The acute effect of food structure on post prandial glucose and subsequent metabolic responses.

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Abstract

Glucose is a major molecule for energy supply. Thus, the movement of glucose from food into tissues is necessary for survival. However, since high levels of glucose are toxic to the body, glucose homeostasis plays a key role in preventing a number of diseases, including type 2 diabetes, obesity, coronary heart disease and many types of cancer.

Empirical evidence has shown that postprandial glucose and insulin sensitivity are not only governed by the content of food but also by other factors that may play a role in the digestive process, including food structure, rheological properties and food breakdown pattern. The combination of different levels of structure—from the molecular to the micro and macro levels—develops the rheological behavior of food that impacts the availability and digestibility of nutrients.

In this work, I brought together nutritional physiology and rheology to explore the impact of peas and chickpeas on postprandial glycaemia and to develop a better understanding of how their physical properties change the physiological response. This in turn will give insight into the effect of food structure and its rheological properties on postprandial metabolic responses.

The main finding of this work highlighted the importance of an intact cell wall in lowering postprandial glycaemia and subsequent metabolic responses when compared to a ruptured structure. Also, this work showed that starch structure played a role in postprandial glycaemia and insulinemia when the cell wall is absent.

Further investigation was performed on the rheological properties of foods with different structures, relating these results with metabolic responses. Results from studies assessing the rheological and mechanical properties of food strongly suggested that they indeed affect the food breakdown pattern during digestion. It was shown that an intact structure's rheological properties slowed the breakdown of food during digestion. Also, different intact structures had different rheological properties that suggested an impact on the food's behaviour during digestion and hence on postprandial metabolic responses.

Throughout this thesis, an indirect relation between food structure, its rheological properties, food breakdown during digestion in relation to postprandial glycaemia and related metabolic responses was realised.

Copyright Declaration

Declaration of Contributions

The majority of the work in this thesis was performed by the author. All collaboration and assistance are described below.

All clinical studies were performed with the help of the research nurses and administration staff at NIHR/welcome Trust Imperial Clinical Research Facility.

Chapter 3:

This study was carried out with the assistance of Lucia Braz. Dietary ingredients were provided by Dr. Cathrina Edwards, Quadram Institute Bioscience, Norwich and Professor Peter Ellis, Kings college London. In vitro testing of starch was conducted with the assistance of Dr. Cathrina Edwards, Quadram Institute Bioscience, Norwich.
Radioimmunoassays were performed with the assistance of Dr. Edwards Chambers and Dr. Claire Byrne.
Rheological tests were performed with the assistance of Muhammad Iqbal and under the guidance of Dr. Ruth Brooker and Mike Rideout, Quadram Institute Bioscience, Norwich.

Chapter 4:

The pea seeds used were provided by Professor. Claire Domoney and the John Innes Centre, Norwich. The pea flour was produced by Campden BRI, Surrey.
This study was carried out in collaboration with Dr. Katerina Petropouloe and Mai Khatib. Nasogastric and nasoduodenal tubes were placed throughout the duration of the study by Dr. Eleutheria Panteliou, Dr. Lilian Mendoza, and Dr. Nicholas Penny.
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Poster presentation

Rasha Alshaalan, Cathrina Edwards, Peter Ellis, Gary Frost, Maria Charalambides.”The impact of the Hummus structure and rheological properties on glycaemic response in humans”. Annual European Rheology Conference, 2018

Manuscripts in preparation detailing the work of study 1 presented in this thesis: The Acute effect of different structures of chickpea hummus on post prandial glucose response

Nomenclature

Adenosine Diphosphate	(ADP)
Adenosine Triphosphate	(ATP)
ADP Glucose pyrophorphorylase	(AGPase)
Body mass index	(BMI)
Cardiovascular diseases	(CVD)
Cell wall	(CW)
Celsius	(°C)
Clinical research facility	(CRF)
Coefficient variation	(CV)
Constant steady shear rate	(CSSR)
Enzyme-linked immunosorbent Assay	(ELISA)
Ethylenediaminetetraacetic acid	(EDTA)
Flour Hummus	(FH)
Gastric emptying	(GE)
Gastrointestinal tract	(GIT)
Glucagon like peptide-1	(GLP-1)
Glucagon-like Peptide-1 receptor	(GLP-1R
Glucose transporter	(GLUT)
Glucose-dependent Insulinotropic Peptide	(GIP)
Glucose-Dependent Insulinotropic Peptic receptor	GIPR)
Glycemic index	(GI)
G-protein coupled receptor	(GPCR)
Incremental area under the curve	(iAUC)
Insulin resistance	(IR)

Kilocalories	(Kcal)
Kilogram	(Kg)
Linear viscoelastic range	(LVR)
Micrometre	(*μ*m)
Millimetre	(mm)
Newton	(N)
Pancreatic polypeptide	(PP)
Pascal second	(Pas)
Radioimmunoassay	(RIA)
Rapidly digested starch	(RDS)
Relative centrifugal force	(RCF)
Research Ethics Committee	(REC)
Resistant starch	(RS)
Short chain Fatty Acid	(SCFA)
Slowly digested starch	(SDS)
Sodium Glucose Co-transporters	(SGLT-1)
Solid modulus	(G')
Starch branching enzyme	(SBE)
Standard error of the mean	(SEM)
Starch synthase	(SS)
Viscous modulus	(G")
Whole hummus	(WH)
Wild type *RR* peas	(*RR* Peas)
World health organisation	(WHO)
Wrinkled *rr* mutant peas	(*rr* Peas)

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CHAPTER 1: Introduction

1.1 Glucose homeostasis

1.1.1 Physiological significance of plasma glucose concentration

Glucose homeostasis is the balance between the rate of glucose entering the circulation by intestinal absorption, glucose production by the liver and the rate of glucose removal from the circulation. Such a balance is important to human health: as glucose is the foundation of our energy supply, the maintenance of a balanced level of glucose in the blood is necessary for survival (Frayn, 2010). This complex mechanism involves multiple organs as well as hormonal, neurohormonal and autoregulatory factors.

Glucose, a carbohydrate, is a nutrient that is used mainly for energy production in the human body. Blood glucose levels in healthy humans should be maintained within a physiological range that is between 4 and 5.9 mmol/l if fasted, and less than 7.8 mmol/l two hours after meals (UK, 2018). These levels are maintained within the normal range by the pancreas and liver, depending on the fed and fasted phase. In the fed phase, glucose is derived by intestinal absorption and thus blood glucose levels will increase. The major determinant of glucose appearance in the circulation is gastric emptying, among other factors (Aronoff et al., 2004). Insulin is secreted from the pancreas by ß-cells in response to elevated blood glucose. Not only does it control the uptake of glucose by peripheral tissues such as muscles and adipocytes, it also promotes the storage of glucose as glycogen in muscles and the liver (Caumo and Luzi, 2004). Glucose molecules are converted to fatty acids, amino acids and glycogen, or they will be oxidised by catabolic pathways (Szablewski, 2011). During the fasted phase, glucagon is released from pancreas ∂-cells to promote production of glucose by glycogenolysis and gluconeogenesis in the liver (Röder et al., 2016). Glucose homeostasis is also controlled by actions of glucoregulatory hormones such as glucan-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP).

1.1.2 Regulation of glucose homeostasis

Glucose homeostasis is controlled by a complex of physiological actions, including hormonal, neurohormonal and autoregulatory signals that regulate digestion, absorption and uptake of ingested nutrients by organs (Röder et al., 2016).

The pancreas, liver and incretins will be discussed in this section.

1.1.2.1 Glucose transport and uptake

When carbohydrates reach the small intestine, hydrolysis occurs, and thereupon monosaccharides, such as glucose, are produced to facilitate transportation across the intestinal mucosa (Frayn, 2010). However, as glucose molecules cannot cross the cell membrane by simple diffusion due to their polar nature and large molecule size, protein transporters are needed. As of now, two main types of glucose transporters have been identified: sodium-dependant glucose transporters (SGLT-1) and facilitated diffusion glucose transporters (GLUT), and both types can be divided into subclasses that are tissue-specific. These two transporters are different in their regulatory mechanisms and distribution (Navale and Paranjape, 2016). SGLT-1 is an active transporter that allows glucose to move up a concentration gradient along with sodium ions (Frayn, 2010). SGLT-1 mediates glucose diffusion across the intestinal brush-border membrane, whereas GLUT2 is a passive transporter that allows the movement of glucose across cell membranes only down a concentration gradient. GLUT2 allows glucose to move from the intestinal epithelial cells into the extracellular medium near the blood capillaries to be ready for absorption. In contrast, GLUT2 in hepatocytes regulates hepatic glucose metabolism. As for GLUT4, which is an insulin-responsive glucose transporter, it can be found in muscles, adipose tissues and the brain to facilitate the uptake of excess glucose from the circulation into cells (Navale and Paranjape, 2016).

SGLT1 also functions as a glucose sensor in enteroendocrine cells, which contributes to the glucose-induced secretion of insulin, GLP-1 and GIP. Experimental studies on mice showed that the responses of plasma incretin hormones (GIP, GLP-1) to an oral glucose load were reduced in SGLT1-deficient mice, which resulted in a decreased insulin response and higher blood glucose. However, GLUT2-deficient mice demonstrated unaltered plasma GIP and GLP-1 in response to an oral high glucose load (Roder et al., 2014).

1.1.2.2 The role of the pancreas in glucose homeostasis

The pancreas is a gland located behind the stomach; it exerts an exocrine function by producing exocrine enzymes to aid digestion and endocrine function, producing hormones to regulate blood glucose. Exocrine cells release digestive enzymes and peptides such as amylase, pancreatic lipase and trypsinogen that help in the breakdown of food during digestion (Röder et al., 2016). Endocrine hormones are

produced from the islets of Langerhans, which contain 5 distinct cell types: α- β- δ- ε- and γ (PP) cells (Jouvet and Estall, 2017). β-cells contain receptors of glucose that sense the elevation of glucose in blood and therefore trigger insulin secretion. α-cells are responsible for the release of glucagon, GIP and GLP-1; δ-cells and ε-cells are responsible for the secretion of somatostatin and ghrelin. Finally, PP cells produce pancreatic polypeptide that acts locally within the pancreas to autoregulate its endocrine function and regulates gastrointestinal secretion (Kojima et al., 2007).

1.1.2.2.1 Insulin secretion

Insulin is a glucoregulatory hormone that is produced by β-cells in the pancreatic islets of Langerhans in response to elevated blood glucose levels of more than 5 mmol/L. In healthy individuals, glucose enters β-cells through GLUT2. Following glucose uptake, glycolysis occurs, starting with an increase in the ATP/ADP ratio that causes the closure of K^+ channels and depolarization of the cellular membrane This causes the opening voltage-gated calcium channels to increase intracellular Ca. The high amount of intracellular Ca will trigger the fusion of insulin granules with the plasma membrane in order to be ready for the hormone release into the circulation (Jouvet and Estall, 2017).

During the fed phase, insulin response occurs in two subphases. The first is a rapid release of insulin, with a peak at around 5 minutes after the glucose stimulus. It is followed by a longer second phase, which is directly related to the degree and duration of the stimulus (Caumo and Luzi, 2004). Insulin controls the uptake of glucose by peripheral tissue such as muscles and adipocytes through the activation of the receptor. Insulin also promotes the storage of glucose as glycogen in muscles and liver by activating glycogen synthase and supressing glucagon secretion (Caumo and Luzi, 2004).

In addition to the high plasma glucose stimulus, insulin secretion is also modulated by the release of two incretin hormones – GLP-1 and GIP – from the gastrointestinal tract (L-cells and K-cells) in response to nutrients available in the GI tract and the stimulation of the vagus nerve by nutrients and gastric relaxation (Szablewski, 2011).

1.1.2.3 The role of gut hormones and their incretin effect in glucose homeostasis

The incretin effect is defined as a phenomenon of increasing the stimulation of insulin secretion in response to oral rather than intravenous glucose administration (Kuhre et

al., 2015). It is thought to be caused by the nutrient-stimulated release of incretin hormones and their insulinotropic action on pancreatic β-cells (Nauck and Meier, 2016). As of now, two incretin hormones have been identified: glucagon-like peptide 1 (GLP-1) and glucose-dependant insulinotropic peptide (GIP). Incretin hormones have low basal plasma concentrations and are released in accordance with an ingested meal that leads to high glucose concentrations, thus stimulating insulin secretion. GIP and GLP-1 account for 25–70% of the total postprandial insulin response, depending on the glucose load (Kuhre et al., 2015).

1.1.2.3.1 Glucagon-like peptide 1 (GLP-1)

GLP-1 is a 30-amino acid peptide that is released from the enteroendocrine L-cells found in the lower small intestine and colon (Holst, 2007). GLP-1 is the product of the post-translational processing of the proglucagon gene. It has two major bioactive forms: $GLP\text{-}1_{7\text{-}36}$ amide, which accounts for 80% of all circulating GLP-1, and $GLP\text{-}1_{17\text{-}37}$ (Lee and Lee, 2017). GLP-1 exerts several effects that are relevant to glucose homeostasis. First, it stimulates the uptake of glucose by muscle tissue and liver. Second, it induces β-cells to secrete insulin and supress glucagon release from α-cells (Woerle et al., 2012). Third, it reduces the secretion of proinflammatory cytokines that help in the restoration of β-cells. Fourth, it controls gastric emptying (Deane et al., 2010) and reduction of energy intake (Naslund et al., 1999) in addition to supressing endogenous hepatic glucose output (Lee and Lee, 2017).

GLP-1 secretion is in response to the ingestion of nutrients – including carbohydrates, fat, and proteins – and occurs through nutrient-sensing mechanisms. It works by binding to the glucagon-like peptide-1 receptor that can be found in the brain, the GI tract and β-cells (Lee and Lee, 2017). Specifically, the action of GLP-1 on β-cells is mediated by the binding of the peptide to a specific, seven-membered transmembrane receptor (GLP-1R), which is a member of the G-protein coupled receptor (GPCR) family. This action will increase the intracellular cAMP concentration and protein kinase A that sensitize β-cells to glucose-stimulated insulin secretion (Doyle and Egan, 2007). However, the half-life of GLP-1 is very short (less than 2 minutes) because of the rapid degradation by dipeptidyl peptidase IV enzyme (DDP-IV), which cleaves off the two NH_2-terminal amino acids (Holst, 2007). GLP-1 has received attention by the research community due to its anti-diabetic therapeutic potential. At present, five GLP-1 R agonists, which are resistant to degradation by DPP-IV, are available for use in

the United States and are approved by the U.S. Food and Drug Administration (FDA) for the treatment of type 2 diabetes (T2DM) ((Tran et al., 2017).

1.1.2.3.2 Glucose-dependant insulinotropic peptide (GIP)

GIP consists of 42 amino acids. It is processed from proGIP in the intestinal K-cells, the majority of which are located in the duodenum and proximal jejunum. It was first introduced as gastric inhibitory polypeptide based on animal studies. Later, the name was changed to glucose-dependant insulinotropic peptide after discovering that GIP can stimulate insulin secretion in animals and humans (Dupre et al., 1973). GIP secretion is species-specific; while carbohydrate ingestion stimulates GIP in pigs and rodents, fat ingestion does so in humans. Moreover, the absorption of nutrients rather than their presence in the small intestine is needed to stimulate GIP secretion (Yip and Wolfe, 1999).

GIP exerts a similar action to GLP-1 on β-cells; however, this action may differ on other tissues. GIP is released from K-cells in response to nutrient ingestion and binds to the GIPR receptor of the seven-transmembrane domain, heterotrimeric G protein-coupled glucagon receptor on β-cells. Similarly to GLP-1, GIP potentiates glucose-dependent insulin secretion through the elevation of intracellular cAMP concentration and the inhibition of ATP-sensitive K+ channels, which increases intracellular Ca^{2+}(Baggio and Drucker, 2007).

The half-life of GIP is reported to be less than 7 minutes in healthy subjects and less than 5 minutes in patients with T2DM (Seino et al., 2010), and similar to GLP-1, it is degraded rapidly by DPP-Vi and therefore loses its insulinotropic effects (Seino et al., 2010). GIP's main physiologic role is to enhance glucose-dependent insulin secretion. Additionally, GIP promotes β-cell survival and proliferation, and anti-apoptotic actions (Baggio and Drucker, 2007).

1.1.2.4 The role of the liver in glucose homeostasis

The liver plays an important role in glucose homeostasis. After consuming a meal, glucose is absorbed through the portal vein and may enter the pathway of glycogen synthesis and storage, whereas glycogen breakdown is suppressed. This process is activated by glucose and insulin. In the fasted phase, the liver is responsible for 80% of endogenous glucose production to the circulation (Ekberg et al., 1999). As tissues and organs such as brain and muscles are still using glucose, the liver produces glucose through glycogenolysis by the activation of glycogen phosphorylase.

Glycogenolysis is the production of glucose from the breakdown of glycogen. When glycogen is depleted in the liver, the liver switches to gluconeogenesis, which is the production of glucose from non-carbohydrate sources in order to maintain glucose levels. Rapid and accurate coordination between glucose, insulin and glycogen is present as insulin is secreted from the pancreas and reaches the liver directly, and glucose arrives directly in the hepatic portal vein; they control hepatic glucose production and promote glycogen synthesis, which helps in the maintenance of normal glucose concentrations in the circulation (Sherwin, 1980).

1.1.3 When glucose homeostasis is disturbed

To maintain a normal glucose homeostasis, 3 mechanisms should normally work: secretion of insulin by β-cells, suppression of hepatic glucose production and stimulation of glucose uptake by muscles and the liver. When glucose homeostasis is disturbed by a sedentary lifestyle or processed diet, many complications may occur that in turn lead to many diseases. One such complication is insulin resistance, a condition in which cells of the body become resistant to insulin; this causes a decrease in glucose uptake, which leads to hyperglycaemia (Riddy et al., 2018). At first, β-cells compensate for insulin resistance by producing more insulin to maintain normal glucose homeostasis. This will cause hyperinsulinemia, but it becomes less effective at lowering plasma glucose levels. This phase may last for years before the diagnosis of T2DM is made (Weir and Bonner-Weir, 2004). Ultimately, β-cells will be unable to respond to insulin, and, consequently, hyperglycaemia develops (Petersen and Shulman, 2002).

The mechanism of insulin resistance is not fully understood, although the literature has identified factors involved in the progression of insulin resistance such as glucose, chemical mediators and disturbances in insulin, glucagon and adipokines secreted by adipose tissues (Schofield and Sutherland, 2012). The increase in insulin resistance is associated with obesity (Petersen and Shulman, 2002), T2DM (Weir and Bonner-Weir, 2004), hypertension and dyslipidaemia (Zivkovic et al., 2007), all of which are risk factors for cardiovascular disease (Bonfante et al., 2015).

As shown in Figure 1.1, as insulin fails to clear glucose from the circulation, hepatic glucose increases, as hepatic glucose uptake is not insulin dependent. Glucose in the liver is converted to free fatty acids or free cholesterol; this will then be increased in the circulation, leading to fat accumulation in organs and a consequent disruption in their function (Zivkovic et al., 2007).

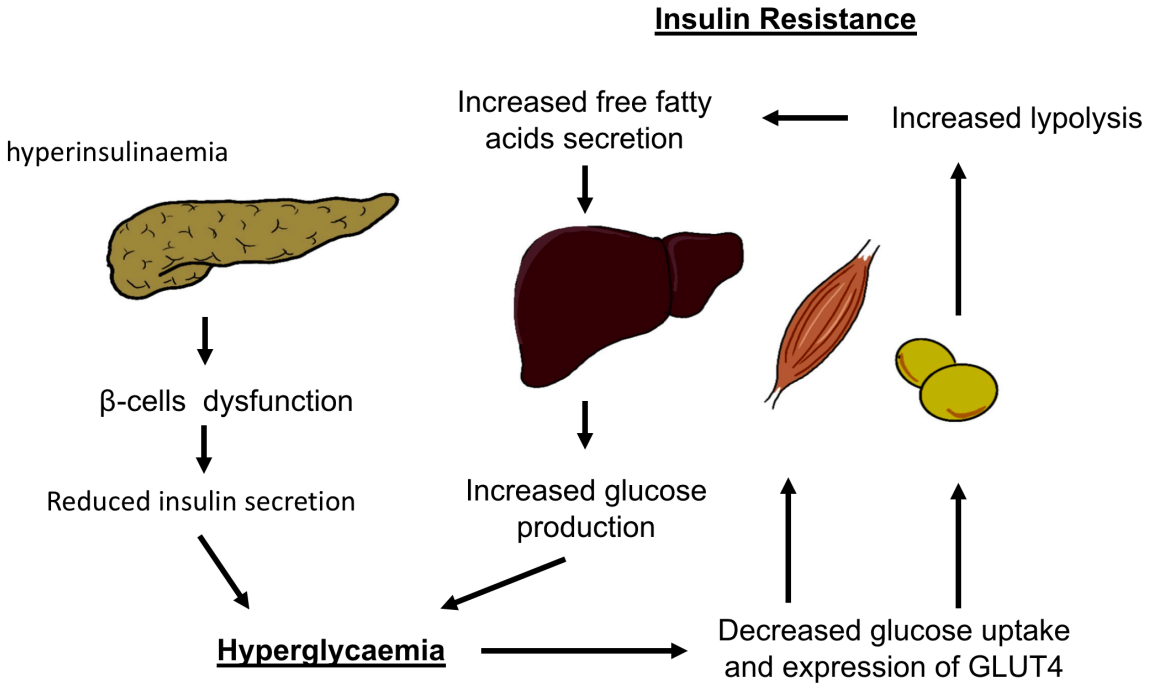

Figure 1.1 Overview of the pathology of insulin resistance that leads to T2DM. adapted from ((Petersen and Shulman, 2002)

1.2 Food structure

Food represents a complex system comprising macronutrients (carbohydrates, proteins and lipids), micronutrients (minerals, vitamins and phytochemicals) and water (Singh and Gallier, 2014). The definition of food structure can be derived from the literature as the cell structure and the spatial arrangement within the cell including cell wall (Gouseti et al., 2019, Aguilera, 2005). Food structure is a combination of different arrangements at different length scales. These arrangements can be seen using visual observation techniques such as electron or light microscopy – i.e. from the molecular (e.g. starch granules, amylose, and amylopectin) to cellular (e.g. cell wall) and macro levels. In addition, its state can vary from liquids to solids (Heertje, 2014, Singh and Gallier, 2014). This combination of different levels of structure develops the rheological behavior and textural properties of food as well as the mechanical properties, consistency, and stability which have an impact on the availability and digestibility of nutrients (Aguilera, 2005, Suman Mishra, 2012).

Food structure can be formed naturally or created artificially by food processing. It contributes to the texture, mechanical properties and appearance of the food matrix

(Waldron et al., 2003). Food structure that occurs naturally can be categorised into four groups: (1) fibrous structures that are assembled from macromolecules into tissue for a specific function, such as muscles; (2) fleshy materials from plants that are composed of hydrated cells that are held together by cell walls and are affected by its turgidity, such as fruits and tubers; (3) encapsulated embryos of plants that contain a dispersion of starch, protein or lipids that is assembled into discrete packets, such as starch in granules; and (4) complex fluids, such as natural milk (Parada and Aguilera, 2007). Although food composition tables provide information about the total amount of nutrients contained within a food, the bioavailability of a nutrient for absorption in the gut is not clear. Nutrient absorption depends on many factors, including food structure, processing and the interaction with other compounds during digestion (Waldron et al., 2003).

1.3 Carbohydrate classifications

Dietary carbohydrate has a complex structure that provide the major exogenous source for glucose, which is the primary source of energy for the cell. Dietary carbohydrate has a defined structure that is organized in a hierarchal order – from polymers to organs – as shown in Figure 1.2.

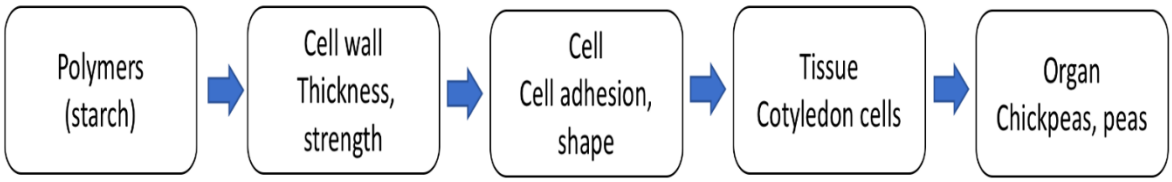

Figure 1.2 Food structure hierarchy.

Several carbohydrate classifications can be found in the literature, including available and unavailable carbohydrates, simple and complex carbohydrates, dietary fibres and resistant starch (Cummings and Stephen, 2007). Carbohydrate classification that is based on chemistry was one of the primary classification methods, and it depends on the character of individual monomers, degree of polymerisation and type of linkage. It can be divided into 3 main groups: sugars (monosaccharides, disaccharides) with 1–2 polymerisation degree(s); oligosaccharides, which consists of short-chain carbohydrates; and polysaccharides, which consist of starch with α-1-4 glucan and 1:6 glucan linkages and non-starch polysaccharides such as the polysaccharides in the plant cell wall (cellulose, hemicellulose and pectin; (Englyst et al., 2007). However, evidence has confirmed that glycaemic response is strongly related to the rate of

digestion rather than to chemical properties alone (Jenkins et al., 1982b), hence the need for digestibility classification for nutritional purposes.

