (MetS)

The cluster of metabolic conditions is sometimes known as metabolic syndrome. The Metabolic syndrome is also termed 'insulin resistance'. Insulin resistance occurs when the body cells become resistant to the hormone insulin. Insulin resistance is a growing major public health problem and clinical challenge all over the world. Insulin Resistance causes a fivefold risk to develop diabetes mellitus (DM) and the twofold of increase of risk cardiovascular disease (CVD) over the next 5-10 years[15, 16]. In addition to this, patients with metabolic syndrome have a two to four fold increased risk of stroke, myocardial infarction and risk of dying compared to those without the metabolic syndrome[15, 16]. DM and CVD are considered in more detail below, with particular reference to obesity.

DM is a lifelong condition in which the blood sugar level in the body is higher than normal. There are two types of diabetes. Type 1 diabetes which is called insulin dependent, whereby the pancreas does not produce insulin and is an autoimmune disease. There is then also Type 2 diabetes, referred to by some as non-insulin-dependent diabetes whereby the pancreas produces a small amount of insulin, which is insufficient for the body's needs and insulin resistance is seen[15, 16]. Gestational diabetes can also occurs during the 2nd half of pregnancy. Obesity has an association with type 2 diabetes and is considered to be a major risk factor. Nearly 90% of individuals diagnosed with T2DM are overweight or obese. Those who are overweight or obese are particularly at higher risk of development type 2 diabetes[30].

Cardiovascular disease (CVD) is the other disease to be considered under the metabolic conditions. This term describes heart or blood vessels diseases. Developing type 2 diabetes is a risk factor of CVD. It has been shown that 58% of diabetes and 21% of ischemic heart disease are attributable to a BMI above 21[30].

Several definitions of MetS have been presented. The most commonly used criteria for definition at the present time are those from the World Health Organization (WHO), the European Group for the study of Insulin Resistance (EGIR), the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III), American Association of Clinical Endocrinologists (AACE), and the International Diabetes Federation (IDF) (table 1)[16].

Table 1: Diagnostic criteria proposed for the clinical diagnosis of the MetS.

Clinical measures	WHO (1998)	EGIR (1999)	ATPIII (2001)	AACE(2003)	IDF(2005)
Insulin resistance	IGT, IFG, T2DM, or lowered insulin Sensitivity a plus any 2 of the following	Plasma insulin >75th percentile plus any 2 of the following	None, but any 3 of the following 5 features	IGT or IFG plus any of the following based on the clinical judgment	None
Body weight	Men: waist-to-hip ratio >0.90; women: waist-to-hip ratio >0.85 and/or BMI > 30 kg/m2	WC ≥94 cm in men or ≥80 cm in women	WC ≥102cm in men or ≥88 cm in women	BMI ≥ 25 kg/m2	IncreasedWC (population specific) plus any 2 of the following
Lipids	TGs ≥150mg/dL and/or HDL-C <35 mg/dL in men or <39 mg/dL in women	TGs ≥150mg/dL and/or HDL-C <39 mg/dL in men or women	TGs ≥150mg/dL HDL-C <40mg/dL in men or <50mg/dL in women	TGs ≥150mg/dL and HDL-C <40mg/dL in men or <50mg/dL in women	TGs ≥150mg/dL or on TGs Rx. HDL-C <40mg/dL in men or <50mg/dL in women or on HDL-C Rx
Blood pressure	≥140/90mmHg	≥140/90mmHg or on hypertension Rx	≥130/85mmHg	≥130/85mmHg	≥130mmHg systolic or ≥85 mm Hg diastolic or on hypertension Rx

Glucose	IGT, IFG, or T2DM	IGT or IFG (but not diabetes)	>110mg/dL (includes diabetes)	IGT or IFG (but not diabetes)	≥100mg/dL (includes diabetes)b
Other	Microalbuminuria: Urinary excretion rate of >20mg/min or albumin: creatinine ratio of >30mg/g			Other features of insulin resistance	

1.2 Food and health

Foods can be broadly categorised into macronutrients (carbohydrate, protein and fat), and micronutrients. Macronutrients are nutrients that, amongst other roles, provide energy that is needed to maintain the functions of the body and perform the activities of daily life[31]

Carbohydrates are considered an energy supply among all macronutrients by the Scientific Advisory Committee on Nutrition (SACN) [32, 33]. It is recommended that the total carbohydrates should provide 50% of total energy intake[32, 34].

The inherent chemical nature of particular carbohydrates and their supramolecular structures with food play a major role in the digestive fate of carbohydrates[2]. Carbohydrates must be broken down to monosaccharide units in order to be absorbed from the gut. Furthermore, a set of hydrolytic enzymes, which is secreted within the mouth and the apical membrane of enterocytes, splits the bonds between sugar residues. Alpha amylase, which breaks down complex carbohydrates in amylose and amylopectin molecules, is a protein enzyme that is secreted by the salivary glands in the mouth and by the pancreas[2].

Carbohydrates can be classified into three categories according to the degree of polymerization: sugars, starches, and fibres[32, 34].

Sugar

This term describes the mono and disaccharides. SACN recommended the definition of ‘free sugars’ as the sugars that are added during food processing and preparation or at table [33]. Furthermore, the ‘free sugars’

term involves also sugars that can be found naturally in honey, syrups and unsweetened fruit juices. However, sugars that are naturally present in milk and dairy products and fresh and most types of processed fruit and vegetables and in cereal grains, nuts and seeds are excluded from the definition because they are still within the cellular structure[33].

Sugars improve the palatability of food and beverages by sweetening their taste. Sugars are a source of energy but in addition to improving flavour also help preserve food and modify palatability, viscosity and texture[33, 35, 36].

Starch

Starch is a polysaccharide that is formed by several glucose units bonded together. It plays a significant role in human nutrition by contributing to the supply of energy required by the body. Starch is the major energy-providing component in cereals[37, 38]. Starches are classified into three categories according to their rate and extent of digestibility; slowly digestible starch, resistant starch and rapidly digestible starch[39].

Starch digestion and absorption occur in three phases: the intraluminal phase, the brush border phase, and the glucose absorption phase. After the ingestion of starches, they are enzymatically hydrolysed and lastly absorbed as glucose for energy metabolism. This process occurs in the gastrointestinal tract[40].

Starch is digested all the way through the GIT. The starch digestion starts in the mouth when lingual alpha amylase is produced by

the salivary glands. In the beginning of starch digestion, Alpha amylase is the most important enzyme and is the most abundant protein in the saliva. The starch digestion that occurs in that mouth is only on average about 5%[41, 42]. Starch hydrolysis continues in the stomach environment though the low pH reduces the alpha amylase activity. Alpha amylase, produced by the salivary glands, digests about 15% of the total starch (5 % in the mouth and 10 % digestion in the stomach)[41, 42]. Gastric emptying (GE), the process of emptying food from the stomach into the small intestine, is the main factor of starch digestion. The mass transfer of starch is reduced with slower gastric emptying[41, 42].

Starches are present in various foods like whole grains cereals and vegetables. The starch compositions of food and starch digestibility are determining factors for the resulting glycaemic response, satiety, and energy intake. Starch consists of amylose and amylopectin. The starches with high amylopectin content are broken down to glucose by digestives enzymes, whereas granular starches that are rich in amylose content tend to be more resistant to digestion[43].

Fibre

Defining ‘fibre’ is somewhat problematic because of the diversity of materials that have been historically considered as dietary fibre, but which have a range of functional consequences following digestion.

Dietary fibre is considered here to be the edible parts present in plant foods (such as whole grain, fruit and vegetables) that are resistant to digestion and absorption in the small intestine and pass relatively

intact into the large intestine[44, 45]. They are then either fermented by the colonic microflora or passed through the colon and bind water, increasing the stool weight. This definition includes the resistant starches (RS), which are a part of starch that is not absorbed in the small bowel and are considered a type of dietary fibre[46].

The dietary fibres are sometimes classified as including insoluble and soluble fibre[33]. Insoluble fibre is defined as the fibre component that does not absorb or dissolve in water. Insoluble fibre passes through the GIT in nearly its original form[44]. Insoluble fibre can be found in whole grains such as wheat, rye, oats and millet[33]. Conversely, soluble fibre is defined as a variety of materials that is soluble in water. Soluble fibre forms a gel-like substance and swells when is mixed with water[44]. Soluble fibre can be found in fruits, vegetable, nuts and some whole grains[33].

Dietary fibres have significant physiological properties with the potential to promote beneficial health[44]. Soluble and insoluble fibre may have different effects on digestion and metabolism[47]. For instance, soluble fibre could increase the viscosity which may benefit the upper GIT. The viscosity that is generated by the soluble fibre relies on factors such as molecular weight and the extent of hydration[47]. The increase in the viscosity has some health benefits[47-49] such as delaying gastric empting and limiting glucose diffusion towards the enterocytes for absorption[50]. This can lower glycaemic response when sufficient quantities are ingested[50]. Dietary fibres may also induce satiety and help control body weight[44, 51].

1.3 Consumption of whole - grain cereals

Worldwide dietary recommendations have encouraged consumers to increase their consumption of whole - grain cereals in their diets.

A grain is a small, hard dry seed. The two major groups producing grains are legumes and cereals. Cereals are from the Poaceae (also known as Gramineae) family. A whole - grain cereal is defined as one which includes the three main components of the grain: the germ, endosperm and bran in contrast to refined grains, which only retain the endosperm. The grain may be milled, but the three components are retained in the product that is consumed[52].

Considering the components of a whole grain in more detail (Figure 1), the inner germ layer consists of the plant embryo. The endosperm is the germ's food supply and provides food for the growing seedling. It is the major energy supply for the embryo for the germination of the seed. The endosperm is a rich source of starch with approximately 50-75% and approximately 8-18% storage of protein. The endosperm also contains some minerals, vitamins, fibre, or phytochemicals. The bran contains antioxidants, fibre and B vitamins and it protects the germ from environment factors (climate, pests, and other microorganisms)[43, 53].

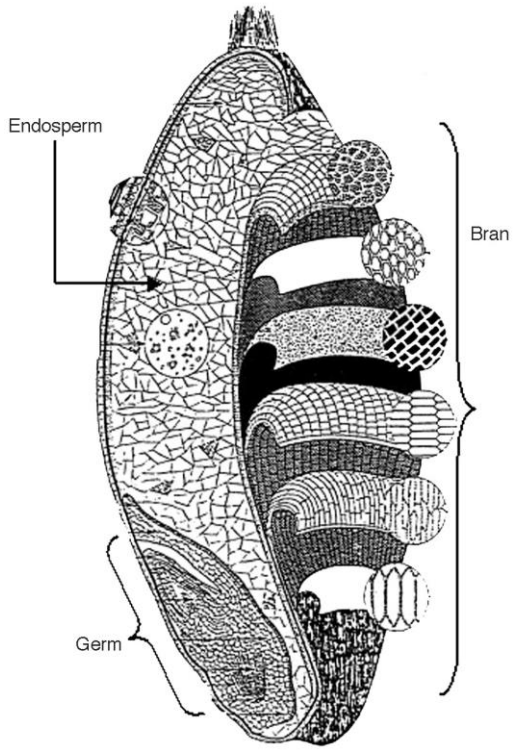

Figure 1: Shows the structure of whole grain cereal. Diagram authored by Slavin [53].

Health benefits of whole grain cereal

Whole - grain cereals provide approximately two-thirds of the energy and protein intake in various countries over the entire world, especially in developing nations[54, 55]. Their consumption is thought to have positive health effects[44, 56, 57] including an association with a reduced risk of obesity and related diseases, potentially via improved energy balance regulation and metabolism[57, 58]. including blunting postprandial glycemic and insulinemic responses[59], lowering blood pressure, improving serum lipid profile[60] and improving long term weight management via satiating properties[53, 61, 62].

The next section will present, in more detail, epidemiological studies that specifically consider wholegrain consumption, obesity and the related diseases, diabetes and CVD.

The Harvard Nurses' Health Study was conducted over a 12 year period in 75,000 women. In this prospective cohort study, those who had consumed whole - grain cereals and high fibre foods consistently weighed less (gained an average of 1.52 kg) compared with those who did not. In contrast, refined flour (white flour) was linked to weight gain over the years. The risk of Weight gain was reduced significantly (by 49%) for women who consumed high fibre products and whole grains in their diets. The assessment of this study was self reported[63]. In another long term study (27,082 men studied over 8 year period) it was shown that there was an inverse relationship between whole - grain cereal intake and with long-term weight gain. For every 40 g/d increment in whole-grain cereal intake from all foods, weight gain was lower by 0.49 kg. This study provided prospective data that links changes in consumption of whole grains to weight gain particularly among men [64].

In diabetes, research has showed an inverse association between whole grain cereal intake and the risk of diabetes. Some studies suggested that the risk of diabetes is reduced by 21% by consumption of whole grains[65, 66]. In a randomized crossover design study, fasting insulin was dropped by 10% after six weeks following consumption whole - grain diet compared to refined - grain diet for over-weight hyper-insulinemic patients, which suggests improvements in insulin sensitivity during this regime[67].

In cardiovascular conditions, the Harvard Nurses' Health study showed that higher intakes of whole - grain foods may help reduce the risk of coronary heart disease[68] . Furthermore, eating the same servings of whole grain foods per day was associated with a lower risk of ischemic stroke[69].

1.4 Breakfast and health

It has been well established that diet plays a significant role in the development of obesity and related chronic disorders, including insulin resistance, T2DM and cardiovascular disease[63-69]. Therefore, attempts should be made to provide evidence based dietary advice resulting in health promoting diets, in patients and the general population.

Breakfast is the first meal of the day, usually eaten before 10am in the morning[70]. The fasting time between dinner on the previous day and breakfast can be long, at approximately 8 hours for many people. As a result, blood glucose can be relatively low when waking up and levels of glycogen stored in the liver can be low too. Therefore, breakfast is considered the most important meal during the day, as it is essential for restoring the body's energy to cope with the morning's mental and physical activities[69].

The consumption of a satiating breakfast will prevent hunger from reaching intense levels in the morning. Consequently, snacking could be reduced and a more moderate energy intake throughout the day could be maintained. The feeling of hunger can be reduced by consuming any type of breakfast, however not all breakfast meals are equally satiating.

The sensory, nutritional and physical characteristics can modulate satiation (the process that leads to terminate eating) and satiety (the feeling of being satisfied after eating for a longer time)[71]. The feeling of hunger, appetite, satiation and satiety will be defined later in this chapter.

1.5 Breakfasts made from different plant grain origin

Cereal grains used at breakfast are a staple source of energy for many populations worldwide and differences between regions tend to reflect the local crops that have been grown historically. Traditionally oats, for example, are more commonly consumed in the English-speaking countries; rye is favoured in the Scandinavian countries whilst millet is very common in Asia and Africa although it contributes minimally to the Western diet[72-74].

Breakfast cereal porridges are made from a variety of whole - grain cereals and might be expected to result in varied complex gastrointestinal, biochemical, and appetitive responses. These responses will depend on the physical and chemical characteristics of the grain (such as macronutrient composition, amylopectin to amylose ratio and fiber content), the preparation and cooking method and the resulting food matrix[75-78], modulating in turn the glycaemic and appetitive responses, and potentially impacting on food intake. There are many varieties of cereal grains. Of particular interest for this project are: oats, rye, finger millet and pearl millet.

1.5.1 Oats

Oats (*Avena sativa*), an annual crop used both for human and animal nutrition grown mostly in cool, moist climates and are adversely affected by hot, dry weather[79].

Oats have numerous uses in food and oatmeal porridge is commonly regarded as a low-cost, healthy food in parts of the western world. Unlike wheat, oats are usually processed as a whole-grain form[80].

Oats are nutritious grains. Oats contain most lipids (including the essential fatty acid linoleic acid)[81, 82]. They are nutritionally rich in protein and in dietary fibre, such as soluble fibre, mixed linkage β- glucan. Oat grot for instance contains a high amount of β - glucan, varying between 2.3 and 8.5 g/100 g[83, 84]. The dietary fibre has been shown to have some benefits in reducing serum cholesterol, a risk factor for chronic heart diseases[51, 80, 85, 86]. In addition, oats contains several antioxidants including vitamin E, phytic acid, phenolic, and avenanthramides, which are unique antioxidants that only present in oats[87, 88].

The food processing methods have effects on the chemical composition and physical properties. The processing methods transform the raw grains into final product with good sensory properties and have a good nutritional value for the consumers. There are various methods used to process raw grains. These can affect substantially the physical form and the nutritional properties of the products hence it is worth consider these briefly [39].

Different production methods can be used to make a range of oat products. These include steel cut oats, large flakes, quick-cooking oats

and instant oatmeal. Steel cut oats are formed by cutting the harvested grout, which is another name for a grain kernel, into two to four pieces laterally. Oat flakes are produced by passing the grouts, which have been steamed, between a pair of rollers. The thickness of the flakes is determined by the gap between these rollers. If the rollers that are used have a wide gap, thick oat flakes, which are called ‘old fashioned’ are made. However, thin flakes, which are known as quick oatmeal, are made using rollers set closer together and this takes more processing time than thick flakes. Instant oatmeal is made from steel cut oats and kilned at higher temperatures than normal steel cut oats, in order to gelatinise the starch (when starches are heated in excess water the granules undergo a characteristic structural reorganization)[39].

The different physical characteristics as a consequence of the different processing methods of these products affect the availability and accessibility of nutrients and thereby, have effects on the physiological responses (glycaemic response, gastric emptying and appetite response). The processing methods greatly affect the structure of these crops and the characteristics of the starch within them[80]. Starch is a main determiner of the available carbohydrates of oats, with the remainder coming from added and endogenous sugar that absorbed in the small intestine[80, 89].

1.5.2 Rye

Rye is a cereal with modest requirements with regards to soil, fertilization and climate. It is mainly cultivated in parts of Northern and Eastern

Europe. Rye is an important source of both dietary fibres (soluble and insoluble). Soluble arabinoxylan is the main dietary fibre component in rye, which seems to have positive health benefits similar to the β - glucan in oats[90]. Starch, dietary fibre, and protein are the macronutrients in rye similar to the other cereals. Rye generally consists of small amounts of starch but has high levels of dietary fibre and free sugars.

Rye is usually consumed as a whole grain and rye porridge is traditionally made with rye flakes instead of flour due to the taste of flakes. Rye porridge is associated with different factors, which have a positive impact on satiety. Firstly, it has high levels of fibre. Secondly, the processing of rye kernels into flakes keeps part of the original botanical structure. According to Isaksson, Rakha, Andersson, Fredriksson, Olsson and Åman [91], the intact structure of rye kernels results in delayed hunger after feeding for about 4-8 hours.

Moreover, the large volume and the low energy density of the porridge results in an improvement in the satiation and in food consumption due its high water content and a food property with potential to increase satiety and decrease food intake[62]. For instance, a randomized, cross-over design study compared porridge made from whole grain rye with iso-energetic refined wheat bread at breakfast. Subjective feelings of appetite ratings were used in this study. A greater feeling of satiety from the rye porridge for up to 8 hours under standardized conditions was seen although there was no significant difference in the subsequent food intake[92]. Late evening meals containing rye kernels reduced the *ad libitum* energy intake by 11%

compared with white wheat bread suggesting consumption of rye may have significant short effects on energy intake[93].

Other two randomised studies also found that whole grain rye crisp bread resulted in lower self – reported hunger and lower fullness as well as a lower energy intake after an *ad libitum* lunch compared with refined wheat bread[94]. In another study, endosperm rye products and whole grain rye breads lowered insulin responses and reduced the blood glucose compared with white wheat bread; this suggests that rye has a positive effect on appetite regulation and metabolism[94].

1.5.3 Millet

Millet is the common name for various species of plants belonging to different *genera* but all within the grass (*Poaceae* or *Gramineae*) family, with small-seeded grains.

Millet grains are nutritious cereal grains and considered to be *Ancient Grains*, as they are grains that have not changed over many hundreds of years. Millet is one of the most drought-resistant crops hence it has high levels of resilience to the potential consequence of climate change (such as reduced rainfall and increased temperature) and it has high nutritional value. Additionally, it has resistance to pests and diseases[95]. Therefore, millet could make a significant contribution to ensuring food and nutritional security in various countries all over the world against a back drop of detrimental climate change and increasing populations which could result in food scarcity and price increases[96].

There are several types of millets; amongst the most commonly used are finger millet and pearl millet. They constitute a staple food for a large number of the population in various countries of Asia and Africa, and these millets grow extensively in these countries

1.5.4 Finger millet

Finger millet is one of the minor cereal grains that is an important food stable source in Asia and Africa, particularly for people with low income[95].

The nutritional composition of finger millet is similar to other grains regarding protein (7.3 g). However, it has the highest calcium content among all cereals. Finger millet is also a rich source of carbohydrate (72 g), starch (65.5 %), free sugars (1.04 %) and dietary fibre (11.5 %). It has a much higher dietary fibre content compared with other millets but is comparable to that of pearl millet. Regarding the fat content, it is reported that the finger millet has lower fat levels (1.3 g) than pearl millet (5 g) and this could contribute to the improved shelf storage properties of finger millets[74]. Whole - grain finger is less attractive with its appearance (a dark colour); however, it is rich in dietary fibres, vitamins and mineral content. In contrast, refined finger millet flour is free from the seed coat hence has a white colour; however, it may have a higher glycaemic response and is nutritionally inferior to consumption of the whole grain.

There is no general consensus over the glycaemic response to finger millet as the number of subjects in most studies was too small and some studies used venous samples instead of arterialised blood or capillary samples[97]. According to FAO/WHO 1998 and VEN 2007, the

difference in glycaemic response between foods is larger and easier to detect statistically using capillary methods, compared with venous samples, because the rise in blood of glucose, in response to the food, is higher and there is a lower variability in capillary samples[74, 95, 98].

1.5.5 Pearl millet

The biological name of the pearl millet is *Pennisetum Glaucum*; other names are Kamboo, Sajjalu, Bajri, and Bajra. Pearl millet is grown on about 30 million hectares (ha) in 30 countries spread across Asia, Africa, the Americas and Australia[99]. The largest producing country for this crop is India (about 8.5 million ha). Pearl millet ranks third in production after wheat and rice and is also a stable food source in economically poor countries[100].

Pearl millet is *an ancient* and small- seeded grain that may have potential health benefits particularly with respect to glucose and insulin metabolism[40, 74, 81, 101]. Pearl millet is nutritionally comparable to major cereals such as wheat. Pearl millet has high levels of energy, dietary fibre and proteins, and also some vitamins and antioxidants[101]. Amylose content of finger millet lower than that of pearl millet by 21%.

It has been suggested that pearl millet may have potential health benefits due to its chemical composition[40, 74, 81, 101]. Pearl millet has in relative terms a higher iron content (8mg / 100g) content than other cereals[102] which may be important where iron deficient anaemica. Pearl millet has also higher zinc content (3.1 mg/100g) than other cereals[102],. Pearl millet has also high amounts of fibre (1.2g/100g); therefore, it can

be widely used to prepare healthy foods which need to have high fibre contents, particularly for disorders such as obesity and constipation.

Pearl millet also includes high levels of antioxidants (51.4mg/100mg) which may have anticancer properties[103]. Pearl millet can also play a significant role in managing the hyperglycaemia in diabetes[101, 104]. Pearl millet has higher amounts of Omega3 fatty acids compared with other grains and this may have additional benefits in treating and preventing metabolic disorders such as CVD, DM, arthritis and certain types of cancer[101, 105]. Pear millet is gluten free and this makes it also ideal for people with gluten sensitivity[106].

Recently novel products containing millet have been marketed as a health-promoting snacks in countries such as India. Considering all these potential health benefits of pearl millet, there are surprisingly few nutritional studies of pearl millet interventions, particularly clinical trials on the impact of this crop on metabolic disorders which are needed clearly and have been recently recommended[101].

In addition to these favorable characteristics, pearl millet has the potential to contribute to the development of a more sustainable and resilient agricultural system, with greater plant and dietary diversity[107]. Millet can be grown in areas where the annual rainfall is unpredictable or low, and in areas which are characterized by low soil fertility and high temperatures[99, 100]. Water scarcity and worldwide population growth increase the necessity for drought-resistant grains that can be grown for consumption in Asia and African countries[96] and potentially distributed worldwide for human consumption.

Additional evidence could lead to more informed dietary advice and potentially support an increased role for of a currently under used, sustainable food resource. Better informed dietary advice may be beneficial not only to patients with poor glycaemic control but also to promote the health of the general population.

Processing of millet grains

The aim of processing is to transform the raw grains into a final product with good sensory properties and a good nutritional value for the consumers. The processing methods have effects on the chemical composition and physical properties. There are various methods used to process raw grains. These can affect substantially the physical form and the nutritional properties of the products, hence it is worth considering these briefly. The processing methods applied to these grains reduce the number of phenolic compounds. For example, the levels of phytochemicals present in millet food and beverage are lower than that in grains[40]. There are four processing methods for the millet grains: milling, decortication, malting and popping methods.

Milling

In some instances the grains need to be milled for the preparation of flour. For example this is commonly done for finger millet as the kernels cannot be cooked in grain form because it has a fragile endosperm with an intact seed coat[40].

Decortication

Previous decortication methods were not effective for finger millet compared to the other grains due its characteristic features (high fragile endosperm and intactness of the seed coat). A new decorticated finger millet process was developed in 2006 by Malleshi. This technique plays an important role in stabilizing the soft endosperm throughout hydration, steaming and drying processes enabling the grain to resist the mechanical impact on the endosperm during the decortication process. Finger millet prepared with this method could be cooked as a discrete grain similar to rice.

Malting

Previous research reported that the finger millet grain has good malting characteristics but the preferred grain for malting is barley for both the brewing and food industries. Additionally, the small size of the finger millet grain is beneficial for obtaining germination and kilning. Malt flour is termed as an amylase-rich food and is utilized in milk-based beverages, confectionaries and cakes. On the other hand, malted cereals consist of highly digestible CHO; therefore; they may not be suitable for the people with metabolic conditions because it may exhibit higher glycaemic responses[40].

Popping

This technique is extensively used to prepare ready-meal products. High Temperature Short Treatment (HTST) is applied using a heat connector

in which the starch of the finger millet becomes gelatinized and the endosperm bursts open. Popped finger millet has a highly desirable taste and a typically pleasant smell. It is consumed as snacks after seasoning to enhance the favour. Popped finger millet flour is a whole grain product rich in nutritious components (e.g. macronutrient, micronutrient and dietary fibre) mixed with vegetable or milk protein sources[40].

1.6 Gastrointestinal tract (GIT) anatomy, function and its response to food

The digestive system

The digestive system is responsible for digestion and absorption. The digestion process starts with the anticipation of consuming food or beverages products and finishes with the excretion of faeces. The food consumed is systematically broken down by a multitude of mechanical and enzymatic processes in order to fully absorb the available nutrients.

Food digestion lies between the food consumption and health benefits. The digestion includes three phases[108] following on from the pre- oral phase. Oral processing, or the first phase represents a key sensory interaction with the food. Furthermore, the particle size of food is reduced and amylase is added in the oral processing stage. The second step of the digestion is the gastric phase. This phase is considered an important stage to understand the connection between food structure and rates of nutrient release. The intestinal phase is the final stage of the digestion[109]. This phase involves both the small and large intestines[108]. Nutrient absorption occurs in the small intestine, with

fluid, salts and fermentation of some unabsorbed dietary components occurring by gut microflora in large intestine[108]

The gastrointestinal tract (GIT) begins at oral cavity (mouth), goes through the oesophagus, the stomach, the small bowel or intestine and the large intestine or colon and ends at the anus (Figure 2).

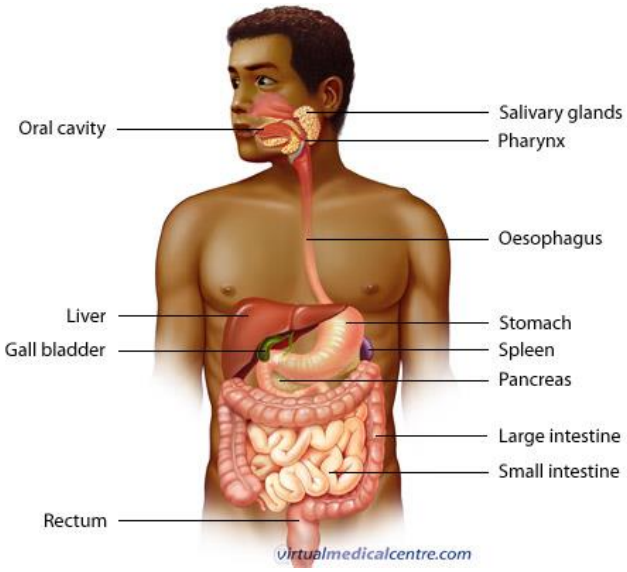

Figure 2: Schematic representation of the gastrointestinal tract. Diagram adopted from http://www.myvmc.com/anatomy/gastrointestinal-system/. Date accessed 06 September 2018.

Oral digestion

Oral ingestion of food is the first step of the movement of food through the GIT[110]. In the oral cavity physical processes occur (mastication) that start to breakdown the food (solid or semi solid) in smaller particles and chemical process occur (enzymatic hydrolysis) to breakdown starch by Alpha - amylase and to breakdown the lipids by lingual lipase. The goal of these processes is to form a bolus, a mass of chewed food. The bolus can be safely swallowed if it contains sufficiently small particles and is

lubricated with saliva. Swallowing is the process that transfers food from mouth to the stomach and it includes the integrated activities of oral cavity, pharynx, oesophagus and orad portion of the stomach[110].