Carbohydrate digestibility classification depends on physical and structural properties such as the type of monosaccharide absorbed, the presence of other components such as fat and protein, degree of solubility in water, gel formation, crystalline structure and their relation to the rate of digestion and absorption (Jenkins et al., 1987). Carbohydrates can be classified into three main groups: rapidly digestible starches (RDS), such as processed foods that contain α-glycosidic bonds that are easily broken-down during digestion by α-amylase, leading to a rapid elevation in blood glucose and usually a subsequent episode of hypoglycaemia. This fluctuation in blood glucose may disrupt the glucose homeostasis, causing stress on the body and thereby leading to tissue or organ damage (Ludwig, 2002). This type of starch poses a health challenge, as its consumption promotes the development of insulin resistance and subsequent metabolic diseases (Shelat et al., 2015).

Slowly digestible starches (SDS) also consist of α-glycosidic bonds, but they offer a slow increase in postprandial glucose levels and sustained blood glucose levels over time. It offers health benefits due to its stabilization and sustaining effect of blood glucose levels (Lehmann and Robin, 2007).

1.3.1 Resistant starch

Resistant starch (RS) is one type of starch that is found in food naturally or by processing. It is the fraction of starch that is not digested or absorbed by the human body. RS is a non-glycaemic carbohydrate that is similar to dietary fibres containing β-glyosidic linkages, and thus unaffected by the enzymatic activity (Aller et al., 2011). This causes RS to pass through the GI tract, reaching the large intestine where it undergoes fermentation that produces short chain fatty acids (SCFA), gases and water (Chambers et al., 2018). SCFAs has been linked to many health benefits, including decreased risk of developing gastrointestinal and cardiovascular diseases, and they also play a role in glucose homeostasis by initiating the secretion of incretins (Chambers et al., 2018).

RS can be divided into five groups (Birt et al., 2013): RS1, which is encapsulated within the food structure that is not accessible for digestion; RS2, which is raw uncooked starch that is resistant to amylolysis; RS3, which is recrystallized starch following thermal processing, such as retrograded starch; RS4, which consists of starch that is

chemically modified to resist digestion; and, finally, RS5, in which starch interacts with lipids to form an amylose-lipid complex that is thermally stable.

1.3.2 Non-starch soluble and insoluble fibres

Fibres were defined previously as the cellular walls of plants which is digested very poorly in humans (Trowell, 1972). Fibres can be classified into soluble and insoluble fibres which are both resistant digestion and absorption in the small intestine. Non-soluble fibres can be derived from fruits and vegetables as part of the plant cell wall structures. As for soluble fibres, they are polysaccharides including pectins that is also contributes to cell wall mechanics and support (Gouseti et al., 2019).

1.3.2.1 Plant cell wall

Plant cell wall (CW), a form of dietary fibre, is the proportion of food that is digested poorly in the human body (Mann and Cummings, 2009). CWs are not only important in maintaining cell structural integrity, but evidence also indicates that dietary fibres have health benefits in reducing the risk of many non-infectious diseases such T2DM, obesity and cardiovascular disease (Mann and Cummings, 2009, Kendall et al., 2010, Anderson et al., 2009). Empirical evidence has demonstrated that intact CWs play a role in controlling the rate and extent of nutrient release, as CWs block access of digestive enzymes to the granules, leading to a slower digestion rate and postprandial plasma glucose response (Berg et al., 2012, Dhital et al., 2016b, Rovalino-Córdova et al., 2018, Wursch et al., 1986b, Ellis et al., 2004, Berry et al., 2008, Edwards et al., 2015a). However, they may be affected by physical disruption such as processing or during digestion (mastication, mechanical force in the stomach or small intestine). The latter will cause either separation or rupture, depending on the CW mechanical properties and the cross-linkage holding CWs together (Waldron et al., 1997).

CWs may also change the meal's rheological properties during digestion such as increasing its viscosity, which may delay glucose and lipid release from the food structure (Capuano et al., 2018). Interestingly, evidence has demonstrated that cell walls can be fermented in the colon by microbiota to produce gasses and free fatty acids that may activate the production of GLP-1 and PYY in the colon in order to reduce appetite (Chambers et al., 2015)

1.3.2.1.1 Cell wall composition

CW composition depends on the type of plant, and it is influenced by the plant development stage. Plant CW consists of starch and non-starch polysaccharides.

Non-starch polysaccharides comprise a network of cellulose and hemicellulose embedded in a matrix of pectic polysaccharides:

Cellulose: this is a polymer of ß-1,4-linked glucan chains that consist of several thousands of glucose residues. Comprising crystalline microfibrils, these microfibril networks are formed by unbranched glucan chains that give cellulose its strong structure, which is resistant to digestion.

Hemicellulose: it consists of ß-1,4-linked backbone. Hemicellulose has an amorphous, semi-rigid structure. Strong hydrogen bonds can be found between specific sections of the hemicellulose and cellulose microfibrils. Xyloglucan (XyG) is the most abundant hemicellulose in dicotyledonous plants, and it is thought that microfibrils are linked by an XyG forming cellulose/Xyg network that is embedded in a pectin matrix (Vogler et al., 2015).

Pectin: it is the major component of the plant CW that it is known for its gelling properties. Pectin polysaccharides can be divided into three types: homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Mohnen, 2008). HG is the backbone of pectin with α-1-4 linked galacturonic acid residues. It is a highly methylesterified polysaccharide that can be de-methylesterified by cell wall protein apoplastic pectin methylesterases (PMEs) during growth, which will affect HG de-esterification patterns (Wolf et al., 2009). Pectic polysaccharides are thought to play a major role in cell adhesion and mechanical characteristics of plant CW (Johnson et al., 2018). It can mostly found in the middle lamella, acting as cement holding CWs together; this gives firmness and elasticity to tissue by modifying cellulose hydration (Terefe, 2011, Redgwell and Selvendran, 1986).

1.3.2.1.2 Cell wall mechanical properties

Mechanical properties of CW are defined by its biochemical composition and its specific interactions between wall polymers. This will also contribute to its anatomical structure, cell-cell adhesion and cell turgidity (Ryden et al., 2003).

Cell-cell adhesion occurs between two cells through the CW, specifically the middle lamella, which is rich in pectin that acts as a glue to hold the cells together (Mohnen, 2008). The modification of pectin, such as pectin de-esterification and calcium-mediated gelling, affects its ability and strength to hold cells. For example, de-esterified HG by PMEs will allow a cross link by calcium, leading to a more rigid structure that will stiffen the cell wall, altering the mechanical properties such that it will result in cell separation rather than breakage (Daher and Braybrook, 2015).

However, another cell wall protein, pectin methyl-esterase inhibitors (PMEIs), also has an effect on the mechanical properties of the cell wall that makes them less adhesive and easily separated (Daher and Braybrook, 2015).

The release of cell content depends on cell rupture rather than cell separation. Cell rupture occurs when the adhering of cells together is stronger than the CWs themselves. Alternatively, if CW is stronger than the forces holding the cells together, the cells will separate (Waldron et al., 2003).

1.4 Factors affecting digestion

In general, during digestion both mechanical and chemical forces work simultaneously to breakdown ingested solid food. First, the size of food particles is reduced by mechanical forces, which allows more surface area to be in contact with enzymes. Second, after the major size reduction, chemical forces, including gastric juices and digestive enzymes, further the breakdown of foods to help release nutrients so that they pass into the bloodstream.

Many factors influence the rate at which starch is digested: extracellular structure (cell wall), starch structural features, amylose: amylopectin ratio, and processing (Cummings and Englyst, 1995, Wursch et al., 1986a). These factors will be discussed thoroughly in this section.

1.4.1 Role of plant cell wall

Two types of digestive patterns may occur, depending on the porosity of cell wall. The first, the ‘inside out’ type, refers to enzymes being able to diffuse into the cell through pores and to start digestion from inside the cell toward the outside (e.g. cereals). The second, ‘outside in’, occurs when pores are not present in cell walls; thus, enzymes start digestion from the outside to the inside (e.g. legumes due to their smooth surface without pores; (Dhital et al., 2017). The latter will slow down digestibility rate and hence lower postprandial glycaemia (Jeong et al., 2019). Moreover, the size and microstructural characteristics of small particles play a role in nutrient absorption, as they affect the digestion kinetics such as the rate of gastric emptying by changing the viscosity and digesta flow in the gastrointestinal tract (Bornhorst and Paul Singh, 2014a).

1.4.2 Starch structure

Starch is considered a major energy reserve in edible plants. It is a glucose polymer composed of mostly linear amylose and highly branched amylopectin molecules

(Lovegrove et al., 2017). Starch structure consists of a hierarchical, complex structure from molecular to granular levels. Both structural levels are thought to play a role in controlling the breakdown of starch during digestion, including granule morphology, amylose: amylopectin ratio and botanical source (Cummings and Stephen, 2007). The structural hierarchy is changed or lost by processing (cooked, milled) or during digestion, affecting its rheological properties and hence increasing or decreasing starch hydrolysis during digestion (Jeong et al., 2019).

1.4.2.1 Amylose: amylopectin ratio

Starch granules comprise uniformly arranged molecules of amylose and amylopectin. Amylose consists of glucose molecules linked with a ∂ 1-4 glycosidic bond, forming a long linear configuration of glucose units, whereas amylopectin is highly branched due to ∂ 1-6 glyosidic bonds in addition to the short chains of ∂ 1-4 linked ∂-D-glucose units (Rao, 2014). Most starches consist of 20–30% amylose and 70–80% amylopectin; however, mutations can result in forms of starch with altered amylose: amylopectin ratios. For example, natural mutations in peas such as wrinkled *rr* peas contain 70% amylose (Lovegrove et al., 2017). Having a higher amylose-to-amylopectin ratio tends to slow down digestion, resulting in slower glucose release in the circulation (Lovegrove et al., 2017). Also, the non-hydrolysed proportion of the starch will travel to the colon for fermentation by the gut microbiota (Chambers et al., 2018).

Plants synthesize starch in semi-crystalline form to minimise storage space and to maximise the energy stored (Tester et al., 2004). Crystallinity can be determined using x-ray diffraction scattering to differentiate the degree of crystallinity. As shown in Figure 1.3, type B consists of double helices amylopectin that are packed in antiparallel, hexagonal mode, whereas amylose is amorphous and scattered among amylopectin molecules. The central channel is surrounded by six double helices filled with water. Type B is commonly found in potato, banana and high amylose starches. Type A is similar to type B but differs in the central channel that is occupied by another double helix, making the packing closer, and it is found in most cereal starches. Type C is a mixture of both A and B and can be found in legumes starches (Cummings and Stephen, 2007).

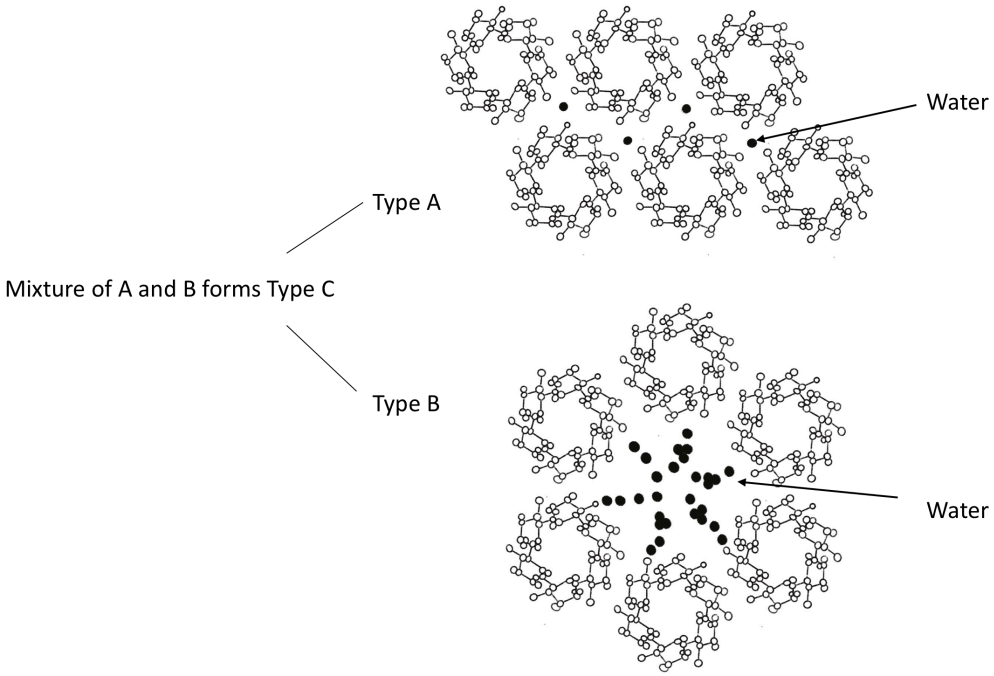

Figure 1.3 Types of crystallinity A and B. Adapted from (Buleon et al., 1998)

1.4.3 Processing

1.4.3.1 Gelatinization and Retrogradation

Gelatinisation of starch is a process that occurs in the presence of water and heat. During this irreversible process, water de-stabilises hydrogen bonds in the amorphous regions of granules, which results in granular swelling. This process leads to the loss of birefringent properties as starch becomes more disordered, and that will change the crystallinity of starch and separate (Matignon and Tecante, 2017). Several factors may control gelatinization such as heat, water and space. Full gelatinisation may occur when there is no limit to heating, hydration and space. The more gelatinised starch that is present, the higher accessibility of the digestive enzymes to the glycosidic bonds, thus increasing the digestibility rate of starch (Jeong et al., 2019). Also, different behaviours of gelatinization can be observed, depending on the starch characteristics, structure and cell wall presence (Edwards et al., 2015c). Legumes are a good example of resisting full gelatinisation during cooking. Legumes contain an intact cell wall that limits the access of water to the starches. This will lessen starch granular swelling, which leads to distorted granules that are resistant to digestion (Dhital et al., 2016a). Moreover, higher amylose content may increase the gelatinisation temperature and viscosity (Ma et al., 2017).

However, if gelatinised starch is cooled down, 'retrogradation' occurs, as shown in Figure 1.4, which involves re-association of starch molecules to form a more ordered structure; this has a major impact on the digestibility rate by making it more resistant to digestion (Patel et al., 2017). Although both amylose and amylopectin tend to retrograde, amylopectin will retrograde to a lesser degree compared to amylose due to its high branching and thus higher penetration of digestive enzymes, leading to a higher digestibility rate (Ottenhof and Farhat, 2004).

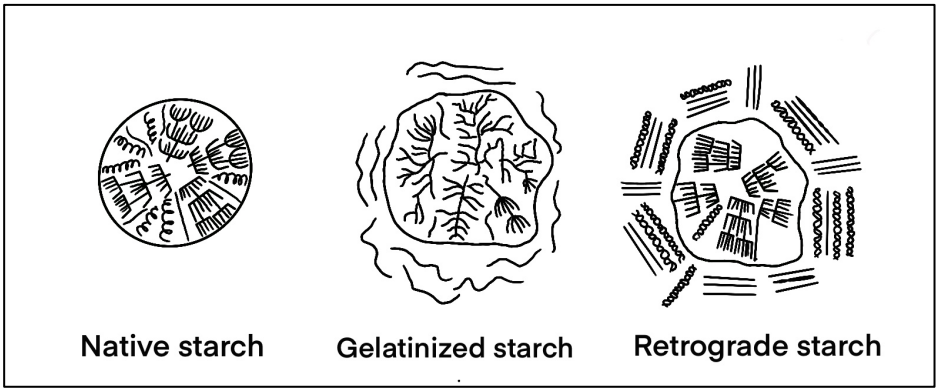

Figure 1.4 Crystallinity changes in starch during gelatinization and retrogradation of starch.

1.4.3.2 Milling

Disruption in food structure can be performed domestically and industrially. Processing such as milling in plant base foods may result in the loss of the protective barrier in starch granules such as the cell wall, proteins and lipids on the starch granule surface and loss of the molecular packing of starch within the starch granule (Mishra and Hardacre, 2012). Milling is the mechanical disruption of food structure using strong shearing forces that break the seed coat and endosperm tissue. This process will result in a form of powder with varying particle size (Oghbaei and Prakash, 2016). The milling process can be performed by either converting legumes into flour without abstracting any parts, or by exposing them to roller milling in order to yield multiple products (Oghbaei and Prakash, 2016). Milling food structure will result in smaller particles that will increase the rapidly digestible starch and decrease the resistant starch (Hallfrisch et al., 2000).

1.5 Food rheology

Rheology is defined as the study of deformation and flow matter (Rao, 2014). Specifically, food rheology measures the food response to a force at the macroscopic level. However, the latter is affected directly by changes at the microscopic level. Food rheology cannot lead to a straightforward physical classification of materials, as real foods are a complex mixture of food ingredients. Therefore, food rheology is defined as the study of rheological characterization that quantifies the functional relationship between stress, deformation and the resulting properties such as viscosity, elasticity and viscoelasticity (Fischer and Windhab, 2011).

1.5.1 Basics in rheology

The mechanical properties of foods depend on their structure, physical state, and rheology, and these properties are affected by chemical composition and hierarchical structural organization. Moreover, properties can be intrinsic – controlled by the food itself, such as viscosity – or responsive – dependent on external conditions, such as flow behaviour depending on mastication (Fischer and Windhab, 2011). Rheology measures the relationship between stress and strain. 'Stress' can be defined as a force acting on food structure, while 'strain' is the change in size or shape of a food in response to the applied force. Rheological tests can be performed to obtain the stress-strain relationship (Steffe, 1996). Ideal solids deform in an elastic matter, while ideal liquids flow in a viscous Newtonian matter. Foods, containing solid bits combined with liquids, have viscoelastic behaviour that is dependent on strain (Fischer and Windhab, 2011).

Foods' mechanical properties may vary drastically depending on the stress application. There are three types of stresses that can be used to measure the mechanical properties of foods (Figure 1.5): tensile, where the force is directed away from the material; shearing, where the force is directed tangentially to the material; or compressive, where the force is directed toward the material (Steffe, 1996). Foods respond to these stresses in three ways: elastic, viscous or (mostly) viscoelastic.

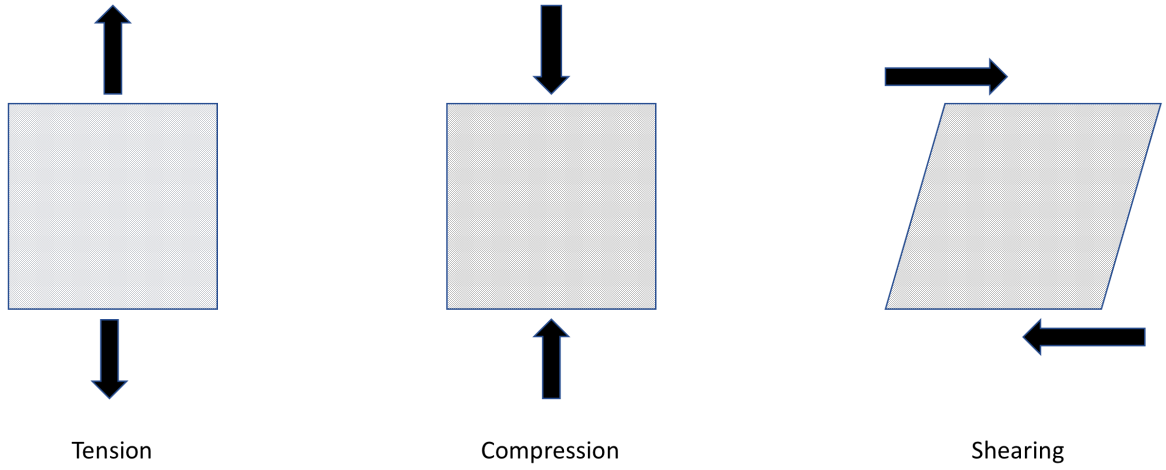

Figure 1.5 Three types of stresses that can be used to measure the mechanical properties of foods.

Different types of rheological behaviour are shown in Figure 1.6. A Newtonian behaviour is defined as a fluid having an ideal viscous flow that is independent from time and force, with a simple linear relation between shear stress and shear rate. A non-Newtonian behaviour, having a non-linear relation between shear stress and shear rate, has a viscosity that is dependent on time or history of deformation, or both. Non-Newtonian behaviour is described as either shear thickening behaviour if the viscosity is increased with the increase in shear rate, or as shear thinning behaviour if the viscosity is decreased when the shear rate is increased (Rao, 2014). For example, hummus that contain a significant amount of dissolved low weight molecular compounds such as sugars and insoluble solids can be expected to exhibit viscous and elastic characteristics, which is a non-Newtonian behaviour featuring shear thinning behaviour (Steffe, 1996).

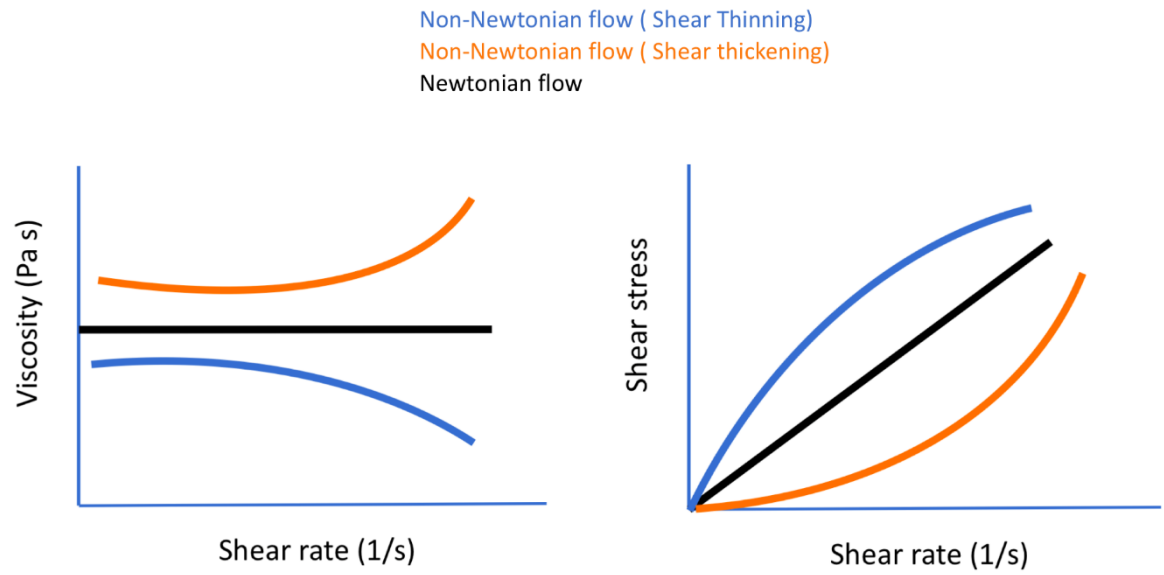

Figure 1.6 Types of flow behaviour: Newtonian and non-Newtonian flow.

1.5.2 Food structure, rheology and digestion

Flow behaviour plays a role in describing food structure before ingestion (manufacturing, kitchen) and during digestion (mastication, nutrient release at specific sites). Digesta flow properties provide important information about food performance and behaviour with regard to a variety of chemical and physical disruptions during digestion. To understand how digestion is optimized or impeded by food structure, it is necessary to know how food structure influences the food flow and mixing under physical and chemical forces during digestion. During digestion, the flow behaviour can influence nutrient release from a food matrix. Flow behaviour is affected by many factors, including a food's mechanical properties, cell-cell adhesion, particle size and fluids. A study by Shelat et al. on pig digesta showed that changes in those properties could alter the digesta viscosity and other rheological characteristics as digesta go through chemical and physical forces affecting the reduction in structuring of macronutrients that will highly change its rheological properties (Shelat et al., 2014).

1.5.2.1 Food structure and early digestion in humans

Digestion is a complex process that works on two levels: mechanical and chemical. The human gastrointestinal (GI) tract consists of a multistage system in which food is processed, dissolved and broken down into small particles in order to be ready for absorption by epithelial cells. The GI tract consists of four compartments: mouth, stomach, small intestine, and large intestine.

Oral phase

This is the first stage of carbohydrate digestion. It is a crucial step for solid foods but has limited role for liquid foods. Food is reduced in size by mechanical forces through mastication. The tongue performs a shearing action to position food under the molars so that it would be ready for compression (bites). This step is rapid and consists of crushing, cutting and grinding, as the initial contact force range of 10–20 N is increased to approximately 50–150 N at the end of the chewing cycle (Norton et al., 2014, Zhong-Rong Zhou, 2013). To make the bolus ready to swallow, food particles should be well lubricated and thus are mixed with saliva and mucous to form a viscous food bolus that is ready for digestion (Kong and Singh, 2008a). in addition to lubrication, saliva also introduces amylase into bolus that initiate the breakdown of dietary starches (Gouseti et al., 2019).

Gastric phase

The food bolus is then transported to the stomach through the oesophagus by a peristaltic wave of contractions in order to push the food into the stomach. The stomach will erode the food and shear it into smaller particles to increase the available surface area by mechanical processes such as contractions and retropulsion (Gouseti et al., 2019). This will result in structure break down and liquification of food particles to allow the access of pancreatic digestive enzymes to the inner layers. During the fasted phase, the stomach has cyclic contractions, while in the fed phase, muscle contractions become continuous, moving food from the top of the stomach to the pylorus and then propelling it back into the stomach's main body by retropulsion. These contractions have a force ranging from 0.2 N to 1.89 N, depending on the stomach phase (Camilleri and Prather, 1994, Norton et al., 2014). The rheological properties of the digested food within the GI tract are changing continuously depending on how food structure is deforming under the chemical and physical forces during digestion. They are crucial in determining motility and transit time. Moreover, gastric emptying depends greatly on food composition, structure and its rheological properties (Bornhorst et al., 2016, Turgeon and Rioux, 2011). Due to these reasons, liquids leave the stomach more quickly than solids or semi-solid foods through the distal part of the stomach, where the pylorus acts as a sieve that only allows particles 1–2 mm to pass through to the small intestine (Juvonen et al., 2009). Continuously, solid foods are emptied from the stomach after approximately 3–4 hours, and chyme then travels to the small intestine, where digestion and absorption continue.