Chewing time, amount of saliva and final bolus particle size are affected by both physiological variables (such as gender, personality type, and dentition status) and food properties (such as composition, physical form and portion size of a food product)[111].

Oesophageal transit

In the oesophagus, the food bolus is propelled from the pharynx to the stomach[110]. Once the bolus is swallowed, oesophageal peristalsis is started. During the oesophageal transits, simple force gravity may have an effect on the bolus movement as long as the subject is not in supine position. Then the bolus passes through lower oesophageal sphincter into the stomach[112].

Gastric digestion

Gastric digestion is complicated process which consists of physical and chemical breakdown. The physical breakdown by the antral contraction waves (ACWs) acts to crush and grind of the food particle size. The chemical breakdown is facilitated by secretion of gastric acid and enzymes (pepsin, lipase). The gastric acid has the effect of softening the food particle texture. The digestive enzymes hydrolyse the nutrients (the pepsin hydrolyses proteins and the lipase hydrolyses lipids) allowing them to be absorbed when they reach the small intestine[113].

The stomach is functionally separated into a proximal part, which includes the cardia, fundus and body, and a distal part, which involves the antrum and the pylorus (Figure3).

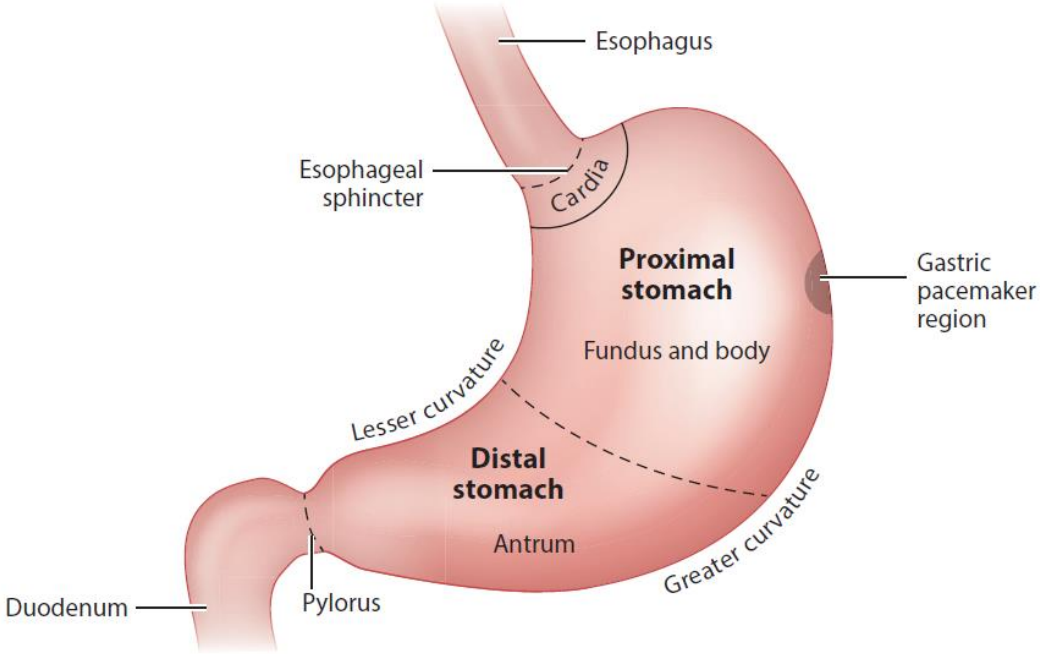

Figure 3: Gastric anatomy. The dashed line in the centre of the stomach represents the approximate division between the proximal and distal stomach regions. Diagram authored by Bornhorst and Paul Singh [111].

The proximal stomach is thought to works as reservoir for food, and is where the ingested food stays before it moves to the distal part of stomach. The cardia contains mucous-secreting cells. The fundus is lined with mucosa forming thick folds called rugae, where HCl is secreted by parietal cells and pepsinogen is secreted by chief cells. The distal part is thought to be the main location for the physical breakdown of the food in the stomach, where the ACWs act to crush and grind food particles until they pass through the pyloric sphincter into the small intestine[111, 112].

The lower oesophageal sphincter and the pylorus play an important role to keep the chyme within the stomach. In addition to the effects of food characteristics on gastric digestion process, gastric secretions, motility and emptying are physiological processes that have an impact on mixing of gastric contents.

Gastric secretions

The stomach can secrete about 2 - 3 L of gastric fluid in a day[112]. The inner cells and musculature of the stomach are protected by a gel – like mucous layer throughout the gastric lumen[111]. Some stomach cells secrete hormones such as somatostatin, gastrin, cholecystokinin, glucagon and intrinsic factor, that mediate the overall digestion process[112].

The gastric glands are located at different areas in stomach. The cardia region contains about 5% of the gastric surface area. The fundus and body regions comprise 75% of the gastric glands[113]. These gastric glands secrete mucus, acid, intrinsic factor, somatostatin, histamine, and pepsinogen. The mucus is also secreted in the antrum[112]. Furthermore, gastrin and somatostatin are secreted in antrum region and they play important role in the regulation of gastric secretion in the fundus and body regions[112].

The gastric glands that located in proximal stomach secret the key components of gastric fluids[113]. The gastric glands consist of mucus cells, chief cells and parietal cells[113]. Mucus cells secrete mucus and they are located at the top of the gastric glands. Chief cells are located at the

bottom of the gastric glands and they secrete pepsinogen and gastric lipase[113]. Parietal cells secrete intrinsic factor and acid. Acid secretion is modulated in the GIT during food intake. The time when acid is secreted and its quantity are regulated to keep the balance between the physiological benefits (such as food digestion) and the potential to harm the tissue[113].

Gastric acid secretion in the fasting state is secreted at basal values of 0–11 milliequivalents per hour to maintain the luminal pH ≤ 3. The regulation of gastric secretion depends also on food related factors as well as individual related factors. For instance, secretion can be affected by factors as food physical form, coffee consumption, caffeine consumption, age, stress, smoking habits and time after meal consumption[113].

Gastric motility

Gastric motility is defined as the movement of the stomach walls and is considered as an important step for the gastric digestion process. The characteristics of the ACWs such as its frequency, duration and intensity have effect on the rate and mechanism of physical breakdown of food and mixing of food in the stomach. As a result, food particles may experience changes in the forces, torques, pressures and flow profiles[111, 112].

The gastric motility of proximal and distal parts of the stomach following a meal may vary. The contractions of the proximal part are tonic contractions, which are sustained with low frequency and amplitude[114].

On the other hand, the contractions of the distal part are phasic, peristaltic muscular contractions[113].

Phasic contractions close up the gastric lumen to a certain degree due to concurrent circumferential contractions of the greater and lesser curvature. The average velocity of phasic contractions is 1.5–3 mm/s and the average frequency is ∼3 cycles/min[111, 115, 116].

Gastric emptying (GE)

Gastric Emptying (GE) is the process of emptying food from the stomach into the small intestine. The rate of gastric emptying is the rate at which food leaves the stomach. The rate of gastric emptying is controlled by various variables including food properties (composition, energy, volume, structure and processing), food breakdown (rheological properties, particle size distribution, gastric mixing), physiological response (gastric secretions, hormonal response), subject characteristics (age, gender and activity levels) (Figure 4)[111]. For instance, the half time of gastric emptying may vary based on gender[117], the amount of exercise (e.g., whether standing at rest or walking on an exercise treadmill at different speeds)[118], energy content of the meal[119], meal composition[120], and meal structure (e.g., whole-food items or a blended soup)[121].

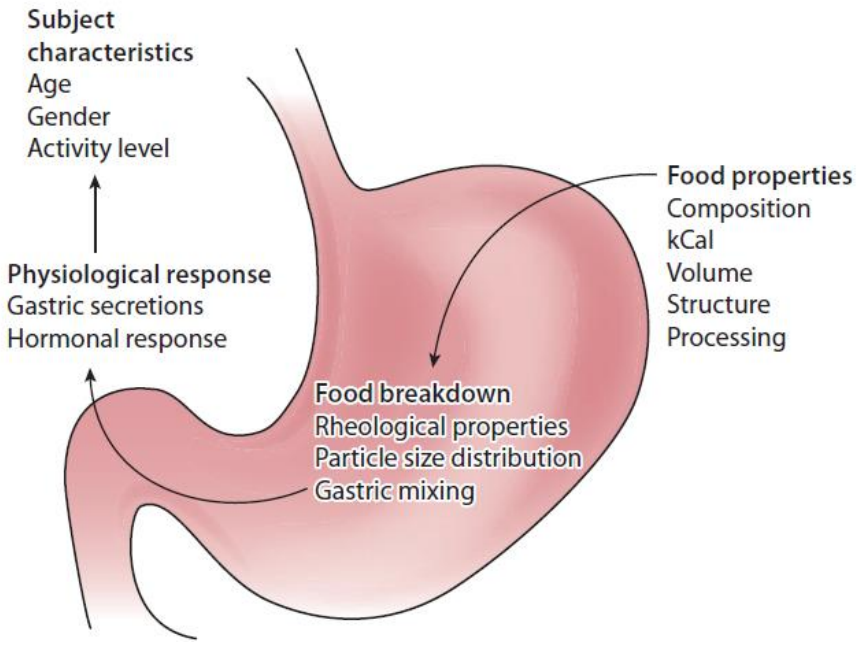

Figure 4: Representation of the food and subject factors that are interrelated in determining the gastric emptying rate of a meal. Diagram authored by Bornhorst and Paul Singh [111].

Although the emphasis on the role of GE in the absorption of nutrients into the blood was limited in the past, the rate of GE plays an important role in determining post prandial glycaemia[122, 123]. For instance, a study aimed to analyse the relationship between post prandial glycaemia at 30, 60, and 120 min and gastric emptying after oral glucose tolerance test (OGTT) in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (T2D). The study found that GE was more rapid and blood glucose was greater at 120 minutes; therefore, there is an inverse relationship between blood glucose at 120 minutes in oral glucose tolerance test and GE[124] in subjects with NGT but not in IGT or T2D. The blood glucose

concentration at 30 min in all three groups and 60 min in IGT and T2D was also related directly to GE[124]. Additionally, another study confirmed the relationship between blood glucose and GE as it reported that hyperglycaemia slows the rate of GE[125].

Small intestine digestion and absorption

The digestion and absorption process in the small intestine rely on the motor function. Contractions in the small intestine are performed in order to achieve three primary functions: mixing of the ingested food materials with digestive secretion and enzymes, dispersion of the nutrients for digestion and absorption and propulsion of the intestinal chyme in the aboral direction[110].

The small intestine extends from the pyloric sphincter into the large intestine and is 6-7 meter long. In the small intestine, chyme is broken down to molecules for absorption by the epithelial cells and these are then transport into the blood stream. The small intestine is separated into three regions namely duodenum, jejunum (2.5 m in length and is the main location for absorption the majority of nutrients), and ileum (3.5 m in length)[111, 112].

Functionally the physical process of the small intestine involves peristalsis and segmentation contractions that move the chyme through the intestine. The peristalsis contractions cause a strong forward movement that propel the chime at a rate of 2-25 cm s^{-1}. The segmentation contractions aim to mix the chyme in order to facilitate the nutrients absorption. The chemical process of the small intestine is the

enzymatic hydrolysis. Lipid breakdown is hydrolysed by lipase and phospholipase, starch breakdown is hydrolysed by either amylase or amyloglucosidase and protein breakdown is hydrolysed by trypsin, chymotrypsin, carboxypeptides and elastase [111, 112].

The starch hydrolysis is continued in the small intestine by the pancreatic alpha amylase[41, 42]. Alpha amylase rapidly converts the starch molecules to oligosaccharides and dextrins. The breakdown of oligosaccharides and dextrins into glucose is controlled by other enzymes such as alpha-limit dextrinase, glucoamylase, maltase and isomaltase[41, 42].In the small intestine, glucose is ultimately absorbed by the epithelial cells[41, 42].

Large intestine fermentation

The large intestine extends from the end of small intestine to the anal canal and is about 1.5 m long. The segmentation and peristalsis result in the movement and mixing of the chime. The large intestine is colonised by 10^{12} microorganisms / g of intestinal contents. These bacteria will ferment the rest of the undigested particles. The large intestine mostly absorbs short – chain fatty acids and water[111, 112].

1.7 Physiological responses to food

Feeding elicits a range of physiological and behavioural responses involving complex neurological and hormonal responses, this section summarises briefly those that are most relevant to this project, which are

the glycaemic, insulinemic, appetitive, the gastrointestinal peptides and gastrointestinal responses.

1.7.1 Glycaemic response (GR)

After ingestion a meal, the glycaemic carbohydrates (available carbohydrates) are absorbed by the intestine and passed into the bloodstream, resulting in an increase in blood glucose concentration. The magnitude of the increase and fall in the blood glucose levels and period over which the blood glucose occurs has been called glycaemic response.

Glucose Metabolism and Regulation

The concentration of blood glucose is a function of rate of glucose entering the blood circulation (glucose appearance) balanced by removal of glucose from the blood circulation (glucose disappearance). The blood glucose concentration is balanced in a narrow range in healthy subjects. Recommended pre-prandial levels are in the range of 4.0 to around 5.6-5.9mmol/L and blood levels return to this value a couple of hours following food intake.

There are three sources responsible for circulating glucose. Firstly, intestinal absorption during food intake. For the intestinal absorption source, gastric emptying rate is considered the major factor as it has an effect on how quickly glucose appears during food intake. The other sources that are responsible for circulating glucose are glycogenolysis, the breakdown of glycogen, and gluconeogenesis, the

process where the glucose forms mainly from lactate and amino acids during the fasting condition [122].

The process of circulating glucose concentrations is maintained in a relatively narrow range by glucoregulatory hormones which involve insulin, glucagon, amylin, GLP-1, GIP, epinephrine, cortisol, and growth hormone [122]. The effects of primary glucoregulatory hormones such as glucagon and insulin are listed in table 2.

Table 2: The effects of primary glucoregulatory hormones such as glucagon and insulin

Hormone	Secreted from	Main function
Glucagon	by alpha-cells of the pancreas	Stimulates the breakdown of stored liver glycogen. Promotes hepatic gluconeogenesis. Promotes hepatic ketogenesis
Insulin	beta- cells of the pancreas	Affects glucose metabolism and storage of ingested nutrients. Promotes glucose uptake by cells. Suppresses postprandial. glucagon secretion. Promotes protein and fat synthesis. Promotes use of glucose as an energy source.

Insulin is a peptide hormone that is secreted by the β cells of the pancreatic islets of Langerhans. Insulin maintains normal blood glucose[126, 127]. Insulin release is slowed when blood glucose falls to normal physiological values[67128].

Glucagon, a hormone that is produced by alpha-cells of the pancreas, has partly controlled the glycogenolysis and gluconeogenesis. Glycogenolysis is the primary mechanism that produces glucose during

the fasting period that ranges from 8- 12 hours. This process is facilitated by glucagon. Over longer period of fasting, gluconeogenesis produces glucose that is released from the liver[122.]

Factors affect glycaemic response

Different carbohydrates have different glycaemic responses. For instance, glucose and cooked potato starch, which are fully and readily digestible carbohydrates, induce rapid rise in the blood glucose concentration and followed by a similarly fast drop. However more slowly digestible carbohydrates produce a slower and more prolonged increase in blood glucose, rising to a lower peak.

The glycaemic response is influenced by two main set of factors: food- related factors and individual – related factors. Food related factors that have an effect on glycaemic response involves firstly nature and amount of available carbohydrates. These factors include the physical form of the carbohydrates (particle size and degree of hydration) and the types of starch (amylose, amylopectin, starch-nutrient interaction and resistant starch).

They also include the monosaccharides content (glucose, fructose, galactose, mannose and tagatose), the disaccharides contents (sucrose, isomaltulose, treehouse, lactulose) and the oligosaccharides content (maltodextrins) [128-131]. Moreover, the nature and amount of available other food components (fat, protein, organic acids, dietary fibres and phytochemicals) may have an influence on glycaemic response[129-131].

In addition to that, cooking and food processing have an influence on glycaemic response. These involves degree of starch gelatinization (degree of cooking), physical form of food (solid versus liquid), particle size and cellular structure[129-131].

Individual –related factors are the second set of factors that have an influence on glycaemic response. These factors are linked to eating behaviour and physiological factors relating to individuals. These include the time and speed of ingestion; the number of meals and snacks consumed. They include also the digestive response, rate of gastric emptying and individual variations[128, 131].

Sampling methods for blood glucose

The World Health Organization (WHO) / Food and Agricultural Organization (FAO) have recommended the capillary blood glucose sampling method (finger prick), as diabetic patients commonly do to monitor their blood sugars[132].

This method is preferred due to three reasons. Firstly it is a simple and relatively cheap technique that makes it easy to obtain results. Secondly, as the rise in capillary blood glucose is greater than that in venous blood glucose and is less variable than the venous one. Thirdly, with this method the detected difference in responses between different foods is larger than with the venous method[37, 133].

1.7.2 Appetitive responses

This part will define firstly the feeling of hunger, appetite, satiation and satiety as well as the satiety cascade.

Hunger is defined as physiological (internal) feeling that drives individuals to eat. Different factors have an effect on hunger such as nutrients in the bloodstream, eating patterns and climate.

Appetite is a psychological (external) feeling that determines the desire to consume a specific food or nutrient, often in absence of hunger. Different external forces have an impact on appetite and appetite can be overwhelming [71].

Satiation is also known as intra-meal satiety and is the process that leads to terminate a meal. In this sensation, hunger is supressed when the brain sends a signal to stop eating. Therefore, satiation is a control mechanism on meal size.

Satiety is also known as inter-meal satiety and is the process that prevents further eating following consumption a meal by increasing the fullness sensation and decreasing hunger sensations [71]. Satiety is known as post-ingestive satiety or inter-meal satiety and is the process that inhibit further consumption after a meal has finished[71].

The “Satiety Cascade” offers a conceptual framework to investigative the effect of foods on satiation and satiety (Figure 5). A variety of factors contribute to the modulation of satiation and satiety. For instance, macronutrients, energy density, physical structure and sensory qualities contribute to the modulation of satiation and satiety[71].

The Satiety Cascade shows how satiation and satiety organize our eating behaviours by controlling the size and frequency of meals. In the beginning, when food and its nutrient content is consumed, the brain is informed by sensory input. The chemo- and mechano-receptors in the gastrointestinal tract monitor physiological activity and pass information to the brain mainly via the vagus nerve. This information forms one class of `satiety signals' and part of the pre-absorptive control of appetite. This will help to identify a post absorptive phase. This phase arises when nutrients have digested and absorbed to enter the circulation [71, 134, 135].

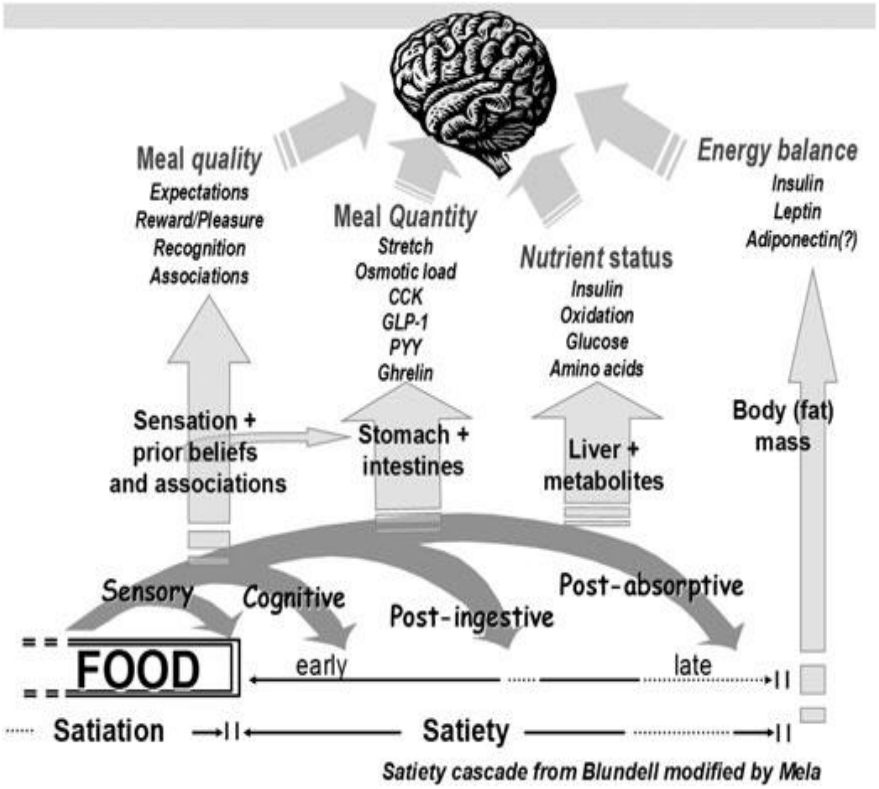

Figure 5: The 'Satiety Cascade' linking the timing and sequence of eating motivations and behaviours to associated cognitive and physiological processes. This figure was adopted from Blundell, De Graaf, Hulshof, Jebb, Livingstone, Lluch, Mela, Salah, Schuring and Van Der Knaap [71]

1.7.3 Gastrointestinal peptides

The functions of GIT are regulated by peptides and a variety of mediators released from the nervous system. All GIT hormones are peptides, however, not all peptides found in the GIT are considered hormones. The gastrointestinal peptides are classified depending on the delivery method of the peptide to its target site. They are classified into endocrines, paracrine and neurocrines[110]. Endocrines describes hormones that are released into the general blood circulation and reach the target cells. Paracrine describes hormones action where released hormones diffuse through the extracellular space to nearby target tissues. Neurocrine hormones are those released by neuroendocrine cells and they are secreted near their target sites[110].

GIT is largest endocrine organ in the body producing hormones which play a major role in regulation of energy balance. Peptide hormones released from the gut and pancreas are considered the most important factors in controlling appetite and satiety. There are many peptide hormones that influence appetite, satiety and glycaemic response[136]. This section will concentrate on 4 hormones that have direct influence appetite, satiety and glycaemic response, namely: peptide tyrosine tyrosine [PYY (3–36)], glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and cholecystokinin (CCK).

PYY

PYY is secreted from Endocrine L cells that are located in the small and large intestines[137]. A small amount of PYY (1-10 %) is found in oesophagus, stomach, jejunum and duodenum. However, high concentration of PYY are found at the terminal ileum, colon and maximum concentration in the rectum[138].

PYY inhibits gastric motility and increases water and electrolyte absorption in the colon. It has been shown to reduce appetite. PYY may act as 'ileal brake', which has a role to slow the gastric emptying and intestinal transit, hence, the efficiency of the digestion and nutrient absorption after feeding would be increased[139].

Plasma PYY levels are decreased at the fasting state and are at their lowest level in the morning. Plasma PYY levels are released in response to feeding (within 30 minutes of nutrients reaching the gut)[140]. The plasma PYY levels are increasing after breakfast, increasing further after lunch and peaking after dinner. PYY release is influenced by the nutrient intake in proportion to the energy contents. PYY is particularly stimulated by fatty meals compared to iso-energetic meals made from carbohydrates and protein[138].

GLP-1

GLP-1 is secreted from L cells of the small intestine and large intestine[137]. Plasma GLP-1 levels are at their lowest level in the fasting state (after overnight fasts). The plasma levels rise rapidly during meals and usually remain above the baseline (the morning levels) between meals[140, 141].

For instance, when healthy subjects consumed 524 kcal breakfasts and 4 h later ate mixed-nutrient lunches containing 511, 743, or 1034 kcal, active GLP-1 rose after 30 minutes from about 5 pM to 8, 12, and 16 pM respectively and then dropped three hours later to about 7 pM (above the baseline)[142].

GLP-1 may contribute to satiety and satiation as the postprandial GLP-1 levels are mostly increased for several hours[141]. Additionally, in a study with healthy subjects who consumed fixed breakfast meals with same energy contents and weights but different energy density, plasma GLP-1 levels at 60-180 min (late satiety phase) were associated with both subjective hunger ratings and size of meal offered at 180 min (neither association was found at early satiety phase 0-60 min after the fixed meal size)[143].

GLP-1 may contribute on meal-related glycaemic control by stimulating insulin secretion, inhibiting glucagon secretion, slowing gastric emptying, and reducing hepatic glucose metabolism. GLP-1 may also have contributions on glycaemic control in the fasting state[144].

GLP-1 release is proportional to energy content and begins within 5-30 min after ingestion. GLP-1 is one of the incretin hormones, which stimulate insulin secretion. GLP-1 is released in response to ingestion of carbohydrates and fats and the release is a slower and more sustained[138].

GIP

The primary role of GIP is that of an incretin hormone, binds to its specific receptor on pancreatic β-cells, and enhances glucose dependent insulin secretion[145]. GIP is secreted from intestinal K-cells that are located primarily in the duodenum and proximal jejunum[145]. GIP is secreted in response to absorption of nutrients particularly glucose and fats. More specifically, GIP release is stimulated by the rate of nutrients absorption rather than the presence of nutrients in intestine [145].

GIP release after fats ingestion is higher than after carbohydrates in humans, whereas in rats GIP release after carbohydrates ingestion is higher than after fats[146]. The plasma GIP levels at baseline in humans range from between 0.06 and 0.1 nmol/L, depending on the assay used to measure total vs intact GIP, and rise to 0.2– 0.5 nmol/L following a meal[147]. The half-life of intact biologically active is about 7 minutes in healthy subjects, 5 minutes in diabetic patients and less than in rodents[147].

GIP is one of the incretin hormones which stimulates insulin secretion in response to glucose. GIP has a role on inhibition gastric acid secretion and regulation of lipid metabolism in adipose tissue, where it stimulates FA synthesis and increases the incorporation of FAs into triglycerides[147].

Cholecystokinin (CCK)

CCK is the first hormone discovered to have an effect on appetite. It is secreted mainly from the duodenum and jejunum[137]. The plasma CCK levels increase for 10- 30 minutes following the start of a meal. This rise is then followed by a gradual fall of plasma levels to the baseline values. This fall may take 3-5 hours depending on the composition of the meal. CCK is released mainly in response to fat and protein and it has only a weak response to carbohydrates. [137]

The main functions of CCK are to facilitate absorption of nutrients, to stimulate gall bladder contraction and to stimulate pancreatic enzyme secretion. Furthermore, CCK has an important role in inhibiting gastric emptying[137]. The half-life of CCK is very short (1-2 minutes), hence the administration of CCK15 minutes or more before a meal may have ineffective role as intervention to reduce food intake

The main function of PYY, GLP-1, GIP and CCK hormones as well their secretion are summarised in table 3.

Table 3: Summary of gut peptides and their main function

Peptide	Secreted from	Main function
PYY	L cells of the small and large intestine.	- To delay gastric emptying. - To increase GI transit time (ileal brake) - Inhibits gastric motility and increases water and

		electrolyte absorption in the colon. -It has been shown to reduce appetite
GLP-1	L cells of the distal small intestine and large intestine.	- It has an incretin effect. - Acts as an ileal brake. - May contribute on meal-related glycaemic control, satiety and satiation.
GIP	K-cells of duodenum and proximal jejunum	- is an incretin hormone -inhibits gastric acid secretion and regulates lipid metabolism in adipose tissue
CCK	I cells of the proximal duodenum and jejunum.	- To stimulate gall bladder contraction. - To stimulate pancreatic secretions. - To delay gastric emptying

1.7.4 Magnetic resonance imaging (MRI): GI function

MRI is a non-invasive medical imaging technique that is frequently used to create detailed image of the organs and tissues within the body. The MRI image depends on the properties of the nuclei of the hydrogen atoms and particularly their spin. The hydrogen nuclei contain of single proton that carries a positive electrical charge. The protons are spinning constantly and therefore their positive electrical charges move around them.

The movement of the electrical charge generates a magnetic moment. The magnetic moments are generally randomly orientated, however when an external magnetic field is applied they can either align parallel or antiparallel to the applied field with a small population difference. The sum of all small magnetic moments results in a net magnetization precessing at a given frequency, also called the Larmor frequency[148].

A radiofrequency (RF) pulse at that frequency is able to disturb the magnetization and transfer energy to the protons[149]. This transfer can only happen when the frequency of the RF pulse is that of the precessional frequency of the protons. This process called resonance and hence the term magnetic resonance imaging[149]. The RF pulse turns the magnetization away from the main static magnetic field and it now possible to detect a signal induced in a receiver[148].

When the RF is switched off, the net magnetization vector loses its energy (relaxation) then it realigns again along the Bo. The amount of magnetization in the longitudinal plane is gradually increased (recovery).

The recovery of the longitudinal magnetization is caused by a process termed T1 recovery. In the same time but individually the amount of magnetization in the transverse plane is gradually decreased by dephasing (decay). The decay of the transverse magnetization is caused by a process called T2 decay[150].

The signal depends on the magnetic field hence it is possible to encode spatial information using three orthogonal magnetic field gradients. The encoded signal can be later post processed thereby reconstructing the MRI image[148].

MRI of GIT function is nearly a quarter of a century old[151]. This imaging technique that has revolutionised diagnostic radiology in every field is particularly suited for the assessment of GIT motor functions. Its many advantages include the absence of ionizing radiation, the richness of soft tissue contrasts available, the speed, resolution and multi-planar capability. Over the years there have been several breakthroughs in hardware, imaging sequences and data processing techniques. The technology is also now diffused, with over 26,500 scanners installed worldwide[152]. These numbers are increasing and a particularly rapid market growth in the Asia East. Clinical translation to help both the patients and the gastroenterologists managing them is round the corner. There are still a few obstacles to overcome.