The small intestine

The rate and pattern of gastric emptying is regulated by a complex of neurohormonal mechanisms that are triggered by the starch digestibility rates and the rheological properties of the contents (Ferrua and Singh, 2010). Small intestine is the site where most digestion and absorption take place. It has two types of wall movement. The first one is segmentation that involves the contractions of the circular muscles of the small intestine. This occurs when the tube becomes full and helps in the mixing process, which allows the nutrients to be released and absorbed. The second wall movement is peristalsis, which is a rhythmic contraction of the longitudinal muscles in the small intestine. This is responsible for movement along the tube, and it is controlled by the mass of the material in the system (Bornhorst et al., 2016). However, during this stage, the breakdown of foods is mostly chemical.

Carbohydrates are broken down in the small intestine to monosaccharides during digestion. Starch digestibility rate can be determined based on granule surface, degree of crystallinity and structure. Having a structure such as intact cell wall that encapsulates starch is highly important as it can prevent or slow the enzymatic penetration leading to slower release of starch to be ready for absorption (Edwards et al., 2015b). Once carbohydrates are digested, the by-products are absorbed and transported by the portal vein to the circulation by SGLT-1 and GLUT-2 transporters. However, not all carbohydrates are absorbed such as resistant starch. The latter will travel further down to the large intestine where fermentation will occur by the gut microbiota or pass in the faeces (Psichas et al., 2015). There is some evidence in the literature showed that high resistant starch consumption may lead to improvements in the post prandial glucose and insulin responses (Robertson et al., 2005, Bodinham et al., 2014)

1.5.2.2 Viscosity and digestion

Viscosity is the resistance of a fluid to flow due to internal friction (Lentle and Janssen, 2008a). Viscosity can be used to measure the behaviour of digesta in relation to a digestion mechanical force. It is thought that GI tract maintains a ‘rheological homeostasis’, which is the ability to maintain the flow properties by changing the amount of fluids and increasing or decreasing motility during digestion (Lentle and Janssen, 2008b). A study by Cameron-Smith et al. on rats using different high soluble fibre-containing meals showed that increasing the viscosity of the meal corresponded

to a decrease in postprandial glucose concentrations; however, although large differences in viscosities of these meals were predicted pre-ingestion, postprandial glucose concentrations were not significantly different between the types of fibres used in each meal. This showed that the GI tract was able to modify the viscosity of these meals, leading to similar postprandial glycaemic responses when fibres are present in meals (Cameron-Smith et al., 1994).

Many factors can affect the properties of digesta at initial digestion such as the liquid phase, particle shape and size. As digesta is changed progressively during digestion, properties will be governed by the strength, elasticity, cohesion and permeability of solid particles (Lentle and Janssen, 2010). Studies on the flow properties of digesta are limited due to the difficulty in obtaining digesta samples. However, physical interactions in digesta could be similar to other particulate systems that are well described in the literature (Lentle and Janssen, 2008b). Viscosity of the digesta liquid phase compared to whole digesta was measured in pigs and dogs; results showed a Newtonian behaviour for the liquid phase digesta when maintained on natural diets, suggesting a proportional movement rate to the applied force. With regard to the whole digesta, it exhibited non-Newtonian behaviour as a result of the particulate matter showing a shear thinning behaviour (Takahashi and Sakata, 2004, Dikeman et al., 2007). It is speculated that a balance is made between the formation of microstructures within the digesta from particle-particle interactions and the erosion of these structures by mechanical and chemical forces during digestion, which will lower the size of the microstructure and hence lower the viscosity.

1.6 Legumes as a model

1.6.1 Health and legumes consumption

Legumes (chickpeas and peas) were chosen in this thesis as ‘food models’ for studying the food structure role in digestion because of their unique structure that may influence starch digestibility. Legumes comprise plant cell wall components that are relatively strong and resistant to digestion in the human body (Tharanathan and Mahadevamma, 2003). Having an intact cell wall encapsulates nutrients within the cell, which hinders the action of digestive enzymes on nutrient hydrolysis (Berg et al., 2012, Dhital et al., 2016a). Previous in vivo studies have also reported the importance of intact structured foods compared to ruptured structures (Golay et al., 1986). Moreover, processed legumes contain RS-3, which contributes to blood glucose

responses that are lower than those of other carbohydrate foods (Jenkins et al., 1980, Madhusudhan and Tharanathan, 1996). Also, the legume starch structure is composed of 30–40% amylose, accounting for their low digestibility (Madhusudhan and Tharanathan, 1995). Additionally, anti-nutritional factors such as phytates and amylase inhibitors may influence the rate and extent of starch digestion. Due to all these reasons, legumes promote slow and moderate postprandial glucose and insulin responses (Nestel et al., 2004) and are thus considered beneficial in the management of diabetes, metabolic syndrome and cardiovascular diseases (Kim et al., 2016, Mollard et al., 2012).

1.6.2 Legumes structure

1.6.2.1 Chickpea structure

Chickpea (*Cicer arietnum L.*) is one type of pulse in the legume family that is consumed worldwide. Chickpea is of a great interest due to its potential nutritional health advantages (Hughes et al., 2009). It can be divided into two main varieties: Kabuli type and Desi type (Wood et al., 2011). Globally, chickpeas can be consumed in many ways through products made from chickpea flour or paste, or whole chickpeas. It was shown that chickpea cells are able to survive domestic cooking due to their structure and remain intact, which is one of the reasons why the chickpea is considered a low glycaemic food compared to other carbohydrate foods (Mishra and Hardacre, 2012).

While it was discussed earlier that legume structure plays a role in the low impact on postprandial glycaemia, studies varied in their speculation as to whether intact legumes have a lower effect on glucose than flour legumes. Many studies showed that legume flour with ruptured structure elicits a higher glycemic response when compared to whole legumes (Golay et al., 1986, Jenkins et al., 1982a). However, one study showed that legume flour, depending on the processing method used, had the same benefits on short-term glucose regulation as whole legumes (Anderson et al., 2014). Moreover, evidence has also shown that hummus – which is a spread made of cooked, puree chickpea mixed with lemon juices and tahini – had a similar effect to whole chickpea in relation to glucose and insulin response (Wallace et al., 2016).

1.6.2.2 Pea structure: RR wild type and *rr* wrinkled peas

Pea seeds (*Pisum sativum*) are one type of legume. Canada is its largest producer,

with around 21% of the world total production (Bekkering, 2014). The species *P. sativum L.* has a C-type crystalline structure arranged (a) peripherally and (b) centrally (Bogracheva et al., 1999). This type of pea has a range of mutants that has been identified through breeding. These mutants have an effect on the starch synthesis pathway (Davydova et al., 1995). In general, the starch biosynthesis process requires three enzymes: ADP glucose pyrophorphorylase (AGPase), starch synthase (SS) and starch branching enzyme (BSE) (Martin and Smith, 1995). The first step in starch biosynthesis is the catalysis of glucose 1-phosphate with ATP to form ADP-glucose by the enzyme AGPase; this is the sugar molecule required to form amylopectin and amylose. The exact proportion of starches depends on the botanical sources. The second step is using ADP-glucose by SS enzymes to add α-D-glucose units at the end of a growing polymer chain to start building up starch molecules by α (1-4) glycosidic bonds. This step is followed by SBE actions on forming branches in starch polymers by hydrolysing α (1-4) glycosidic bond and forming α (1-6) glycosidic bonds (Ball and Morell, 2003).

When a natural mutation is present in peas, SBE is absent; this will cause hydrolysing α (1-6) bonds and the breaking apart of branches in the polymer chains, forming more amylose than amylopectin (Bhattacharyya et al., 1993). Wrinkled *rr* peas is an example of a natural mutation product. These are genetically different and thus produce different granular morphologies and characteristics compared to smooth RR peas. In wrinkled peas, the activity of BSE enzyme is only 14% of that seen in smooth peas (Smith, 1988). Therefore, *rr* peas have a reduced starch level compared to *RR* peas. In addition, the amylose content is 60–76% compared to the 30–40% content found in *RR* peas (Colonna and Mercier, 1984). Granule characteristics are also different between the two types. It is observed that starch granules in *RR* peas are oval or round and large in size, whereas *rr* peas starch granules are smaller and appear as a mixture of simple and compound granules (Bhattacharyya et al., 1993).

The differences in the macroscopic structure between the *RR* and *rr* peas, as shown in Figure 1.7, are also due to the inefficient conversion of sugar to starch during development. This leads to sugar accumulation in the seed of the wrinkled pea that increases the water uptake osmotically, leading to the seed swelling to a greater size. When dried, more water is lost, and wrinkles appear. In contrast, the seeds of the smooth pea have less water to lose when dried due to having less sugar in the seed, so the smooth appearance remains (Wang et al., 1987).

Figure 1.7 Difference in the appearance between *RR* and *rr* peas.

1.7 Thesis summary

The purpose of this thesis is to investigate the interplay between intact structure, rheological properties and food breakdown during digestion, and their effect on postprandial glycaemia and subsequent related metabolic responses. With this project, there is a great opportunity to connect information from food rheology and human physiology. This will give a better understanding on how food is broken down during digestion and hence postprandial glycaemia.

Many factors influence the digestion of carbohydrates, including food structure and its rheological properties. For example, an intact cell wall prevents enzymes from entering the cell and hence keep nutrients encapsulated during digestion. Rheological properties, as affected by food structure, also play a role in the breakdown and digestion of food. Changes in food structure will affect the rheological properties of food (break point or viscosity) which in turn causes alterations in gut motility and mixing (rate of gastric emptying). Changes in the flow behaviour of food during digestion may influence nutrient absorption in the small intestine. All these factors are discussed in this thesis using legumes (chickpea and peas) as food models.

1.7.1 Thesis hypothesis

I hypothesised that intact food structure would reduce post prandial glycemia and subsequent metabolic responses compared to ruptured structure. Moreover, different food structure would result in different rheological properties that would have an effect on the breakdown behaviour during digestion.

1.7.2 Thesis aims and objectives

1. To investigate the acute effect of different food structures on glucose homeostasis using:
   I. Chickpeas: intact vs flour during a mixed meal test as hummus that is controlled for starch and calories.
   II. Genotypes of peas and flour peas using *RR* peas and *rr* peas.

2. To explore the association between food rheological properties and postprandial blood glucose level
   I. Examine the rheological properties of test meals (whole chickpea hummus and flour chickpea hummus) before ingestion using the rheometer.

II. Explore the mechanical properties of different genotypes of cooked peas before ingestion using Instron universal testing systems for a better understanding of the effect of mastication on the breakdown of food.

III. Explore flow behaviour by measuring viscosity of aspirated small intestinal digesta of different genotypes of peas.

IV. Explore the breakdown behaviour of aspirated small intestinal digesta of different genotypes of peas using light microscopy.

CHAPTER 2: Materials and methods

2.1 Introduction

This project combines in vivo and in vitro methods in order to improve our understanding of the breakdown of food during digestion. These methods were used to compare the acute effect of food structure on postprandial metabolic responses and to explore the relationship between the rheological properties and the breakdown of food.

This project includes two investigations: study 1 comprises a clinical trial, in vitro kinetics of starch digestion and rheology tests, using chickpeas as the main ingredient in the test meal (hummus). Hummus meals were composed of the same ingredients blended with different chickpea structure (ruptured or intact). Study 2 comprises a clinical trial and rheology tests using two genotypes of peas (wild type *RR* and wrinkled *rr* mutant peas) in different forms.

This chapter will present the materials and methods used in this thesis for clinical trial recruitments, volunteers' screenings, collection and analysis of blood samples and rheological tests. The methodology will be described in detail for each part. Subsequent chapters will refer to the materials and methods in this chapter.

2.2 Food materials

2.2.1 Hummus preparation from chickpeas for study 1

All meals were prepared on the same day of the study in the research kitchen at Imperial College London, using a blender to mix all ingredients together and to give the hummus its creamy look (Figure 2.1). Carrots and red peppers (40 g) were served to help participants eat the hummus as a dip. A detailed recipe of hummus can be found in Appendix 1.

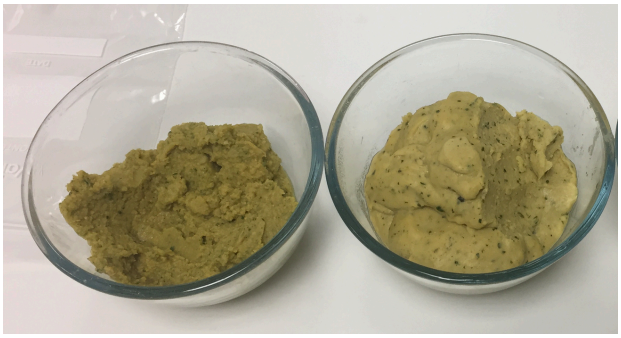

Figure 2.1. Test meals for investigation 1: (right) flour chickpea hummus, (left) whole chickpea hummus.

All meals contained the same ingredients and calories, as shown in Table 2.1, and were controlled for starch (described in Chapter 3, section 3.1.1) but were different in chickpea structure. The first meal had as its chickpea ingredient a commercially canned chickpea (Sainsbury's Chickpeas in water 400 g), and the second, flour chickpea. It was provided by Dr. Cathrina Edwards, Kings College London, and was prepared by dry milling, where chickpea was first crushed with a cutting mill and subsequently ground with a pin disc impact mill once to obtain chickpea flour with particle sizes of <150 μm.

Table 2.1 Test meal ingredients for chickpea study

	WHOLE CHICKPEA HUMMUS			**CHICKPEA FLOUR HUMMUS**		
	Weight (g)	Energy (Kcal)	Starch (g)	Weight (g)	Energy (Kcal)	Starch (g)
CHICKPEAS	157.1	191.6	25.2	76.5	250	25.2
WATER	26.7	0	0	289.7	0	0
LEMON JUICE	13.4	>1	>0.5	13.4	>1	>0.5
SPICE MIX	4	12.7	>1	4	12.7	>1
TAHINI PASTE	13.4	88	>0.5	13.4	86	>0.5
SALT	2	0	0	2	0	0
OLIVE OIL	20.1	165	0	20.1	161	0
FRESH CORIANDER	3.3	>1	0	3.4	>1	0
SERVING PORTION	240.0	459.1	**26.8**	423.0	510.8	**26.8**

2.2.2 Peas preparation for study 2

Test meals consisted of peas that were provided by John Innes Centre as follows: wild type whole peas (*RR*), whole wrinkled peas (*rr*), flour wild type peas (*RR*) and flour wrinkled peas (*rr*).

Pea seeds were analysed nutritionally by Eurofins testing laboratory group, as shown in Table 2.2.

Table 2.2 Nutritional information analysis for pea seeds

PER 100 G DRY WEIGHT	*RR* peas	*rr* peas
ENERGY VALUE (KCAL)	286	295
PROTEIN (G)	21.3	23.8
CARBOHYDRATE (AVAILABLE) (G)	32.3	28
TOTAL FAT (G)	<1	1.7
DIETARY FIBRE (G)	36	36.3

50 g of peas were soaked for 24 hours prior to the study visit and then cooked for 1 hour. Whole peas were served as green peas, and pea flour was served at pea soup.

2.3 Experimental methodology to all investigations

2.3.1 Ethical approval

Investigation 1 was approved by the Stanmore Research Ethics committee (REC reference 16/LO/0209) and registered on clinicaltrial.gov as NCT03424187. Investigation 2 was approved by South East-Coast Surrey Research Ethics committee (REC reference 15/LO/0184).

2.3.2 Participant recruitment

The same recruitment strategy was used for all investigations. Participants were recruited using the volunteer database *The Healthy Volunteer Panel at the National Institute for Health Research (NIHR)/Wellcome Trust Imperial Clinical Research Facility (CRF)*. For study 1, recruitment was also through study advert posters placed in hospitals and universities. Participants who expressed an interest in taking part in the study were provided with the volunteer Information Sheet (PIS) (Appendix 2). Participants' inclusion was based on two screening phases. The first phase was filling in a pre-screening questionnaire (Appendix 3) by email or telephone in order to identify participants who fit the inclusion and exclusion criteria. Potentially eligible participants were then invited to attend a health screening visit to further assess their eligibility and to provide written, informed consent (Appendix 4). During the health screening, participants were asked questions about their health history, anthropometric measurements were taken, blood samples were collected, an echocardiogram (ECG)

was performed and blood pressure (BP) was measured as well. All results were checked by a research doctor before potential participants' enrolment could take place.

2.3.3 Inclusion and exclusion criteria

Investigation 1 and 2 inclusion and exclusion criteria are shown in Table 2.3 based on the approved protocol for each study. Any volunteer with any of the exclusion conditions below would already have an altered pattern of hormone and glucose response that may give misleading results.

Table 2.3 Inclusion and exclusion criteria for Investigations 1 and 2

	CHICKPEA STUDY **INVESTIGATION 1**	**PEAS STUDY** **INVESTIGATION 2**
INCLUSION CRITERIA	- Age, 18–65 years old - Normal BMI, 20–29.9 kg/m^2	- Age between 18–65 years - Body mass index (BMI) of 20–35 kg/m^2
EXCLUSION CRITERIA	- Thyroid defect - Undergoing hormone or steroid therapy - Pregnant or lactating - Had given birth within the past year - Use of medications or natural remedies that affect appetite or plasma glucose - Excessive alcohol intake - Had blood donation within 12 weeks prior to the study start date - Smokers - Psychiatric illness - History of any disease with unknown outcome - Diabetes - Nut allergy	- Weight change of >3 kg in the preceding 2 months - Current smokers - Substance abuse - Excess alcohol intake - Pregnant or lactating. - Diabetes - Cardiovascular disease - Cancer - Gastrointestinal disease - Kidney disease - Liver diseases - Pancreatitis - Use of medications likely to interfere with appetite and plasma glucose

2.3.4 Randomisation

Following the health screening, eligible participants were randomised into the study. Randomisation was performed using an online software application called Sealed Envelope (sealedenvelope.com).

2.4 Blood samples analysis

2.4.1 Collection and preparation of samples

Samples were collected by different methods depending on the analysis. For each analysis, different additives were added before the collection of the samples and were centrifuged immediately or after clotting (10 min), as shown in Table 2.4. Samples were centrifuged at 2,500 relative centrifugal force (RCF) at 4 °C for 10 min. Samples were then aliquoted into Eppendorf tubes and immediately frozen at -80 °C until analysis.

Table 2.4 Methodology for blood samples collection and processing

ANALYSIS	SAMPLE TYPE AND COLLECTION TUBE	ADDITIVES	PROCESSING
PARACETAMOL	Serum SST vacutainer	Clot activator Gel for separating	Clotting for 10 min at room temperature before centrifugation
GLUCOSE	Plasma Grey vacutainer	Potassium oxalate/sodium fluoride	Immediate centrifugation
INSULIN	Serum SST vacutainer	Clot activator Gel for separating	Clotting for 10 min at room temperature before centrifugation
GIP	Plasma Lithium heparin tube	Lithium heparin Aprotinin[1]	Immediate centrifugation
GLP-1	Plasma Lithium heparin tube	Lithium heparin Aprotinin[1]	Immediate centrifugation

[1] Aprotinin (Trasylol, Nordic Pharma): a pancreatic trypsin inhibitor; Trasylol = 20 μL/ml of whole blood.

2.4.2 Glucose analysis

Glucose analysis was performed in the Department of Biochemistry at Hammersmith Hospital using an Abbott ci8200 analyser (Abbott Diagnostics, USA). In summary, glucose is phosphorylated by hexokinase, and during this reaction, one micromole of NADH is produced for every micromole of glucose consumed. The resulting NADH absorbs light at 340 nm and can be detected by spectrophotometry. Based on the absorbance, the analyser gives back direct readings of glucose concentration. The limit of detection is 0.139 mmol/L.

2.4.3 Paracetamol analysis

Paracetamol analysis was performed in the Department of Biochemistry at St Mary's Hospital using an Abbott Architect ci8200 analyser (Abbott Diagnostics, USA). Paracetamol concentration can be used to estimate gastric emptying for the liquid phase. Paracetamol is not absorbed in the stomach, but it is rapidly absorbed in the small intestine. Upon absorption, paracetamol can be measured in the plasma using peak concentration to estimate gastric emptying. Although the paracetamol method is not the gold standard for gastric emptying estimation as it only provides an indirect estimation for liquid phase and not solids, it is an inexpensive alternative to the more invasive methods, including scintigraphy, and therefore it has been widely used in clinical trials (Willems et al., 2001).

In summary, amide bonds of the acetaminophen are cleaved by the enzyme aryl acylamidase to form *p*-aminophenol and acetate. In the presence of manganese ions, *p*-aminophenol reacts with 8-hydroxyquinoline-5-sulfonic acid to form a coloured compound. The analyser reads the absorbance at 615 nm, which is directly proportional to the concentration of acetaminophen in the sample. The reportable range of the assay is 3 to 377 ug/mL.

2.4.4 GIP analysis

GIP analysis was performed using Human GIP (total) ELISA kit (MerckMillipore Corporation, Blillerica, USA) based on the manufacturer's specified protocol. This assay is a sandwich ELISA that cross reacts to human GIP (1-42) and GIP (3-42). It is based on the competitive inhibition enzyme immunoassay technique. In summary, a competitive inhibition reaction is launched between labelled and unlabelled GIP with the pre-coated antibody specific to GIP microplate. After incubation, the unbound conjugate is washed off and streptavidin-Horseradish peroxidase conjugate (HRP) is added to each microplate well. After incubation, the amount of bound HRP conjugate is reversed proportional to the concentration of GIP in the sample. After addition of the substrate solution, the intensity of colour developed, which represents the concentration of GIP in the sample. It is measured spectrophotometrically at 450 nm and 590 nm. The CVs were 3.4% and 1.8% for study 1 and study 2, respectively.

2.4.5 Radioimmunoassay

2.4.5.1 Principles of radioimmunoassay

In summary, a fixed concentration of radiolabelled tracer antigen is incubated with a constant dilution of antiserum, which limits the number of antigen binding sites on the antibody. Unlabelled antigen is added, which then competes with the labelled tracer for binding sites on the antibody. As the amount of unlabelled antigen present increases, the amount of tracer bound to antibody decreases. After centrifugation, the amount of radioactivity is counted by a gamma counter for the supernatant containing the free tracer and the pellet containing the antibody bound tracer. The standard curve is set up using an increased concentration of unlabelled antigen, which allows for the quantification of antigen in the unknown samples.

2.4.5.2 Insulin analysis

The insulin RIA was performed using Millipore Human Insulin Specific RIA Kit (Millipore Corporation, Blillerica, USA) based on the manufacturer's specified protocol with a 50-ul sample. It uses ^{125}I-labelled Human Insulin and a Human Insulin antiserum. The assay was incubated for 20–24 hours at room temperature before separation of the free from antibody bound level by secondary antibody. Pellets were counted using a gamma counter. Insulin assay %CV was 8% and 4% for study 1 and study 2, respectively.

2.4.5.3 GLP-1 analysis

GLP-1 concentrations were measured using a specific and sensitive in-house RIA (Kreymann et al., 1987). The antibody cross-reacted with all amidated forms of GLP-1, but no with glycine extended forms (GLP_{1-37} and GLP_{7-37}) or any other known pancreatic or gastrointestinal peptide. The assay was incubated for three days at 4 °C before separation of the free from bound label by charcoal adsorption. A %CV below 10% was accepted for all the GLP-1 assays.

2.5 Microscopic analysis

All images were visualised using light microscopy (Carl Ziess microscope Axio Scope. A1 HAL 100, LLC, USA) at the Department of Mechanical Engineering at Imperial College London. For study 1, a drop of water was added to the hummus sample for dilution, and a cover slip was used. Images were obtained from three different batches of hummus.

For study 2, aspirated small intestinal samples were visualised without dilution using a plate and a cover slip. Samples were obtained from three participants and were visualised at the following time points: 30, 60, 120 and 180 min. This was performed to explore the kinetics behaviour of peas genotypes (*rr* peas and *RR* peas).

2.6 Rheological tests

Rheological and mechanical properties were investigated to explore the interplay between food structure, rheological properties and food breakdown. A good understanding of the relationship between food structure and its rheological properties with digestion is important, as the rheological properties of digesta can be used to quantify the overall breakdown of food particles. Further understanding of the food breakdown during digestion may help in controlling the release of nutrients to specific areas in the gastrointestinal tract and, hence, the activation of gut hormone signals to control postprandial glycaemia levels.

Attempts to measure the mechanical properties of the small particles within the pea digesta were included using a preliminary new developed technique to measure the penetration force in digesta.

2.6.1 Tests on chickpea samples for study 1

2.6.1.1 Dynamic oscillatory test

This test is commonly used to provide more information on the food's structure and its rheological response to deformation, including the viscoelastic nature of foods and food stability (Steffe, 1996). Food structure and its properties play a role in the kinetics of digestion, as it affects food disintegration. Food is broken into small particles in response to physical forces (mastication, peristaltic contractions in stomach) and chemical forces such as enzymes and gastric juices dilution ((Kong, 2009, Kong and Singh, 2008b). The dynamic test is performed by applying a small sinusoidal strain and measuring the resulting stress (Steffe, 1996). This test is a two-step experiment (Figure 2.2A). First, a strain sweep must be performed at low frequency to determine the linear viscoelastic region (LVR). LVR can be defined as constant values of G' (solid modulus) and G" (viscous modulus) over a range of shear strain, as shown in the graphs of G' and G" versus applied shear strain (Figure 2.2B). Secondly, using the same settings (temperature, sample freshness, composition), a frequency sweep can be achieved using a strain within the viscoelastic limit. The frequency sweep can

provide more information about the effect of forces on the sample and the interactions among particles within the sample, which all contribute to the stability of food.