MRI can assess several gastrointestinal functions without ionizing radiation. MRI can contribute to the understanding of many aspects of digestion including gastric emptying, mixing, gastrointestinal fluids and also intra-gastric appearance of meal and layering. Gastric emptying can

be measured by the reduction in the volume of gastric contents over time on anatomical MRI scans, gastric motility can be assessed using cine imaging and gastric secretion can be estimated by monitoring the dilution of the meal[153].

Furthermore, MRI has been proposed to study the fate of gastrointestinal contents such as relaxometry (chime T2, 'viscosity') [154, 155]. The MRI relaxometry method represents another method of quantitative MRI analysis and refers to study of relaxation times from the MRI images. It is also possible to create a relaxometry image map based on relaxation times [156].

Of particular relevance to this project, MRI can visualize the chime in the stomach and assess both its volumes and also its motion inside the stomach in terms of mixing. It can also look at fluid volumes in the small bowel in response to meals.

Gastrointestinal volumes

A single breath-hold is usually sufficient to acquire image data from a whole abdominal volume (Figure 6), allowing measurements of organ volumes. Gastric volumes have long been measured with MRI including validation studies[157] and assessment of reproducibility[158].

A typical application is the assessment of gastric emptying; the literature includes many examples investigating the gastric emptying of a wide range of foods and beverages using MRI. Recent focus included intragastric distribution and emptying of fat emulsions[159] and aerated drinks[160] and their relationship with postprandial symptoms. The effects

of gastric secretion have been studied[161] and the data analysis can be semi-automated[162] shortening considerably the time required to process the data compared to manual segmentation of the images. It is possible to check the participants' compliance with overnight food restrictions by visualizing the volumes of resting juices in the gastric lumen at fasting baseline[163].

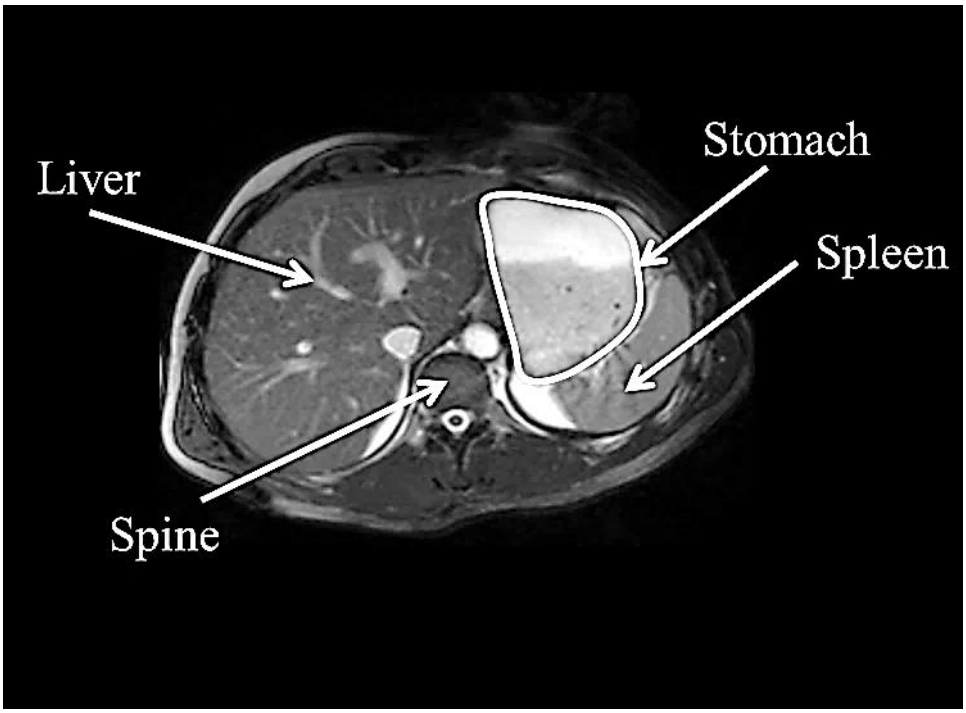

Figure 6: Representative example of axial MRI images of the abdomen of a healthy volunteer fed with pearl millet porridge (PMP).

Gastrointestinal mixing

Gastric mixing is defined as the combination of substance with another in the stomach[113]. There are several types of mixing such as solid-solid mixing, solid-liquid mixing and liquid-liquid mixing (Figure 7).

An example of the solid- solid mixing is the mixing meal components with each other. An example of the solid- liquid mixing is the combination of solid meal with gastric secretions. An example of the liquid - liquid mixing is the combination of liquid meal with gastric secretions[113].

In the solid – solid mixing, the mixing would be forcefully driven by antral contraction. However, the other types mixing (solid-liquid and liquid – liquid mixing types) would be influenced by both the external forces and diffusion. The diffusion during the mixing process may play a limited role in the distal part of the stomach due to the antral movements, however, the diffusion may play primary role during the mixing in the proximal part of stomach because the meal may stay longer in this part of stomach and experience only tonic contractions[113].

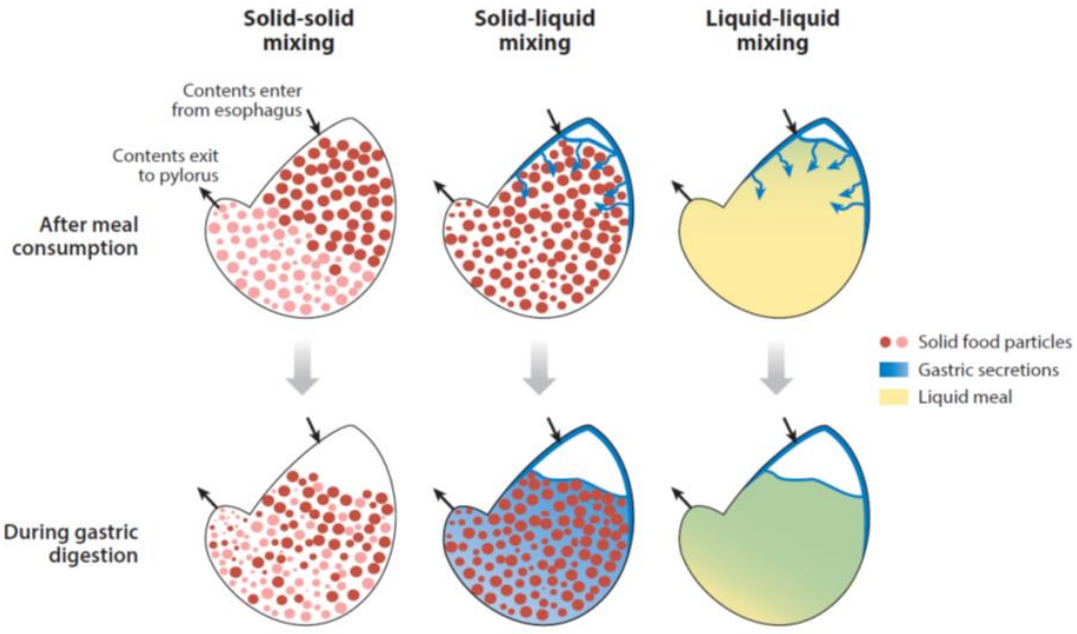

Figure 7: Diagrammatic representation of solid-solid, solid-liquid, and liquid-liquid mixing processes during food digestion. The representations shown here are for example only; these processes

vary on the basis of meal properties and rates of gastric secretion, emptying, and motility. This diagram authored by [113]

The applications of MRI methods to study the motion of gastric contents have been so far very limited. Previous research has used a MRI tagging technique to look at the gastric motion after consumption of porridge meal[164]. The tagging method will be explained in more detail in Chapter 4. Briefly, use of the tagged MR imaging method here was chosen because it provides an easy-to-interpret 'motion map' that can be used to assess the movement happening inside an organ or a sample. The method is based on a spatial modulation of the magnetization that is currently widely applied in cardiac imaging[165] but is relatively new in the gastrointestinal field. Other MRI techniques that have been proposed to study the fate of gastrointestinal contents such as relaxometry [154, 155] do not capture the motion of the gastrointestinal chime and therefore were not used here.

There is clearly synergy between this kind of MRI capability and the interests of this project to better understand gastric handling of breakfast porridges hence the project aims to use the tagging method to look at mixing of breakfast porridges in the stomach.

Small bowel water content (SBWC)

The secretion and absorption of small bowel water play a key role in the digestion of nutrients. The chyme which leaves the stomach contains protein, fat, sugar and starch. In the small intestine, the chime is also

digested by pancreatic enzymes and bile to break it down to osmotically active small molecules, which are then absorbed. Up to 2 to 3 litres of combined fluid secretions (salivary, gastric and pancreatic fluid) flow into the small intestine each day, in addition to any water ingested[166].

The MRI provides a unique tool to assess the fluid volumes in the small bowel as it is non-invasive tool, has no ionizing nature and has good sensitivity to mobile or free water, which is water not mixed with intestinal chyme[166-168]. My contemporary review of this field is now published in Neurogastroenterology and Motility[169].

The small intestinal secretory and fluid response to breakfast porridges is unknown, there are particulates that could stimulate secretions like bran does[167], and hence these measurement methods will be considered too in this project

1.8 Aims and hypothesis

Based on the background knowledge and literature summarised above, little is known about possible differences in glucose responses, relationship with gastric emptying and appetite in response to breakfast porridges made from different grains. Of particular interest to this study are recent suggestions that different grains, and particularly millet grains, may have positive health benefits with respect to glucose and insulin metabolism.

The hypothesis underpinning this proposed work is therefore that breakfast porridges made from different cereal grains may produce

different postprandial glucose levels and that these will be associated with gastric emptying and appetite in healthy volunteer participants

The aims of the project are therefore to carry out three studies. Namely:

1. To investigate the glycaemic, gastrointestinal and satiety responses to breakfast porridge made from different grains
2. To carry out an in-depth study of the physiological responses to the best performer from the pilot study using also the techniques developed in the developmental study.
3. To investigate the feasibility of using a continuously tagged magnetic resonance imaging sequence to monitor and assess gastric handling (such as mixing) of breakfast porridge meals in healthy subjects.

This work is relevant as an improved knowledge of the physiological and appetite responses to breakfast porridges could lead to more evidence based dietary advice. The breakfast porridge intervention is based on relatively cheap and sustainable grains and could help reduce the burden of obesity and related metabolic disorders worldwide.

1.9 Thesis outline

The work described in this thesis was conducted at the Sir Peter Mansfield Imaging Centre located at the University of Nottingham from October 2015 to September 2018. The research described in this thesis is original, unless otherwise stated.

The layout and content of the thesis chapters is as follows.

Chapter 1: This chapter contains the introduction of the thesis.

Chapter 2: This chapter describes a pilot study which investigates the glycaemic, gastrointestinal and appetite responses to breakfast porridges from ancient cereal grains (AMORE study).

Chapter 3: This chapter contains the main study that was designed based on the best performer from the pilot study using also the techniques developed in the developmental study The main study investigates the glycaemic, gastrointestinal, hormonal and appetitive responses to pearl millet and oats porridge breakfast: a randomized, crossover trial in healthy humans (MOM study). This study was designed based on the experience gained from the pilot study (AMORE)

Chapter 4: This chapter describes a MRI developmental study aims to enable assessment of the motion of gastric contents in healthy subjects using MR tagged imaging.

Chapter 5: This chapter contains an overall conclusion, and discussion of the work carried out. It also describes the research impact of the project and an outlook on possible future work.

2. Assessment of Millet, Oat and Rye Porridge Breakfasts Glucose and Gastric Emptying (AMORE)

2.1 Introduction

It has recently been suggested that different cereal grains, particularly millet grains, may have enhanced health benefits in terms of glucose and insulin metabolism[74, 81, 101]. However, limited studies have been conducted to investigate the physiological and gastrointestinal responses to these grains, particularly the blood glucose and appetite responses to the millets.

Magnetic resonance imaging (MRI) provides a unique tool to investigate gastrointestinal handling of food[169]. Furthermore, the small intestinal secretory and fluid response to breakfast porridges is unknown. After milling, intact particles can exert effects through mechanical stimulation of the small intestine, as we showed using bran and plastic particles[168], and MRI provides a non-invasive means to assess gastrointestinal fluid responses[167].

2.1.1 Hypothesis

We hypothesised that breakfast porridges made from different varieties of cereal grains would produce different postprandial glucose responses, gastric volumes and subjective appetite scores in healthy participants.

2.1.2 Aims

This pilot study was therefore designed to collect initial data on postprandial glucose levels following consumption of isoenergetic

breakfast porridges made from finger millet, pearl millet oats and rye. Secondly, the study aimed to compare postprandial gastric volumes, small bowel water content and subjective appetite for these meals. It also aimed to explore possible correlations between blood glucose levels, gastric volumes, and subjective appetite outcomes.

2.2 Methodology

2.2.1 Population

Participants were recruited from the student and staff population of the University of Nottingham via a poster advertisement. This was a pilot study and we did not have own data to speculate on the sample size required. Within the study period a total of 17 healthy participants were screened. One did not attend screening so 16 healthy subjects (ten female and six male, 20.9 (SD 0.9) years old, BMI 22.1 (SD 2.9) kg/m^2 participated. A full dataset for all four meals was obtained for seven participants who were then included in subsequent analysis. This included four females and three males, aged 21 (SD 1) years old, and with a BMI of 22 (SD 2) kg/m^2.

A subgroup analysis was also considered for ten participants who consumed all of the SOP, RP and PMP meals. The remaining nine participants were excluded from the analysis either because they did not attend visits or because they were unable to consume all of the test meal on one or more visits (Figure 8).

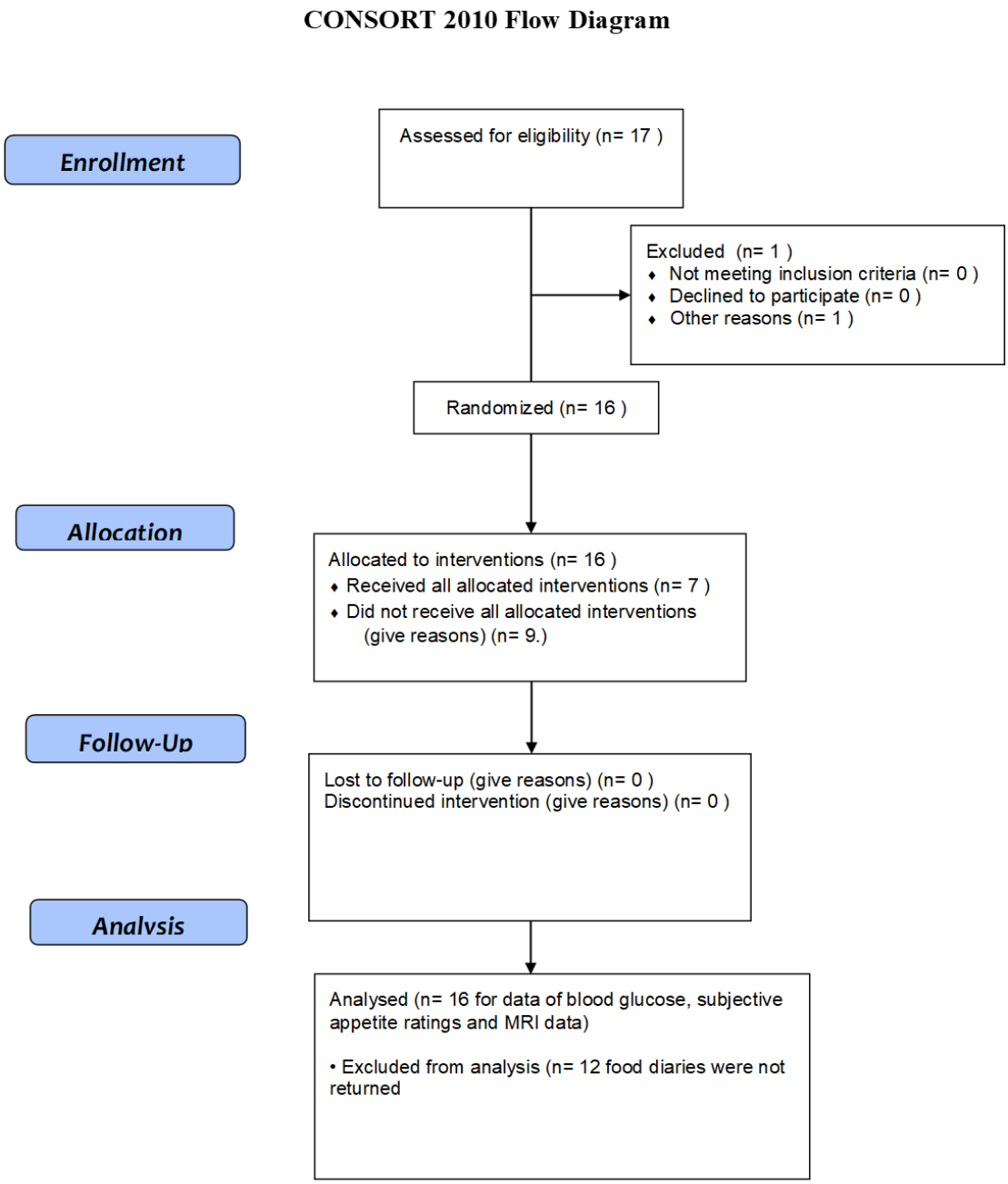

Figure 8: Study participant flow diagram.

Eligibility

Those who expressed interest were invited to a screening session to establish whether they met the study inclusion criteria.

Inclusion criteria:

- Age 18 - 65 years.
- Healthy volunteer, male or female.
- BMI ≥ 18 and ≤30 kg/m^2.
- Able to give informed written consent.

Exclusion criteria:

- Using medication which interferes with study measurements.
- Participating in another nutritional or biomedical trial three months before the pre-study examination or during the study.
- Not being a habitual breakfast consumer.
- Not usually eating at least three meals a day.
- Reporting participation in night shift (between midnight and 6.00 am).
- Doing strenuous exercise for >10 h/week.
- Consuming of ≥21 alcoholic drinks in a typical week.
- Following a medically or self-prescribed diet during the two weeks prior to the pre-study examination and until the end of the study.
- Contraindications for MRI scanning (e.g. presence of metal implants, infusion pumps and pacemakers) as assessed by standard MRI safety questionnaire.
- Pregnancy.

- Inability to lie flat and exceeding the scanner bed weight limit of 120kg.

The study was conducted at the Sir Peter Mansfield Imaging Centre at the University of Nottingham. Informed written consent was obtained from each participant before the trial. A site master file and case report forms were kept according to good clinical practice.

All procedures in this study involving human participants were approved by the University of Nottingham, Medical School Research Ethics Committee (F14072015). The study was registered within Clinical Trials.gov (NCT02653274). The trial registration name was ‘Assessment of Millet, Oat and Rye Porridge Breakfasts Glucose and Gastric Emptying (AMORE)’.

2.2.2 Study design

This study was randomized, four way crossover design. Participants attended the laboratory on four separate days, approximately 1 week apart, in order to consume four different porridge breakfasts in a randomized order. Participants consumed their habitual diet between each visit. Each visit lasted from 8:00 am until approximately 1:00 pm. The porridge meals differed in appearance and taste hence participants could not be blind although they were not informed which porridge they were consuming on each visit.

2.2.3 Screening

All potential participants attended a screening visit to establish that they met the study inclusion criteria for the study. Height and weight were measured and the Body Mass Index (BMI) was calculated as weight divided by the square of height.

2.2.4 Laboratory visit protocol and procedures

Figure 9 shows the study day protocol. The participants were asked to fast overnight (for at least ten hours). A glass of water was permitted on waking. On arrival they completed the study eligibility check questionnaire to ensure adherence to the study day restrictions such as the overnight fasting. Baseline measurements (defined as t = 0 min) were then made. These included fasting blood glucose, participant completion of paper based subjective visual analogue appetite score (VAS, described below) and a MRI scan. The participants were then requested to eat the given porridge within a maximum time of 15 min. This was followed by an immediate postprandial (defined as t = 20 min) measurement of blood glucose, followed by VAS completion and a MRI scan. Blood glucose was subsequently measured every 15 min until t = 60 min, then every 20 min until t = 120 min, followed on each occasion by completion of VAS. MRI scans were undertaken hourly from t = 20 min up to t = 140 min. Participants were given a blank food diary and instructed to complete it over the remainder of the day.

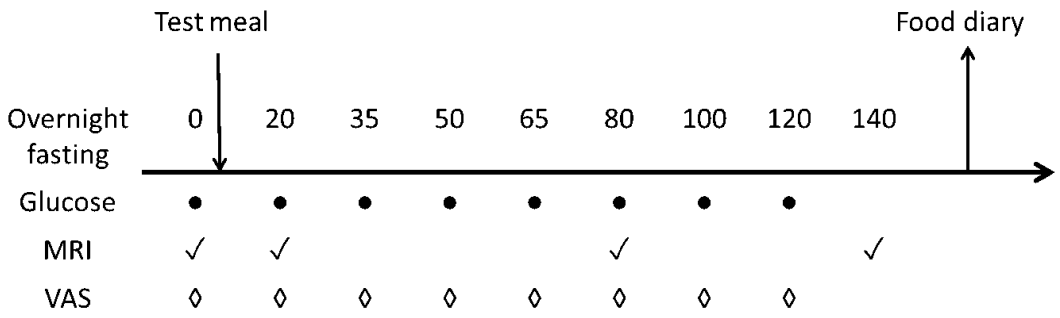

Figure 9: Diagram of the study day protocol

2.2.5 Breakfast porridge intervention

Four breakfast porridges were made from Scottish oats (Asda, United Kingdom, Figure 10.A), rye (buywholefoodsonline.co.uk, Canterbury, United Kingdom, Figure 10.B), finger millet (Top-Op Foods Ltd., Stanmore, United Kingdom, Figure 10.C) and pearl millet (Herbs n Spice it, India, Figure 10.D).

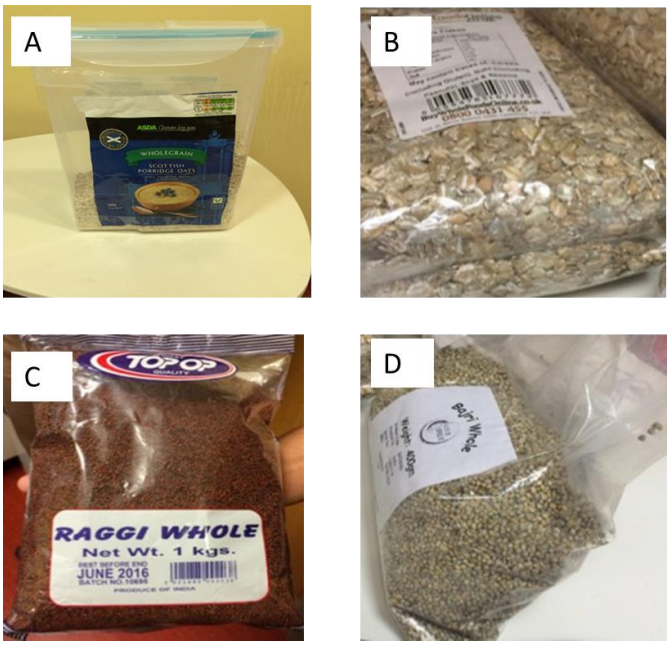

Figure 10: The oats (A), rye (B), finger millet (C) and pearl millet (D) grains used in the study

The oats and rye were steam rolled flakes. Rye flakes were larger than the oat flakes. The millets were plain dehulled grains ground to a flour using a spice grinder in our lab. The cooked products were analysed for macronutrient composition and total energy by Campden BRI, Chipping Campden, Gloucestershire, UK (Table 4) so that the four breakfast meals given to the participant could be made isoenergetic (220 kcal each).

Table 4: Macronutrient composition of the breakfast meals. The values are shown total for each cooked product as served.

	SOP	RP	FMP	PMP
Weight (g)	400	297	432	311
Energy (kJ)	920.0	920.0	920.0	920.0
Energy (kcal)	220.0	220.0	220.0	220.0
Protein (Kjeldahl, g)	7.2	4.2	3.5	5.3
Total carbohydrate (by difference, g)	42.0	49.3	53.1	45.6
Carbohydrate(Avail, g)	34.0	39.8	43.6	38.8
Total sugars (enzymic, g)	1.6	5.9	1.7	0.3
Fructose (enzymic, g)	0.4	0.3	0.4	0.3
Glucose (enzymic, g)	0.8	0.3	0.4	0.3
Maltose (enzymic, g)	0.4	0.3	0.4	0.3
Sucrose (enzymic, g)	0.8	5.9	1.7	0.3
Fat (Weibull-Stoldt, g)	4.4	2.7	1.3	3.1
Saturates (g)	0.8	0.6	0.4	0.6
MUFA (cis, g)	2.0	0.6	0.4	0.9
PUFA (cis)	1.2	1.2	0.4	1.2
Trans fatty acids (g)	0.4	0.3	0.4	0.3
Total fiber (AOAC, g)	8.0	6.5	13.8	6.8
Sodium(ICP-MS)	24.4	17.8	28.5	19.5
Moisture (Oven102°C)	345.2	240.0	372.4	255.6
Ash(@525C)	1.2	1.1	1.4	1.0
Protein N Factor	6.25	6.25	6.25	6.25
Equivalent Salt	0.4	0.3	0.4	0.3

The grains were cooked in water in two separate aliquots using two microwaves (900 watts, Figure 11).

Figure 11: The two microwaves used in this study

The aim was to achieve an acceptable final product hence the grains were subjected to different preparation protocols. The oats were simply heated with water, the rye was soaked for half an hour in boiled water then heated; the millets were ground prior to cooking using a spice grinder for 30 s. The study meals were consumed with 240 ml of water in a glass and on each of the four occasions, the participants were asked to consume the entire portion with the water drink within 15 min. Other meal characteristics such as, volume and weight necessarily differed between meals SOP 400 g; RP 297 g; FMP 432 g; PMP 310 g). The appearance of the porridge breakfasts was also different (SOP Figure 12.A, RP Figure 12.B; FMP Figure 12.C; PMP Figure 12.D)

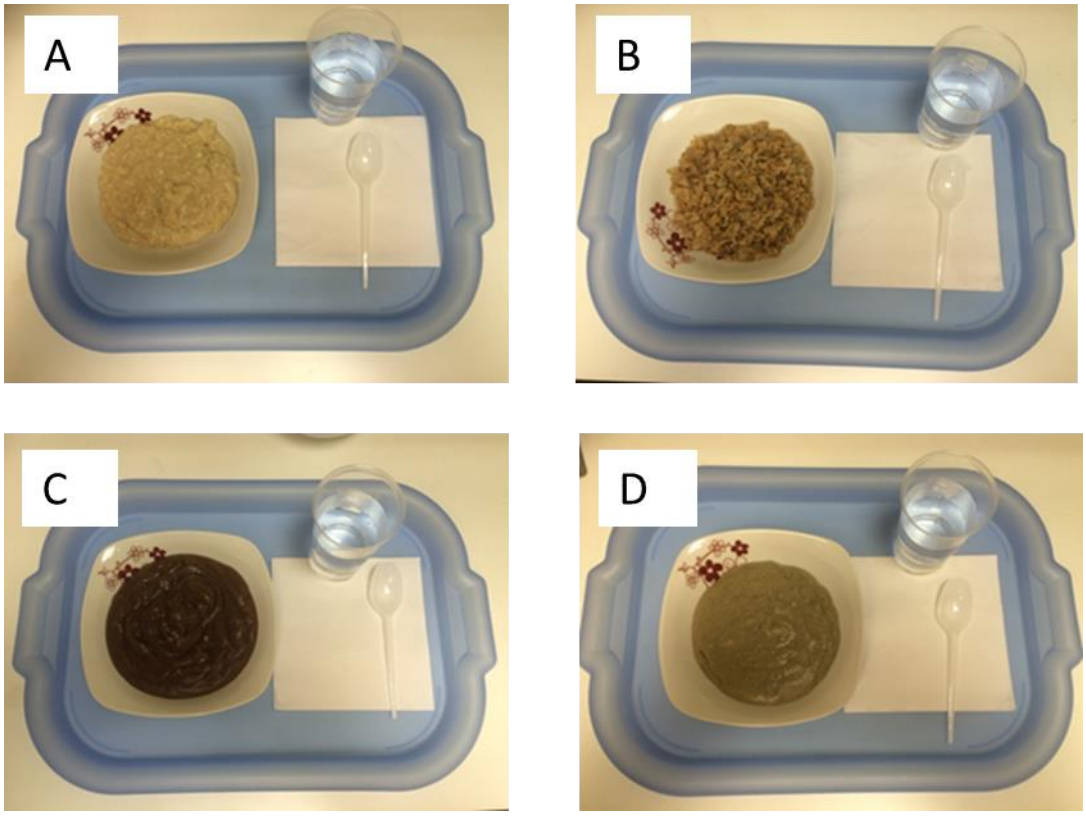

Figure 12: Scottish oats porridge (A), rye porridge (B), finger millet porridge (C), pearl Millet porridge (D).