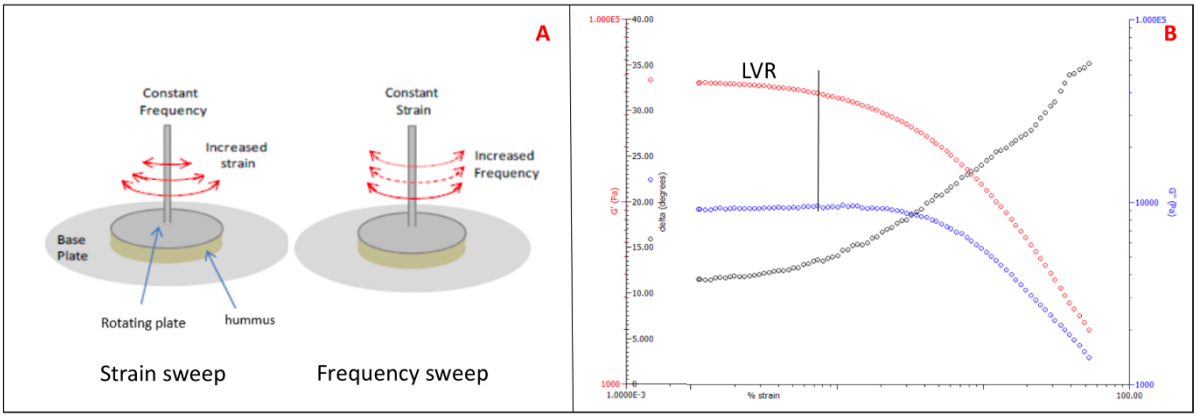

Figure 2.2 A: strain sweep and frequency sweep (Mohammed, 2012); B: linear viscoelastic region on the graph.

Hummus samples were prepared the same way as the test meals in the clinical trial. Measurements were conducted in triplicate. Strain sweep and frequency sweep were obtained using a parallel plate geometry on a controlled stress rheometer with a gap bigger than the size of the microstructure of the sample (AR 2000, TA Instruments, Delaware, USA). A 16, 25-mm diameter plate with gap variation between 2500-3800 micrometres at room temperature were used. For each test, the sample was transferred to the rheometer plate, and excess material was wiped off carefully so as not to disturb the sample. The sample was left to relax for 30–50 min or until the Normal Force is approximately 0 to minimise its effect on results. The sample was subject first to a strain sweep using 1 Hz frequency to obtain the LVR. After that, a frequency sweep was conducted over the frequency range of 0.1–10 rad/s by applying a shear amplitude from inside the linear viscoelastic domain 0.05% strain. Values of LVR and frequency sweeps were obtained from the computer software supplied by the manufacturer (Advanced/Universal Analysis software).

2.6.1.2 Constant Steady Shear rate (CSSR)

The constant steady shear rate (CSSR) test gives more information on the breakage point of the material. Breakdown food into small particles in the mouth and stomach will increase the surface area for digestion and hence nutrient absorbance (Kong and Singh, 2008a). The CSSR test involves applying a constant shear rate on a sample, leading the sample to continuously deform until particle breakage occurs (Gunasekaran and Ak, 2000). This test was conducted only on hummus samples using

a controlled stress rheometer, as samples from peas and digesta are heterogenous and very small in volume, so it was not possible to obtain repeatable test data.

Similar to the strain sweep sample preparation, the sample was transferred onto the rheometer plate and left to relax for 30–50 min or until Normal Force was approximately 0. As shown in Figure 2.3, CRRS was measured at strain rates of 0.1 and 0.05 (1/s) using a gap range of 2500–3000 micrometres with a duration time of 10 min at room temperature. The test was conducted in triplicate for each test meal using a new sample each time.

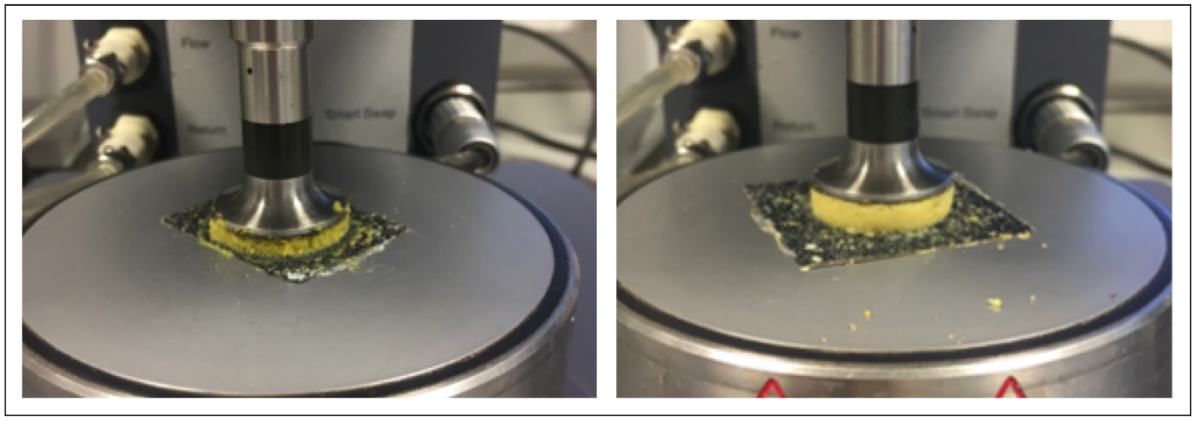

Figure 2.3 Chickpea hummus (left) and Chickpea flour hummus (right) for CSSR tests.

2.6.2 Tests on cooked peas for study 2

2.6.2.1 Dynamic oscillatory test

Peas were first cooked and then broken down using a garlic press (Figure 2.4). Samples were prepared using a circular mould (Figure 2.4). However, data results were inconsistent due to the heterogeneity of the sample.

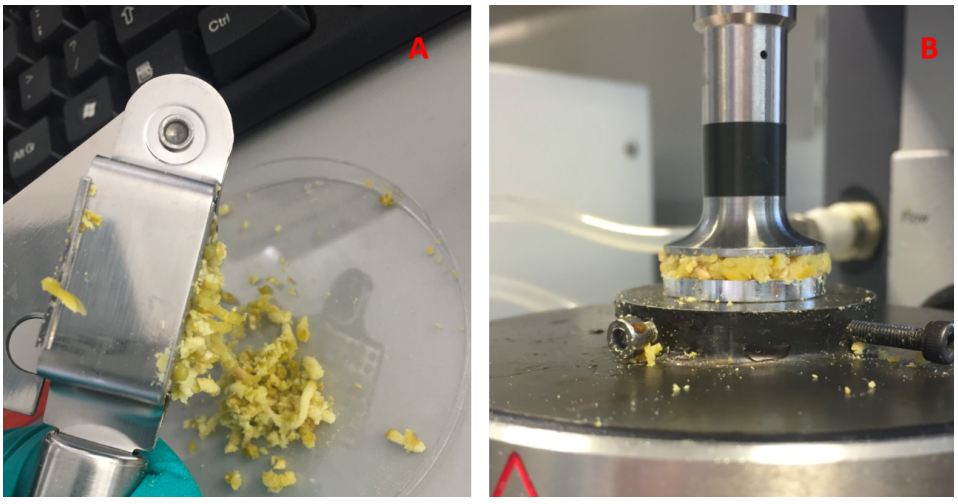

Figure 2.4 Preparing pea sample using garlic press; B: nonhomogeneous pea sample during a strain sweep.

2.6.2.2 Compression test

Since we were unable to test the peas' rheological properties using the rheometer, a compression test was conducted to measure the mechanical properties of the sample. A compression test is used for the evaluation of the material behaviour under uniaxial compression load. It may help in gaining a better understanding of the relationship between the food's structure and its properties (Tabilo-Munizaga and Barbosa-Cánovas, 2005). The degree of fragmentation during digestion is significantly affected by the physical and mechanical properties. One of the factors affecting the rate of particle size reduction in the mouth is the food's resistance to fracture at first bite (Singh et al., 2015).

An Instron machine 5540 was used to conduct the test with a 10 N load cell, model 2530-428, and was connected to *Bluehill3* software for the collection and analysis of the results.

Peas (*rr* pea seed and *RR* pea seed) were tested, using the same cooking method as the clinical trial. Ten seeds from each type using three different batches were measured for length (mm) and height (mm) using digital Vernier calliper to ensure similar geometry between peas. To conduct a test, a sample seed was placed in the most stable position prior to testing. A flat plate attached to Instron was used to apply load to the seed, as shown in Figure 2.5. The compression test was performed at speeds of 1 mm/s and 15 mm/s. The force versus deformation curves were obtained until rupture of the seed.

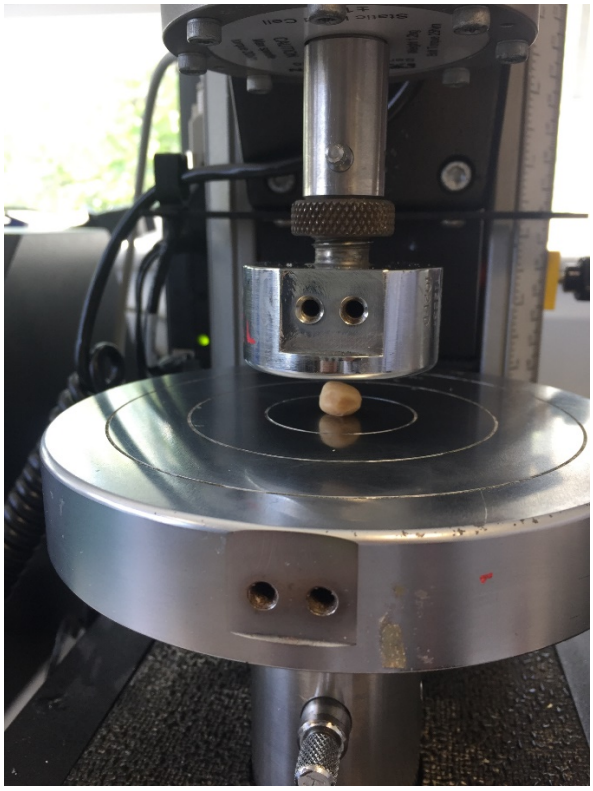

Figure 2.5 Compression test setting for whole peas.

2.6.3 Test on pea digesta

2.6.3.1 Dynamic oscillatory test

Because of the nature of digesta comprising liquid and various particles sizes, it was not possible to perform this test.

2.6.3.2 Compression test on digesta small particles

A technique was developed to measure the penetration force on small particles within the digesta. This may help in understanding the effect of the physical forces during digestion on the breakdown of food. The preliminarily designed probe used for the test is shown in Figure 2.6. A needle was attached to an adaptor in the Instron machine. The sample was put in Eppendorf tubes after removing the liquid from the sample and was attached to the base of the machine using a reusable adhesive. The experiment was performed first on crushed apples to mimic small particles in digesta. It was performed with an insertion speed of 1 mm/s at an insertion angle of approximately 90 degrees. The parameters of the test were set up to collect data with a penetration length of 1 mm. The penetration procedure starts and stop automatically when the

probe reaches the 1-mm length into the sample. The data was collected using the *Bluehills3* software.

We were unable to validate this technique due to the inhomogeneity of the sample obtained from digesta. Moreover, Instron machine was not able to detect forces less than 0.03 N. For these reasons, further research is needed.

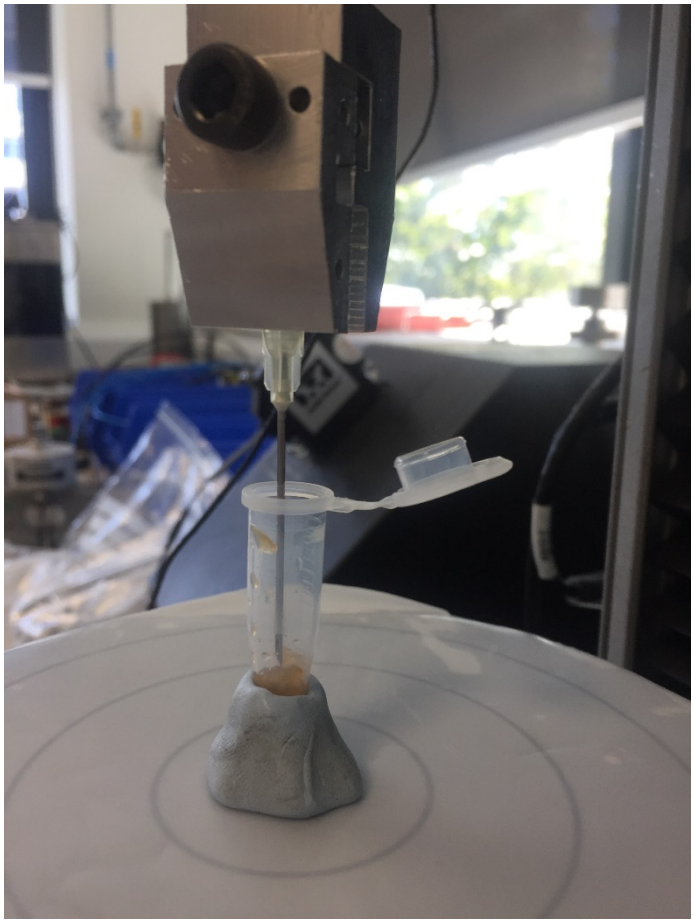

Figure 2.6 small particles from digesta compression test setting.

2.6.3.3 Viscosity

The rheological properties of digesta have a major impact on the mixing, transport and hydrolysis of nutrients within the gastrointestinal tract (Lentle and Janssen, 2008a). Digesta viscosity, in particular, is thought to play a role in gastric emptying and nutrient absorption (Shelat et al., 2015). Viscosity is a measure of the material resistance to gradual deformation by shear stress. Early investigations measured the viscosity of the liquid phase of digesta (Razdan and Pettersson, 1996, Johansen et al., 1996), whereas a more recently performed study by Takahashi and Sakata on pigs digesta showed that the removal of solid particles changed the properties from a non-Newtonian to a Newtonian fluid (Takahashi and Sakata, 2002). Therefore, the solid

phase of digesta is important to identify the rheological characteristics of digesta. However, the measurement of viscosity of whole digesta is difficult in humans because of the following:

- Composition of digesta: it is a heterogonous material that has particles of different sizes within the sample, along with liquid such as enzymes, gastric juices and mucin.
- Unlike obtaining digesta samples in animal studies after euthanasia, human digesta particles are constantly moving, making samples prone to errors from settling.
- Rheological methods to test viscosity require a large volume of sample (more than 15 ml), which is difficult to obtain from humans.

For these reasons, viscosity was only measured for the liquid phase of digesta using four time points (at 30, 60, 120 and 180 min) to compare rheological behaviour between whole *RR* peas and whole *rr* peas, using viscosity parameter for comparison. During this test, shear rate ranged between1 and 10 s^{-1}, based on reported values for shear rates generated by intestinal contractions (Hardacre et al., 2016, Takahashi and Sakata, 2004, Dikeman et al., 2006). Measurements were only conducted for the small intestinal samples, as the stomach samples were heterogenous with various particles sizes; hence, it was not possible to obtain reliable results. Moreover, results for time points 120 min and 180 min were not reliable due to having different results each experiment. This may be because to the non-homogenous samples and thus were not reported in this thesis.

2.6.3.3.1 Sample preparation

Samples that were collected from the clinical trial were frozen. It was not possible to make measurements on fresh samples, as the CRF and rheology lab are at different campuses of Imperial College London. However, Shelat et al.'s study on pigs digesta showed that thawed and fresh digesta samples had similar viscosity behaviour, but with slightly lower values after freezing and thawing (Shelat et al., 2014).

Digesta samples were left at room temperature for 30 min to defrost and settle (Figure 2.7A). The liquid phase of digesta was then transferred to a controlled stress rheometer (AR 2000, TA Instruments, Delaware, USA) using a pipette dropper to remove bigger particles. It was then left to relax for 10 min to minimise the normal force effect.

2.6.3.3.2 Viscosity measurement

A 25-mm diameter plate with a gap variation between 700–1900 micrometres was used at room temperature (Figure 2.7). Tests with a shear rate range of 10300 (1/s), 10 points per decade and a maximum time of 1 min were conducted in triplicate for each pea type.

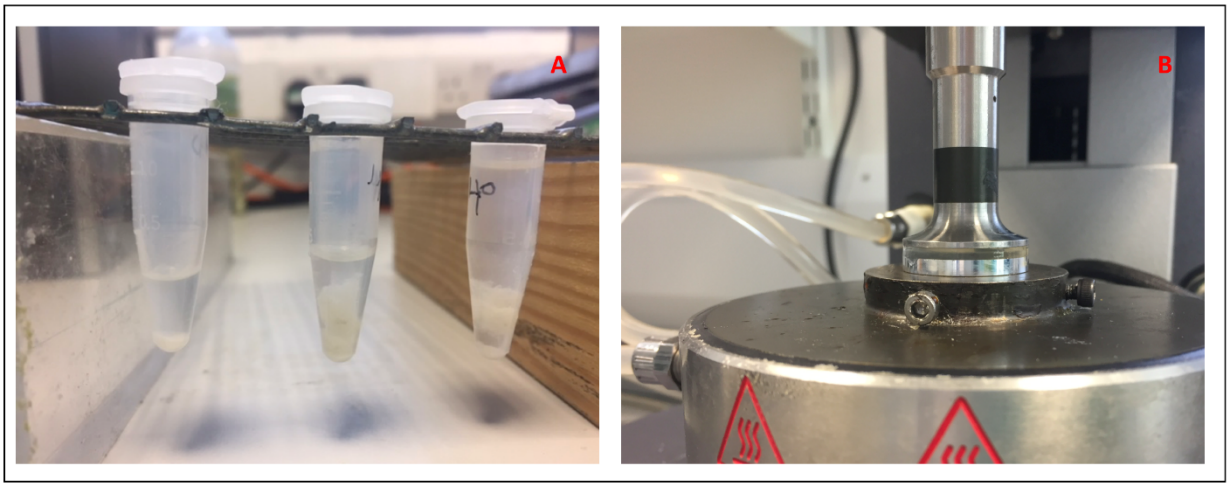

Figure 2.7 Digesta sample preparation for viscosity test.

2.7 Statistical analysis

2.7.1 Sample size

Both studies were preliminary studies to refine the design and evaluate accessibility, feasibility and cost. These studies were also required to calculate the sample size required for the main trial. For these reasons, no power calculation was done.

2.7.2 Statistics

Statistical calculations were performed using SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA), Microsoft Office Excel 2007 and GraphPad Prism software (Prism 6.03, GraphPad Software Inc., CA, USA).

The Shapiro-Wilk test was used to check the normality of the data ($p \geq 0.01$). Data were expressed as mean ± the standard error of the mean (SEM), unless otherwise stated. In cases of non-parametric data, log transformation was performed before carrying out parametric statistical tests. In the case of missing values, they were replaced with the average of the time points adjacent to the missing time point. The average of the two baseline measurements (-10 and 0 minutes) were used to give a new baseline value. Time series data were analysed using repeated measures analysis of variance (ANOVA) with post hoc Fisher LSD tests. Incremental area under

the curve (iAUC) was calculated using trapezoidal rule. Rheological results and iAUCs were compared using paired *t*-tests; $P = 0.05$ was considered statistically significant.

CHAPTER 3: The acute effect of intact structure on post prandial glucose response

3.1 Introduction

As previously discussed in section 1.2, the definition of food structure can be derived from the literature as the cell structure and arrangement of structural elements within food material at the molecular, micro and macro levels. Food structure influences texture, mechanical properties and the process of digestion (Waldron et al., 2003, Parada and Aguilera, 2011). It also plays a role in the rate of carbohydrate digestion by either encapsulating nutrients within the cell and preventing enzymes from entering the cell, or by the molecular structure being resistant to digestion, hence slowing the rate of starch hydrolysis and absorption, and thus altering glycaemic response (Noah et al., 1998). A lower glycaemic response has many health benefits, including improved insulin control (Sievenpiper et al., 2009a), reduced blood lipid levels (Ha et al., 2014) and improved satiety (Clark and Duncan, 2017). A meta-analysis confirmed that the consumption of low glycaemic response diets, including legumes, has a favourable impact on markers of metabolic diseases such as type 2 diabetes (Bhupathiraju et al., 2014) and coronary heart disease (Mirrahimi et al., 2012). Moreover, it has been established that legumes, including chickpeas, have a positive effect on glycaemic response (Jenkins et al., 1983, Sievenpiper et al., 2009b). Furthermore, processing techniques such as blending and mixing may not change legumes' beneficial effects. A randomized clinical study indicated that hummus, which is a blended chickpea spread, is similar to whole chickpea in its effect on postprandial glucose concentrations and is also lower than the white bread effect (Augustin et al., 2016). Many factors contribute to the health benefits of chickpeas, including food structure and chemical composition such as bioactive compounds that may hinder the actions of digestive enzymes (Ellis et al., 2004, Noah et al., 1998, Tovar et al., 1991). The focus of this chapter will be on examining food microstructure, specifically intact cells, and its effect on blood glucose concentrations as well as exploring its associated rheological properties.

3.1.1 Chickpeas as a model system to explore the impact of food structure on digestion

Chickpea seed consists of two cotyledons covered by a seed coat (Wood et al., 2011). The physical structure of legumes is one of the many factors responsible for the lower postprandial glycaemic response that is elicited following its consumption (Sievenpiper et al., 2009b, Clark and Duncan, 2017). I chose chickpeas because they are known to

have a relatively thick and strong cell wall structure (Wood et al., 2011). Furthermore, due to their strong physical structure, hydrothermally treated chickpea cells separate rather than rupture during digestion; this encapsulates the starch and prevents the access of digestive enzymes (Dhital et al., 2016b). Moreover, it has been reported that chickpea starch content ranges between 18% and 45%, of which resistant starch (RS) content varied (3–40%) depending on the methodology and differences in the time periods of hydrolysis (Hughes et al., 2009, Chung et al., 2008).

3.2 Gastric emptying

GE is a complex process that is regulated by central nervous system, gastric smooth muscle cells, and gut hormones including GLP-1, Peptide Tyrosine Tyrosine (PYY), Gherlin, and Cholecystokinin (CKK) (Hellström et al., 2006). Promotion or inhibition of GE will control food transit to optimize digestion and absorption of nutrients.

GE is also influence by dietary factors such as the food composition, food volume, food structure and the form of food (Bornet et al., 1990). As for solid foods, the breakdown starts in the mouth by mechanical forces. Food will travel to the stomach for further mechanical and chemical breakdown. The stomach is a muscular organ that works as food storage and the remaining food breakdown. It consists of four main parts: the fundus, corpus, antrum, and pylorus (Hellström et al., 2006). Grinding, and mixing with gastric juices and enzymes occur in the antrum by peristaltic contractions to reduce particle sizes to approximately 1-2 mm to be ready to travel to the small intestine as the pyloric sphincter allows only liquid and particle sizes less than 1-2 mm to pass through (Mackie et al., 2013). This process is called GE.

3.2.1 Gastric emptying measurements

Scintigraphy is considered the gold standard for measuring gastric emptying rate which offers a real-time imaging of the progress of a meal in the GI tract. However, due to the high cost and risk of exposing participant to radiation, it has been considered unsuitable for research purposes. Other non-invasive methods have been proposed that are validated against scintigraphy. Paracetamol absorption was validated for the liquid phase of digesta and it measures gastric emptying rate using the peak concentration of blood paracetamol (Willems et al., 2001). Another method is the non-radioactive ^{13}C stable isotope using breath samples. After the consumption

of a ^{13}C isotope labelled test meal, rapid absorption will occur that can be measured by $^{13}CO_2$ in the expired breath (Bornet et al., 1990).

3.3 Purpose of the study

This chapter focuses on a clinical trial that was conducted using hummus in two test isocaloric meals that were controlled for starch content. The difference between the two meals was the physical structure of the main ingredient, with chickpea flour having predominantly ruptured cells, and whole chickpea having predominantly intact cells. Chickpea was then blended with the rest of the ingredients to give the creamy texture of hummus. Light microscopy was used to confirm the difference in the microstructure between whole chickpea hummus (WH) and chickpea flour hummus (FH), as shown in Figure 3.1.