2.3 Outcomes

2.3.1 Gastrointestinal response measured by MRI

Magnetic resonance imaging (MRI) was carried out on a research-dedicated 1.5 T Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands, Figure 13). Participants were in the supine position with a 16 element receiver coil wrapped around their abdomen. Gastric volume was measured using a balanced turbo field echo (bTFE) sequence. A total of 25 axial images were acquired with the following sequence parameters: the field of view (FOV) 400 mm ×320 mm ×250 mm, acquired resolution 2.01 ×1.76 mm^2, slice thickness 10 mm, repetition time (TR) 2.8 ms, echo time (TE) 1.4 ms, no slice gap, flip angle

(FA) 80^{o} and one breath hold for 10 s. Gastric volume was measured manually by one operator using Analyze9 software (Mayo Foundation, Rochester, MN, USA).

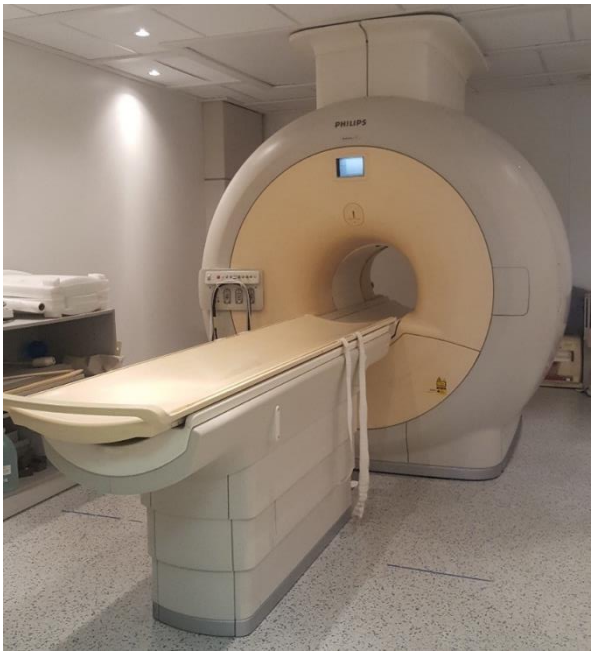

Figure 13: The 1.5T Philips Achieva MRI scanner at the Sir Peter Mansfield Imaging Centre (SPMIC) at the University Park Campus, University of Nottingham.

The water content of the entire small bowel (SBWC) was measured using a single–shot, fast spin echo sequence (rapid acquisition with relaxation enhancement), which shows high intensity signals from areas with free mobile fluid and dark signals from other body tissues. A total of 24 coronal images were obtained using the following sequence parameters: FOV 400 mm × 400 mm, acquired resolution 0.78 × 0.78 mm^2, slice thickness 7 mm, TR 8000 ms, TE 320 ms, no slice gap, and one breath hold for 24 s.

The SBWC was assessed using in-house software which was previously validated[166]. Briefly, bright signals from organs other than small bowel water (e.g. stomach, gall bladder) were segmented out manually, and then integrating total volume over pixels with intensity values above the calculated threshold. The total AUC for gastric volume and for small bowel water were calculated.

2.3.2 Glycaemic response

The glycaemic response was measured in capillary blood samples[170] using the protocol described by Brouns et al[132] which is in line with techniques recommended by the World Health Organization (WHO) / Food and Agricultural Organization (FAO 1998). The capillary blood samples were collected by finger prick using single-use lancets (Unistix Owen Mumford, Oxfordshire, United Kingdom). The capillary blood glucose was measured using Accu-check (Roche Diagnostics, USA, Figure 14).

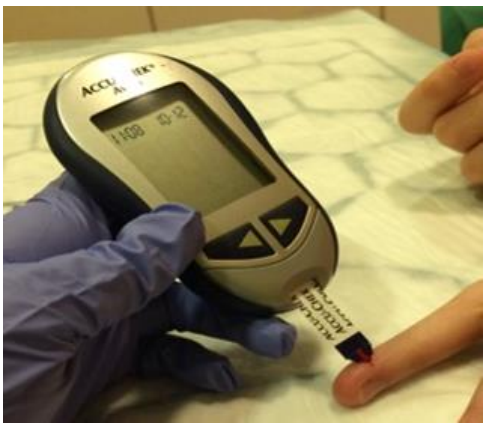

Figure 14: Blood glucose monitor used in this study

Participants were requested to warm their hands before the finger prick in order to increase the blood flow. To extract the blood, the fingertips were gently massaged from the base of the hand, moving towards the tips in order to minimise the plasma dilution. Incremental area under the glucose curve (iAUC) and peak blood glucose response to the test products were calculated according to Brouns et al[132] Wolever and Jenkins [171]. iAUC was obtained using the trapezoid rule and ignoring the area beneath the baseline.

2.3.3 Subjective appetite ratings

100 mm VAS were used to measure the subjective feelings of hunger, satisfaction, fullness, desire to eat and prospective food consumption[172]. When outside the MRI scanner, the participants were requested to make a vertical mark on each scale at the point that best matched how they felt at that time. Each end of the line was anchored by statements expressing the extreme for the sensation. For example, 'not hungry at all' and 'more hungry than have ever been'. To avoid bias from previous answers the participants were presented only with a new VAS sheet at each time point and this was removed immediately after completion. The VAS appetite ratings were determined by measuring (in millimetres) the distance from the left side of the line to the vertical mark.

The average Appetite score was calculated for each individual at each time of measurement, for each test meal, using the formula:

Average appetite score = [hunger + (100 – satisfaction) + (100 – fullness) + desire to eat + prospective consumption]/5[173, 174]. The Average

Appetite scores at each time point were used for the statistical analysis. The range for the appetite score was between 0 and 100 with 0 representing the minimum appetite sensations and 100 representing the maximum appetite sensations. Total AUC for average appetite score were calculated[71].

2.3.4 Food diaries

Food diaries were given to the participants before discharge. They were requested to provide a detailed record of food and beverages consumed over the remainder of the day, once they had left the unit. They were required to include information such as portion sizes, product brand names, and cooking and preparation methods. Furthermore, if the participants prepared composite dishes at home, then they were requested to provide the recipe and portion size.

Nutritics software (Nutritics Ltd, Dublin, Ireland) was used to analyse the food intake from the food diaries. Some food items were added manually to the database using the information on nutrition labels.

2.3.5 Sample size and statistical analysis

Descriptive and statistical analyses were undertaken using Prism version 6.07 (Graph Pad Software Inc., La Jolla, CA). All data are presented as mean ± SEM unless otherwise indicated. Data were assessed for normality using the Shapiro-Wilk's test. Normally distributed data were analysed using parametric methods; non-normally distributed data were analysed using non-parametric methods.

Differences in glycaemic response, gastric volume, SBWC and appetite score were assessed using one-way repeated-measures analysis of variance followed by Tukey's post hoc test.

Correlations between blood glucose, gastric volume and appetite scores were assessed using Pearson's correlation. Differences were considered significantly different at $P < 0.05$.

2.4 Results

In this pilot study, the effects of different porridges on postprandial glycaemic response, gastrointestinal response (gastric volume and SBWC) and subjective appetite were measured.

Seven participants failed to consume all of the finger millet test meal hence they were excluded from the per protocol analysis. When asked, palatability was reported as the main problem. Two more subjects did not attend one of the study session hence were also excluded. The results presented are thus shown as per protocol analysis with n = 7 (four females and three males of normal weight) who consumed all of the four study meals (Table 5). Additionally, a subgroup analysis (n = 10 for SOP, RP and PMP) was considered in this pilot study (Table 6).

Table 5: Blood glucose, time to peak, gastric volumes, small bowel water content and average appetite sensations measured from n = 7 healthy participants who were fed four different breakfast porridges (Mean values with their standards errors) n = 7

	SOP		RP		FMP		PMP		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	1-way ANOVA
IAUC glycaemic response mmol/min (over 120 min)	131	28	119	27	145	23	110	29	0.5
Glucose peak, mmol/l	7.2	0.3	7.2	0.5	7.7	0.4	6.7	0.3	0.2
Gastric volume at T = 20	505	26	384	22	548	48	532	23	0.007
AUC Gastric volume ml/min	50324	2696	41644	2892	56606	3832	58684	3339	0.003
AUC small bowel water content ml/min	1611	429	1303	360	735	259	2157	499	0.06

Table 6: Sub-analysis for blood glucose, time to peak, gastric volumes, small bowel water content and average appetite sensations measured from n = 10 healthy participants who were fed three different breakfast porridges. (Mean values with their standards errors) n = 10.

	SOP		RP		PMP		
	Mean	SEM	Mean	SEM	Mean	SEM	1-way ANOVA
IAUC glycaemic response mmol / L (over 120 min)	134	27	102	21	107	21	0.6
Glucose peak, mmol / L	7.1	0.2	7.0	0.4	7.0	0.2	0.7
Gastric volume at t = 20	535	23	407	29	544	17	0.0008
AUC Gastric volume mL / min	41519	1978	34751	2249	59454	2499	0.0001
AUC small bowel water content mL / min	1302	255	1123	332	1713	275	0.18

2.4.1 Appearance of the gastric content and total gastric volumes

Figure 15 shows the appearance of the gastric content for each of the porridges immediately after consumption (t = 20 min). With SOP, two layers can be seen in the stomach, a bottom layer providing a lower signal (appearing darker in the figure) and a top layer providing higher signal (appearing brighter in the figure), whilst RP remains in a distributed form in the stomach. FMP and PMP produced multiple layers in the stomach.

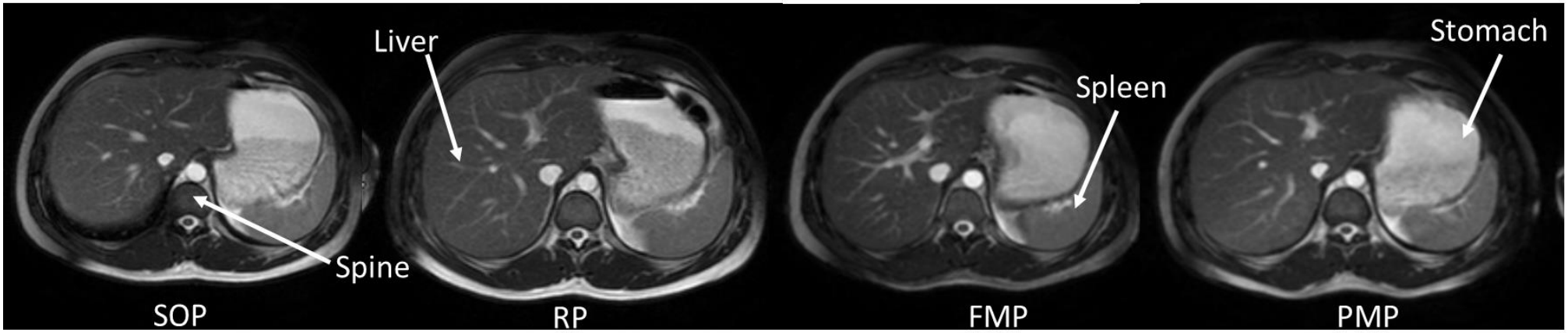

Figure 15: Representative example of axial MRI images of the abdomen of a healthy participant fed with , Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP); Pearl millet porridge (PMP) on four different occasions. Images were taken at t = 20 min.

There was no significant difference in fasting baseline gastric volumes between the test days as expected. Gastric volumes rose on consumption of the porridges and declined with time as shown in Figure 16.

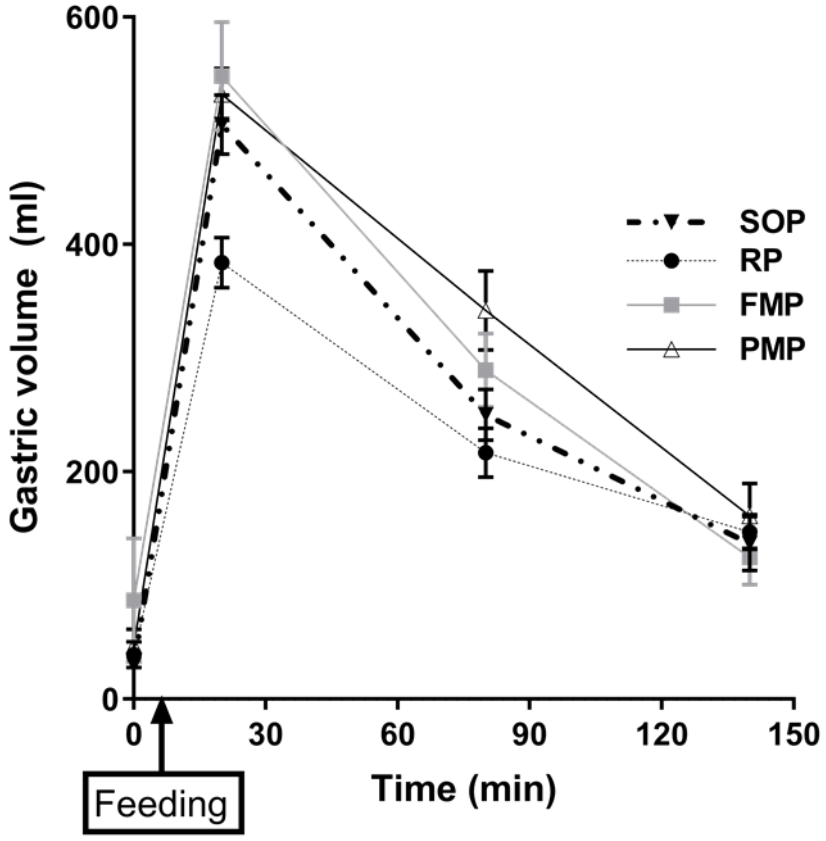

Figure 16: Plot of the volume of the gastric contents for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP); Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

The immediate postprandial gastric volumes (t = 20 min) were significantly different between the four breakfast meals (*P* = 0.007) in keeping with the initial meal volume differences. Overall AUC 2 h gastric volumes showed a significant difference between the study meals (*P* =

0.003). There was a significant difference in gastric volume AUC between the RP and FMP (P = 0.04) and a difference in gastric volume AUC between the RP and the PMP (P = 0.002). The subgroup analysis (n = 10) also showed significant difference immediate postprandial gastric volumes (20 min) and AUC gastric volumes between the test meals as shown in Table 4.

2.4.2 Small bowel water content

SBWC data are shown in Figure 17; the mean fasted SBWC was 23.1 ml (SD 6.4 mL) for the four study porridges. All the meals induced an initial drop in SBWC after feeding followed by a rise at t = 80 min, but the differences were not statistically different. There was a significant difference in the SBWC between the meals (P = 0.009).

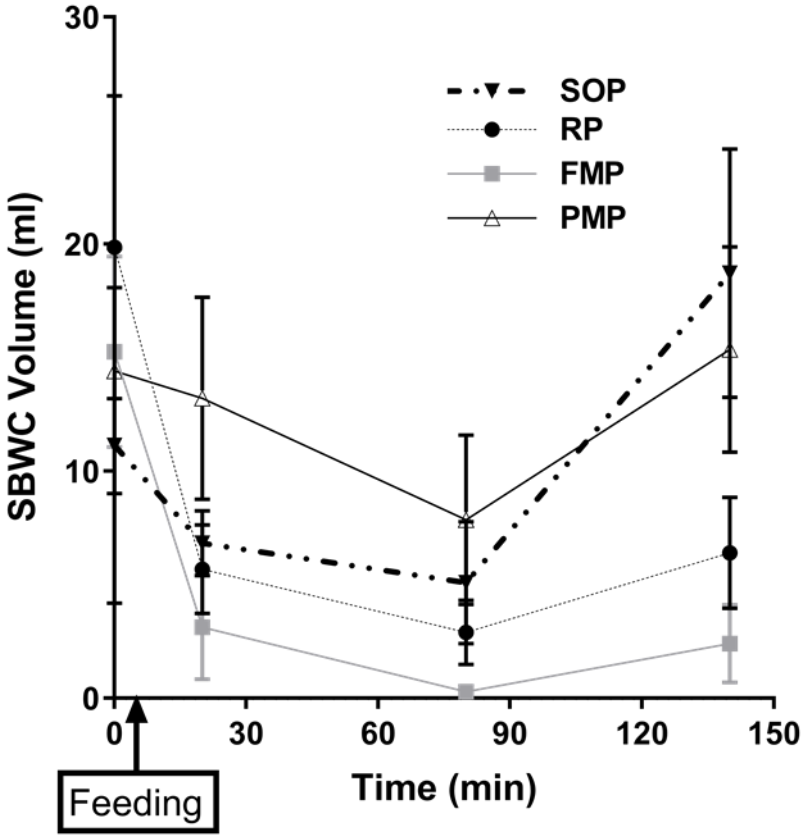

Figure 17: Plot of the volume of the small bowel water content (SBWC) for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP); Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

2.4.3 Glycaemic response

The glycaemic responses to all the porridges were in the normal range for non-diabetic subjects. There were no differences in fasting glucose values, and the expected post consumption increase in blood glucose was seen in all cases. Figure 18 shows the iAUC for all participants. The highest mean peak glucose was measured following FMP at 7.8 mmol/mL compared with following SOP, RP and PMP at 7.1 mmol / mL,

6.8 mmol / mL and 6.9 mmol / mL respectively. For the n = 7 analysis, glucose iAUC 0-2 h was also the lowest after PMP (109.6 mmol / L 120 min) compared with following SOP, RP and FMP (131.1 mmol / L 120 min, 119.5 mmol / L 120 min and 145.4 mmol / L 120 min respectively. These differences were not statistically significant.

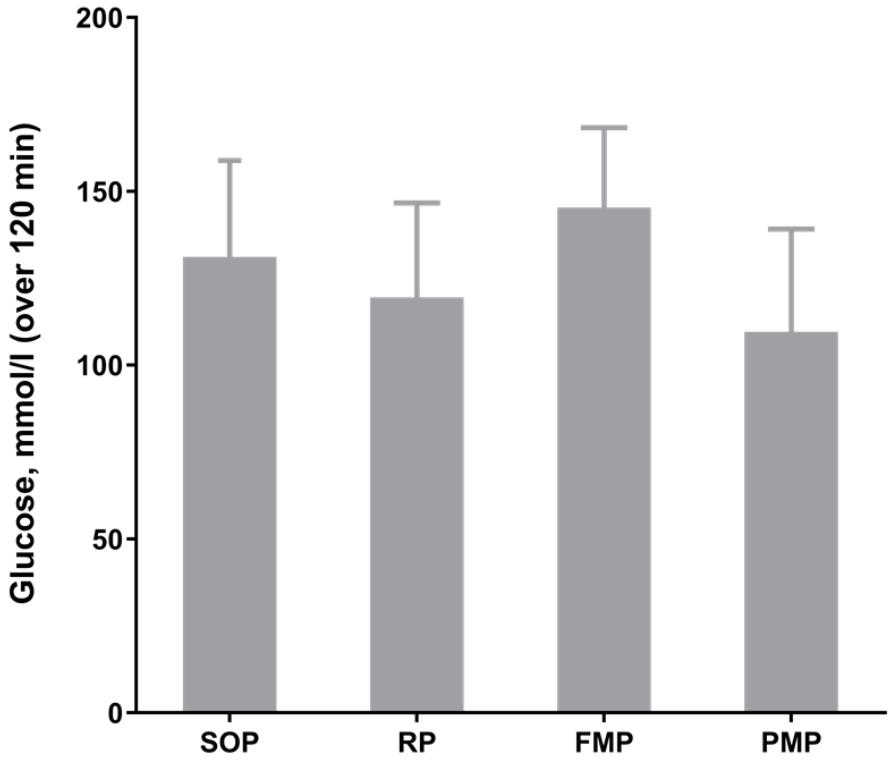

Figure 18: Incremental area under the glucose curve (iAUC) for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP); Pearl millet porridge (PMP). Values are mean ± SE, n=7.

The n = 10 subgroup analysis showed that PMP and RP had similar peak blood glucose level at 7.0 mmol /mL, whereas peak blood glucose of SOP was 7.1 mmol / mL. There was no significant difference in IAUC glucose between the test meals (Table 4).

2.4.4 Subjective appetite ratings

The areas under the curves of the subjective appetite ratings are summarized in Table 5 and 6 the impact of the four breakfast porridges on the scores for hunger, satisfaction and average appetite are shown in Figures 20 – 24. As expected, for all interventions, the scores for hunger, desire to eat and prospective food consumption decreased after consuming the breakfast before returning to baseline, whilst fullness and satisfaction initially decreased and then increased again in all cases. The AUC for the sense of hunger of the subgroup analysis showed a significant difference between the test meals (P = 0.017, Table 8). The average appetite score was the lowest the after consuming the millet (Figure 19), but the AUC for this score was not significantly different between the three porridges (Table 7).

Table 7: Participants' (n = 7) area under the satiety curve from the visual analogue scales for hunger, satisfaction ,fullness, desire to eat, prospective food consumption and average appetite score. (Mean values with their standards errors) n = 7.

	SOP		RP		FMP		PMP		
Appetite sensations variables	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	1-ANOVA
Hunger (mm/min)	6325	461	7717	636	5378	1196	5465	1030	0.08
Satisfaction (mm/min)	6877	537	5920	716	8636	1164	7849	917	0.08
Fullness (mm/min)	6881	580	6149	754	8385	1117	8238	1102	0.3
Desire to eat (mm/min)	6776	526	7643	832	5549	1349	5992	1003	0.5
Prospective food consumption (mm/min)	7092	502	7984	788	5618	1359	5938	1067	0.3
Average appetite sensations	6887	463	7855	710	5505	1220	5862	991	0.5

Table 8:Participants' (n = 10) area under the satiety curve from the visual analogy scales for hunger, satisfaction, fullness, desire to eat, prospective food consumption and average appetite score. (Mean values with their standards errors) n = 10

	SOP		RP		PMP		
Appetite sensations variables	Mean	SEM	Mean	SEM	Mean	SEM	1-way ANOVA
Hunger (mm/min)	5274	673	6606	770	4996	842	0.01
Satisfaction (mm/min)	7989	695	7062	785	8121	672	0.22
Fullness (mm/min)	7976	733	7235	799	8502	811	0.43
Desire to eat (mm/min)	5737	688	6535	829	5538	835	0.18
Prospective food consumption (mm/min)	6131	690	6836	833	5498	887	0.36
Average appetite sensations	5835	672	6736	786	5482	782	0.22

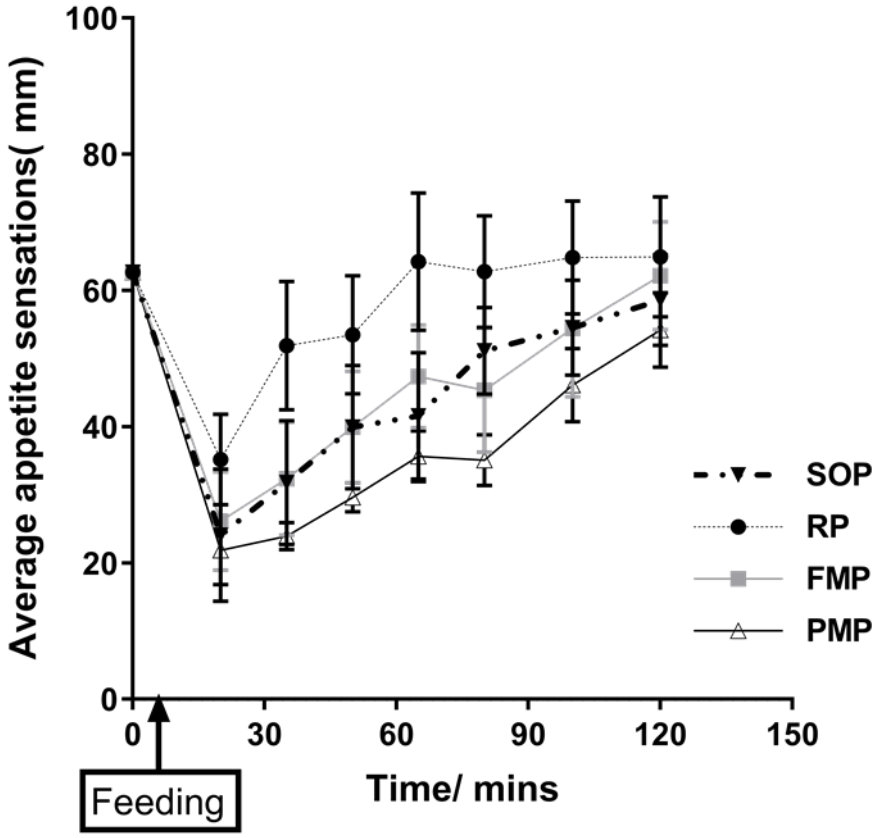

Figure 19: Plot of the average appetite sensations for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP) and Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

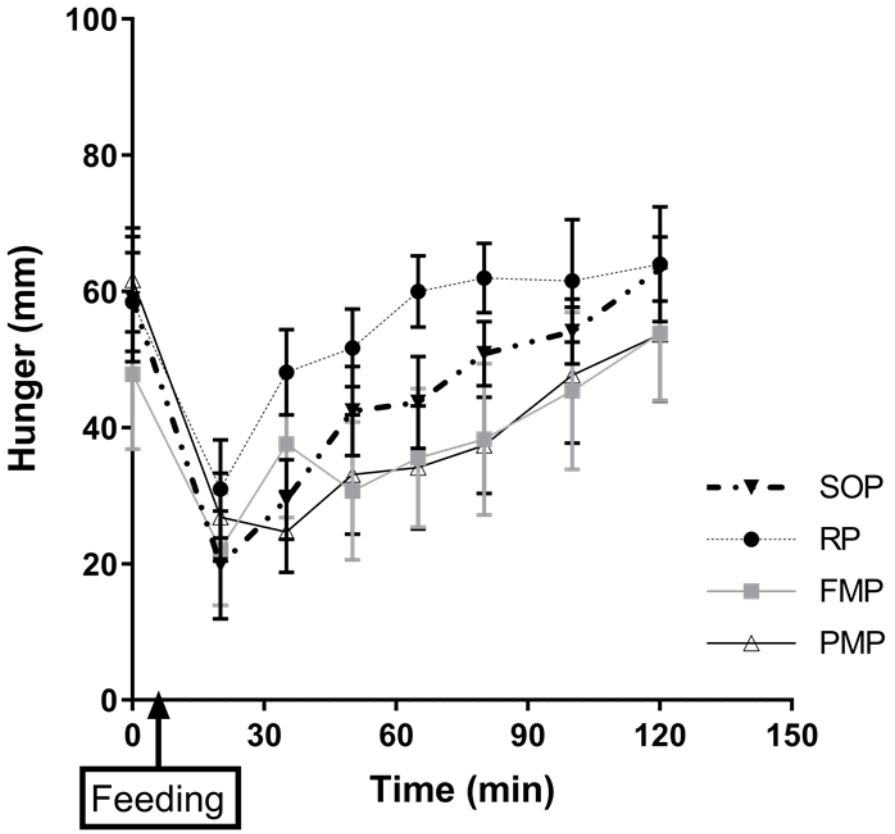

Figure 20: Plot of the hunger for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP) and Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

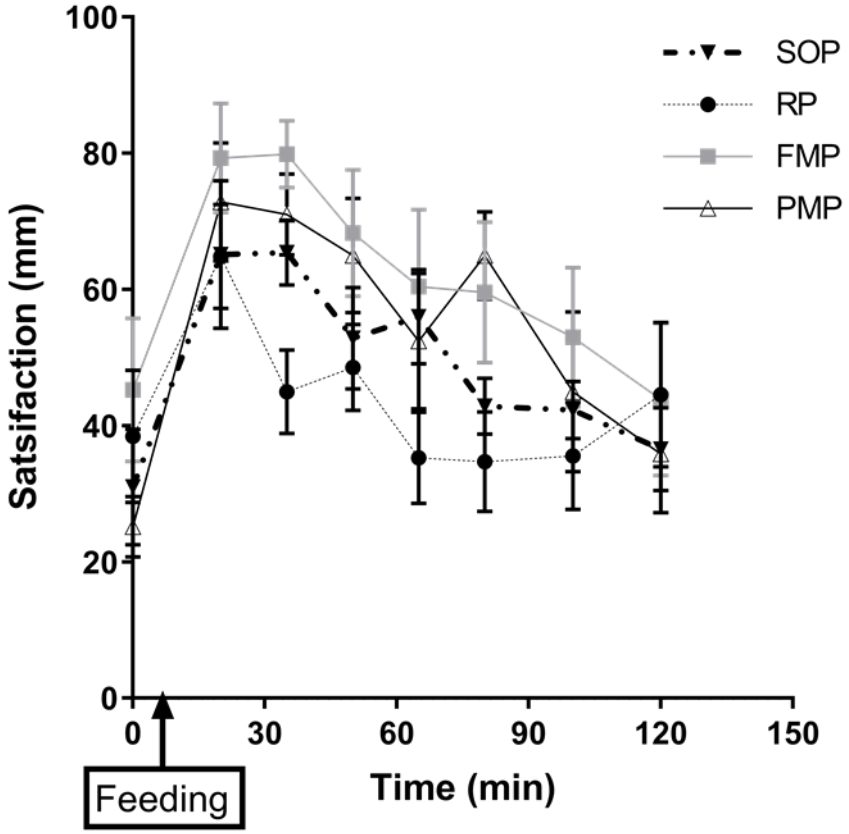

Figure 21: Plot of the satisfaction for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP) and Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