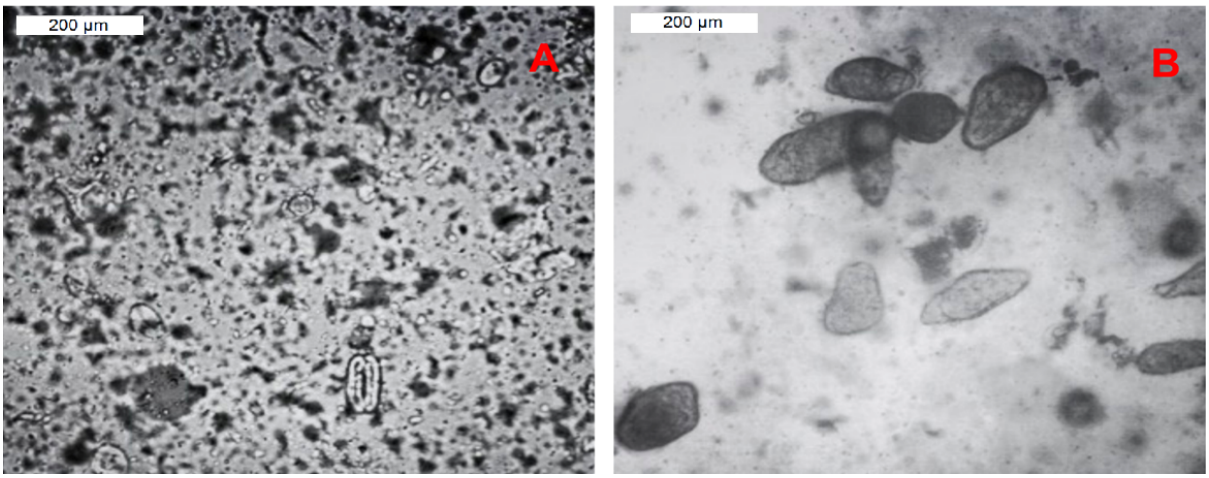

Figure 3.1 Representative microscopy images of hummus meals. Difference in microstructure of blended hummus between (A) chickpea flour hummus showing no intact cells and (B) whole chickpea hummus showing the presence of intact cells.

3.3.1 Hypothesis

It was hypothesised that hummus prepared with blended whole chickpea (WH) would result in lower postprandial plasma glucose concentrations compared to chickpea flour hummus (FH) due to the inaccessibility of starch and hence lower availability of glucose for absorption.

3.3.2 Aim of the study

The aim of the present study was to understand the mechanisms behind the acute effect of food microstructure on postprandial metabolic responses, mainly glucose, insulin and GIP, using hummus for comparison. A secondary aim was to explore the

rheological properties of hummus with different microstructure and to correlate it with glucose concentration.

3.3.3 Outcome measures

The **primary outcome** measure was glucose concentrations in vivo and in vitro, which can be used as an indication of starch release from food structure for digestion (Edwards et al., 2019). Also measured were postprandial metabolic responses, including insulin, GIP and GLP-1. **Secondary outcomes** assessed the effect of food microstructure on gastric emptying and the food's rheological properties.

3.4 Methods

Meal preparation, recruitment, blood preparation and collection, rheological tests and statistical analysis are explained in detail in Chapter 2.

3.4.1 In vitro starch kinetics

An in vitro digestibility assay was conducted on chickpea hummus to examine the extent of starch digestibility between both meals. Tests were performed in the Quadram Institute Bioscience, Norwich, UK, with the assistance and under the guidance of Dr Cathrina Edwards.

Hummus samples were freshly prepared as per the human study on the same day of testing. Two tests were conducted to calculate the rate of starch digestibility as per sections 3.3.1.1 and 3.3.1.2.

3.4.1.1 Total starch assay

In general, this method relies on the solubilisation and enzymatic conversion of starch into glucose through an enzyme cascade, wherein starch is hydrolysed by α-amylase into maltodextrins that are subsequently enzymatically cleaved into glucose by an amyloglucosidase, such that the quantity of glucose detected is proportional to the amount of starch in the original food sample. Starch content was measured using a modified version of the Official Analytical Chemists (AOAC 996.11) Megazyme kit (Megazyme International) method (Megazyme, 2018), carried out by using the Dimethyl sulfoxide (DMSO) format. The protocol was performed on 1/20th of the original scale and modified by increasing the duration of the DMSO heat solubilisation step to 16 min. The reason for modifications was to allow sufficient time to complete solubilisation of starch in pulses, because the original method was found to

underestimate total starch content in these samples. The procedure was conducted as described below.

A triplicate of a 50 ± 10-mg sample was weighed directly using a centrifuge tube. Next, 200 µL DMSO was added to the pellet and vortexed until mixed. Tubes were placed in boiling water for 16 min and vortexed every 2–3 min. After setting the boiling water to 90 ºC, 300 µL of 1:30 diluted thermostable *∂*-amylase was added to all tubes, vortexed and returned to the water bath for 60 min. The tubes were then transferred to a 50 ºC water bath for 2 min. After that, 5 µL amyloglucosidase was added, vortexed and incubated for 30 min. Tubes were centrifuged at 12,000 g for 1 min. Deionised water was added to the samples for dilution; 1 ml glucose oxidase/peroxidase (GOPOD) reagent was then added to the diluted sample, vortexed and incubated in a 50 ºC water bath for 30 min. Following this step, colour development should be noticed and read at Abs 510 nm in a microplate reader (Bio-Rad Benchmark Plus, Waukegan, Illinois, USA). The final step was to calculate starch content according to the Megazyme protocol.

A flow diagram for the starch assay is shown in Figure 3.2. The full protocol can be found in Appendix 7.

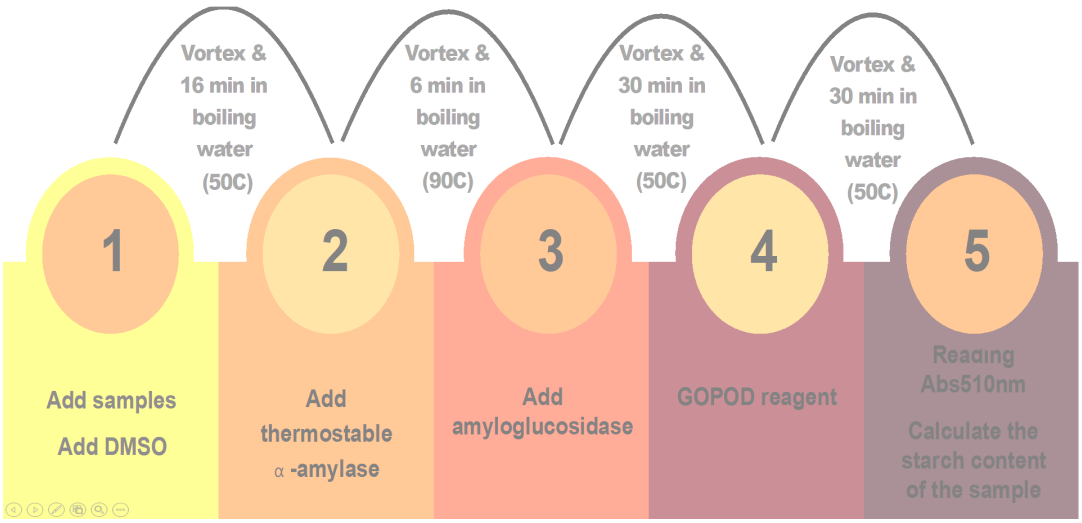

Figure 3.2 Total starch assay flowchart.

3.4.1.2 Starch digestibility test

Starch contents for hummus samples were standardised to correspond to the served portion size in the human study. This test determines starch digestion performance. Many factors may affect starch digestibility such as particle size, microstructure (flour vs whole), moisture content and starch type.

The digestibility test was conducted following the digestion protocol established by Edwards et al. (Edwards et al., 2014). It involves sample digestion with porcine pancreatic ∂-amylase and quantification of digestion using PAHBAH assay (Figure 3.3).

Three identical samples from each test meal were weighed into tubes and suspended in 5 ml phosphate buffered saline (PBS, pH 7.4). Samples were then allowed to equilibrate at 37 ºC for 15 min at 30 RPM on a rotary mixer (Stuart rotator SB3). A blank aliquot of 100 µL from each tube was taken into 1.5-ml microfuge tubes. Amylase was then added to all tubes using a start-up counter to measure the time points 0–90 min. It was previously shown that a 90-min hydrolysis value would be enough to estimate in vivo glycaemic response (Goñi et al., 1997). Centrifugation at 15,000 G for 5 min was done on all tubes to spin down any starch remnants. PAHBAH assay was then used to quantify the concentration of reducing sugars. Samples were diluted in deionized water, and 20 µL of the diluted sample was transferred to a clear plastic, flat bottom 96-well plate. Twenty µL of PAHBAH reagent was added to the samples. Standards containing known concentrations of maltose were included as well. The plate was then incubated at 100 ºC for 5 min, followed by 10 min at room temperature to cool down. Absorbance measurements at Abs 405 nm was read using a microplate reader (Bio-Rad Benchmark Plus, Waukegan, Illinois, USA). Reducing sugars were expressed as maltose equivalents by reference to a standard curve. Figure 3.3 shows the flow diagram for the starch digestibility assay.

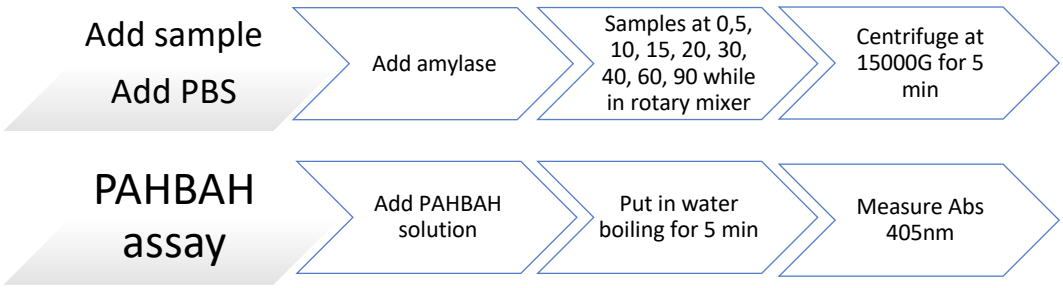

Figure 3.3 Digestibility test flowchart.

Measuring moisture content is an important value for calculating the starch digested in a sample. Hummus samples were weighed into microtubes and dried by using pre-dried aluminium pans that were heated at approximately 103 ± 2 ºC. The moisture contents were then calculated from the difference between dried weight and fresh weight of the sample.

3.4.1.3 Calculations for rate of digested starch

Total starch content

Total starch content (percentage fresh weight basis) was calculated as follows:

Total starch in sample size = sample absorbance Abs 510 nm × Glucose conversion of Abs of unknown sample to glucose × Dilution Factor × Correction Factor

Where the:

- Dilution factor is an intrinsic part of the assay that considers the dilution sample for the GOPOD assay;
- Conversion factor is calculated from the absorbance of glucose standard, calculating the conversion from Abs to glucose; and
- Correction factor is an adjustment for the loss of water during hydrolysis.

Starch digestibility test

Starch digestibility was calculated as follows:

[(Reading absorbance at time point – Y-intercept) / Slope] / [dilution stop solution × standard dilution]

Where the:

- *Y*-intercept and Slope values were taken from Log scale calculations obtained from the absorbance values for time points 0–90 min; and
- Standard dilution is an intrinsic part of the assay.

Rate of starch digested

Percentage of starch digested was calculated as follows:

(Maltose produced / Maltose initial from total starch assay) × 100

Where the:

- Maltose produced was obtained from the digestibility test; and
- Maltose initial was obtained from the total starch assay.

3.4.2 Clinical trial: study visit overview

The clinical trial was a randomised, single blind, crossover study and involved 3 study visits. Information on randomisation can be found in section 2.3.4.

This study consists of 3 study days, 7 days apart. The first visit is the acclimatisation visit, followed by two separate study visits. All study days were conducted at the NIHR/Wellcome Trust Imperial Clinical Research Facility (CRF) between July 2016 and February 2019.

3.4.2.1 Study visit preparation

Participants were instructed to avoid alcohol, excessive exercise and caffeine in the 24 hours prior to the study day. Participants were also instructed not to start any new diet or exercise regimes during the study to avoid conflicting results.

A standard meal of the participant's choice was consumed the evening before each visit to maintain consistency between visits. Participants were also asked to fast for 12 hours prior to the start of the study visit.

3.4.2.2 Acclimatisation study visit

It was shown that research study participation and venepuncture are stressful and hence will affect gut hormone levels and glucose homeostasis (Chandarana et al., 2009). An acclimatisation visit was added to the study design so that participants would be introduced to the study protocol, with the aim of reducing the stress factor on the results. It was similar to the other visits (section 3.3.1.3) except for the blood collection; this involved taking a small amount of blood (1 ml) at each time point, after which the sample was immediately discarded.

3.4.2.3 Study visit protocol

Following their arrival and after checking if they were still willing to proceed with the study, participants were weighed to ensure that a constant variable was controlled and that the data retrieved from the study were comparable and unbiased. After that, a cannula was inserted into the forearm to collect blood samples based on the time point schedules.

An overview of the study design is shown in Figure 3.4. A test meal was prepared according to the participant's randomisation: chickpea flour hummus (FH; 510 kcal, 25.2 g Carb, 30 g Fat per serving size of 224 g) or whole chickpea hummus (WH; 460 kcal, 25.2 g Carb, 29 g Fat per serving size of 240 g). The meal was served as breakfast at 0 minutes, and participants were asked to consume it within 10 minutes. A total of 1.5 g of paracetamol was given orally after finishing the meal to measure gastric emptying rate. Ten-ml blood samples were collected at frequent time points in different vacutainers, as shown in section 2.5 ('Blood sample preparations'). During the study visit, participants were asked to minimise their physical activity. Water was allowed ad libitum. After the last time point for blood sample collection, the cannula was removed, and the participant was discharged from the CRF.

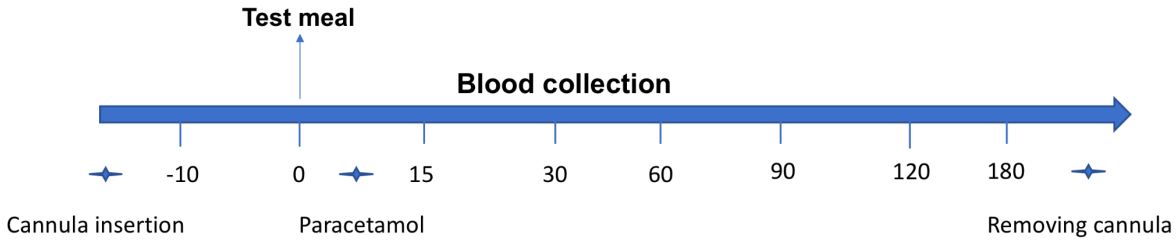

Figure 3.4 Study visit overview. Overview of timing of blood sampling, Paracetamol administration and test meal introduction.

3.4.3 Rheological tests

Strain sweep and frequency sweep were conducted as explained previously in section 2.3. A power law model was used to define the rheological properties of the material using the equation

$$G'(t) = G'(1)\,\omega^{n},\ G''(t) = G''(1)\,\omega^{n}.$$

Where:

- G' is the solid modulus
- G" is the viscous modulus

It is helpful to obtain numerical values that can describe the response of the material tested for comparison with other food materials (Rao, 2014). This may help in categorising food materials that may behave similarly during digestion based on their rheological properties.

3.5 Results

3.5.1 In vitro kinetics

3.5.1.1 Total starch assay

Total starch content of WH and FH were (mean ± SEM) 11 ± 0.4 and 10 ± 0.2 expressed mg/100 mg fresh weight basis, respectively.

3.5.1.2 Rate of starch digested

Moisture content for WH and FH were (percentage ± SEM) 63.6 ± 0.040% and 72.2 ± 0.278%, respectively. Digestibility test calculations were modified accordingly. WH, which consists of intact cells, was digested significantly slower than FH between 0 and 90 min (P = 0.005), as shown in Figure 3.5.

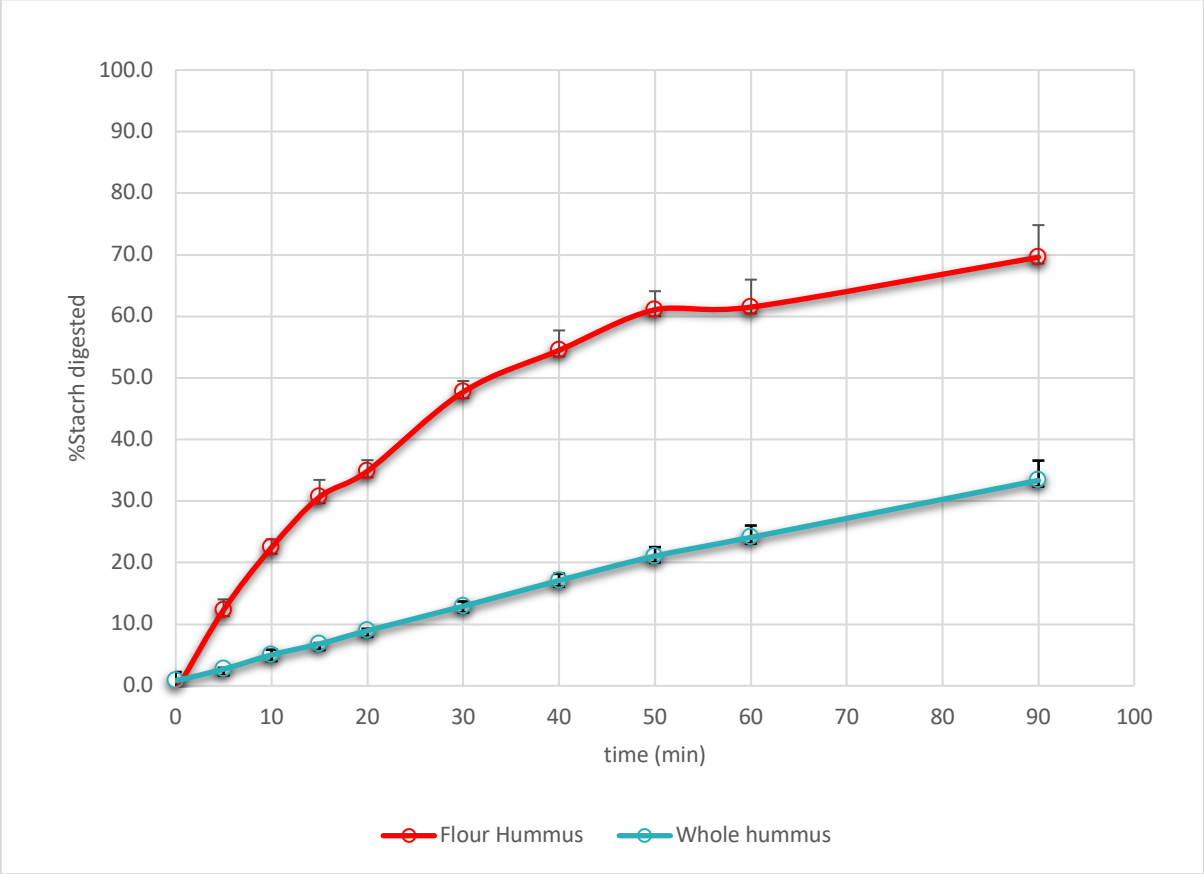

Figure 3.5 In-vitro starch digestibility curves obtained for blended whole chickpea hummus (green) and blended chickpea flour hummus (red). Samples digested using porcine ∂-amylase. Data expressed as mean ± SEM, compared using unpaired samples *t*-test.

3.5.1.3 Microstructural analysis after the in vitro digestibility test

Figure 3.6 shows images of hummus (whole and flour) before and after the in vitro digestibility test. Results showed a clear difference in microstructure, with some visible remaining intact cells in the whole chickpea hummus compared to chickpea flour hummus after 90 min of the test.

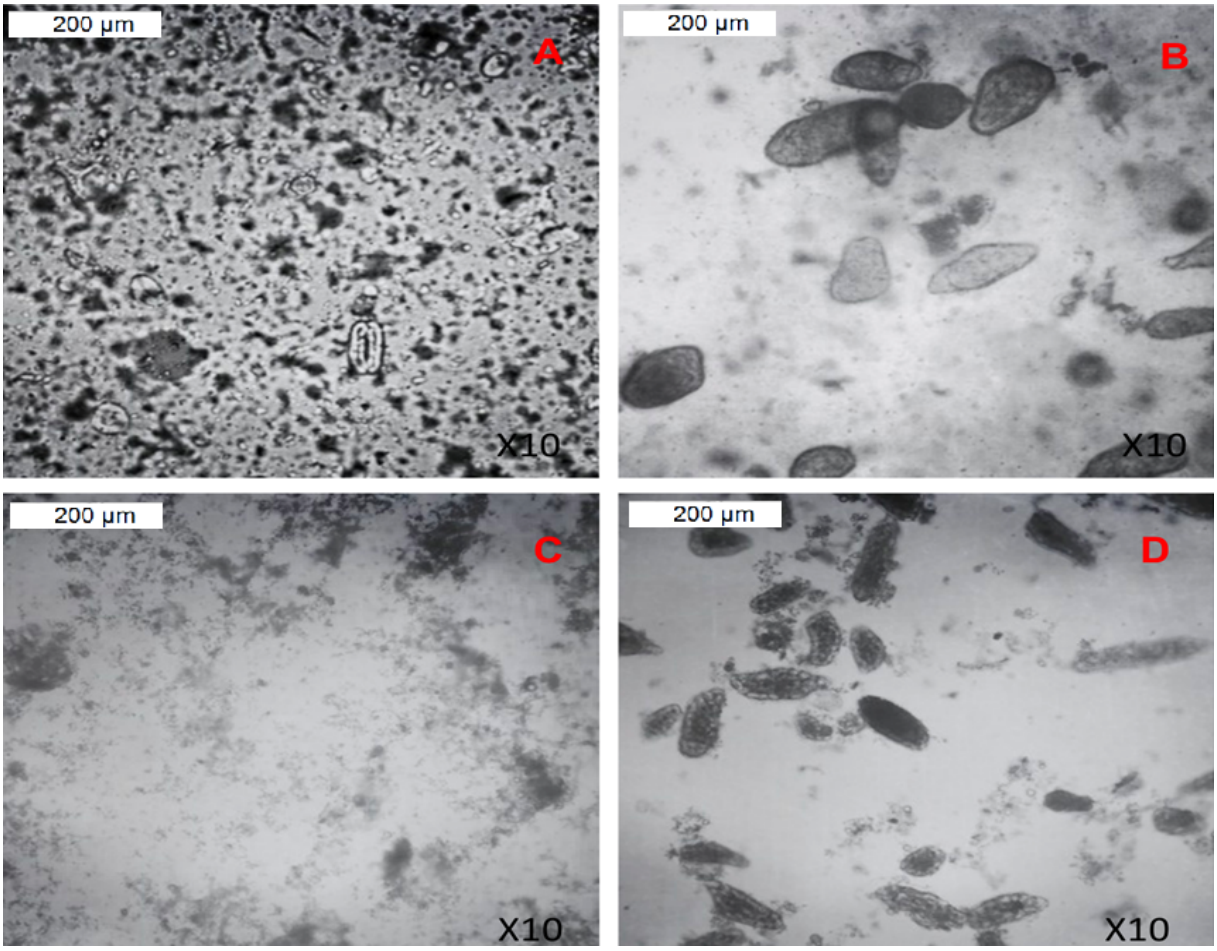

Figure 3.6 Light microscopy images for (A) (C) FH before and after in vitro digestibility test, respectively; (B) (D) WH before and after in vitro digestibility test, respectively. Microscopic images confirm the presence of intact cells after 90-min in vitro starch digestibility assay. Figure is not representative of whole sample. These images are used to detect the trace of intact cells in the sample after the in vitro digestibility test.

3.5.2 Baseline characteristics of participants

As shown in Figure 3.7, a total of 29 adults were screened for this study, with 15 participants completing the entire protocol.

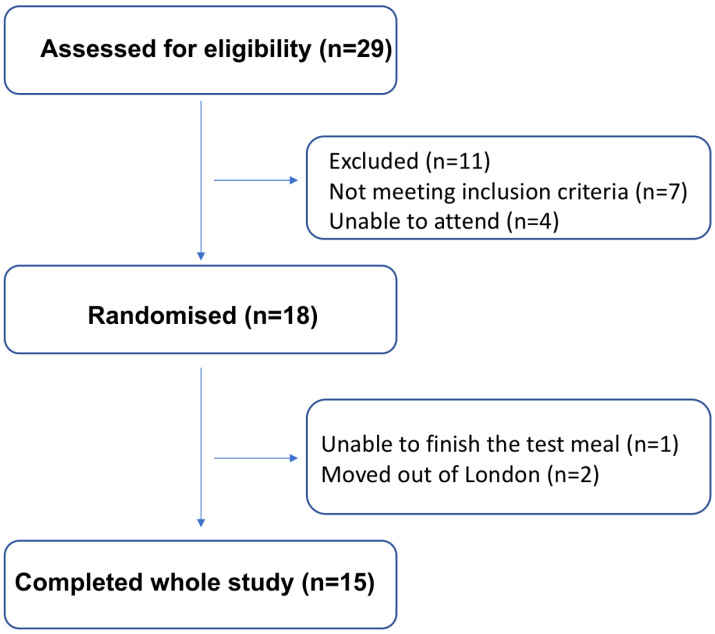

Figure 3.7 Flowchart describing the recruitment of volunteers.

The baseline characteristics of the 15 participants who completed all three study visits are described in Table 3.1.

Table 3.1 Participant characteristics

VARIABLE	ALL PARTICIPANTS
N	15
AGE (Y)	33 ± 3.0
WEIGHT (KG)	68.6 ± 2.5
BODY MASS INDEX BMI (KG/M^2)	24.9 ± 0.6
BODY FAT (%)	26.6 ± 1.57

Note: Continuous variables are presented as mean ± SEM. Abbreviation: y = years.

3.5.3 Paracetamol profile

Time course and iAUC data for paracetamol concentrations are shown in Figure 3.8. Repeated measures ANOVA analysis showed no significant difference in paracetamol concentrations (P = 0.6). Blood paracetamol iAUC values between 0 and 180 min were not significantly different between meals (P = 0.6).