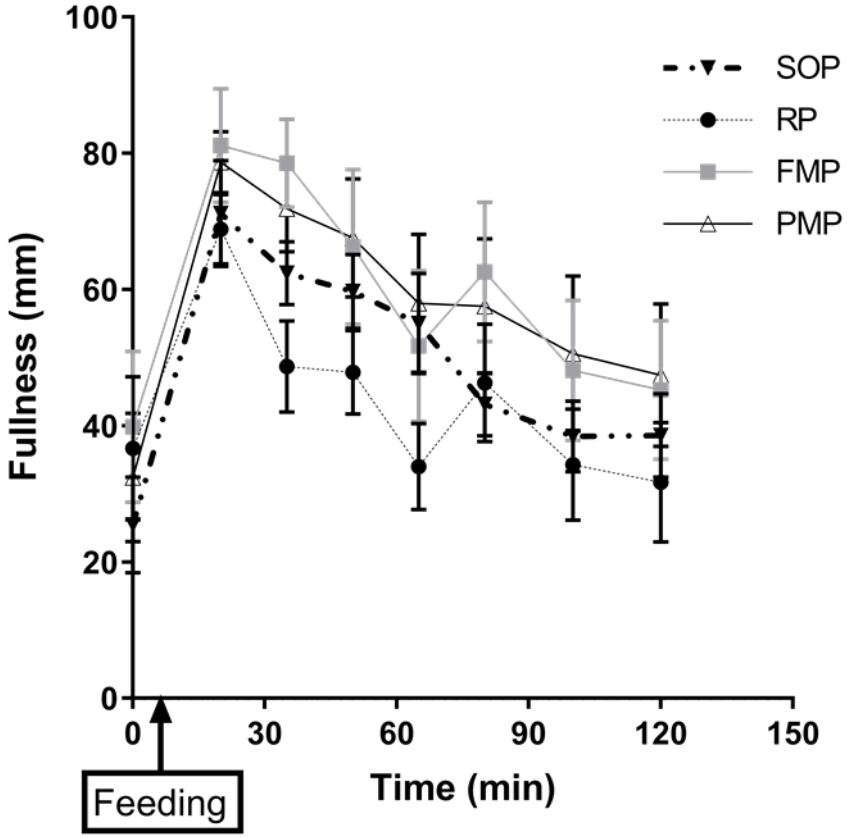

Figure 22: Plot of fullness for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP); Finger millet porridge (FMP) and Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

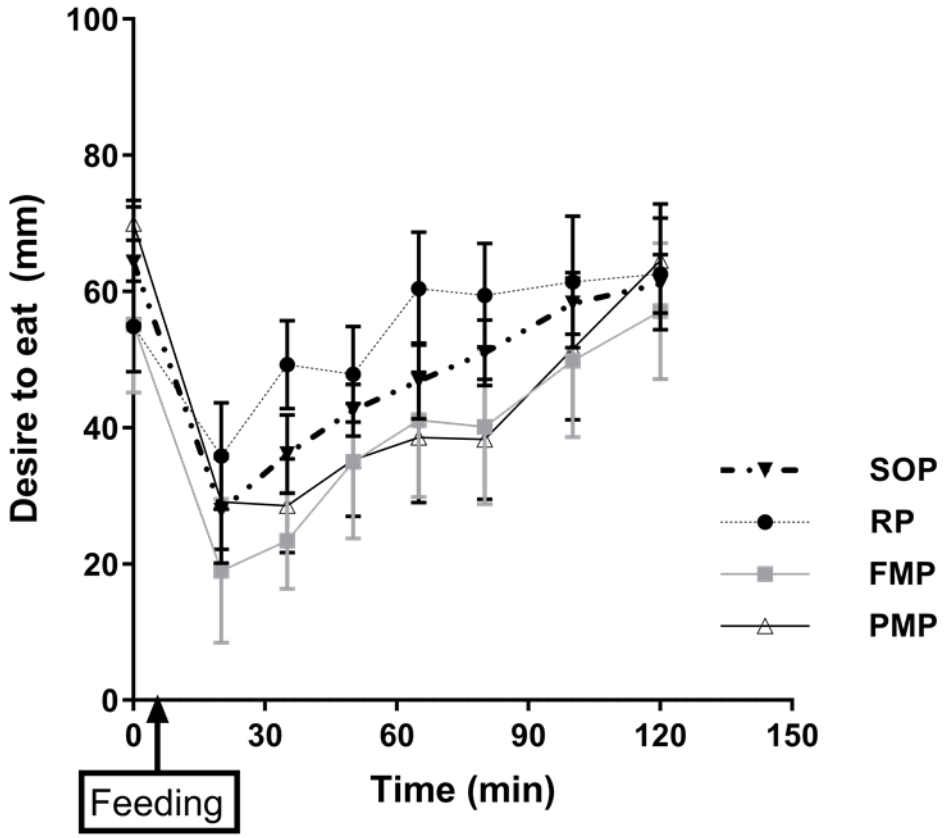

Figure 23: Plot of desire to eat for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP), Rye porridge (RP), Finger millet porridge (FMP), Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

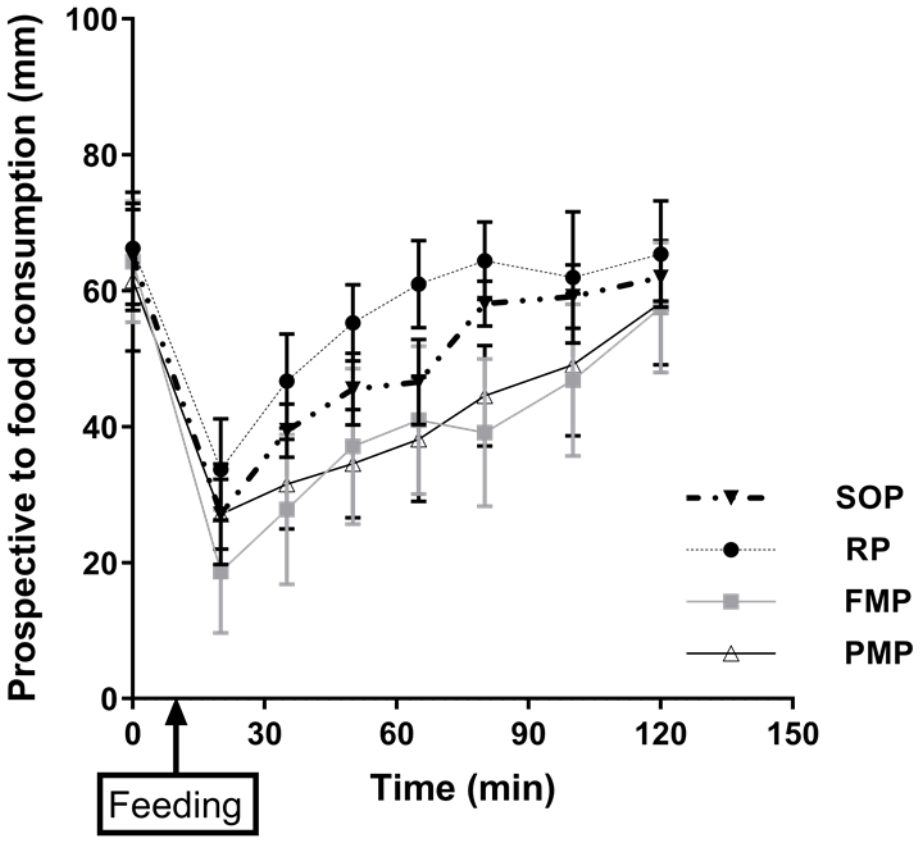

Figure 24: Plot of prospective food consumption for healthy participants after they consumed the four different study porridges. Scottish oats porridge (SOP); Rye porridge (RP), Finger millet porridge (FMP), Pearl millet porridge (PMP). Values are mean ± SE, n=7. The arrow on the horizontal axis indicates the meal start time.

2.4.5 Food intake record

Four food diaries were not returned (1 for SOP, 2 for RP and 1 for FMP) so these data cannot be presented as per protocol. Data are presented as mean and standard error of the mean. The self-reported daily energy intake records following consumption of the SOP, RP, FMP and PMP were 1747 ± 158 kcal / d, 2332 ± 369 kcal / d, 1694 ± 100 kcal/d and

1754 ± 322 kcal / d respectively, with the differences being not statistically significant.

2.4.6 Correlations

There was a significant correlation between total gastric volume AUC and average appetite AUC r = -0.47, $P < 0.010$, but not between gastric volumes and iAUC glucose ($P < 0.3$). The subgroup analysis with n =10 showed also a similar significant negative correlation between total gastric volume AUC and average appetite AUC r = -0.465, $P < 0.01$.

2.5 Discussion

This is the first pilot, *in vivo* imaging study assessing the glycaemic, gastrointestinal and appetite responses to porridges made from different 'modern' and 'ancient' cereal grains in healthy, normal-weight participants. The pilot project is a small-scale study conducted to gain experience and allow appropriate sample size calculations for future studies[175].

In this current study, some participants were unable to consume some of the breakfast meals in full, particularly the FMP. Future studies need to review the way in which the product is prepared and consumed in order to ensure better compliance with the protocol. Exclusion of those participants who had not consumed all porridges reduced the sample size to n = 7. Ten subject completed oats, rye and pearl millet providing a second post-hoc analysis.

Among those who were able to consume all four porridges, isoenergetic breakfast porridges made from different grains induced different gastrointestinal and physiological responses, although for the sample size used in this pilot study only gastric volumes were significantly different. After consumption of the pearl millet porridge, there was a trend for the glucose response to decrease, gastric volume to increase and appetite to increase compared with the other porridges some of which failed to reach significance potentially because of small numbers. The secondary analysis with n = 10 showed similar trends with other outcome differences reaching significance such as Hunger.

The immediate post prandial gastric volumes were significantly different as would be expected given that the isoenergetic portions had different volumes. Although the total energy of a meal has an effect on the gastric emptying, in this study the four breakfast meals were isoenergetic, suggesting that the gastric volume was affected by other factors such as particle size, viscosity and the meal volume, which is a key regulator of gastric emptying[176]. Furthermore, separation of liquid and solid parts (known as sieving) could affect the gastric emptying rate as the liquid part would be absorbed quickly in the early phase[121]. In this study, SOP emptied faster compared with the other porridges, which may be due to the separation between liquid part and oat flakes as shown in Figure 15. On the other hand, the liquid and solid phase were combined in the PMP and this may limit the sieving and delay emptying of the meal[177]. Although the volume of PMP porridge was lower than that of

SOP and FMP, PMP emptied at a slower rate, which could account for the smaller rise in blood sugar of this millet[122].

The appearance of the SBWC resembles that reported previously[166, 178]. The postprandial SBWC initially fell during the 'gastric phase' after feeding and the rose during the 'intestinal phase'. The early decrease in the SBWC is possibly related to the absorption of the readily available nutrients in the liquid phase. The later rise of the SBWC is likely to be related to the increased pancreatobiliary and enterocyte secretion after a mixed liquid/ solid meal and possibly also the effect of particulates[167, 168, 179].

All the grains had a relatively low glycaemic index[74, 101, 180, 181]. The present study is in agreement with other studies that have shown that rye can induce a lower and prolonged blood glucose response[181, 182]. The rye grains were soaked in water before heating in the microwave in this study, which could have an effect on the gelatinisation of rye starch and as a result modify the glucose response[183]. The glucose response after consuming the RP remained lower. The glucose response after consumption of oats was also in agreement with other studies. A study on rolled oats showed that after consumption a similar peak blood glucose value of 7 mmol / L, suggesting that our results are in line with the literature[180]. However, the results in relation to the finger millet were inconsistent in terms of the higher blood glucose in comparison with other studies[74, 184]. However there is little information available about the physiological and gastrointestinal responses to millet grains, especially pearl millet, limiting our knowledge about their potential health benefits.

The differences in the glycaemic response seen between these grains could be due to the processing of cereals which alters the digestion of the cereal grains; this is a considered a major determinant of the glycaemic response[185, 186] and also of the impact on appetite[62]. Oat and rye are steamed rolled flakes which can keep the endosperm intact and ultimately limit accessibility of amylase to the starch oats[40]. Our finger and pearl millet, on the other hand, were milled to flour which offers a higher surface area to digestion. This possible explanation is supported by a systematic review investigating the effects of different processing methods on glycaemic responses, in which it is shown that a smaller particle size caused greater gelatinisation and a heightened glycaemic response[80, 86]. In addition, another study has found that different milling methods may have effects on glycaemic response of foods made with finger millets flour[187]. The difference in the glucose response between the two millets could be due to the different amount of carbohydrate content which is potential an important determinant of the glucose response[188, 189]. In this study, FMP had the highest carbohydrate content (53.1 g) compared with PMP (45.6 g). This could explain the greater rise of FMP compared to the other porridges.

The current study indicated that the millet porridges may have prolonged satiating properties compared with the oats and the rye porridges. The increase in satiety following the millets could be related to the delay in decline of the gastric volume for these grains causing prolonged distension of the stomach and delayed delivery of nutrients into the small intestine[109, 155, 190, 191]. Furthermore, the reduced rate on

gastric emptying following consumption of the PMP could account for the blunted glycaemic response of the pearl millet[192]. This study did not measure duodenal motility hence it is not possible to comment on possible differences in motility between meals and the impact that this may have on gastric emptying[193, 194].

There were several limitations to the current study including the fact that the test meals were physically different, two were steam rolled flakes and two plain grains ground to a flour. The test meals were prepared slightly differently to obtain a more acceptable final product which may have altered the bioavailability of carbohydrates. Seven participants found the palatability of finger millet poor and could not finish all the test meal. The isoenergetic portions were of different volume. Use of capillary blood glucose does not represent arterial blood however it is a close approximation[132]. Also, future studies should measure insulin. Some food diaries were missed limiting our opportunity to assess the impact of the porridge consumed as a breakfast on 24 energy intake. Appetite ratings are a proxy measure for what people will actually eat. This leads us to suggest that this will be better assessed in future studies by providing lunch using an *ad-libitum* objective test meal thereby providing a more accurate and objective measure of actual food consumption at midday. Another suggestion for future work is to manage more closely the return of the food diaries.

In conclusion, this pilot study was our first experience of working with whole grain, porridge breakfast interventions. Although the study did not offer many significant differences in the physiological and

gastrointestinal responses after consumption of the four breakfast meals, valuable experience has been gained in the implementation of the protocols and provided useful directions for further studies.

Finally, the use of in vivo imaging can increase our knowledge of the behaviour of these meals in the gastrointestinal (GI) tract. This will facilitate an understanding of the interface between the input of a given feeding stimulus and various physiological and behavioural consequences. This will help us to improve our understanding of the effect of physical properties of food on digestion and appetite, engineer foods with the desired in vivo behaviour and develop more relevant *in vitro* / *in vivo* food digestion models.

3. Gastrointestinal Responses to Millet and Oats Breakfast Interventions Assessed by MRI

3.1 Introduction

The pilot study described in the previous Chapter has been very informative for the subsequent development of this project. It provided a better understanding of issues related to cooking, acceptability the meals, food diaries return and physical form of the products. Furthermore, the preliminary data collected from this pilot study was used to power the main physiology study. Appetite ratings are only a proxy measure for what people will actually eat later in the day. This led us to introduce a better food intake assessment by way of providing an *ad-libitum* objective test meal. This yields a single number and is a more objective measure of actual food consumption at midday. Also, it was recognised that the following study should measure insulin and related gut hormones such as GLP-1, GIP and PYY.

In the previous pilot study, a pearl millet breakfast porridge appeared to induce a lower postprandial blood glucose response and appetite scores compared to other grains, although the differences were not conclusive and the physical form of the test meals was not comparable[195].

3.1.1 Hypothesis

The hypothesis underpinning this study was that a pearl millet porridge breakfast will cause a smaller rise in blood sugar compared to an iso-

energetic and iso-volumetric breakfast meal made from Scottish oats porridge.

3.1.2 Aims

This larger study therefore aimed to investigate further the glycaemic, hormonal and appetitive responses to consumption of breakfast porridges made from a novel pearl millet flake compared to common porridge oat flakes for which the nutritional composition, as eaten, had been measured.

3.2 Methodology

3.2.1 Population

Healthy subjects, aged 18-65 years old with a BMI ≥ 18 and ≤30 kg/m^2 were recruited between Oct 2016 and May 2017 from the University campus population.

The sample size was calculated using finger prick glucose pilot data from the previous study on similar porridge breakfasts[195]. Using a crossover, paired design it would be possible to detect a change of 27.4 mmol·min/L (or 33%) in iAUC2h blood glucose with alpha=0.05 and a power of 80% using n=26 participants. This change is of the same order of magnitude as that reported in a published study comparing a rye versus an oat breakfast.

Exclusion criteria included not being a habitual breakfast consumer, not usually eating at least three meals a day, consuming ≥21

alcoholic drinks in a typical week, following a therapeutic diet and exhibiting any contraindications for MRI scanning.

A total of 34 healthy volunteers were initially assessed for eligibility (Figure 25). Seven participants were not eligible; another participant, although initially eligible, did not meet the criteria on the study day. Therefore, 26 participants, 17 females and 9 males, aged 28.5 (SD 9.6) years old, and with a BMI of 23.4 (SD 3.2) kg/m^2 were enrolled and studied. All participants gave written, informed consent.

The study was approved by the institutional Ethics Committee (F12072016) and registered on ClinicalTrials.gov with identifier NCT03068039. The trial registration name was ‘Millets and Oats MRI (MOM)’.

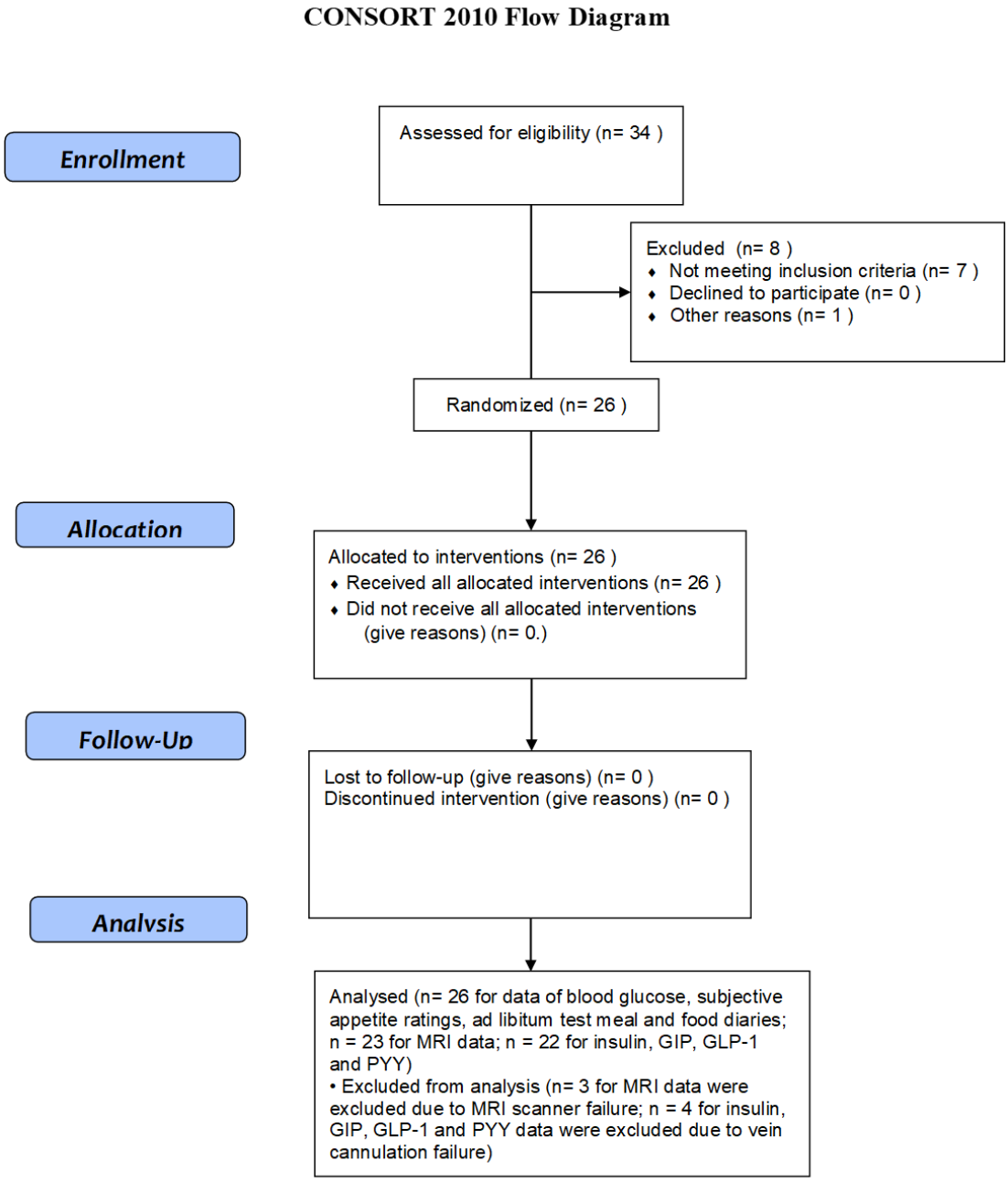

Figure 25: Study participant flow diagram

3.2.2 Experimental design

This study used a single-centre, randomised, two way crossover design that consisted of two separate test days, approximately 1 week apart. Participants consumed their habitual diet between each visit. The randomization scheme was generated with the use of the Second Generator Plan from www.randomization.com. Each study visit lasted from 08:00 am until approximately 13:30. The porridge meals differed in appearance and taste hence participants could not be blind to the intervention although they were not informed of which porridge they were consuming on each visit.

The participants were asked to fast overnight (for at least ten hours) but a glass of water was permitted on waking. On arrival they completed the study day eligibility check questionnaire to monitor adherence to the study day restrictions, such as overnight fasting. An MRI scan was done to collect baseline images and to ensure that the participants' stomach was actually empty at baseline. Measurements were taken at baseline and for up to 2 hours post consumption for gastric emptying, blood glucose, insulin, PYY, GIP, GLP-1 and paper based subjective visual analogue appetite scales were completed (Figure 26).

Participants were then given an *ad libitum* test lunch meal to measure intake. After this, but before discharge, they received instructions on how to record in a food diary that was provided their food and drink intake over the remainder of the day.

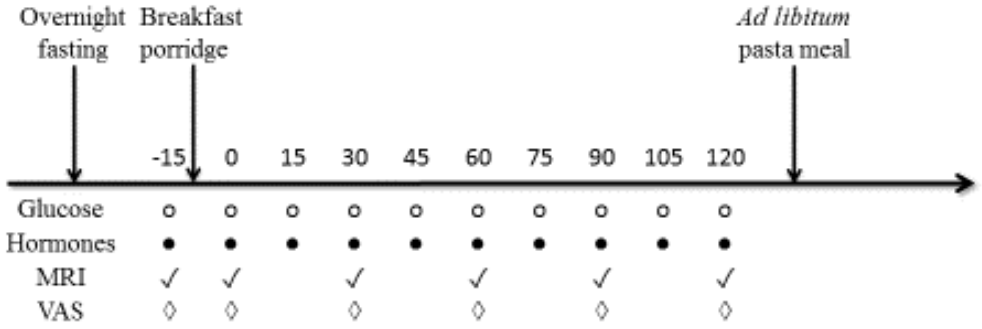

Figure 26: Diagram of the study day protocol.

All the data save the glucose values was blinded prior to analysis and the blind code was broken only after a blind data review was conducted. The outcome assessor was the one carrying out the finger-prick test so the glucose data could not be blinded.

3.2.3 Breakfast porridge intervention

The two breakfast porridges were made from either Scottish oats commonly sold in a supermarket chain (Asda, United Kingdom) or a novel pearl millet flake (supplied by Unilever, Sharnbrook, UK, under a Material Transfer Agreement). Both products were in the form of steam rolled flakes. The cooked products were analysed for protein, fat, moisture and fibre composition. Available carbohydrate was calculated as the difference between total carbohydrate and fibre (measured by the AOAC method). Total carbohydrate per 100g was calculated by difference (100 - (moisture / 100 g + ash/100g + fat / 100 g + protein / 100g). The total energy was calculated assuming that the energy provided by protein, fat, available carbohydrate and fibre is 4 kcal / g, 9

kcal / g, 4 kcal / g and 2 kcal / g respectively (analysis and estimations provided by Campden BRI, Chipping Campden, UK). The composition of the two products is detailed in Table 9.

Table 9: Breakfast porridge test meal characteristics per served portion.

	SOP	PMP
Weight (g) of cooked product served	400	415
Volume of Water drunk with cooked product served (mL)	240	304
Total volume (mL) = volume of cooked product served + water drunk (mL)	640	640
Energy (kJ)	920	920
Energy (kcal)	220	220
Protein (Kjeldahl, g)	7.2	6.6
Total carbohydrate (by difference, g)	42.0	44.4
Carbohydrate (avail, g)	34.0	37.4
Total sugars (enzymic, g)	1.6	1.7
Fat (Weibull-Stoldt, g)	4.4	3.3
Saturates (g)	0.8	0.8
MUFA (cis, g)	2.0	0.8
PUFA (cis)	1.2	1.7
Trans fatty acids (g)	0.4	0.4
Total fibre (AOAC, g)	8.0	7.5
Moisture (oven102°C)	345.2	359.4
Ash (at 525°C)	1.2	1.1
Protein N Factor	6.3	6.3
Equivalent salt (g)	0.4	0.4

The test meals prepared for the study were iso-energetic (220 kcal each) and iso-volumetric (640 mL each). Both porridges were cooked in the same way, in that 40 g of flakes were placed in an open glass bowl, gently mixed with 270 mL of room temperature water and heated in a

900W microwave. This procedure was repeated in parallel using an identical second open glass bowl and a second identical microwave. The porridges were heated for 2 minutes at full power, stirred gently with a spoon and left to rest for one minute, heated again for 2 minutes at full power, stirred gently with a spoon and left to cool for 6 minutes. By this point the water from the cooking had all being absorbed into the cooked product. The contents of the 2 bowls were then combined before a set weight of porridge was given to the participants to eat, namely 400g for SOP and 415g for PMP. This was done to match the energy content of the cooked product, flakes plus cooking water, to 220kcal.

The study meals were consumed with a glass of water at room temperature and the volume of water provided to the participants was used to compensate for volume differences in the cooked iso-energetic product portions. Therefore 240 mL of water was provided in a glass with SOP and 304 mL of water was provided in a glass with PMP, making the total volumes matched to 640 mL. The drinking of the glass of water was not standardised in aliquots, but the participants were asked to consume all of the porridge and all of the drink within 15 minutes. The manner and timing of the way the participants drank the water was not formally recorded but they mostly drank the water whilst eating the porridge, not all of it at the end. Other meal characteristics such as appearance and weight necessarily differed between meals**.**

3.2.4 Outcomes

Finger-prick blood glucose

Capillary blood samples were collected by finger prick using single-use lancets (Unistix Owen Mumford, Oxfordshire, United Kingdom). The capillary blood glucose was measured using a hand-held device (Accu-check, Roche Diagnostics, USA)[170] at the fasting baseline (t = -15), immediately after feeding (t = 0) and every 15 minutes thereafter until t = 120 min.

The glycaemic response was calculated using the protocol described by[132] which is in line with techniques recommended by the World Health Organization (WHO) / Food and Agricultural Organization (FAO 1998).

MRI of gastric volumes

MRI scans were collected on a 1.5T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands), in the supine/oblique position, at baseline (t = -15 min), immediately post-consumption (t = 0 min) and at 30 minutes intervals until t = 120 min.

A balanced turbo field echo (bTFE) sequence was acquired across the abdomen with a breath hold of 10 seconds. Gastric volume was manually measured from the scans by tracing a region of interest around the meal within the stomach[161]. The gastric half emptying times (T50%) were calculated for each individual and then averaged by fitting the data to a previously published equation [196]: $V(t) = V_0 [f (1+ kt / t_{emp}) e$

– t / t_{emp} +(1 – f)(1 – Gt)], where V0 is the gastric volume at time 0 and f, k, tempt and G are fitting parameters.

Volumes of the different gastric content layers (brighter and darker layers) were measured separately by Analyze 9.0 software (Biomedical Imaging Resource, Mayo Foundation). The measurements were done for two time points (t = 0 and t = 15 min) by tracing manually a region of interest around each area and summing the volume across slices.

The gastric empting rate for 1 hour was also calculated using the initial volume 640 ml and the equation (640 - V60) / 60.

Blood sampling and analysis of peptides

The sampling and assay protocols were similar to previous work [140]. Briefly: on arrival, a 20-G cannula (Intron Saety 3, B Braun Melsungen AG) was sited in a forearm vein of the participants to allow serial blood sampling (Figure 27).

Figure 27: Cannula inserted for blood sampling.

Blood samples were collected at fasting baseline (t = -15), immediately after feeding (t = 0) and every 15 minutes thereafter until t = 120 min for serum insulin, plasma GLP-1, plasma GIP and plasma PYY.

The initial 2 mL dead-space blood sample was discarded to avoid contamination with the saline flush and the 6 mL experimental sample was then drawn into a vacutainer tube (K2E EDTA, BD, UK) containing 0.5 ml of aprotinin (3-7 TIU / mg protein, A6279 Sigma Aldrich, UK) added on the morning of the test. The cannula was flushed with 5 mL 0.9% Sodium chloride (BD PosiFlush^{TM} SP, UK). Blood samples were centrifuged for 10 minutes before being stored on ice. The plasma was immediately aspirated from the centrifuge tubes and divided into 3 aliquots that were stored in a (-20°C) freezer within 2 h of being taken and transferred to a -80°C freezer at the end of the MRI study day until subsequent analysis.

Serum insulin and PYY concentrations were measured using RIA kits (Millipore, Missouri 63304 USA). Total GLP-1 and total GIP concentrations were each measured with the use of a specific ELISA kit (both kits from EMD Millipore Corporation, Missouri 63103 USA).

Appetite ratings

Subjective feelings of hunger, satisfaction, fullness, desire to eat and prospective food consumption ratings were assessed using paper-based 100 mm VAS[71, 172].

Each end of the line was anchored by statements expressing the extreme for the sensation. For example, ‘not hungry at all’ and ‘more

hungry than have ever been'. To avoid bias from previous answers the participants were presented with a new VAS sheet at each time point, and this was removed immediately after completion. Every time they came out of the MRI scanner room, the participants were requested to make a vertical mark on each scale at the point that best matched how they felt at that time.

A composite satiety score was calculated for each individual at each time point, without adjusting for baseline, using the formula:

Composite satiety score = [hunger + (100 – satisfaction) + (100 –fullness) + desire to eat + prospective consumption]/5.

The range for the composite satiety score was therefore between 0 and 100 with lower composite scores being in the 'positive' direction (low hunger, high fullness, low desire to eat) and higher composite scores being in the 'negative' direction (high hunger, low fullness, high desire to eat) in this context[173, 174].

Ad libitum test meal

A pasta based test meal consisting of a single large quantity was served at lunch time to assess *ad libitum* food intake[197]. The *ad libitum* meal consisted of tomato and mozzarella pasta bake (Tesco, United Kingdom, Figure 28).

Figure 28: Tomato and mozzarella pasta bake meal.

The nutritional composition table indicated that it had 129 kcal per 100 gram provided by 5.5 protein, 17.0 g carbohydrate, 3.6 g fat and 3.0 g of fibre.

Three semi-fresh pasta bake packs (450 g each) were heated in a microwave (900 W) at full power for a total of 10 minutes and stirred at the end of the period. Participants were given a single weighed portion of approximately 1300 g and a 200 mL glass of water. They were told that this portion was deliberately much larger than that normally consumed, and to eat from the bowl until satisfied. They were also told to drink the water when they wanted with the pasta but that they had to finish the entire amount of water. The amount of pasta left over was removed and weighed and the energy intake was calculated from the amount consumed as an objective measure of food consumption[197].

Food diaries

The subjects were instructed to provide a detailed record of food and beverages consumed over the remainder of the day. They were required

to include information such as recipes, portion sizes, product brand names, and cooking and preparation methods.

Nutritics software (Nutritics Ltd, Dublin, Ireland) was used to analyse the food intake from the food diaries, with manual additions for food items and recipes not on the database.

3.2.5 Statistical analysis

Prism version 6.07 (Graph Pad Software Inc., La Jolla, CA) was used to undertake descriptive and statistical analyses. All data are presented as mean ± SEM unless otherwise indicated. The data were assessed for normality using the Shapiro-Wilk's test. Most data were normally distributed and were analysed using parametric methods; the GLP-1, insulin and composite satiety data were non-normally distributed and were analysed using non-parametric methods.

Values for the iAUC blood glucose, gastric volumes, gut hormones and appetite ratings were calculated with the use of differences from baseline. Values were considered positive when they were greater than baseline values and considered negative when they were less than baselines values. The area above or below baseline was calculated with the use of the trapezoid rule[198].

Comparisons of iAUC blood glucose, gastric volume, the gut hormones, the composite satiety score, intake of the ad libitum test meal and self-reported daily energy between SOP and PMP were made with the use of Student's paired t test.

Two-factor repeated-measure ANOVAs (factor 1: meal, 2 levels; factor 2: time, 10 levels) were used to for blood glucose, gastric volumes, the gut hormones and the composite satiety score. When an interaction was identified, simple main effects were explored with the use of pairwise comparisons for the different time points, and a one way ANOVA for within each treatment. When no interaction was seen, main effects were compared.

An exploratory investigation of correlation was undertaken between gastric volume and glycaemic and insulinemic responses, gut hormones, and appetite scores. Differences were considered significant at $P < 0.05$.

3.3 Results

In this study, the effects of porridges made from pearl millet and oats, on gastrointestinal (gastric volume), glycaemic, hormonal (insulin, GLP-1, GIP and PYY) and appetite responses were measured. The study procedures were well tolerated and all 26 subjects completed the two study days. There were no adverse events during the study. The MRI scanner broke down (quenched) causing exclusion from analysis of n = 3 MRI data sets. Failure to sample bloods caused exclusion of n = 4 peptide data sets.

3.3.1 Glycaemic response

Fasting baseline glucose levels between study arms were not significantly different, as expected. Glucose level rose rapidly after feeding and declined towards baseline level at t = 120 min (Figure 29).

There was no significant difference between the meals for iAUC glucose (paired t test, $P > 0.05$), which was the primary outcome for this study.

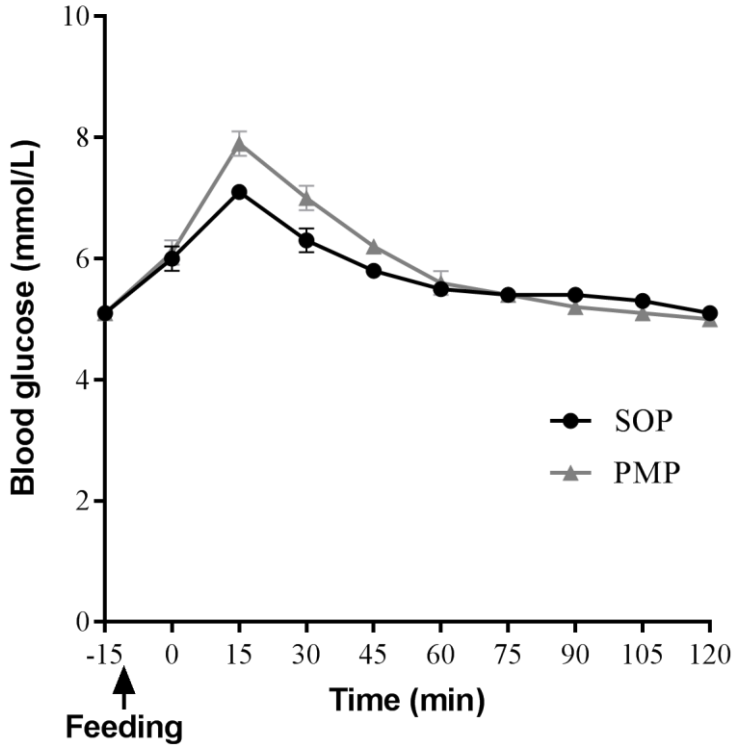

Figure 29: Plot of the blood glucose for healthy participants after they consumed two different breakfast porridges. ●— , Scottish oats porridge (SOP) and ▲— , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26. There were no significant differences in glucose iAUC 2h between the meals (paired t test, *P* > 0.05). The glucose levels at t = 15 min and at t = 30 min were significantly higher for the PMP breakfast meal (ANOVA, *P* < 0.05). Significant difference between SOP and PMP, *P* < 0.05.

The glucose levels peaked at 7.9 ± 0.2 mmol / L for pearl millet and 7.4 ± 0.1 mmol / L for oats porridge, a modest but significant difference (paired t test, $P < 0.05$). There was a significant interaction (ANOVA, $P <$

0.05) for glucose levels at t = 15 min and at t = 30 min which were higher for the PMP breakfast meal.

3.3.2 Appearance of the gastric content and gastric volumes

Figure 30 shows the appearance of the gastric content for SOP and PMP immediately after consumption (t = 0 min). Both porridges showed clear layering (phase separation), with a brighter layer on top (consistent with a more liquid phase in this type of moderately T2-weighted images) and a darker layer at the bottom (consistent with thicker / particulate material in this type of moderately T2-weighted images). The two layers were present also at t = 30 min. However, at later time points (t = 60 min to t = 120 min) the top layer was no longer visible.

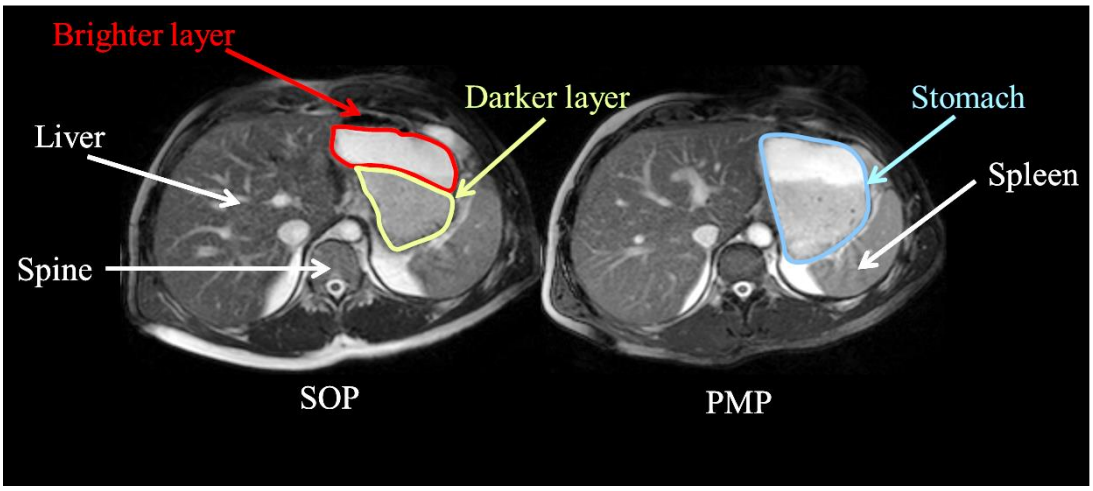

Figure 30: Representative example of axial MRI images through the same location in the abdomen of a healthy participant who consumed Scottish oats porridge (SOP) or pearl millet porridge (PMP) test meals on two different occasions. Images were taken at t = 15 min after feeding. Anatomical landmarks such as the liver, spine and spleen are indicated by the white arrows, whereas the stomach is circled in blue on the panel on the right. Both porridges showed clear layering (phase separation), with a darker layer at the bottom of the stomach (circled in yellow on the panel on the left) and a brighter layer at the top of the stomach (circled in red on the panel on the left.

The brighter (liquid) upper layer volume

The PMP meal had a significantly higher liquid volume than that of SOP at t = 0 and t = 30 min (figure 32A). Similarly the AUC 30 min showed a higher volume of the liquid for PMP than for the SOP (table 8).

The darker (thicker / particulate material) lower layer volume

There was no significant difference after t = 0 and t = 30 min in the darker layer between the meals at (figure 32B). There also no significant difference in the dark layer between the meals in AUC 30 min (table 10).

Table 10: Post-prandial brighter and darker layers gastric volumes measured by MRI in healthy participants who were fed two different breakfast porridge test meals. All values are mean ± SEM. n = 23. SOP, Scottish oats porridge and PMP, pearl millet porridge.[2] Paired t test of difference between SOP and PMP.

	SOP	PMP	*P* < [2]
Bright layer volume			
At t = 0 min (mL)	93 ± 10	178 ± 16	0.001
At t = 30 min (mL)	49 ± 9	84 ± 13	0.012
AUC 30, mL / min	2127 ± 268	3925 ± 416	0.001
Darker layer volume			
At t = 0 min (mL)	351 ± 17	319 ± 11	0.052
At t = 30 min (mL)	219 ± 15	226 ± 13	0.872
AUC 30, mL / min	8549 ± 427	8166 ± 300	0.329

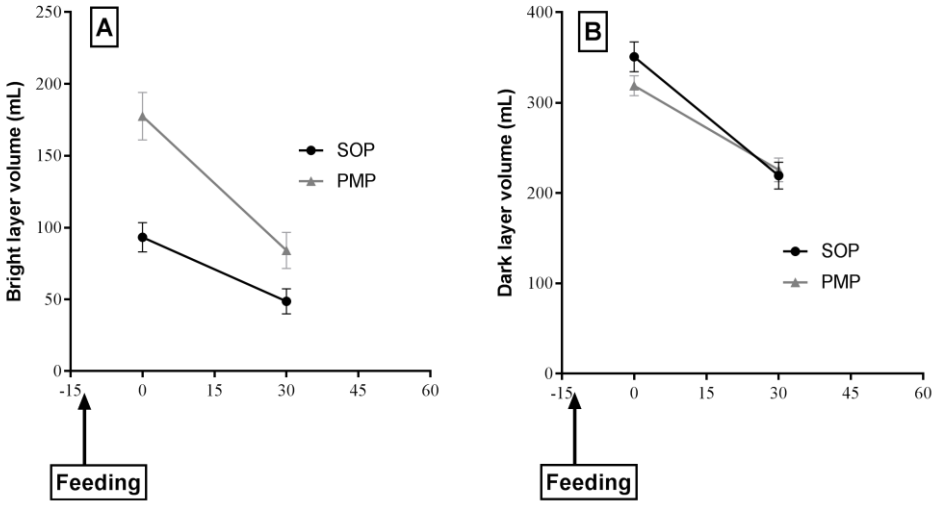

Figure 31: Plot of the upper brighter (or more liquid) layer volume (A) and the lower darker (more viscous/particulate) layer volume at t = 0 and t = 15 min for healthy participants after they consumed two different breakfast porridge test meals, Scottish oats porridge (SOP) and pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 23. There was a significant differences in gastric volume AUC30 between the meals (paired t test, P < 0.05).

Gastric volumes at fasted baseline (t = -15) for both meals were not significantly different, as expected. Gastric volumes rose immediately after feeding for both meals and then the volumes declined with time (Figure 32).

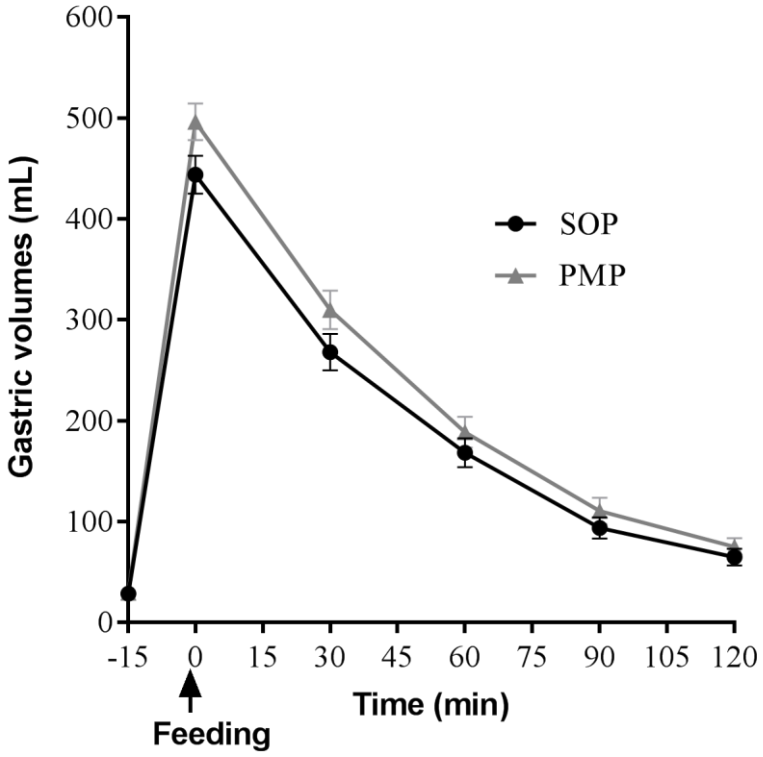

Figure 32: Plot of the gastric volume with time for healthy participants after they consumed two different breakfast porridge test meals. ●— , Scottish oats porridge (SOP) and ▲— , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 23. There was a significant differences in gastric volume iAUC 2h between the meals (paired t test, *P* < 0.05).

There was a significant interaction (ANOVA, $P < 0.05$) for gastric volumes at t = 0 min and at t = 30 min, which were higher for the PMP breakfast meal. The iAUC for gastric volumes were significantly different between the meals, although both meals were iso- volumetric at ingestion (paired t test, $P < 0.05$). PMP meal had larger gastric volumes compared with SOP (Table 11). The half gastric emptying time ($T_{50\%}$) of

SOP and PMP were however similar at 45 ± 17 min and 47 ± 18 min respectively (paired t test, $P > 0.05$).

Table 11: Post-prandial gastric volumes measured by MRI in healthy participants who were fed two different breakfast porridge test meals[1].[1] All values are mean ± SEM. n = 23. SOP, Scottish oats porridge and PMP, pearl millet porridge.[2] Paired t test of difference between SOP and PMP.

	SOP	PMP	$P <$ [2]
The half gastric emptying time, $T_{50\%}$ (min)	45 ± 17	47 ± 18	0.918
Gastric volumes iAUC 2h (mL min)	23340 ± 1639	26779 ± 1774	0.045

The gastric empting rate for 1 hour was 7.9 ± 0.2 mL/min for oats and 7.5 ± 0.3 mL/minfor pearl millet.

3.3.3 Blood peptides

Insulin

Serum insulin concentrations increased markedly after both PMP and SOP up to t = 30 min and declined afterwards towards baseline at t =105 min (Figure 33). There were no significant differences either by iAUC or ANOVA between porridges for insulin concentration between PMP and SOP ($P > 0.05$).

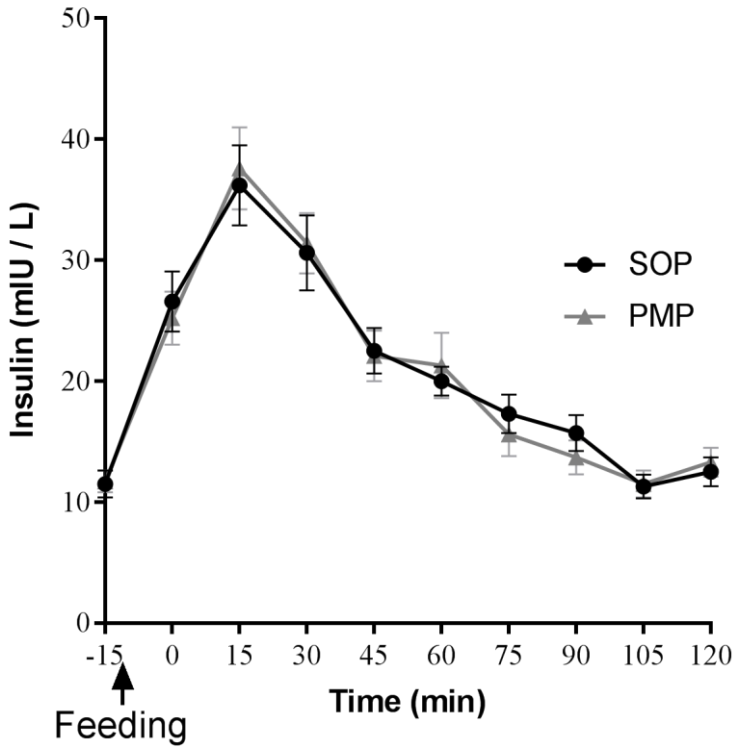

Figure 33: Plot of the plasma insulin concentrations with time for healthy participants after they consumed two different breakfast porridge test meals. ●— , Scottish oats porridge (SOP) and ▲— , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22.

Total GLP-1

Plasma GLP-1 concentrations following SOP rose quickly at t = 0 min compared with PMP. Thereafter, at t = 15 min, the concentration declined below the fasting value (Figure 34). There were no significant differences either by iAUC or ANOVA between porridges for GLP-1 concentration between SOP and PMP ($P > 0.05$).

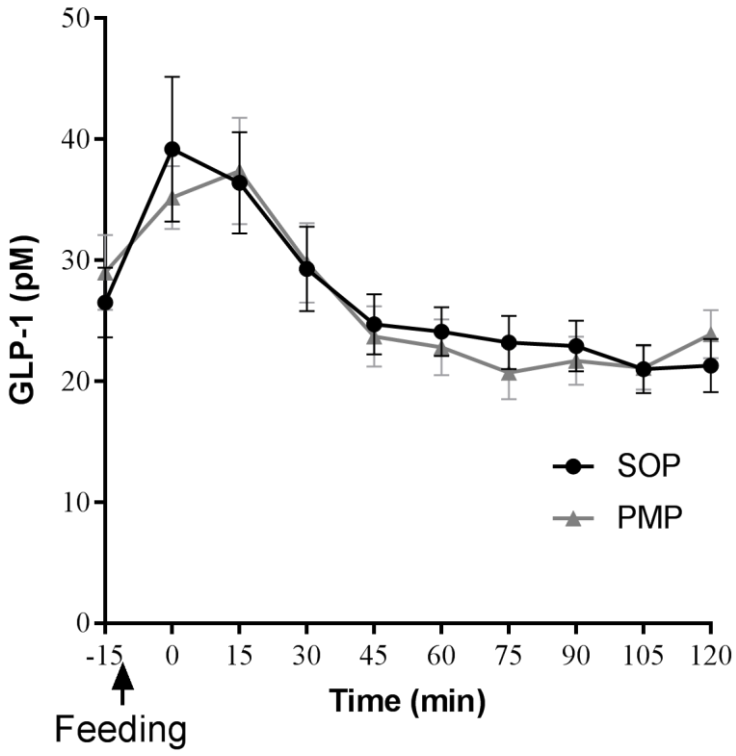

Figure 34: Plot of the plasma GLP-1 concentrations with time for healthy participants after they consumed two different breakfast porridge test meals. ●— , Scottish oats porridge (SOP) and ▲— , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22

Total GIP

Plasma GIP concentrations rose rapidly from baseline after feeding for both SOP and PMP. At t = 15, the two curves separated with the peak GIP for SOP being 23% higher than for PMP. GIP remained higher for SOP than for PMP throughout the remainder of the sampling period, the difference being significant (ANOVA, $P < 0.05$) (Figure 35).

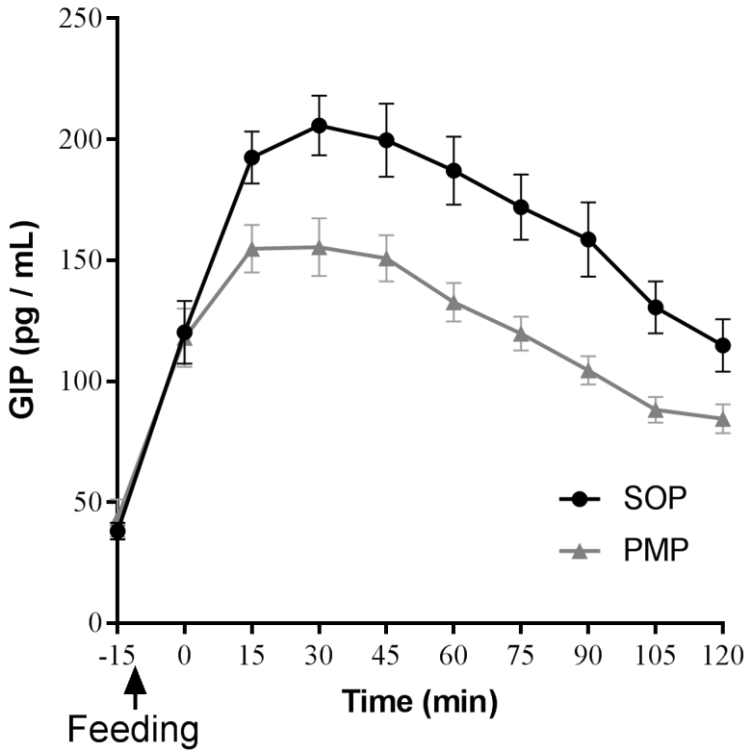

Figure 35: Plot of the plasma GIP concentrations with time for healthy participants after they consumed two different breakfast porridge test meals. ●— , Scottish oats porridge (SOP) and ▲— , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22. There was a significant difference in GIP iAUC 2h between the breakfast meals (paired t test, *P* < 0.05).

Accordingly, there was a significant difference in iAUC 2h GIP concentration between the two porridge breakfasts (paired t test, *P* < 0.05) with SOP being higher. There was a significant interaction for GIP at t = 15 min to t = 120 min between the meals (ANOVA, P < 0.05).

PYY

Plasma PYY concentrations for SOP increased slightly from baseline upon feeding at t = 0 min and remained at the same level until t = 75 min,

then dropped to baseline level (Figure 35). Plasma PYY concentrations for PMP remained at the same level as baseline, until t = 15 min when the concentration increased rapidly, before returning to the baseline values at t = 45 min (Figure 36).

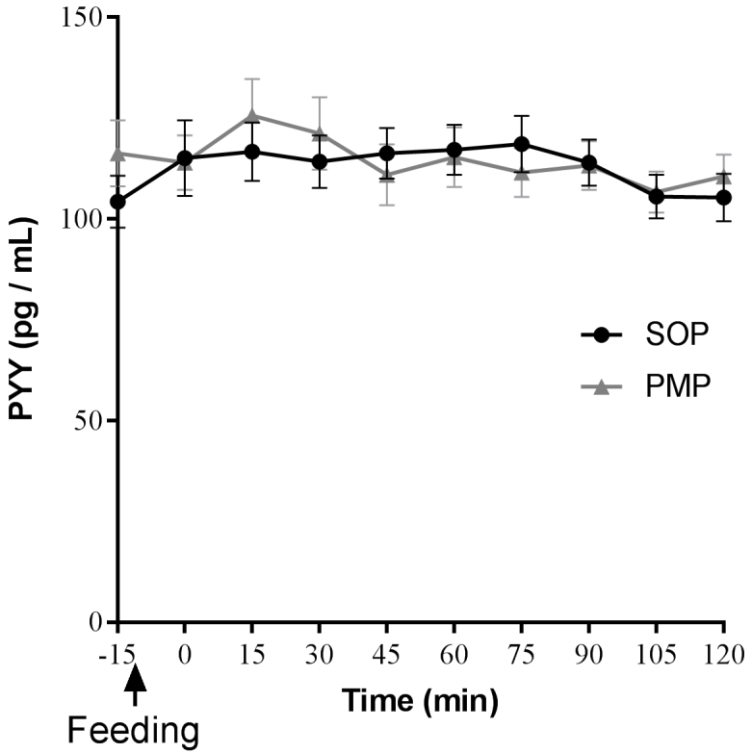

Figure 36: Plot of the plasma PYY concentrations with time for healthy participants after they consumed two different breakfast porridge test meals. ● , Scottish oats porridge (SOP) and ▲ , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22.

There were no significant differences either by iAUC or ANOVA between porridges for PYY concentration between SOP and PMP ($P > 0.05$, Table 12).

Table 12:Glucose, insulin, GIP, GLP-1 and PYY concentrations measured from healthy participants who were fed two different breakfast porridge test meals .[1]All values are mean ± SEM. n = 26 for blood glucose, n = 22 for insulin, GIP, GLP-1 and PYY concentrations. SOP, Scottish Oats porridge and PMP, pearl millet porridge.[2] Paired t test of difference between SOP and PMP.

	SOP	PMP	P < [2]
Fasting glucose (mmol / L)	5.1 ± 0.1	5.1 ± 0.1	0.627
Glucose peak (mmol / L)	7.4 ± 0.1	7.9 ± 0.2	0.01
Glucose iAUC 2h (mmol/L min)	100 ± 11	125± 14	0.106
Insulin iAUC 2h (mIU/L·min)	2885 ± 189	2759 ± 202	0.503
GIP iAUC 2h (pg / mL·min)	21643 ± 1375	15796 ± 858	0.001
GLP-1 iAUC 2h (pM·min)	3670 ± 370	3467 ± 334	0.121
PYY iAUC 2h (pg / mL·min)	15337±811	14971 ± 956	0.127

Appetite ratings

As predicted, the feelings of hunger, desire to eat and prospective food consumption all decreased from the fasting baseline following consumption of the breakfast porridges and returned to baseline two hours later, whereas the feeling of fullness and satisfaction increased after feeding and returned to baseline after two hours. There were no significant differences either by iAUC or ANOVA between porridges for the specific appetite ratings ($P > 0.05$). The composite satiety scores for both meals were not statistically different (Figure 37) either by iAUC or ANOVA. The iAUC for the subjective appetite rating are summarized in Table 13.

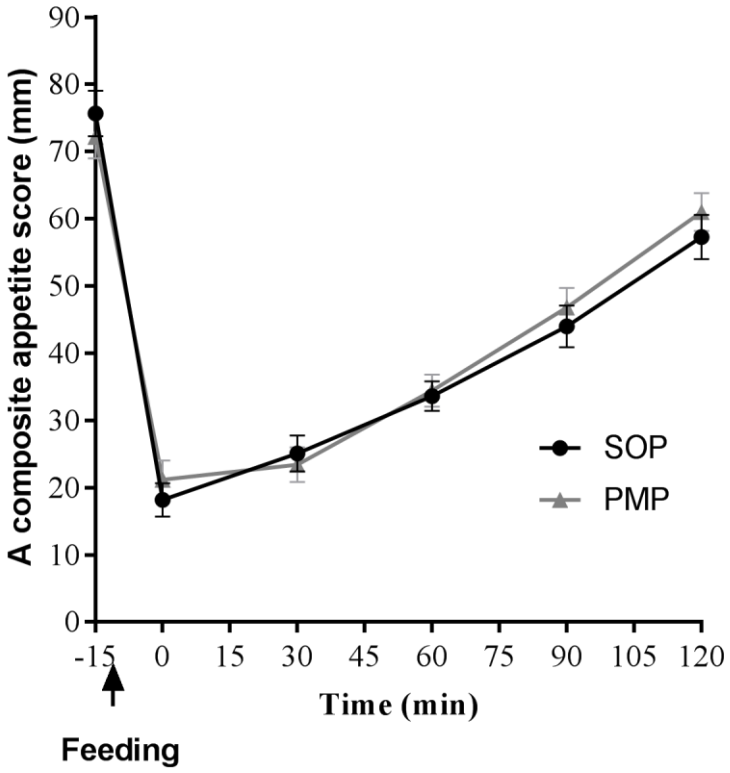

Figure 37: Plot of the composite appetite score with time for healthy participants after they consumed two different breakfast porridge test meals. —●— , Scottish oats porridge (SOP) and —▲— , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.