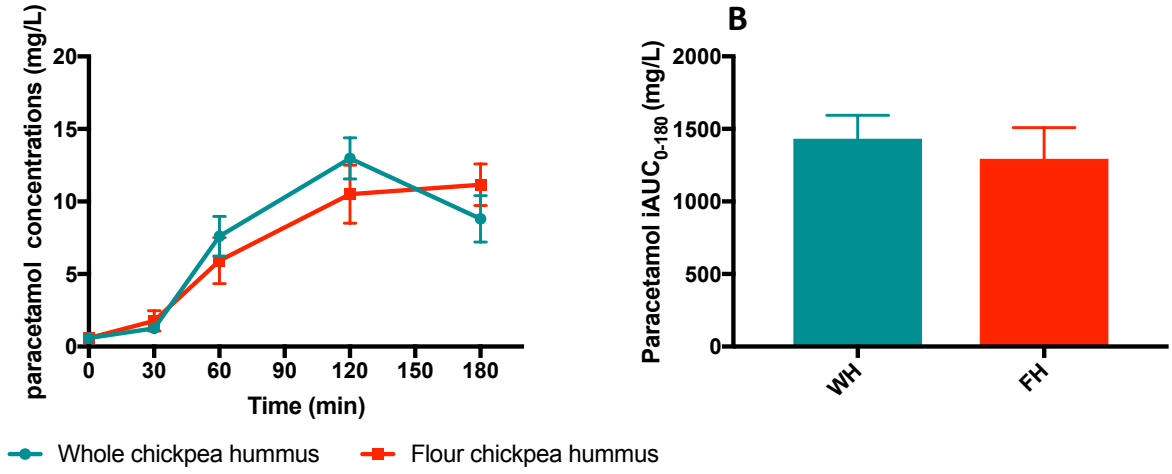

Figure 3.8 Serum paracetamol concentrations following consumption of whole chickpea hummus or chickpea flour hummus. Data represents mean ± SEM (A) serum paracetamol concentrations over time and (B) $iAUC_{0–180}$ for serum paracetamol concentrations between 0 and 180 min following consumption of whole chickpea hummus or chickpea flour hummus (*n* = 15)

3.5.4 Blood glucose and insulin profiles

3.5.4.1 Plasma glucose concentrations

Time course and iAUC data for plasma glucose concentrations are shown in Figure 3.9. Repeated measures ANOVA analysis showed no significant effect on glucose concentrations (*P* = 0.6). Blood glucose iAUC values between 0 and 180 min were not significantly different between meals (*P* = 0.4).

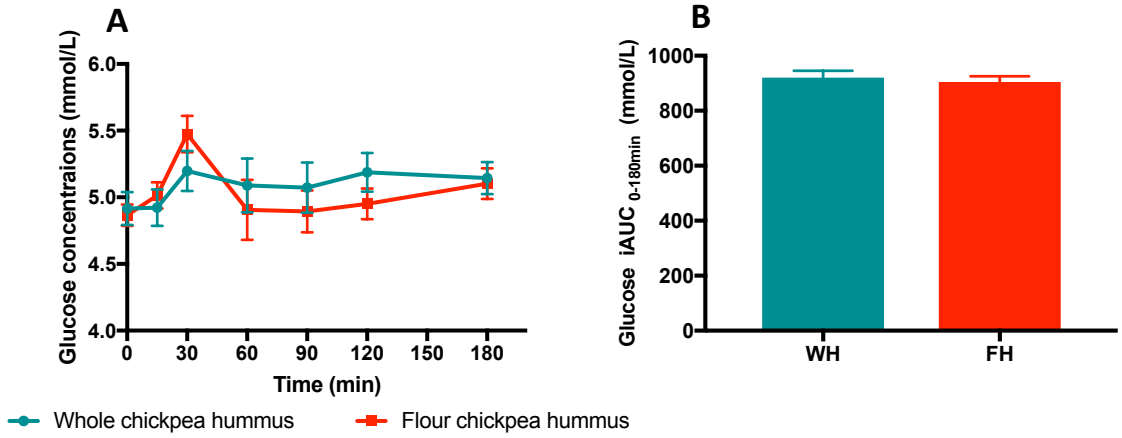

Figure 3.9 Plasma glucose concentrations following consumption of whole chickpea hummus or chickpea flour hummus. Data represents mean ± SEM (A) plasma glucose concentrations over time and (B) $iAUC_{0–180}$ for plasma glucose concentrations between 0 and

180 min following consumption of whole chickpea hummus or chickpea flour hummus (n = 15).

3.5.4.2 Serum insulin

Time course and $iAUC_{0–180}$ data for plasma serum insulin concentrations are shown in Figure 3.10. Repeated measures ANOVA analysis showed a significant interaction between meal and time (P = 0.02). Post hoc tests revealed a significant difference between both meals on insulin concentrations at time points 30 and 120 min for blended whole chickpea hummus (P = 0.02 and P < 0.001, respectively). Blood insulin iAUC values between 0 and 180 min were significantly different between meals at the main effect level (P = 0.01).

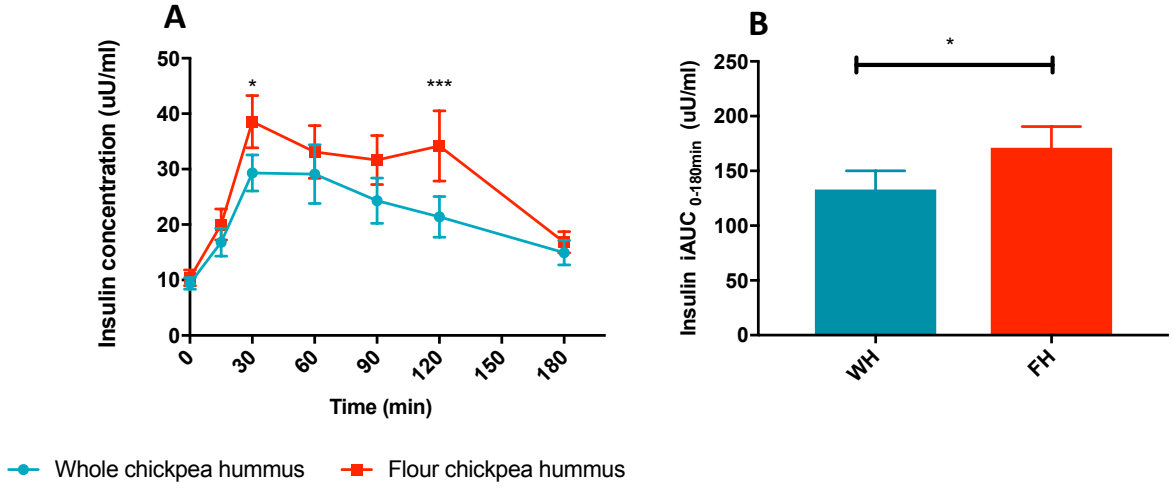

Figure 3.10 Serum insulin concentrations following consumption of whole chickpea hummus or chickpea flour hummus. Data represents mean ± SEM (A) serum insulin concentrations over time and (B) $iAUC_{0–180}$ for serum insulin concentrations between 0 and 180 min following whole chickpea hummus or chickpea flour hummus (n = 15).

3.5.5 GIP profile

Time course and $iAUC_{0–180}$ data for plasma GIP concentrations are shown in Figure 3.11. Repeated measures ANOVA analysis showed a significant interaction between meal and time (P = 0.03), but no significant difference for main effect of chickpea (P = 0.1). Post hoc tests revealed a significant difference between both meals on GIP concentrations at time points 15, 30 and 60 min for blended whole chickpea hummus (P = 0.001, P = 0.02 and P = 0.03, respectively). There was a trend for a difference in blood GIP iAUC values between 0 and 180 min (P = 0.06).

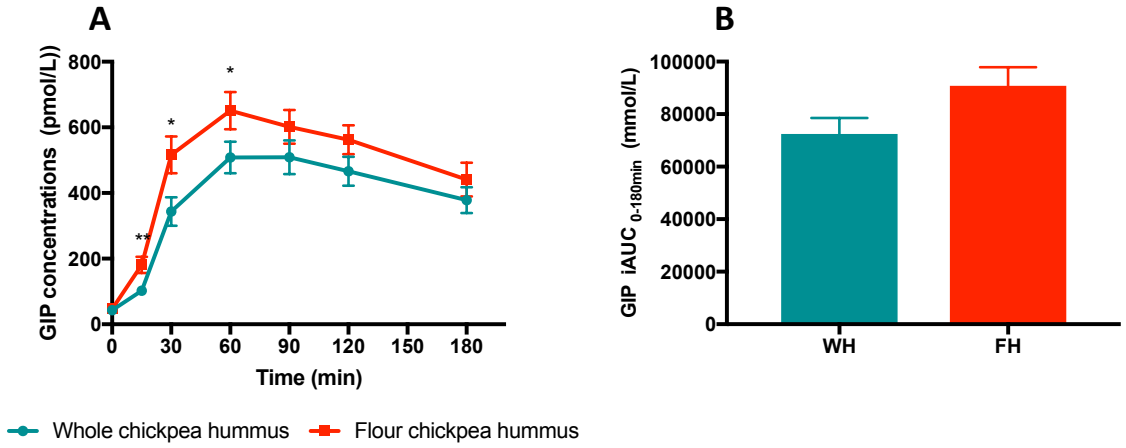

Figure 3.11 Plasma GIP concentrations following consumption of whole chickpea hummus or chickpea flour hummus. Data represents mean ± SEM (A) plasma GIP concentrations over time and (B) $iAUC_{0\text{-}180}$ for plasma GIP concentrations between 0-180 min following whole chickpea hummus or chickpea flour hummus (*n* = 15).

3.5.6 GLP-1

Time course over time and iAUC data for plasma GLP-$1_{0–180}$ concentrations are shown in Figure 3.12. Repeated measures ANOVA analysis showed no significant difference in GLP-1 × time interaction (*P* = 0.5) or main effect of chickpea meals (*P* = 0.1). Blood GLP-1 iAUC values between 0 and 180 min were not significantly different between meals (*P* = 0.4).

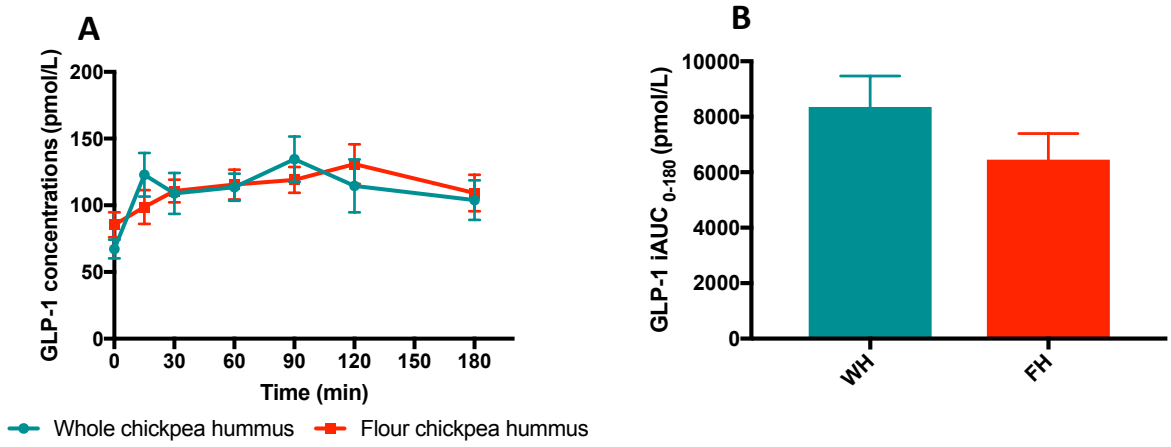

Figure 3.12 GLP-1 concentrations following consumption of whole chickpea hummus or chickpea flour hummus. Data represents mean ± SEM (A) GLP-1 concentrations over time and (B) $iAUC_{0\text{-}180}$ for GLP-1 concentrations between 0-180 min following whole chickpea hummus or chickpea flour hummus (*n* = 15).

3.5.7 Shear rheometry tests outcome

3.5.7.1 Shear strain sweep and shear frequency sweep

As previously discussed in 2.5.2.1, shear rheometry tests are used to provide more information on the viscoelastic nature of foods and food stability. Results are means of three replicates from three different batches of hummus ± SEM. Strain sweep results showed a gel behaviour for both meals (Figure 3.13A) having solid modulus (G') > viscous modulus (G") throughout the linear viscoelastic region (LVR). This indicates the low contribution of the viscous component (G") to the viscoelastic properties of the meal. WH was shown to have higher G' and G" values than FH in the LVR, as shown in Figure 3.13A. This may indicate a more rigid network in WH compared to FH, as G' is directly related to the cross-link density of the network in a gel, which can be explained by the presence of intact cells (Ferry, 1980). However, the extent of LVR for FH is longer than that for WH, which is a result of the structure cohesiveness of FH.

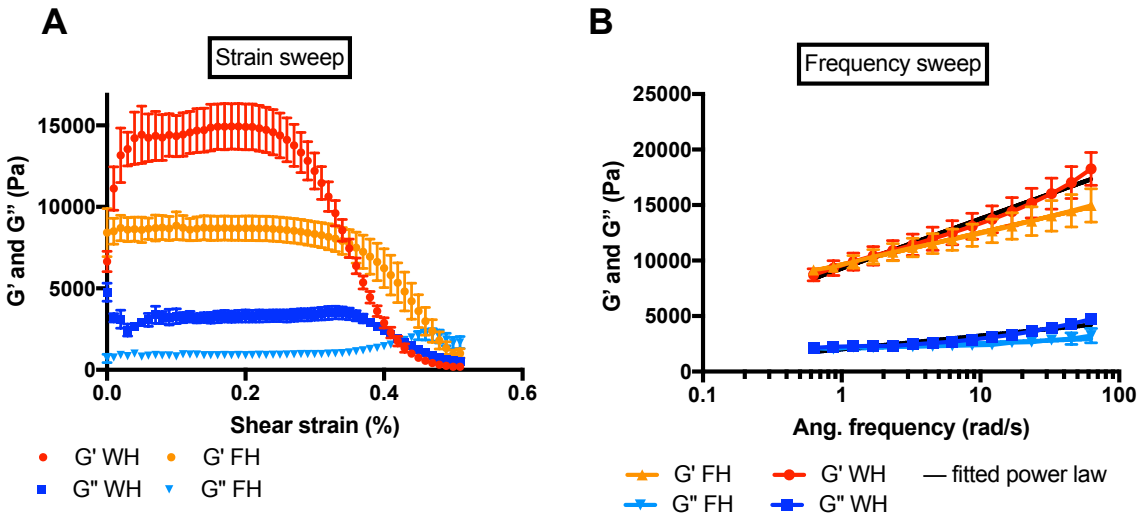

Figure 3.13 (A) Linear viscoelastic region for whole chickpea hummus and chickpea flour hummus by applying a small sinusoidal strain at 1 Hz frequency and measuring the resulting stress. (B) Frequency sweep at 0.05% strain. Data compared using a paired *t*-test.

As shown in Figure 3.13B, a weak, gel-like behaviour was observed for both meals having the (G') greater than (G") in the entire frequency range tested, with no significant difference in the frequency sweeps between FH and WH in G' and G" (P = 0.5 and P = 0.7, respectively). The same behaviour was reported for industrial

sunflower tahini (Muresan et al., 2014) and chickpea gel developed with different formulations (Canet et al., 2015).

Note that the almost linear curves of *G'* and *G"* in Figure 3.13B can be represented by a power law equation. A comparison with different foods in literature is shown in Table 3.2. It can be observed that the power law constant of FH is in agreement with the Spanish hummus more so than that of WH (Alvarez et al., 2017). However, WH has a similar LVR limit to dough and Spanish hummus.

Table 3.2 Range of power law constants obtained from literature for comparison with WH and FH

		G'(T)		G" (T)		LVR
	Temp (C°)	***n***	***G'* (1) (Pa)**	***n***	***G"* (1) (Pa)**	
WH	25	0.2	7620	0.2	1725	0.01–0.2
FH	25	0.1	8141	0.1	648	0.01–3.0
SPANISH HUMMUS (ALVAREZ ET AL., 2017)	25	0.1	5067	0.1	917	0.001–0.1
WHEAT FLOUR DOUGH (MOHAMMED, 2012)	22	0.2	5912	0.1	67	0.01–0.1

Note: G' (1), solid modulus constant; *G"* (1), viscous modulus constant; *n*: power law constant; LVR, Linear viscoelastic region.

3.5.7.2 Constant steady shear rate (CSSR)

This test also gives an indication of the stability of the material and its break point using a large deformation range, unlike the oscillatory tests described above that are performed at small strains. As shown in Figure 3.15, the CSSR result showed that FH had an approximate shear fracture stress of 1500 Pa and a shear strain fracture of 0.5. Cracks were visually observed on the edge of the sample in Figure 3.14B (2), which indicates the break point. It was also noticed that slippage of the sample after the break point caused the sample to get ejected from the geometry gap, as shown in Figure 3.14B FH (3). This has caused the drop of the shear stress, as shown in Figure 3.14A.

WH behaved differently than FH, with an early break point – as shown in Figure 3.14B (2) – due to its non-cohesive structure. This explains the constant response afterwards

exhibiting a steady state shear stress in the range of 500 Pa, as shown in Figure 3.14A. A similar behaviour for both meals was also observed for different shear strain rates of 0.1/min and 0.01/min.

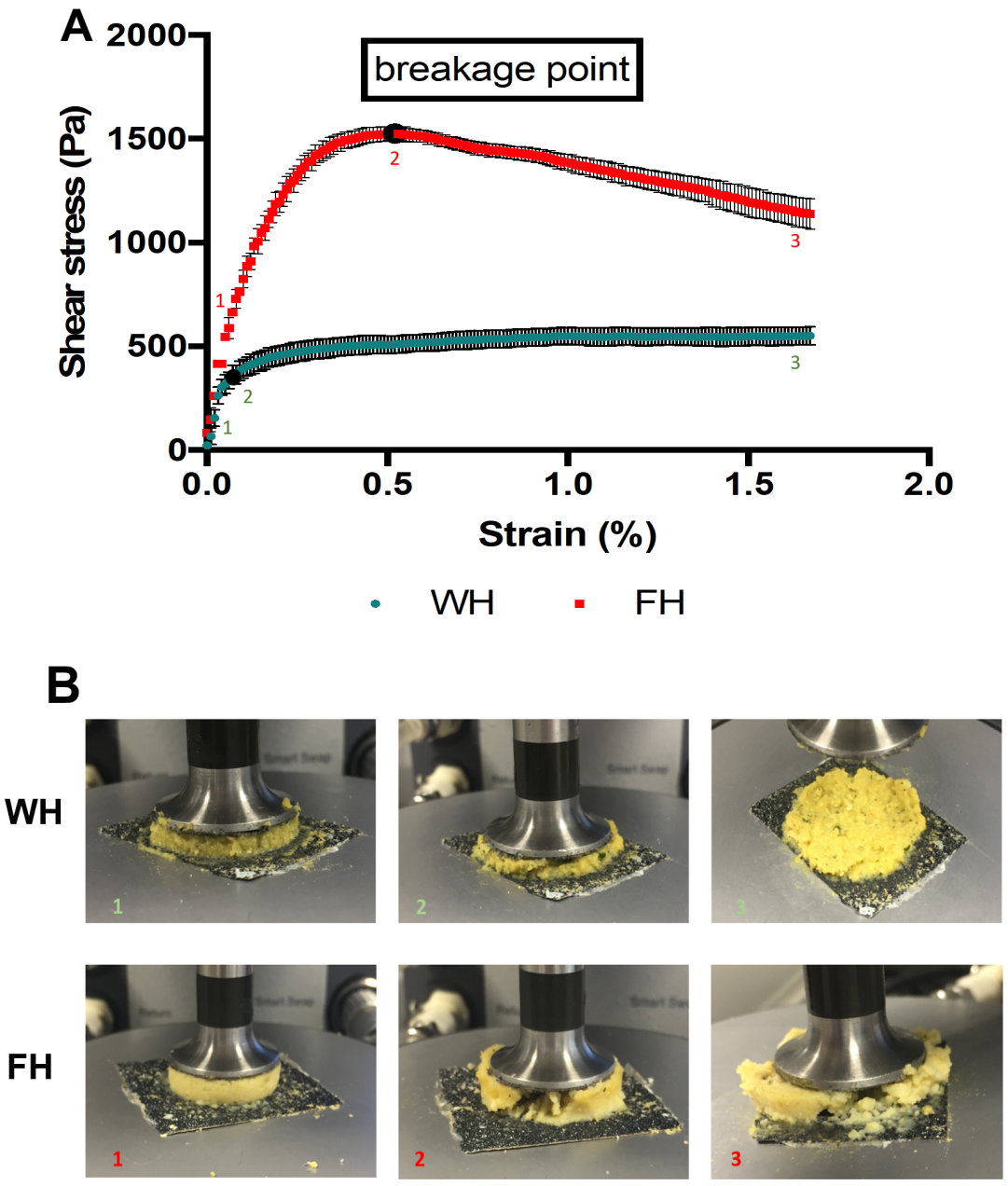

Figure 3.14(A) Constant steady shear rate results. (B) Sample deformation shear tests at 0.05/min: (1) start of the test, (2) the break point and (3) end of the test. FH sample has observable cracks compared to WH.

3.6 Discussion

3.6.1 Summary of findings

- Paracetamol concentrations were not significantly different between meals, indicating no differences in gastric emptying rate in the liquid phase.
- In vitro digestibility test analysis showed the hydrolysis of starch was lower when intact cell walls are present.
- Light microscopy confirmed the presence of some intact cell walls in WH samples after 90 min in vitro digestibility test.
- There was no significant difference in glucose concentrations following the consumption of both meals.
- Insulin and GIP concentrations were significantly lower after consuming WH than FH.
- There was no significant difference in GLP-1 following the consumption of FH and WH.
- The extent of the linear viscoelastic range is larger for FH than for WH. However, storage modulus for WH is higher than for FH.
- WH break point is at smaller strains compared to FH.

3.6.2 Detailed discussion

The aim of conducting this pilot study was to understand the mechanisms behind the acute effect of food structure on postprandial glucose and subsequent metabolic responses – mainly insulin and GIP – using chickpea hummus in two different forms: whole and flour. The starch content for both meals was controlled, as described in section 3.3.1.1. It was confirmed by light microscopy that the microstructure between meals is different, as the WH sample showed the presence of some intact cells after blending, while the FH sample showed ruptured cells. This correlates with previous findings, confirming that blending and mixing do not affect the microstructure of foods (Rovalino-Córdova et al., 2018). Moreover, there was no significant difference in gastric emptying rate in the liquid phase, as observed by blood paracetamol concentrations, and hence all results are assumed to be a consequence of the difference in the food structure and its rheological properties. This is in line with previous study on yogurt with different viscosities (high and low) but similar form. It showed that both had similar gastric emptying rate. However, both yogurts had different behaviours when entering in the small intestine (Menard et al., 2018). This

may indicate that breakdown pattern is different depending on the structure and content of the meal.

The rate of in vitro starch digestion is a measurement of the rate at which foods release their products of digestion. It has been previously shown that there is a positive relationship between the rate of starch hydrolysis and glycaemic response (Jenkins et al., 1982b). In this thesis, the in vitro study on chickpea hummus was conducted to examine the extent of starch digestibility between WH and FH. Results showed that the amount of starch digested was statistically different (P = 0.005) after 90 min between both meals, with a higher amount after FH compared to WH. Microscopy images obtained were consistent with the in vitro digestibility analysis, confirming the resistance of WH cell walls to the digestive enzyme; as a result, intact cells remained after 90 min of the experiment. This is in line with a previous in vitro study that showed the intactness of the legume cell wall throughout cooking and in vitro digestion (Do et al., 2019). However, postprandial glucose response from our clinical trial was not significantly different after the consumption of both meals. Yet, it was observed that FH had an immediate release of glucose, resulting in a blood glucose peak value at 30 min. It can be assumed that incretin molecules rapidly removed glucose from the bloodstream into cells before our measurement took place, and thus we did not see any significant difference in glucose concentrations between both meals.

In this study, more glucose was available in the bloodstream after FH, as observed at time point 30 min, causing the trigger of insulin secretion to move glucose rapidly into cells and hence the sharp drop of glucose concentrations at time point 60 min. Moreover, GIP release from intestinal cells is in response to glucose load, thus signalling insulin release (HLAD et al., 1964). The greater availability of glucose after FH compared to WH explains the significantly higher GIP concentration for FH at time points 15 min (P = 0.001), 30 min (P = 0.02) and 60 min (P = 0.03) compared to WH. Higher GIP concentration after the consumption of FH may partly explain the second peak in insulin at 120 min, as GIP and GLP-1 are responsible for 70% of postprandial insulin secretion (Campbell and Drucker, 2013). Our results correlate with previous findings showing that microstructure, including intact cell walls, play a role in encapsulating nutrients, which restricts starch hydrolysis and hence the lower metabolic response after a meal (Golay et al., 1986, Ramdath et al., 2018, Tovar et al., 1991).

GLP-1 concentration did not differ after the consumption of both meals. As discussed in section 1.2, gut hormones are thought to be secreted in two phases. The main agent for GLP-1 secretion is the second phase, where fermentable carbohydrate – the intact cells and fibres that are not digested in the small intestine – produces short chain fatty acid (SCFA) in the colon that stimulates the release of GLP-1 (De Silva and Bloom, 2012). It would be interesting to sample for longer than 180 min in order to observe a difference in gut hormones later on, as L-endocrine cells that are responsible for GLP-1 hormone secretion are located predominantly in the ileum and colon, and food may take more than 180 min to reach these cells (De Silva and Bloom, 2012). Moreover, there was no difference in the first phase of GLP-1 secretion that is mediated by a neural mechanism in the upper gastrointestinal tract, as both meals contained a similar number of ingested calories (510 kcal for WH and 490 kcal for FH). It was previously shown that the levels of GLP-1 are proportional to the calories ingested (Todd et al., 1998).