Table 13: Subjective appetite scores by question, energy intake from *ad libitum* meal and daily energy intakes from healthy participants who were fed two different breakfast porridge test meals. All values are mean ± SEM. n = 26 for appetite scores, energy intake from *ad libitum* meal and self-reported daily energy intakes. SOP, Scottish oats porridge and PMP, pearl millet porridge. [2] Paired t test of difference between SOP and PMP

	SOP	PMP	P < 2
Hunger iAUC 2h (mm / min)	4049 ± 356	4484 ± 289	0.271
Satisfaction iAUC 2h (mm / min)	8311 ± 330	8137 ± 334	0.685
Fullness iAUC 2h (mm / min)	8487 ± 347	8261 ± 314	0.412
Desire to eat iAUC 2h (mm / min)	4708 ± 375	4722 ± 357	0.812
Prospective food consumption iAUC 2h (mm / min)	5630 ± 387	5711 ± 332	0.985
A composite appetite score iAUC 2h (mm / min)	4918 ± 296	5066 ± 274	0.708
Energy intake from ad libitum meal (kcal)	863 ± 78	900 ± 76	0.328
Self-reported energy intake over the remainder of the day (kcal)	1166 ± 105	1076 ± 106	0.468
Self-reported protein intake over the remainder of the day (g)	53 ± 7	50 ± 7	0.408
Self-reported fat intake over the remainder of the day (g)	45 ± 4	40 ± 6	0.353
Self-reported carbohydrate intake over the remainder of the day (g)	132 ± 14	117 ± 11	0.394
The total daily energy intake (kcal)	1753 ± 138	1818 ± 135	0.506

Data for hunger, fullness, satisfaction, desire to eat and prospective food consumption are shown in figure 38 – 42.

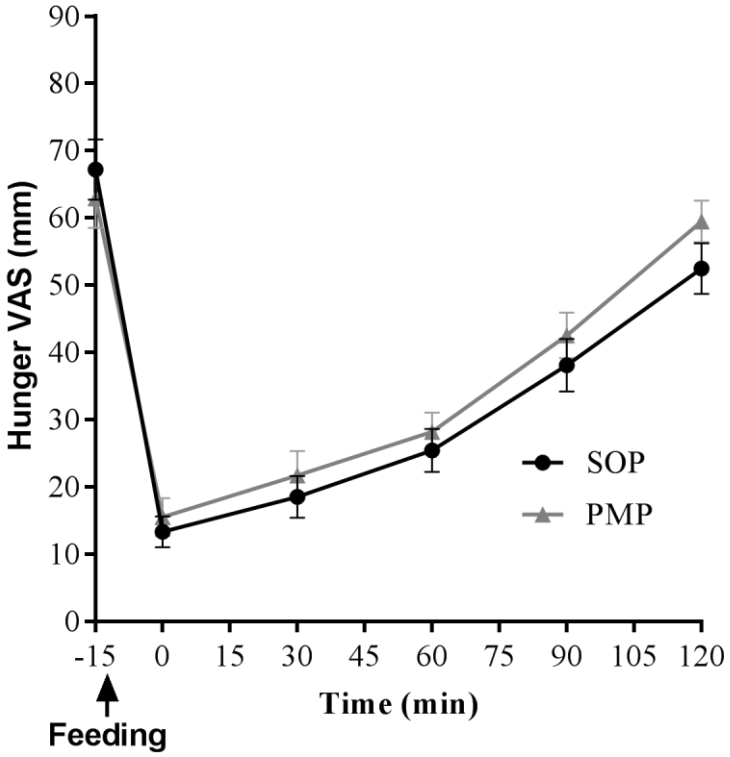

Figure 38: Plot of hunger for healthy participants after they consumed two different study porridges. ●— , Scottish oats porridge (SOP) and ▲— , Pearl millet porridge (PMP). Values are mean ± SEM, n =26. The arrow on the horizontal axis indicates the meal start time.

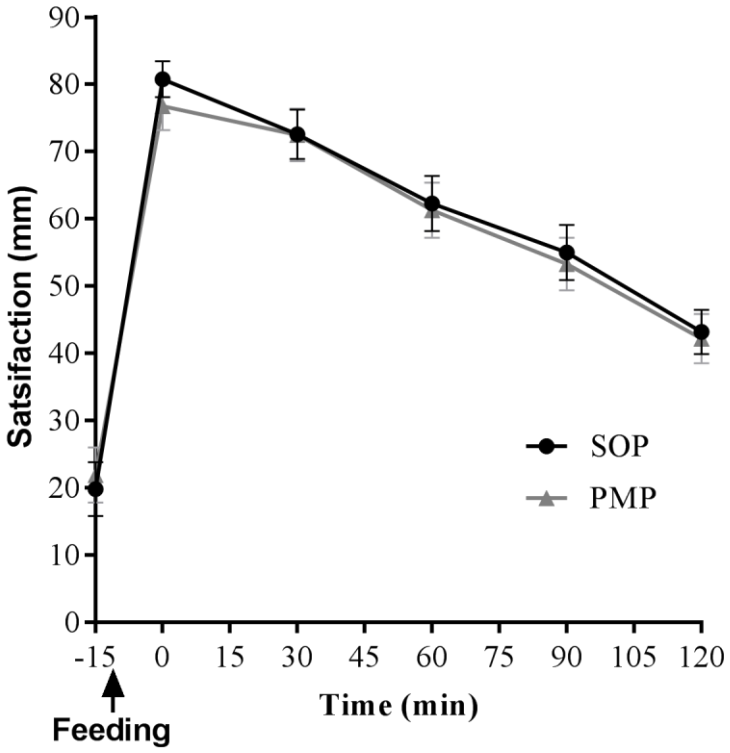

Figure 39: Plot of satisfaction for healthy participants after they consumed two different study porridges. ●, Scottish oats porridge (SOP) and ▲, Pearl millet porridge (PMP). Values are mean ± SEM, n =26. The arrow on the horizontal axis indicates the meal start time.

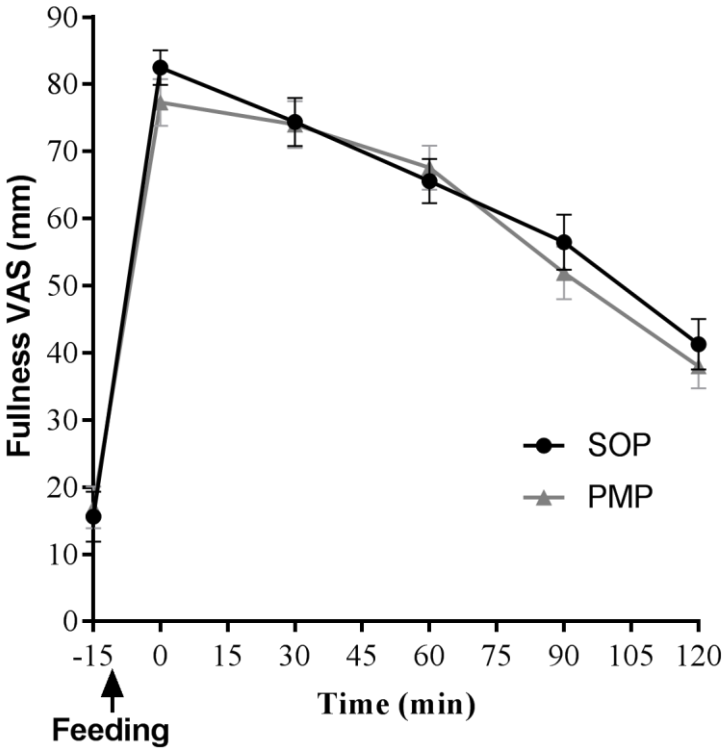

Figure 40: Plot of fullness for healthy participants after they consumed two different study porridges. ●— , Scottish oats porridge (SOP) and ▲— , Pearl millet porridge (PMP). Values are mean ± SEM, n =26. The arrow on the horizontal axis indicates the meal start time.

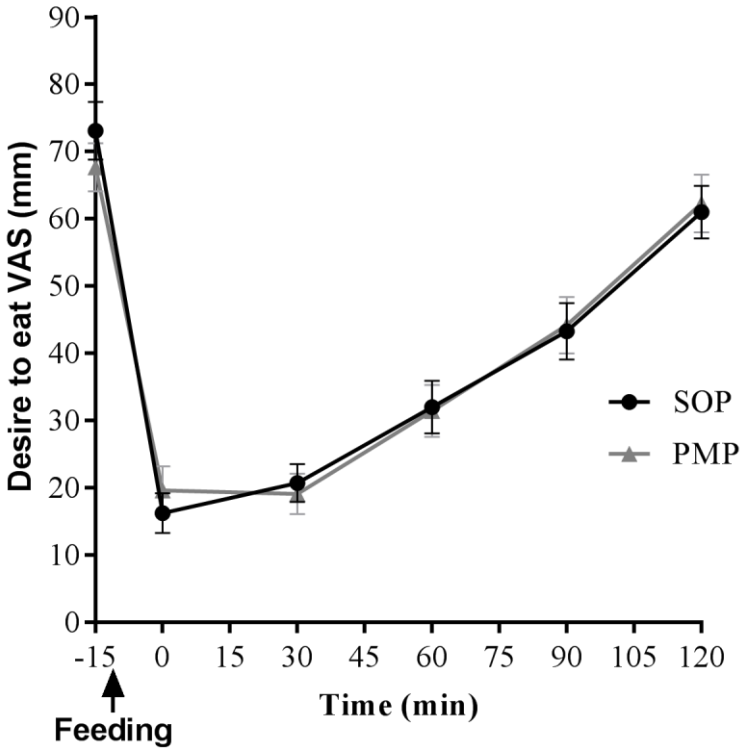

Figure 41: Plot of desire to eat for healthy participants after they consumed two different study porridges. , Scottish oats porridge (SOP) and , Pearl millet porridge (PMP). Values are mean ± SEM, n =26. The arrow on the horizontal axis indicates the meal start time.

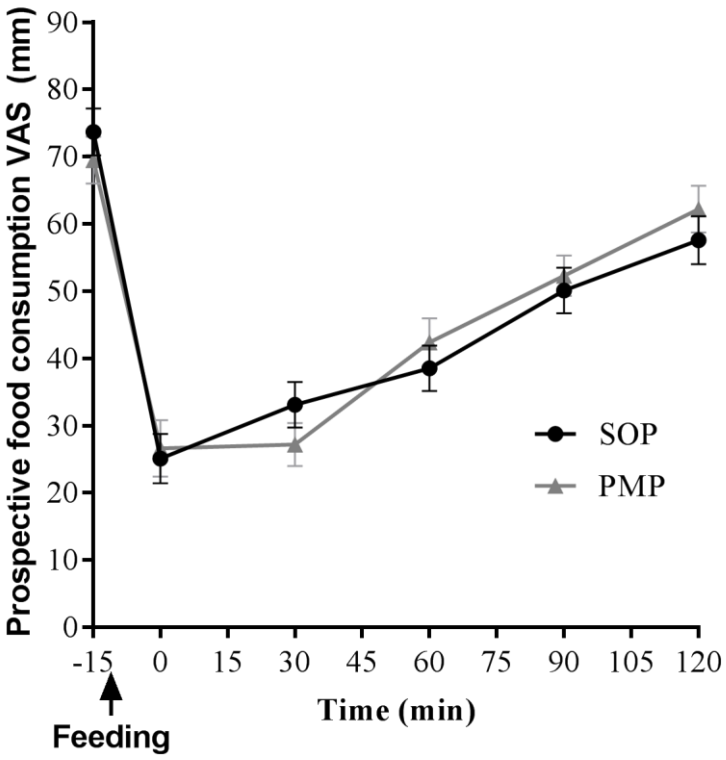

Figure 42: Plot of prospective food consumption for healthy participants after they consumed two different study porridges. , Scottish oats porridge (SOP) and , Pearl millet porridge (PMP). Values are mean ± SEM, n =26. The arrow on the horizontal axis indicates the meal start time.

3.3.4 *Ad libitum* test meal

There was no significant difference in the energy intakes from the *ad libitum* pasta bake meal following consumption of the pearl millet and oats (paired t test, $P > 0.05$) (Table 10).

3.3.5 Food intake

The recorded intake of food consumed during the remainder of the day (Table 10) was not significantly different between the two arms of the study ($P > 0.05$). There were no significant differences in the self-reported percentage of total energy from carbohydrate, protein and fat following the two meals (paired t test, $P > 0.05$). The total daily energy intake including the porridge breakfast, *ad libitum* pasta meal and recorded intake for the reminder of the day (Table 10), was again not significantly different (paired t test, $P > 0.05$).

3.3.6 Correlations

For PMP there was a significant correlation between gastric volume iAUC and the iAUCs for satisfaction ($r = 0.49$, $P = 0.03$), fullness ($r = 0.48$, $P = 0.04$) and desire to eat ($r = -0.54$, $P = 0.02$). For SOP there was a significant correlation between gastric volume iAUC and the iAUCs for fullness ($r = 0.47$, $P = 0.04$) and desire to eat ($r = -0.53$, $P = 0.02$).

3.4 Discussion

This integrated study has assessed the nutritional composition, glycaemic, gastrointestinal, hormonal and appetitive responses of iso-energetic and iso-volumetric breakfast porridge meals made from pearl millet or oats. Unilever specially produced for us commercial quality, steam rolled, novel pearl millet flakes ensuring a valid comparison between our new intervention and commercial oat flakes. Oats were chosen as they are a common breakfast grain with recognised health-

promoting characteristics[84, 199]. This study is the first randomised controlled trial of a pearl millet breakfast intervention.

The nutritional composition of the two porridges, as served, was measured in order to ensure that the energy content of the two meals was identical and to evaluate possible differences in macronutrient and fibre composition.

No significant differences were seen in the glycaemic responses between PMP and SOP either in terms of capillary blood glucose, or insulin response. The glycaemic response is influenced by many factors such as the amount of available carbohydrates, physical form of the carbohydrates and cooking and food processing methods. In this study there were similar glucose and insulin iAUC responses between PMP and SOP. Pearl millet showed a higher glucose peak value than oats, although the difference was modest.

Considering that the two meals were well matched for energy and volume and that most of the other responses were very similar, one could speculate that the smaller particle size of the PMP flakes compared to SOP flakes may have offered an increased surface area to digestion[80, 186]. Other factors, such as fibre content, were fairly similar, although the grains may have contained different types of fibre, resulting in a slightly different physiological response[200, 201]. The macronutrient composition of both meals was comparable (Table 7). The glycaemic response after oats is in agreement with many studies that have shown similar peak blood glucose value around 7 mmol / L[180], which is also in agreement

with our pilot studies. To our knowledge these are the first human data on the glycaemic response after pearl millet in the form of flakes.

The gastric appearance of both meals was similar with two separated layers being apparent immediately after feeding. The layers comprised of an upper liquid phase and a lower solid/viscous phase that could be seen in the stomach. The PMP meal had a significantly higher liquid volume than that of SOP immediately after feeding and half an hour later. However, the emptying rate of PMP was quicker than that of SOP. The fasting emptying rate following PMP matched the lower nutrient absorption that was indicated by the lower of GIP. An hour later, the liquid phase was no longer visible for both meals, suggesting that gastric sieving promoted the emptying of the liquid component of the stomach contents[196]. These results with flakes are similar to those reported by Mackie et al[186]. The half gastric emptying times were also similar for SOP and PMP. This could well relate to the iso-energetic nature of both meals, as energy content may drive gastric emptying to a greater extent than volume[119, 202].

Gastric Emptying is the process of emptying food from the stomach into the small intestine. The rate of gastric emptying is controlled by various variables including food properties, food breakdown, physiological response and subject characteristics[111]. Although both meals were iso-energetic and iso-volumetric, iAUC gastric volumes after PMP were significantly higher than after SOP. This is counter-intuitive because the total meal volume was matched by requiring the participants to consume more water volume with PMP because the cooked volume

of the iso-energetic pearl millet porridge product was smaller. The water was not blended into the cooked porridge because of the desire to keep a more ecological validity with participants able to drink with a meal. Blending would have also required additional stirring with possible changes in the food matrix. The volume measurement of the different upper and lower layers of the meal showed that a large part of the additional water volume ingested with PMP has sieved rapidly from the stomach as one could have expected. Despite this, the total PMP gastric volumes did not fall below the SOP volumes and remained slightly higher than SOP throughout the rest of the gastric emptying time. Larger gastric volumes after PMP could well be due to the characteristics of the meal. Millet has higher carbohydrate and lower energy, protein, total lipid and fibre per 100g compared to oats[203]. It may also be possible that the PMP flakes underwent some further absorption of water or secretion in the gastric lumen, thus causing some additional swelling of the PMP volume, though from the MRI images it was not possible to dissect this. The gastric volume results are in keeping with the previous pilot study, which showed a significant difference in gastric volume between different porridges[195]. The reasons for this remain to be understood. Increased wall stretch and tension is known to result in increased feeling of fullness[204] which correlates with gastric volumes[196] and reduces short-term food intake. Here we found indeed some positive significant correlations between gastric volumes and appetite ratings.

The plasma GLP-1, PYY and GIP concentrations were measured due to their direct physiological effect on gastric emptying, glycaemic

response and appetite[136-138, 141]. However, others not measured like CCK, active form of GLP-1 or active form of GIP.

GLP-1 is an incretin hormone released from L cells located in both the small and the large intestine in response to food intake[141]. Plasma GLP-1 levels are at their lowest level in the fasting state (after overnight fast). The plasma levels rise rapidly during meals and usually remain above the baseline (the morning levels) between meals[140, 141]. PYY is secreted from Endocrine L cells that are located in the small and large intestine[137]. PYY inhibits gastric motility and increases water and electrolyte absorption in the colon. It has been shown to reduce appetite[137]. In this study the GLP-1 responses were consistent with serum insulin concentrations which were comparable following both meals. PYY was not significantly different between the two meals.

The differences in GIP responses between meals are instead marked, with GIP being significantly lower after pearl millet compared with oats. GIP is secreted from intestinal K-cells[145] in response to the absorption of glucose and fat. More specifically, GIP release is stimulated by the rate of nutrient absorption rather than the presence of nutrients in the intestine[145]. The primary role of GIP is that of an incretin hormone, in that it binds to its specific receptor on pancreatic β-cells, and enhances glucose-dependent insulin secretion[145]. Although some studies reported that plasma GIP profiles are consistent with insulin profiles, in the current study we found that GIP profiles behaved differently. Insulin concentrations were comparable between meals, however, GIP was significantly different between meals. GIP in combination with

hyperinsulinemia and hyperglycaemia has been shown to promote triacylglycerol absorption in adipose tissue[205], with high plasma levels of GIP associated with unhealthy body fat distribution[206]. The lower GIP response from the PMP meal may therefore suggest an added health benefit if taken on a regular basis, although further studies would be needed to confirm this.

The subjective appetite responses, the *ad libitum* pasta meal intake and the food intake for the reminder of the day were similar. Therefore the two porridges had similar effects on appetite and satiety in this acute test day setting.

The strengths of the study included the direct analysis of the porridge meals, as served, having carefully controlled for differences in the degree of processing including manufacturing a novel pearl millet steamed rolled flake. Both grain flakes were cooked identically and in plain water as different cooking methods may have an effect on the degree of starch gelatinization[207, 208] and also to avoid macronutrient confounders from added milk or jam. The fully integrated nature of the study allows a unique exploration of pre and post absorptive variables, and to relate this to subsequent perceptions and behaviours which is unique in relation to the study of millet. It is worth noting that pearl millet is poorly characterised in terms of β-glucan content, though some of its properties are similar to those of sorghum[209]. Kodo millet is reported to have 37–38% of dietary fibre and foxtail millet 22%. There is also an aspect of digestive health for millets: foxtail, kodo and banyard millets are

rich in soluble dietary fibre which may have advantages for patients with constipation[210, 211].

Although the participants were of different body sizes, and hence would have had different energy requirements, the test meal portion given was the same for all participants and so would have been a higher proportion of total energy intake for some. This may have reduced the potential for differences in energy intake at the lunch in the participants with a lower energy requirement. Matching for energy, rather than other micronutrients, meant that slight differences in, for example, fat composition may have confounded the results. However this was felt to be the most clinically relevant approach.

In conclusion, this trial has investigated, for the first time, the glycemic, gastrointestinal, hormonal and appetite responses of a pearl millet breakfast porridge intervention compared with a common oats porridge. PMP elicited glycemic, insulinemic, GLP-1, PYY and appetite responses comparable to a known breakfast grain with recognised health-promoting characteristics. In addition, PMP had a larger iAUC gastric volume and a lower GIP responses compared with that of SOP. Pearl millet could therefore represent an alternative breakfast food with similar beneficial effects to those of oats and is a currently underexploited source of nutrition with excellent sustainability and resilience credentials.

4. Assessment of motion of gastric contents in healthy subjects using MR tagged imaging

4.1 Introduction

It became clear from the experience with these feeding interventions that the intragastric appearance of our porridge breakfast meals varied considerably, leaving open the question of how the stomach is mixing them. It would be interesting to be able to investigate this in more detail and we have the appropriate technology.

MRI is non-invasive and an ideal tool for the evaluation of gastric physiology of the stomach, allowing understanding of many aspects of digestion including gastric emptying[154, 155], gastric motility[212-215], mixing[164] and also gastrointestinal fluids[157, 158, 161]. MRI has also the ability to investigate motion and flow in the stomach, something that has been shown in the past, but only to a limited extent[164, 215].

Several MRI techniques have been used to assess the gastric motion such as pulse gradient flow sensitive MRI and tagging[212-214]. The pulse gradient flow sensitive MRI for instance has been used to assess the flow in the gastric antrum[215.] Nottingham was first to propose tagging in 1994 to look at a porridge meal in the stomach though that was a small proof of principle study[164]. It was chosen here to use the tagged MR imaging because it provides an easy-to-interpret 'motion map' that can be used to assess the movement happening inside an organ or a sample. The method is based on a spatial modulation of the magnetization that is currently widely applied in cardiac imaging[165] but is relatively new in the

gastrointestinal field. Other MRI techniques that have been proposed to study the fate of gastrointestinal contents such as relaxometry [154, 155] do not capture the motion of the gastrointestinal chime and therefore were not used here. The development of this new tool could provide new information on the intragastric handling of foods which would be valuable not only for this thesis work but also for future studies

This final part of the work aims therefore explore the ability of MRI in investigating the post-prandial motion of the breakfast porridge interventions in the stomach.

Continuously tagged MR imaging or SPAMM (spatial modulation of the magnetization) is a motion-sensitive MRI technique [165]. The MRI tagging method provides direct imaging of motion by spatially modulating the magnetization prior to imaging. Briefly, the tagging method involves superimposing a series of magnetic tags in the form of parallel black lines to the anatomy. This is carried out at a pre-defined amount of time (hundreds of milliseconds) prior to acquiring an MRI image. Tissues and materials that are static will not deform the pre-applied black lines (tags) which will therefore look straight in the image, demonstrating no motion. Tissues and materials that moved between the application of the magnetic black lines and the acquisition of the MRI image will show instead a deformation of the lines (tags) in the direction of motion, with the deformation being proportional to the amount of motion that occurred. Therefore the tagging images provide an easy-to-interpret ‘motion map’ that can be used to assess the movement of the gastric contents (Figure 43).

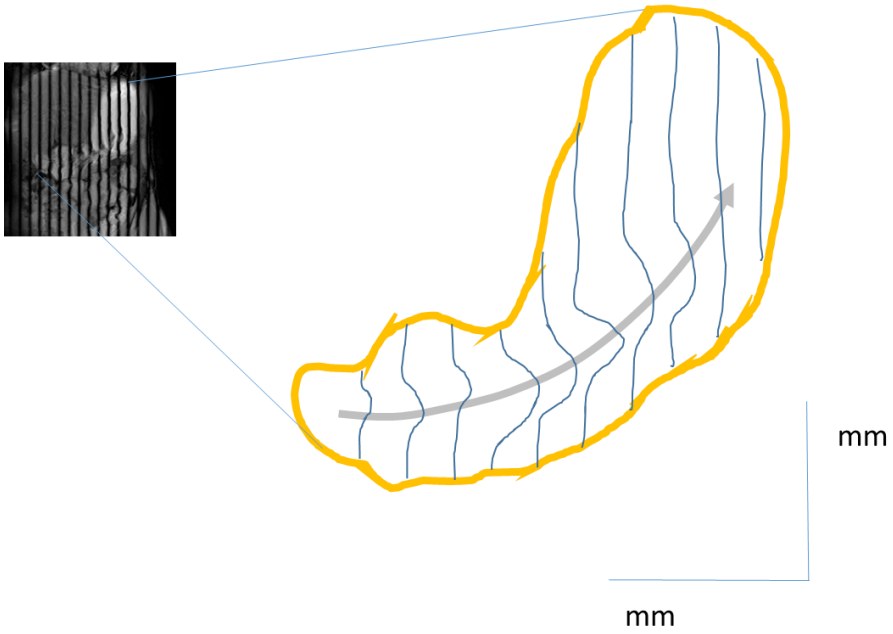

Figure 43: shows the tag movements

In the case of the stomach this is illustrated schematically in Figure 26. The images would show all tags remaining straight if no movement of gastric contents occurred (Figure 44. A)[216]. However, the tag line pattern would ‘bend’ where any movement of gastric contents has occurred in the delay between the application of the tag and acquisition of the image (Figure 44. B).

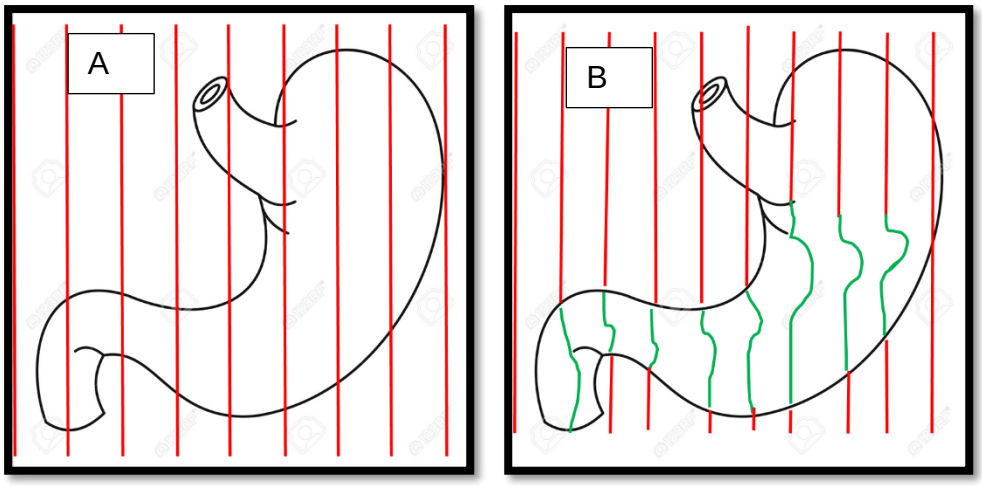

Figure 44: (A) is the no- motion situation and (B) is when motion is present.

In more technical detail, the tagged MRI sequence comprises of two non-selective RF pulses separated by magnetic field gradients. This combination produces a periodic spatial modulation of the magnetization (SPAMM) prior to imaging (Figure 45). Adding the prepulse and a delay between the prepulse and actual imaging sequence, the tissue motion can be recorded before the image is acquired, causing changes in the tags pattern. The tagged images show a tag pattern or periodic bands of zero signal due to the modulation. The tissue motion can therefore be recorded inside the MRI image[217].

Figure 45: Schematic representation of the SPAMM sequence consisting of a prepulse. Diagram authored by Sprengers et al [218].

Different MRI techniques have been used to evaluate gastric motility[212-214]. MRI Tagging has also been used to study gastric motility (more in terms of the stomach wall motion than in terms of intragastric chime motion) following a porridge meal[164]. It has also been applied previously to monitor small bowel motility[218, 219]. More recently, the tagged technique has been used by our group to visualize and assess the degree of fluid movement within the human ascending colon[216] and that initial work is expanded and translated here to the stomach environment.

Using this technique to look at the gastric contents may help to understand the mechanism of food movement, mixing and grinding within the stomach.

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4.1.1 Hypothesis

The development of this new tool could provide new information on the intragastric handling of foods which would be valuable not only for this thesis work but also for future studies.

4.1.2 Aims

Therefore, this study aimed to investigate the feasibility of using a continuously tagged magnetic resonance imaging sequence to monitor and assess gastric handling (such as mixing) of breakfast porridge meals in healthy subjects

4.2 Methodology

This study was separated into two parts. The first part of this study was optimization work, which involved *in vitro* and *in vivo* scanning. The second part involved scanning 17 healthy volunteers after they consumed two different breakfast porridges.

4.2.1 The Optimization work

The optimization work involved both *in vitro* and *in vivo* experiments.