Interestingly, glucose response after both meals was much lower than would be expected for an equivalent dose of available carbohydrates (Lee and Wolever, 1998, Brouns et al., 2005). This suggests that some factors play a role in slowing down the breakdown of WH as well as FH in vivo that were not reproducible in the in vitro experiments, such as GI tract motility, chewing and gastric juices. These may affect the rheological properties of WH and FH, resulting in more resistance to digestion in both meals and hence a lower glycaemic response than that in the literature. For example, it can be assumed that FH resisted forces by forming a stronger gel network between the finer hydrating particles at higher stress compared to other food materials in the literature. This caused a low glycaemic response compared to the literature (Lee and Wolever, 1998).

Our rheological results are in line with those of previous studies on Spanish hummus, chickpea gels and sunflower tahini with a similar gel behaviour, with G' dominating over G" (Canet et al., 2015, Muresan et al., 2014, Alvarez et al., 2017). However, the results from this work showed that WH has a higher storage modulus compared to FH. This is attributed to the presence of intact cells in the sample, reflecting denser and less swollen walls compared to FH (Gartaula et al., 2019). This is in agreement with previous studies that showed the increase in large particle sizes increased the plateau modulus (Moelants et al., 2014). The power law index was less than 1 in both meals, which indicates a shear thinning behaviour for both samples. This is in line with

previous studies showing a shear thinning behaviour less than 1, such as dough and Spanish hummus (Alvarez et al., 2017, Mohammed, 2012).

It was demonstrated earlier that rheological behaviour depends on many factors, including particle size, gelatinisation behaviour, and concentration (Ravi and Bhattacharya, 2004). FH, with its more unified structure due to milling and smaller particle size, resulted in a longer LVR and resistance to shearing compared to WH. However, CSSR testing showed that WH, with its intact larger particles, ruptured earlier than FH, suggesting a stronger gel behaviour compared to FH (Basu et al., 2017). Strong gel behaviour contains strong network between its molecules that slow the action of the digestive enzyme and hence slower release of nutrients. From the work that was conducted in this chapter, it can be suggested that such WH intact structure formed strong gel that may change its rheological properties during digestion, which resulted in resisting forces, physical and chemical, hence resulted in lower glycaemia and insulinemia responses compared to FH.

Unfortunately, we were not able to further investigate the effect of intact cell walls on flow behaviour, as it was challenging to test rheological properties in complex foods with diverse components present (Ravi and Bhattacharya, 2006).

Strengths, weaknesses and future recommendations

The major strength of this study is that we compared food structure on postprandial metabolic responses and supported this by in vitro starch digestibility and rheological testing to provide a complete picture of the impact of food structure and rheological properties on digestion. Another strength is that we conducted a randomized crossover trial and used isocaloric meals with the same ingredients that were controlled for the amount of starch.

The limitations of the present study should be noted. This was an acute study that cannot present a long-term effect on consuming chickpeas. Additionally, our findings cannot be generalized to individuals with chronic conditions such as type 2 diabetes, as this study was conducted on healthy volunteers. Furthermore, the study design would benefit from adding a control meal to the clinical trial for comparison. Moreover, we used paracetamol to measure the gastric emptying rate in the liquid phase of the meal. Although it is not the gold standard to measure gastric emptying, it is a non-invasive method to measure the liquid phase of a meal. As for rheological tests, such tests at a smaller length scale are needed to investigate the microstructure effects on digestion as well as bolus and aspirated digesta. The link between the initial structure

– rheology – and postprandial metabolic responses is complex. Future studies are needed to investigate the effect of the digestion process on food structure breakdown and its flow behaviour by conducting experiments on particles size. This may help to further explore the possible correlation of different food structure and flow behaviour along with absorption level.

3.6 Conclusion

In conclusion, these results provide evidence that food microstructure (intact cell walls) and its rheological properties can affect the rate of starch bioaccessiblity and therefore might explain the difference in the time course of glucose absorption into the portal blood between both meals. Increasing whole foods that contain structure in our diet has a beneficial effect on postprandial metabolism. This study may help in the development of functional foods using legumes that control the release of nutrients to specific locations in the gastrointestinal tract.

CHAPTER 4: The impact of food structure on early digestion and metabolic responses

4.1 Introduction

For the purpose of this study, wild type *RR* peas and wrinkled *rr* peas were used as a model in order to better understand the effect of food structure on early digestion and hence postprandial metabolic responses. As mentioned previously in Chapter 1, section 1.4.2.2, the pea (*Pisum sativum L.*) is one type of legume having a range of mutants that has been identified through breeding. These mutants have an effect on the starch synthesis pathway (Davydova et al., 1995). *rr* pea seeds have a defective starch branching enzyme I (BSEI) that is involved in amylopectin synthesis (Smith, 1988). This causes many changes in the seeds' molecular structure compared to *RR* peas. Starches in *rr* peas have more amylose content (70%) than *RR* peas, which makes them more resistant to digestion (Bhattacharyya et al., 1993). Experimental studies on rats showed that rats who consumed a high amylose diet improved glycaemic control, which resulted in a significant reduction in body weight, as opposed to rats that consumed a normal diet (Zhu et al., 2012, Aziz et al., 2009). Moreover, the appearance of *rr* peas shows wrinkles due to the inefficient conversion of sugar to starch during development, causing more sugar accumulation in the seed. This will cause more water uptake osmotically, leading to swelling of the seed; when dried, more water is lost, and wrinkles appear (Wang et al., 1987). All these changes affect the starch granule characteristics, such as the shape of the granule, and gelatinization properties (Bhattacharyya et al., 1993, insitute, 2016). Edwards et al.'s (2018) study compared the in vitro starch hydrolysis rate between *RR* peas and *rr* peas after milling to determine the effect of the *r*-mutation on starch digestion. It was found that *rr* peas contained a proportion of starch that is completely resistant to α-amylase hydrolysis. This fraction is thought to occur as a result of processing or to be formed during hydrolysis of susceptible starch (Edwards et al., 2018). Escaping digestion has a beneficial effect on the body. Undigested carbohydrates will travel to the colon, where fermentation occurs to produce short chain fatty acids (SCFAs). SCFAs lead to physiological and metabolic benefits that have a major positive impact on human health (Ríos-Covián et al., 2016).

4.1.1 Mechanical properties of food

The food's mechanical properties are a result of food structure, the physical state of food and rheological properties (Rahman, 2009). Mechanical properties can be determined objectively using instruments with controlled deformation using many

types of mechanical loading, such as puncture, shearing and compression (Rahman and McCarthy, 1999). Compression tests are used in food industrial research to determine the behaviour of material such as hardness, cohesiveness and fracturability under a compressive load (B. Dobrzański, 2013). A major factor affecting foods' mechanical properties is the moisture content. A study conducted on fava beans with different moisture contents showed that beans with less moisture content needed greater forces to rupture (Altuntaş and Yıldız, 2007). Information on foods' mechanical properties can be used as an indirect indication of the behaviour of food during digestion, such as the foods' resistance to physical forces and GI tract motility rate. This will give further insight into the role of food structure stability in maintaining glucose homeostasis.

4.1.2 Rheological properties of digesta – viscosity

Digestion is a process of food structure breakdown by mechanical and chemical forces to release nutrients to be ready for absorption. The absorption of nutrients during digestion can be influenced by food structure that will affect the flow, including viscosity and mixing of digesta. Viscosity is the resistance of a fluid to flow due to internal friction (Lentle and Janssen, 2008a). It has been postulated that higher food viscosity may lower the absorption of nutrients by limiting the diffusion of nutrients across the brush border membrane in the small intestine. Another mechanism is that higher viscosity may increase the bulking effect, leading to the formation of a barrier that limits hydrolysis and thus modifies hormonal feedback. Viscosity of a meal can also disrupt the rheological homeostasis of the GI tract (Lentle and Janssen, 2008b).

Having a viscous meal can defeat rheological homeostasis by influencing small intestine motility, which will result in slower digestion and hence slower absorption. Therefore, measuring digesta viscosity can give an indication of nutrient absorption.

The measurement of human digesta is complicated for many reasons. The human digesta is a heterogeneous suspension of partly or fully digested food particles suspended in fluids (Roger G. Lentle, 2011). As the particles in human digesta samples are recovered from the living gut, these samples are not settled due to continuous digestion process while sampling and, hence, are prone to errors. Particles in digesta exhibit a wide range of size distribution, depending on mastication and the physical structure of the food. Moreover, most rheological tests require a large sample size, which is difficult to obtain from humans (Lentle and Janssen, 2008a). Previous studies of different species investigated digesta and found that the liquid phase digesta

alone behaves as a Newtonian fluid (Razdan and Pettersson, 1996, Johansen et al., 1996) while the whole digesta exhibits a decrease in viscosity with increased shear, eliciting a non-Newtonian shear thinning behaviour (Takahashi and Sakata, 2002). This gives an indication that there is less time for new entanglements to form with increasing shear, leading to a reduction in viscosity and, hence, the breakdown of food during digestion.

The molecular structure of peas may play a role in viscosity, as cell wall composition and the conformation of amylose and amylopectin may change the properties of starch and their action during digestion, and thus result in a change in rheological properties.

4.2 Purpose of the study

4.2.1 Hypothesis

I hypothesised that whole *rr* peas and flour *rr* peas would have less of an effect on post-plasma glucose concentrations compared to *RR* peas and flour *RR* peas due to a slower breakdown during digestion due to its structure and its rheological properties limiting starch digestion.

4.2.2 Aims

The overall aim of this exploratory study was to investigate the effect of food structural differences of *RR* peas and *rr* peas, using flour and whole, on their behaviour during early digestion in relation to postprandial metabolic responses.

4.2.3 Outcome measures

The primary outcome measures are:

- Blood glucose concentration
- Insulin, GIP and GLP-1 concentrations
- Mechanical and rheological properties of peas before and after ingestion
- Pea disintegration as assessed by light microscopy.

4.3 Methods

Information on meal preparation is mentioned in Chapter 2, section 2.3. Meals contained 50 gm dry weight peas either *RR* peas (143 kcal; protein, 10.6 g; fat, <.05; carbohydrate, 17.6 g; dietary fibre, 18 g) or *rr* peas (147 kcal; protein, 11.9 g; fat, <.85; carbohydrate, 14 g; dietary fibre, 18.1 g) in two different forms: whole or flour. Pea moisture content, measured only for whole peas, was 57% for *RR* peas and 66% for

rr peas. As for the flour peas, we speculate that they would have a similar amount of moisture content since the flour was produced by dried peas.

Recruitment, blood collection, preparation and analysis, and rheological tests are explained in detail in Chapter 2.

4.3.1 Clinical trial: study visit overview

The clinical trial is a randomised, crossover study. Information on randomization can be found in Chapter 2, section 2.3.4.

4.3.2 Study visit preparation

Participants were instructed to avoid alcohol, excessive exercise and caffeine in the 24 hours prior to the study day. Participants were also instructed not to start any new diet or exercise regimes during the study to avoid conflicting results.

A standard meal of the participant's choice was consumed the evening before each visit to maintain consistency between visits. Participants were also asked to fast for 12 hours prior to the start of the study visit.

4.3.1.2 Study visit protocol

This clinical trial comprises of one study visit consists of 4 days/3 nights held in the NIHR/CRF. Following an overnight fasting for 12 hours, participants were asked to attend the CRF at 9:00 am. Upon arrival, a cannula was inserted for blood sampling, and then nasogastric and nasoduodenal tubes were inserted through the nostrils by a doctor using the CORTRAK system (COPRAK MedSystems, Wheeling, Illinois, USA). The system consists of a receiver and a guide wire, as shown in Figure 4.1. The wire is inserted in the feeding tube to track the position of the tube during placement. The receiver is attached to a computer that records the magnetic signals from the wire to show the tube's path in real time. Tube placement was also confirmed by pH strips of fasting aspirated samples. Tubes remained in place for the whole study visit.

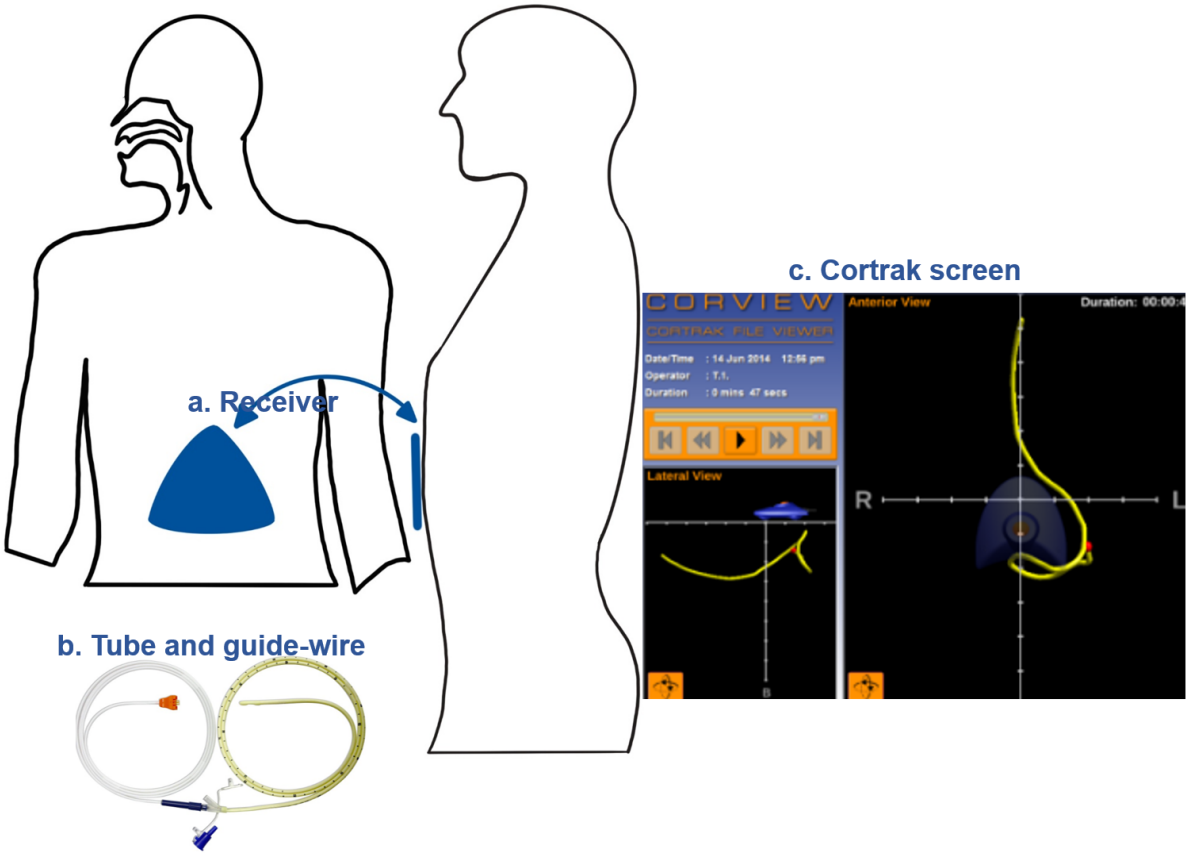

Figure 4.1 CORTRAK system: a. Receiver; b. Tube and guide wire; c. Corpak screen. Adapted from (Taylor et al., 2017)

Participants were allowed to calm down for 30 min before the start of the study on day 1. A test breakfast meal was given at 0 min based on the randomisation, and participants were asked to consume it within 15 minutes. Postprandial blood samples were taken (10 ml) in parallel with gastric and duodenal aspirated samples at time points 15, 30, 60, 90, 120, 150 and 180 min. A schematic representation of the study can be found in Figure 4.2. At the end of the study (4th day), the nasogastric and nasoduodenal tubes as well as the cannula were removed, and participants were discharged from the CRF. Please refer to Methods sections 2.3 and 2.4 for sample preparation and collection in detail.

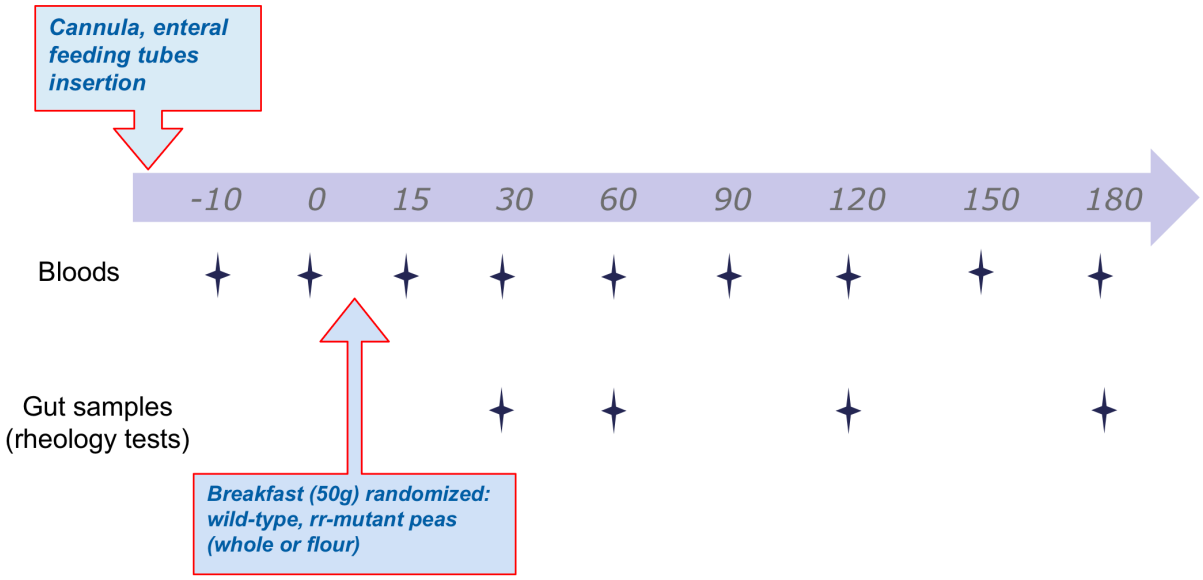

Figure 4.2 Study flow. Overview of timing of blood sampling, gut sampling and test meal introduction.

4.4 Results

4.4.1 Baseline characteristics of participants

Twelve healthy volunteers were recruited and completed the 4 interventions. One of them could not complete one day of the 4-day visit; therefore, only baseline samples have been collected. Participants characteristics are described in Table 4.1.

Table 4.1: Participant characteristics

VARIABLE	ALL PARTICIPANTS (*N* = 12)
GENDER (M: F)	7:5
AGE (Y)	45.8 ± 4.4
WEIGHT (KG)	69.5 ± 1.9
BODY MASS INDEX BMI (KG/M^2)	24.4 ± 0.6
BODY FAT (%)	22.9 ± 2.4

Note: Continuous variables are presented as mean ± SEM. Abbreviation: y = years.

4.4.2 Comparison between whole peas and flour peas

Please refer to Chapter 2, section 2.7.2, for information on statistical analysis.

4.4.2.1 Blood glucose concentration

Time course data and $iAUC_{0–180}$ for plasma glucose concentrations are shown in Figure 4.3 between whole *RR* and flour *RR* peas, and whole *rr* and flour *rr* peas.

Repeated measures ANOVA analysis showed a significant difference for plasma glucose concentrations after the consumption of whole peas compared to flour peas for both *RR* and *rr* peas ($P = 0.0001$ and $P = 0.002$, respectively).

Post hoc analyses showed that whole *RR* peas had lower glucose concentrations at time points 15 and 30 min ($P = 0.001$ and $P = 0.001$, respectively) and higher at time points 120, 150 and 180 min ($P = 0.05$, $P = 0.003$ and $P = 0.001$, respectively) compared to flour *RR* peas. Blood glucose $iAUC_{0-180}$ was significantly different between whole and flour *RR* peas ($P = 0.002$).

As for *rr* peas, post hoc analyses showed that whole *rr* peas had a significantly lower glucose concentration at time points 15, 30 and 60 min ($P = 0.03$, $P = 0.01$ and $P = 0.0001$, respectively), and a higher glucose concentration at time points 120 and 180 min ($P = 0.04$ and $P = 0.02$, respectively) compared to flour *rr* peas. Blood $iAUC_{0-180}$ was significantly different between whole and flour *rr* peas ($P = 0.01$).

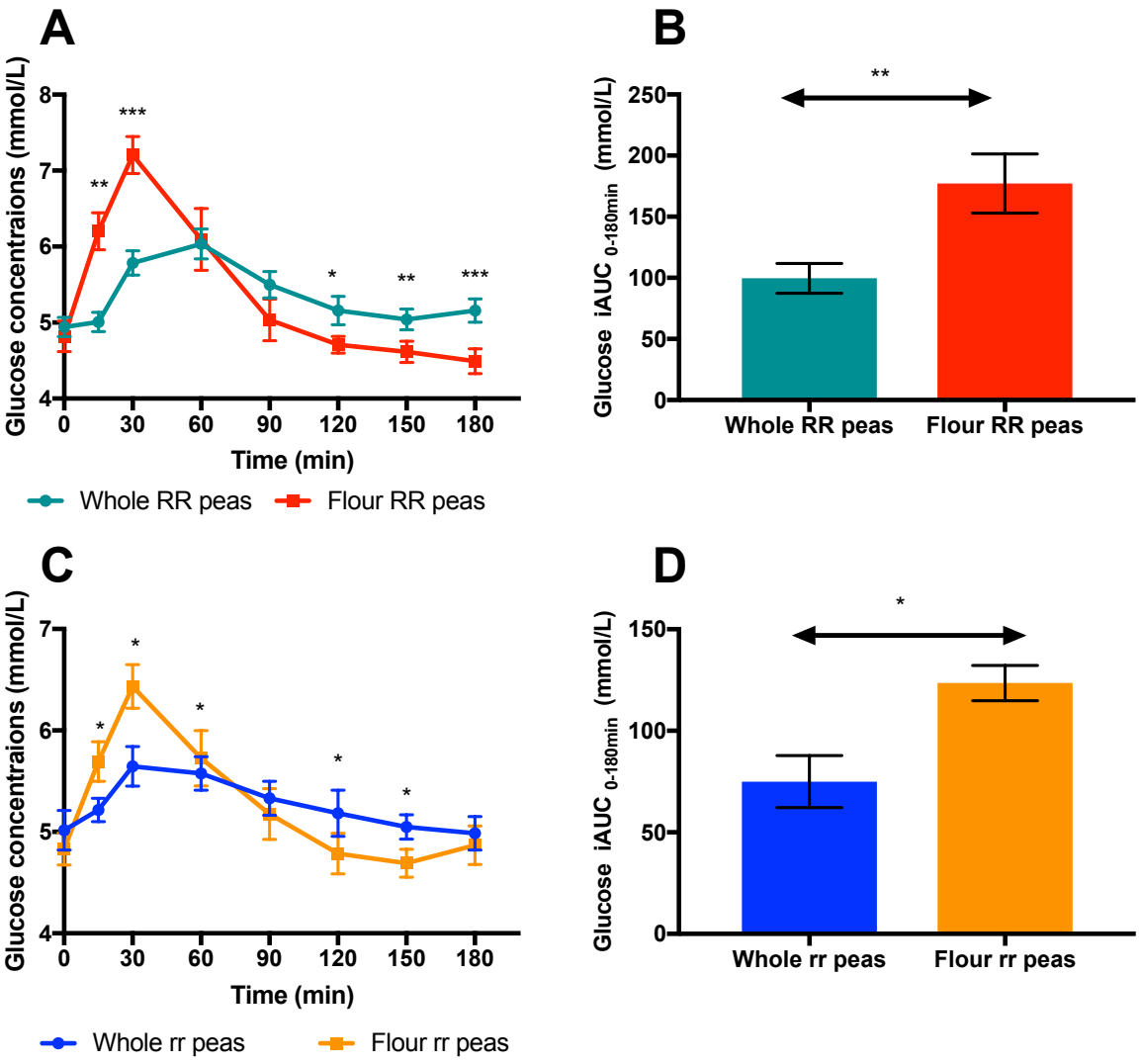

Figure 4.3 Plasma glucose concentrations following consumption of whole *RR* peas against flour *RR* peas (A, B), and whole *rr* peas against flour *rr* peas (C, D). Plasma glucose concentrations over time (A, C). $iAUC_{0–180}$ shown in (B, D). Data represents mean ± SEM (*n* = 11).

4.4.2.2 Insulin concentration

Time course data and $iAUC_{0–180}$ for insulin concentrations are shown in Figure 4.4 between whole *RR* and flour *RR* peas, and whole *rr* and flour *rr* peas.

Repeated measures ANOVA analysis showed a significant interaction between meal and time on serum insulin concentrations after the consumption of whole peas compared to flour peas for both *RR* and *rr* peas (*P* = 0.01 and *P* = 0.01, respectively). Post hoc analyses showed that whole *RR* peas had lower insulin concentrations at time points 15 and 30 min (*P* = 0.004 and *P* = 0.003, respectively) and higher insulin concentrations at time points 90 and 120 min (*P* = 0.01 and *P* = 0.001, respectively)

compared to flour *RR* peas. Blood glucose $iAUC_{0-180}$ was significantly different between whole and flour *RR* peas ($P = 0.001$).