In vitro experiments

To carry out meaningful tagging studies the system being examined needs to provide good contrast between the meal and the tag lines. The first experiment was done to assess this. The breakfast porridges was kept in a container inside the scanner.

The second experiment was done to determine whether is there were differences in the signal intensity and contrast between breakfast porridge made from oats or pearl millet. Both porridges were cooked in the same way and each porridge was placed in a glass jug. Water (as a reference) was also kept in a third jug. The three jugs were placed in water bath with the temperature set to a physiological level of 37°. When

the temperature of the three samples equilibrated, the MRI study started. A range of different delay times between tag placement and imaging was explored (300, 400, 500, 600, 700 and 900 ms), Figure 44. Two calculations were done after data collection to look at T1 recovery

1. Meal signal - tag signal.
2. (meal signal - tag signal) / meal signal.

In vivo optimisation

After the in *vitro* study, an in *vivo* optimisation phase was carried out. Breakfast porridge made from oats was used to assess which was the best position to put the participants in inside the MRI scanner: supine, prone or semi oblique. Also, a further optimisation of the timing delay between tagging and imaging was carried. Using a long tag delay allows for more motion to occur and be captured but also there is more signal recovery of the tag lines which loses contrast between the meal and the tags. Excessive delay can also cause smearing of the tag lines. Another point of interest for this optimisation phase was to assess if and how stomach wall motion could be visualised and correlated to chime motion inside the lumen.

4.2.2 The healthy volunteer study

Participants

17 participants (10 females and 7 males), aged 31.0 (SD 11.2) years old, and with a BMI of 23.9 (SD 3.1) kg/m^2 were included in the data analysis.

The data of the participants were collected from the previous study (MOM). Informed written consent was obtained from each participant before the study.

Experimental design

This study used a randomised, two way crossover design that consisted of two separate test days, approximately 1 week apart. An MRI scan was done to collect baseline images and to ensure that the participants' stomach was actually empty at baseline. After this MRI tagging assessment of the motion of the gastric contents was done immediately after feeding (T = 15 min) and 30 minutes later at T = 45 min.

The MRI data was blinded prior to analysis and the blind code was broken only after a blind data review was conducted.

Test meals

The two breakfast porridges were made from either Scottish oats commonly sold in a supermarket chain (Asda, United Kingdom) or a novel pearl millet flake (supplied by Unilever, Sharnbrook, UK, under a Material Transfer Agreement). Both products were in the form of steam rolled flakes. The test meals prepared for the study were iso-energetic (220 kcal each) and iso-volumetric (640 mL each). Both porridges were cooked in the same way,

Outcome measures

MRI was carried out on a research-dedicated 1.5T Philips Achieva MRI scanner (Philips Healthcare, Best, and The Netherlands). A cine-MRI tagged bTFE single slice sequence [219] positioned coronally oblique through the body and antrum of the stomach was used to visualize the motion of the gastric contents, at 15 and 45 minutes after ingestion of the breakfast porridges made from SOP and PMP. Delays of 300 and 600ms between the application of the tag lines and acquisition of the image were used (following optimisation) to allow visualisation of the motion of the gastric contents. The other parameters of the two scans were similar.

Both scans had TR/TE 3/1.15 ms, FA 60°, with a single coronal slice, thickness 15 mm, FOV 400 mm (FH), 400 mm (RL) These scans were acquired within a 20 second breath-hold with 20 cine frames (temporal resolution 1s).

Four regions of interests were outlined using Analyze9™ (Mayo Clinic, Rochester, USA): proximal stomach, distal stomach, whole stomach and liver.

Data analysis

Movement of the gastric content was visualized and assessed by the co-efficient of variance (CoV) method as previously described[216]. The series of tagged images of the stomach provided a method to visualize and assess movement of the gastric content. If no movement of gastric contents, or adjacent organs occurred during the breath hold scan (20 seconds), the tagged images would be identical with all tag lines

remaining straight. However, the position of tag lines would change if any movement of gastric contents occurred during the scan. The movement of the gastric content, as motion, will lead to changes in signal intensity in the tagged gastric contents from frame to frame. The variation in the signal intensity is the basis for this method to assess motion with gastric contents.

The co-efficient of variance method (CoV) calculated both the mean signal intensity (MI(x, y)) and standard deviation (STDEV(x, y)) on a pixel by pixel basis through the dynamic frames. The mean and standard deviation were calculated using custom-written software IDL® (Research Systems Inc, Boulder, CO, USA) resulting in maps of both mean intensity and standard deviation (Figures 46 A and B). In the standard deviation maps, those voxels whose intensity is changed during the scan due to movement the gastric wall, or by the movement or smearing tagged lines have a larger standard deviation, whereas static structures such as liver has a smaller standard deviation.

The average coefficient of variation (%COV) for the tagged scan is then estimated from the following equation:

$\%COV = 100 \times STDEV_R / MI_R$

($STDEV_R$) is the average STDEV and (MI_R) is the average mean intensity within that region

The observations of tagging movies were done to see gastric motion the anterograde and retrograde and percentages were calculated.

Statistics

Prism version 6.07 (Graph Pad Software Inc., La Jolla, CA) was used to undertake descriptive and statistical analyses. All data are presented as mean ± SEM unless otherwise indicated. The data were assessed for normality using the Shapiro-Wilk's test. All data were normally distributed and were analysed using parametric methods.

Comparisons of the mean values for the CoV in 4 different regions (distal stomach, proximal stomach, whole stomach and liver) for the t = 15 time and t = 45 minutes with delay 300 and 600 ms of SOP and PMP were made with the use of Student's paired t test (2 tailed). If the individual meals were not significantly different, the data of both meals will be pooled.

4.3 Results

The in vitro optimisation work showed that the contrast between the two meals and tags were very similar at the two time delays 300 and 600 ms (table 14 and table 15). The *in vitro* optimisation work showed also that as the delay time was increased, the signal intensity within the tag-lines decreased (due to T1 relaxation).

Table 14: tag signal/ meal signal

Meals	delay 300 ms	delay 600 ms
SOP	0.103	0.334
PMP	0.111	0.290

Table 15: (meal signal - tag signal) / meal signal

Meals	delay 300 ms	delay 600 ms
SOP	0.897	0.666
PMP	0.889	0.710

The *in vivo* optimisation work showed that as the delay time was increased, the signal intensity within the tag-lines decreased (due to T1 relaxation), however a longer delay between tag and image allowed for slower motion to be detected (Figure 46). The longer delay times resulted in more 'smearing' effects in the images.

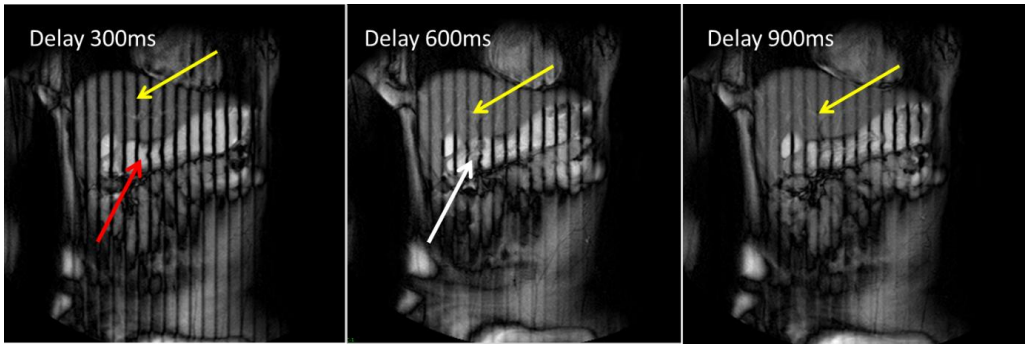

Figure 46: shows three images with different delay times (300, 600 and 900 ms) between tag and image. The red arrow indicates distortion of the tag line which shows slight bulk movement of the contents. The white arrow shows that less coherent motion causes smearing of the tag lines. Yellow arrows show that the tag lines become less dark with the longer delays as seen in the liver.

The *in vivo* optimisation work also showed the antral contractions moving forwards in the stomach is not related to the motion of the contents inside. However, this observation was based only on one healthy volunteer study.

In this study, displacement and smearing of the tag lines were observed in the stomach regions in the processed images, whereas the tag lines remained intact in the liver region (Figure 47).

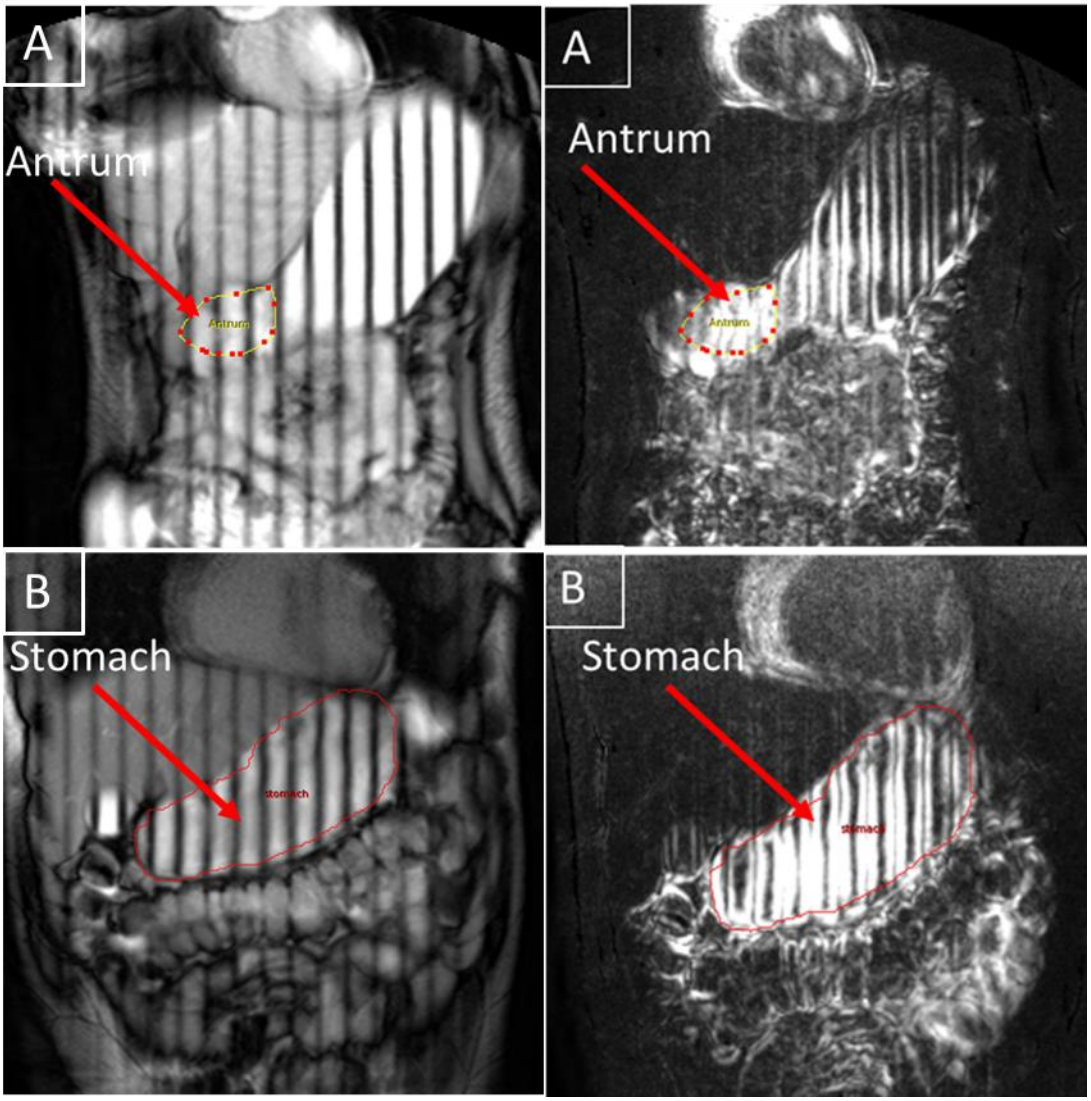

Figure 47: Processed images from tagged cine data showing motion in the antral region only (panel row A) and motion across the whole stomach (panel row B). The left hand side images show the pixel-by-pixel mean data calculated from cine frames across a breath hold, the right hand side of the panel shows the pixel-by-pixel SD data. The liver is almost black on the SD images as there is almost no motion in this organ.

4.3.1 COV values

The displacement and smearing of the tag lines resulted in higher CoV values. The mean values for the CoV in the 4 different regions (the distal and proximal part of stomach, whole stomach and liver) for the t = 15 and t = 45 minutes are shown in Tables 16- 19.

There was no significance difference in COV values between both meals at both times (t = 15 and t = 45 minutes). There was also no significance difference in the COV values between both meals with the two delay times (300 and 600 ms) for both meals (Table 19).Therefore, the COV values will be presented for both meal combined.

Table 16: The mean values for the CoV in the 4 different regions (the distal and proximal part of stomach, whole stomach and liver) for breakfast porridge made from Scottish oats (SOP).

	Time points		Distal stomach		Proximal stomach		Whole stomach		Liver	
			Delay 300	Delay 600	Delay 300	Delay 600	Delay 300	Delay 600	Delay 300	Delay 600
SOP	t = 15	Mean	31	27	24	21	26	22	9.9	6.9
		SEM	2	2	2	2	2	2	0.5	0.3
	t = 45	Mean	26	22	26	20	25	20	9.9	7
		SEM	2	2	2	1	2	1	0.5	0.3

Table 17: The mean values for the CoV in the 4 different regions (the distal and proximal stomach, whole stomach and liver) for breakfast porridge made from pearl millet (PMP).

	Time points		Distal stomach		Proximal stomach		Whole stomach		Liver	
			Delay 300	Delay 600	Delay 300	Delay 600	Delay 300	Delay 600	Delay 300	Delay 600
PMP	t = 15	Mean	31	25	25	21	26	21	9.5	6.7
		SEM	2	2	2	2	2	1	0.6	0.3
	t = 45	Mean	25	21	26	21	25	21	10.3	7
		SEM	2	2	3	2	2	2	0.6	0.4

Table 18: The mean values for the CoV in the 4 different regions (the distal and proximal part of stomach, whole stomach and liver) for both porridges combined (SOP and PMP combined).

	Time points		Distal stomach		Proximal stomach		Whole stomach		Liver	
			Delay 300	Delay 600	Delay 300	Delay 600	Delay 300	Delay 600	Delay 300	Delay 600
	t = 15	Mean	31	26	24	21	26	22	9.7	6.8
SOP and		SEM	2	1	1	1	1	1	0.4	0.2
PMP_combined	t = 45	Mean	25	22	26	20	25	20	10.1	7.0
		SEM	1	1	2	1	1	1	0.4	0.3

Table 19: difference in the COV values between SOP and PMP at t = 15 and t = 45 min for the 4 different regions the distal and proximal stomach, whole stomach and liver) with two delay times (300 and 600ms).

	Proximal stomach	Distal stomach	Whole stomach	Liver
Difference between 300 and 600 delay at t = 15 after SOP and PMP combined	0.0001	0.0001	0.0001	0.0001
Difference between 300 and 600 delay at t = 45 after SOP and PMP combined	0.0001	0.4	0.0001	0.0001
Difference between t = 15 and t = 45 at delay 300 after SOP and PMP combined	0.659	0.0001	0.7	0.267
Difference between t = 15 and t = 45 at delay 600after SOP and PMP combined	0.642	0.0001	0.107	0.163

Distal stomach (Antrum)

The CoV value for the distal part of stomach with 300ms delay time was significantly higher than that with 600ms delay time at t = 15 for both meals combined (Table 19). At t = 45 min, there was no significant difference in the CoV values between both delay times (300 and 600 ms) in the distal part of stomach both meals combined (Table 19).

Proximal stomach (Fundus and body)

The CoV value for the proximal part of stomach with 300ms delay time was significantly higher than that with 600ms delay time at t = 15 and t = 45 for both meals combined (Table 19). There was no significant difference in the CoV values between t = 15 and t = 45 min for both meals combined in both delay times in the proximal part of stomach (Table 19).

Whole stomach

The CoV value for the whole stomach with 300ms delay time was significantly higher than that with 600ms delay time at T15 and T45 min for both meals combined (Table 19).There was no significant difference in the CoV values between t = 15 and t = 45 min in both delay times (300 and 600 ms) for the whole stomach at for both meals combined (Table 19).

Liver

The CoV values of liver with 300ms delay time was significantly higher than that with 600ms delay time at t = 15 and t = 45 min for both meals

combined (Table 19). The significant difference between 300 ms and 600 ms in liver region is related to the reduction in contrast from the tag lines. There was no significant difference in the CoV values between t = 15 and t = 45 min at both delay times (300 and 600 ms) in the liver for both meals combined (Table 19).

4.3.2 Tagging movies

The observations of tagging movies showed that postprandial movement was observed in subjects. About 38% of motion is antegrade. Twice as much (75%) is retrograde with about 38% showing both movements (antegrade and retrograde). The tagging movies showed that the motion is mostly along the central axis of the stomach. A central 'magenstrase' was observed in the stomach but motion seems to be twice as often retrograde than antegrade (Figure 43).

4.4 Discussion

This study focused more on developing MRI methodology and investigated the feasibility of continuously tagged imaging in the stomach using a bTFE acquisition to monitor intragastric handling of breakfast porridge made from oats or pearl millet.

The MRI tagging sequence is ideal to visualize and assess the movement of the stomach contents. In this study, chyme movement was seen for all the subjects using this method. The contrast between both meals and tags were also similar at the two time points (T15 and T45) and at both delay times (300 and 600 ms). Both meals had a sufficiently

long T1 to let the tag lines remain dark in the stomach for delay times up to 1 s.

The CoV method of analysis provides information that is easy to visualize and interpret. The shorter delay times resulted in higher CoV. This may be for two reasons: (1) the darker tag lines in the 300 ms delay images would give a larger CoV for the motion of the stomach contents[216] (2) The longer delay allows more time for the contents to move and may result in more smearing of the tag-lines, reducing the variability between cine frames[165].

The defined regions in the stomach showed higher CoV compared to the liver which indicates that the contents of stomach were moving and mixing during the acquisition time, whereas the tag lines remained intact in the liver region. The lower Cov of liver is due to the liver is a static structure or motionless.

As the delay time was increased, the signal intensity within the tag-lines decreased (due to T1 relaxation). A longer delay (600ms) between tag and image allowed for slower motion to be detected. However, the longer delay times resulted in more ‘smearing’ effects in the images if larger scale motion is present.

In conclusion, tagged cine-MRI can be used to visualize and assess the movement of stomach contents following a porridge breakfast. Further analysis of the tag line deformation on a frame-by-frame basis will provide more information on the movement of the contents. In future work this technique could be applied to different meals to observe gastric mixing processes and increase our knowledge of its relation with gastric

emptying, glycemic and appetite responses. This method is relatively easy to implement and could provide an additional parameter to add to the scan card for future studies.

5. Conclusion and future directions

5.1 Conclusion

Obesity, the prevalence of which is increasing globally, is associated with an increased risk of developing chronic diseases such as type 2 diabetes and cardiovascular disease. Diet, amongst other lifestyle factors, potentially contributes to the development of obesity.

Whole - grain-based breakfast porridge consumption has been associated with beneficial health effects with respect to obesity, and related disease risk factors.

Breakfast porridges made from different plant grain origin are commonly consumed throughout the world. Whilst some data about post ingestive physiological responses are available for varieties that are popular in the Western world, such as oats and rye, other *'ancient'* grains used in the Asia and in Africa such as millets have been minimally studied. This is despite suggestions that they may have beneficial health effects, such as modulation of appetite and circulating blood glucose responses. These grains can be grown in areas with harsh climate and could contribute positively to both the health and food security agendas.

The PhD project therefore included three studies: a pilot human feeding study, the main human feeding study and MR developmental study. The aim of the studies was to test the hypothesis that breakfast porridges made from different cereal grains may produce different physiological responses.

The first study aimed to collect pilot data on postprandial glucose levels, gastric emptying and satiety following consumption of isoenergetic breakfast porridges made from oats, rye, finger millet and pearl millet. This study was the first pilot, in vivo imaging study assessing the physiological responses to porridges made from different 'modern' and 'ancient' cereal grains in healthy participants. In this pilot study, the breakfast porridges from 'ancient' varieties of millet grains showed physiological responses that were comparable with those from common Western varieties known to have beneficial health effects. The pilot study also showed that pearl millet appeared to induce lower postprandial blood glucose response and appetite scores though small numbers did not allow conclusive inferences against other grains.

There were several limitations to the study including the fact that the test meals were physically different, two were steam rolled flakes and two plain grains ground to a flour. The test meals were prepared slightly differently to obtain a more acceptable final product which may have altered the bioavailability of carbohydrates. Seven participants found the palatability of finger millet poor and could not finish all the test meal. The isoenergetic portions were of different volume. Use of capillary blood glucose does not represent arterial blood however it is a close approximation[132]. Also, future studies should measure insulin. Some food diaries were missed limiting our opportunity to assess the impact of the porridge consumed as a breakfast on 24 energy intake. Appetite ratings are a proxy measure for what people will actually eat. This leads us to suggest that this will be better assessed in future studies by

providing lunch using an *ad-libitum* objective test meal thereby providing a more accurate and objective measure of actual food consumption at midday. Another suggestion for future work is to manage more closely the return of the food diaries.

This pilot study was our first experience of working with whole grain, porridge breakfast interventions. Although the study did not offer many significant differences in the physiological and gastrointestinal responses after consumption of the four breakfast meals, valuable experience has been gained in the implementation of the protocols and provided useful directions for further studies.

This pilot study has been very informative for the subsequent development of this project. As a consequence, we better understood issues related to cooking, acceptability the meals, food diaries return and physical form of the products. Furthermore, the preliminary data collected from this pilot study was used to power properly the main physiology study. The pilot study showed also differences in intragastric appearance of these meals and a question was raised about using MRI to investigate the stomach handling of these meals such as mixing and motion. This led to the last study, focussing more on MRI and methods development for future studies than basic physiology.

Following this rationale and building on the previous pilot study, we planned a single-center, randomised, two- arm crossover trial in healthy volunteers. Accordingly, this main study aimed to compare the glycaemic, gastrointestinal, hormonal and appetite responses to a pearl millet and an oat porridge breakfast in healthy volunteers.

In this study, three peptide hormones were measured PYY, GLP-1 and GIP. These peptides were chosen because they influence appetite, satiety and glycaemic response. Moreover, an *ad-libitum* objective test meal were provided to the volunteers at lunch thereby providing a more accurate and objective measure of actual food consumption at midday.

The latter study was the first RCT of a pearl millet breakfast intervention in healthy humans. The study showed that PMP elicited glycaemic, insulinemic, GLP-1, PYY and appetite responses comparable to a known breakfast grain with recognised health-promoting characteristics. In addition, PMP had a larger iAUC gastric volume to that of SOP. Pearl millet also elicits significantly lower GIP plasma values, with GIP having been linked to triacylglycerol absorption in adipose tissue, a possible new added health benefit.

Pearl millet is an under-studied, resilient, ancient grain variety that grows with little water in harsh climates. It is currently underexploited as a human food despite potentially meeting both food security and health agendas. Pearl millet could therefore represent an alternative breakfast food with similar beneficial effects to those of oats and also sustainable and resilient agricultural credentials.

The strengths of the study included the direct analysis of the porridge meals, as served, having carefully controlled for differences in the degree of processing including manufacturing a novel pearl millet steamed rolled flake. Both grain flakes were cooked identically and in plain water as different cooking methods may have an effect on the degree of starch gelatinization[207, 208] and also to avoid macronutrient

confounders from added milk or jam. The fully integrated nature of the study allows a unique exploration of pre and post absorptive variables, and to relate this to subsequent perceptions and behaviours which is unique in relation to the study of millet

In this study, MRI data of three participants were excluded from analysis due to MRI scanner failure and blood data (insulin, GIP, GLP-1 and PYY data) of 4 participants were excluded due to vein cannulation failure.

Although the participants were of different body sizes, and hence would have had different energy requirements, the test meal portion given was the same for all participants and so would have been a higher proportion of total energy intake for some. This may have reduced the potential for differences in energy intake at the lunch in the participants with a lower energy requirement. Matching for energy, rather than other micronutrients, meant that slight differences in, for example, fat composition may have confounded the results. However this was felt to be the most clinically relevant approach.

Lastly, the MRI developmental study demonstrated the feasibility of continuously tagged imaging in the stomach. The MRI tagging method provides direct imaging of motion by spatially modulating the magnetization prior to imaging. The tagged MR imaging method was chosen because it provides an easy-to-interpret ‘motion map’ that can be used to assess the movement happening inside an organ or a sample This study aimed to explore the ability of MRI in investigating the post-prandial motion of the breakfast porridge interventions in the stomach.

The tagged MRI imaging was used to visualise and assess the movement of the stomach contents following breakfast porridge meals. A cine-MRI tagged bTFE sequence was used in this study with two different delay times 300 ms and 600ms.

The study showed that shorter delay times result in higher CoV. The defined regions in the stomach showed higher CoV compared to the liver which indicates that the contents of stomach were moving and mixing during the acquisition time.

The method developed is relatively easy to implement and could provide an additional parameter to add to the scan card for future studies In future work this technique can be applied to different meals to observe gastric mixing processes and to increase our knowledge of its relation with gastric emptying, glycaemic and appetite responses.

Improved knowledge of the effects of different cereal grains could help direct dietary advice. The breakfast porridge intervention is relatively cheap compared with other interventions and could help reduce the burden of obesity and related metabolic disorders worldwide.

5.2 Research impact

The stakeholders who would benefit from this work include the general population, overweight, obese and diabetic individuals, farmers, food scientists, food manufactures and companies.

5.2.1 Economic impact

- The breakfast porridge intervention is relatively cheap and could help to reduce the burden of obesity and related metabolic disorders worldwide
- Pearl millet is one of the most drought resistant grains in commercial production. It is able to grow in areas that experience frequent periods of dry weather. Pearl millet can grow in areas where other grains such as oats, wheat and rice are difficult grow.
- Pearl millet appears to be more tolerant of sandy and acidic soils than other summer grain crops.
- Food manufactures may produce new products made from pearl millet such as snacks.

5.2.2 Societal impact

- Improved knowledge of physiological and appetitive responses to breakfast porridges could direct dietary advice based more strongly on evidence in order to benefit patients and the general population.
- Pearl millet is nutritionally comparable to major cereals. It is a rich source of minerals especially iron and zinc.

5.2.3 Academic impact

- Novel use of in vivo imaging techniques for different porridges will increase our knowledge of the behaviour of these meals in the gastrointestinal (GI) tract. This will facilitate an understanding of

the interface between the input of a given feeding stimulus and various physiological and behavioural outputs. This will help us to improve our understanding of the effect of physical properties of food on digestion and appetite, engineer foods with the desired in vivo behaviour and develop more relevant *in vitro* / *in vivo* food digestion models.

- Improved knowledge of the relationship of MRI data other physiological responses could help scientists to design their feeding studies using these methods.
- Improved knowledge of under-researched ancient grains fitting the sustainability agenda and the health agenda

5.3 Future directions

The initial work described above is based on one single acute exposure. The effect of repeated consumption of pearl millet as a breakfast intervention is not known and would be interesting to study

Consumption of low glycaemic Index (GI) food has positive effects on the glucose response and these positive effects can persist beyond this period. This effect is known as the second meal effect, the bioavailability glucose has a positive effect on the glucose tolerance of the subsequent meal. [220-222].

Furthermore low GI foods often increase colonic fermentation because of the presence of fibre and resistant starch. Colonic fermentation of indigestible carbohydrates could be a further mechanism involved in reduced glycaemia during the second meal[220]. However, limited studies have been conducted to investigate the second meal physiological and gastrointestinal responses to these grains and also the chronic effects to these meals.

We are designing the next study. This will be a three-way crossover design study. Using our previous data, twenty-six healthy volunteers will participate in this three-way crossover design study. The participants will be without medical, behavioural or food allergy/intolerance issues that would prevent their participation in the study or affect the interpretation of results.

Three breakfast porridges made from pearl millet, white rice and brown rice will be used. The breakfast meals will have same energy value and have the same volume. The porridges will be cooked in water and

served with jam and glass of water. They will be cooked in the same way. A standardised lunch will be provided approximately four hours after the breakfast on the test days.

The participants will undergo two 7-day diet periods separated by a minimum 4-week washout in a randomized crossover study. The day before (visit 1) and the day after each diet period (visit 2), participants will attend the study centre for a test day, collecting data at baseline, during the morning after the breakfast intervention and also for 4 hours after the second meal.

Gastric emptying, small bowel water contents and colonic volumes will be measured using MRI. Blood will be collected for measurement of glucose, insulin; related gut hormones and nonesterified fatty acids (NEFAs). Breath hydrogen will be measured as a marker of colonic fermentation. The feeling of hunger, fullness, satisfaction; desire to eat and prospective food consumption will be assessed using visual analogue scales, followed by an objective *ad-libitum* pasta meal.

The smaller particle size of the PMP flakes compared to SOP flakes may have offered an increased surface area to digestion[80, 186]. Therefore, it would be interesting to do also an *in vitro* study to investigate the effects of different particle size for oats and pearl millets.

Further work needs to be done to automate more the tagging analysis and also to display the results in more user – friendly way than using the coefficient of variance. MRI is a promising tool to study flow and mixing and clearly more work needs to be done.

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