As for *rr* peas, post hoc analyses showed a significantly lower glucose concentration at time point 60 min ($P = 0.01$) and a higher glucose concentration at time point 180 min ($P = 0.04$) compared to flour *rr* peas. Blood $iAUC_{0-180}$ was significantly different between whole and flour *rr* peas ($P = 0.005$).

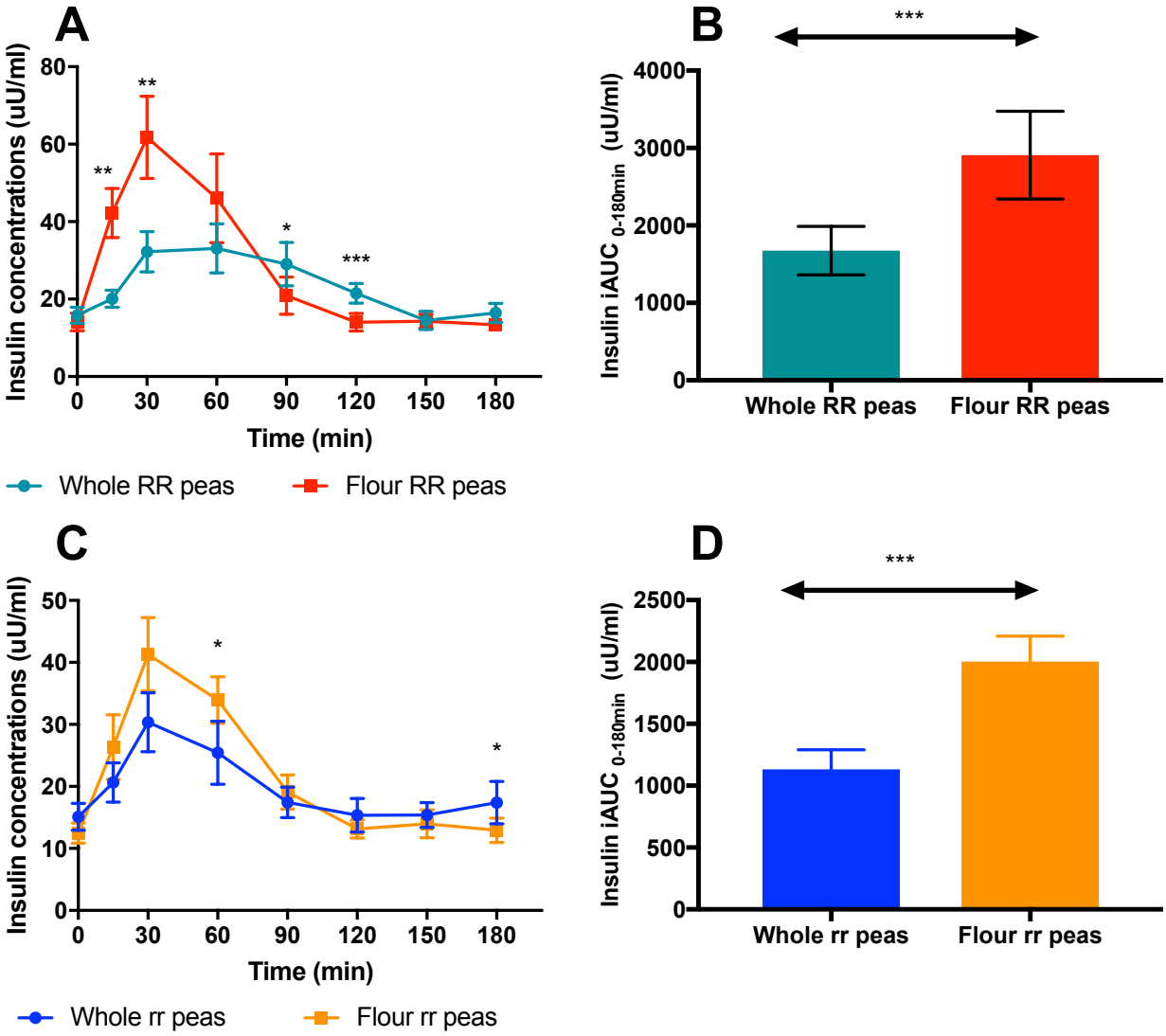

Figure 4.4 Serum insulin concentrations following consumption of whole *RR* peas against flour *RR* peas (A, B), and whole *rr* peas against flour *rr* peas (C, D). Serum insulin concentrations over time (A, C). $iAUC_{0-180}$ shown in (B, D). Data represents mean ± SEM ($n = 11$).

4.4.2.3 GIP concentrations

Time course data and $iAUC_{0-180}$ for GIP concentrations are shown in Figure 4.5 between whole *RR* and flour *RR* peas, and whole *rr* and flour *rr* peas.

Repeated measure ANOVA analysis showed a significant difference for serum GIP concentrations after the consumption of whole peas compared to flour peas for both *RR* and *rr* peas ($P = 0.002$ and $P = 0.006$, respectively).

Post hoc analyses showed that whole *RR* peas had lower insulin concentrations at time points 15, 30, 60 and 180 min ($P = 0.001$, $P = 0.001$, $P = 0.001$ and $P = 0.03$, respectively) compared to flour *RR* peas. Blood glucose $iAUC_{0-180}$ was significantly different between whole and flour *RR* peas ($P = 0.002$).

As for *rr* peas, post hoc analyses showed a significant lower glucose concentration at time points 15, 30, 60 and 90 min ($P = 0.01$, $P = 0.009$, $P = 0.001$ and $P = 0.008$, respectively) compared to flour *rr* peas. Blood $iAUC_{0-180}$ was significantly different between whole and flour *rr* peas ($P = 0.001$).

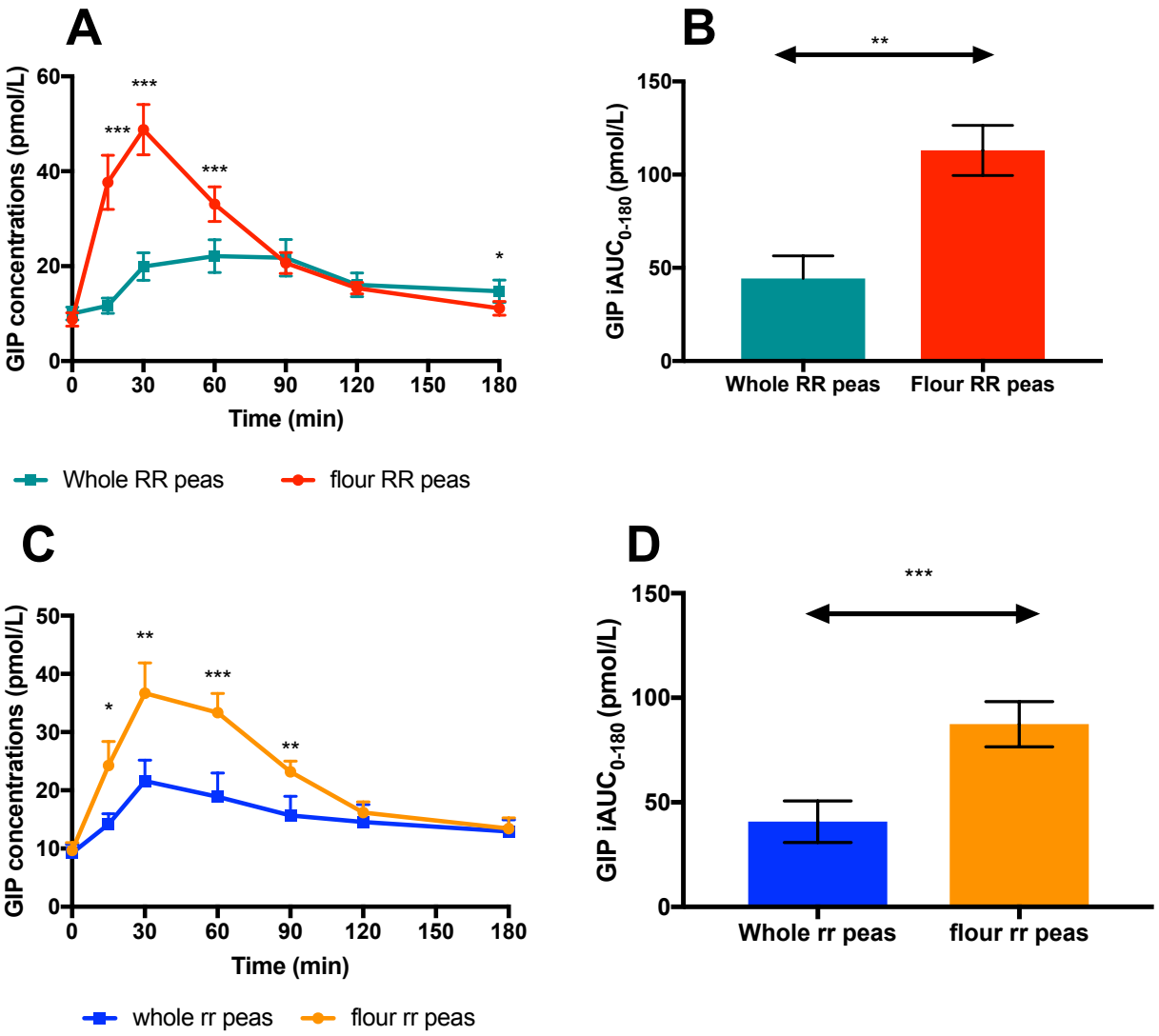

Figure 4.5 Plasma GIP concentrations following consumption of whole RR peas against flour RR peas (A, B), and whole rr peas against flour rr peas (C, D). Plasma GIP concentrations over time (A, C). $iAUC_{0–180}$ shown in (B, D). Data represents mean ± SEM (*n* = 11).

4.4.2.4 GLP-1 concentrations

Time course data and $iAUC_{0–180}$ for GLP-1 concentrations are shown in Figure 4.6 between whole *RR* and flour *RR* peas, and whole *rr* and flour *rr* peas.

Repeated measures ANOVA analysis showed a significant GLP-1 concentration after the consumption of whole peas compared to flour peas for *RR* (P = 0.02) but not *rr* peas (P = 0.4). Post hoc analyses showed that whole *RR* peas had lower GLP-1 concentrations at time point 15 (P = 0.01) compared to flour *RR* peas. Blood glucose $iAUC_{0–180}$ was not significantly different between whole and flour *RR* peas and *rr* peas (P = 0.8 and P = 0.2, respectively).

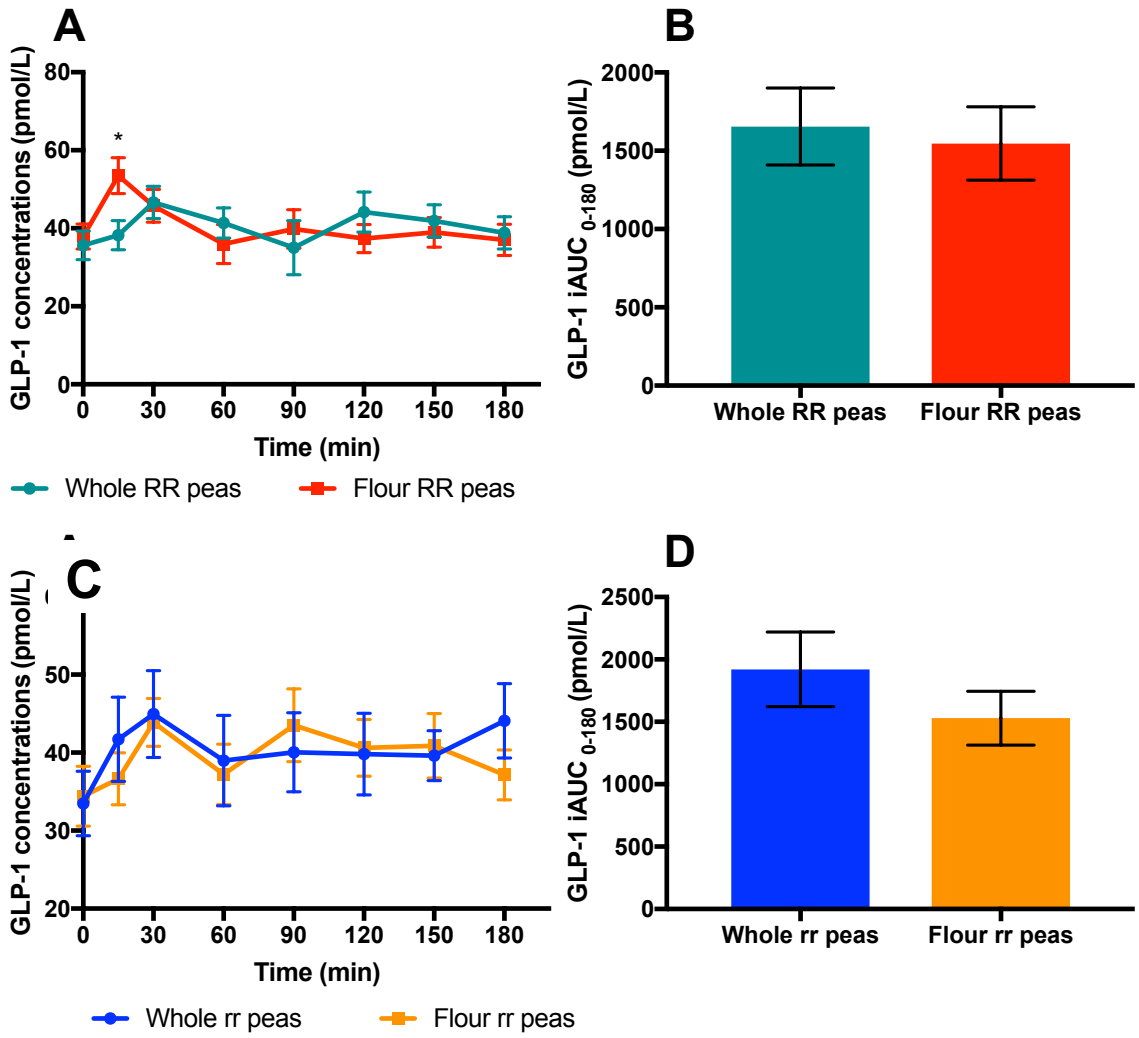

Figure 4.6 GLP-1 concentrations following consumption of whole *RR* peas against flour *RR* peas (A, B), and whole *rr* peas against flour *rr* peas (C, D). GLP-1 concentrations over time (A, C). $iAUC_{0–180}$ shown in (B, D). Data represents mean ± SEM (*n* = 11).

4.4.3 Comparison between whole *RR* peas against whole *rr* peas

Please refer to Chapter 2, section 2.7.2, for information on statistical analysis.

4.4.3.1 Blood glucose concentration

Time course data and $iAUC_{0–180}$ for plasma glucose concentrations are shown in Figure 4.7. Repeated measures ANOVA analysis showed a significant difference in glucose concentrations (P = 0.01). Post hoc analysis indicating a difference at 60 minutes post ingestion with *rr* peas exhibiting lower glucose release as opposed to *RR* peas (P = 0.007). Interestingly, there was an effect at 15 min post ingestion showing that *rr* peas resulted in higher levels of glucose as opposed to *RR* peas. This effect

might be attributed to the mutation resulting in higher sucrose levels (P = 0.04). Blood glucose $iAUC_{0–180}$ was not significantly different between whole *rr* peas and whole *RR* peas (P = 0.09).

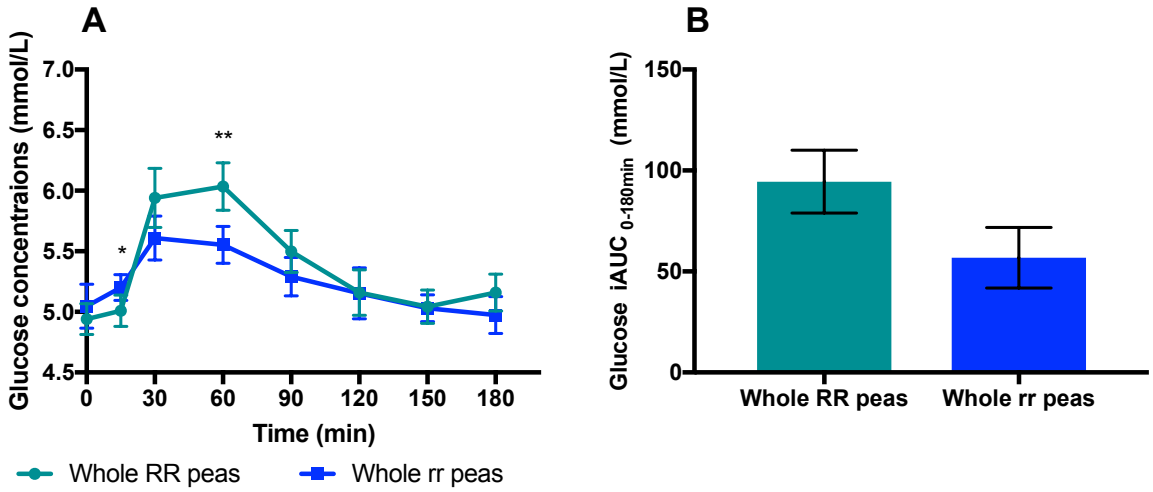

Figure 4.7 Plasma glucose concentrations following consumption of whole *RR* peas or whole *rr*-mutant peas. Data represents mean ± SEM (A) plasma glucose concentrations over time and (B) $iAUC_{0–180}$ for plasma glucose concentrations following consumption of whole *RR* peas or whole *rr* peas (n = 12).

4.4.3.2 Insulin concentration

Time course data and $iAUC_{0–180}$ for serum insulin concentrations are shown in Figure 4.8. Repeated measures ANOVA analysis showed a significant difference in insulin concentrations (P = 0.01). Post hoc analysis showed a significantly lower insulin concentrations after *rr* peas compared to *RR* peas at time points 90 and 120 min (P = 0.005 and P = 0.003, respectively). Blood insulin $iAUC_{0–180}$ was significantly different between whole RR peas and whole *rr* peas (P = 0.03).

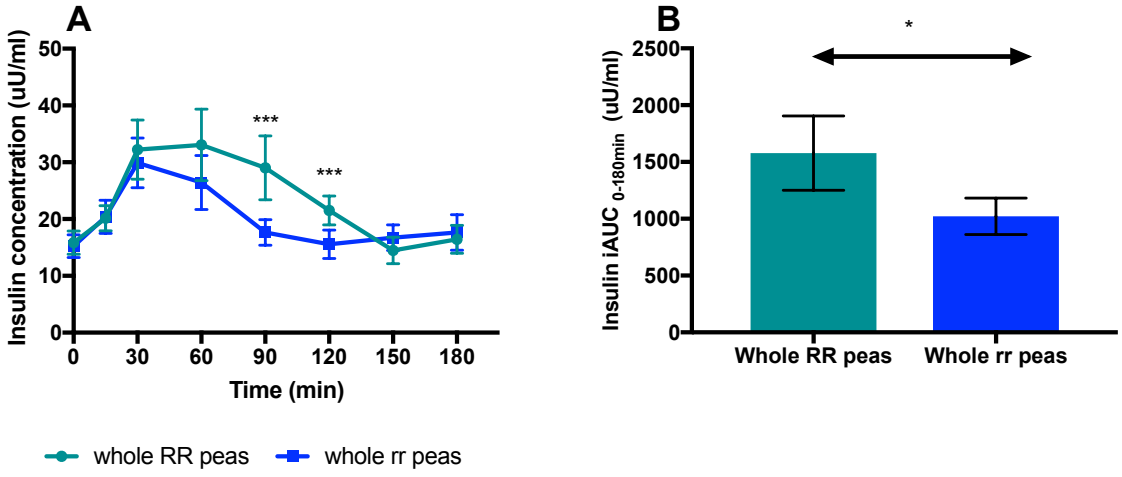

Figure 4.8 Serum insulin concentrations following consumption of whole *RR* peas or whole *rr*-mutant peas. Data represents mean ± SEM (A) serum insulin concentrations over time and (B) $iAUC_{0–180}$ for serum insulin concentrations following consumption of whole *RR* peas or whole *rr* peas (*n* = 12).

4.4.3.3 GIP concentration

Time course data and $iAUC_{0–180}$ for plasma GIP concentrations are shown in Figure 4.9. Repeated measures ANOVA analysis showed a significant difference in GIP concentrations (*P* = 0.04). Post hoc analysis showed significantly lower insulin concentrations after consumption of *rr* peas compared to *RR* peas at time points 90 and 180 min (*P* = 0.04 and P = 0.04, respectively). Blood GIP $iAUC_{0–180}$ was not significantly different between whole *RR* peas and whole *rr* peas (*P* = 0.2).

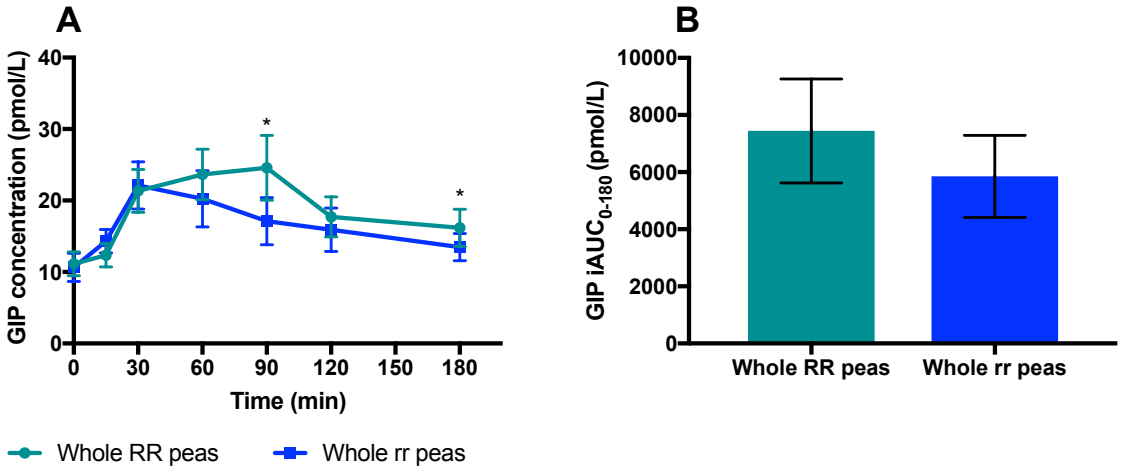

Figure 4.9 Plasma GIP concentrations following consumption of whole *RR* peas or whole *rr*-mutant peas. Data represents mean ± SEM (A) plasma GIP concentrations over time and (B) $iAUC_{0–180}$ for plasma GIP concentrations following consumption of whole *RR* peas or whole *rr* peas (*n* = 12).

4.4.3.4 GLP-1 concentration

Time course data and $iAUC_{0–180}$ for plasma GLP-1 concentrations are shown in Figure 4.10. Repeated measures ANOVA analysis showed no significant difference in GLP-1 concentrations (*P* = 0.5). Blood GLP-1 $iAUC_{0–180}$ was not significantly different between whole RR peas and whole rr peas (*P* = 0.4).

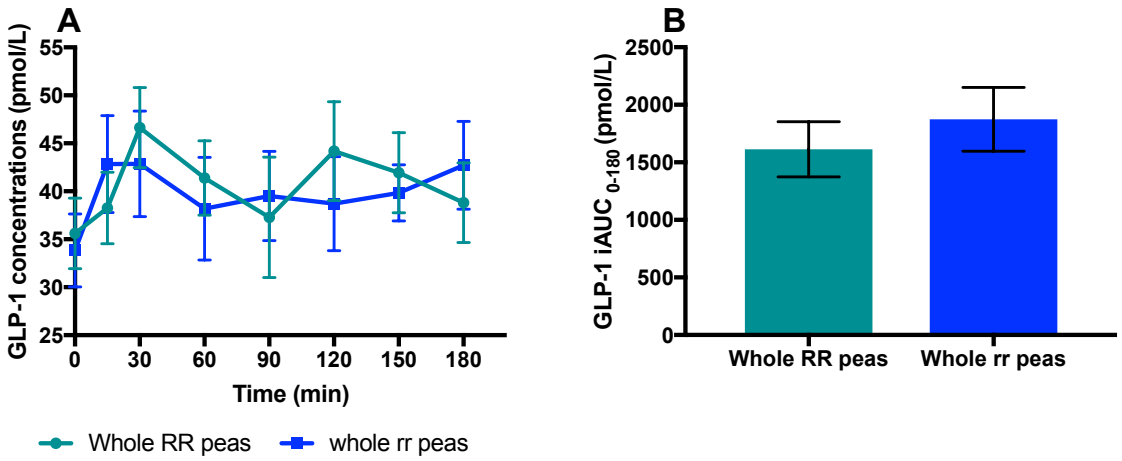

Figure 4.10 Plasma GLP-1 concentrations following consumption of whole *RR* peas or whole *rr*-mutant peas. Data represents mean ± SEM (A) plasma GLP-1 concentrations over time and (B) $iAUC_{0–180}$ for plasma GLP-1 concentrations following consumption of whole *RR* peas or whole *rr* peas (*n* = 12).

4.4.4 Comparison between flour *RR* peas against flour *rr* peas

Please refer to Chapter 2, section 2.7.2, for information on statistical analysis.

4.4.4.1 Glucose concentrations

Time course data and $iAUC_{0–180}$ for plasma glucose concentrations are shown in Figure 4.11. Repeated measures ANOVA analysis showed a significant difference, with a lower glucose concentration after the consumption of *rr* flour peas (P = 0.009). Post hoc analysis showed a significant difference at time points 15 and 30 min (*P* = 0.03 and *P* = 0.02, respectively). Blood glucose $iAUC_{0–180}$ was not significantly different between four *rr* peas and four *RR* peas (*P* = 0.2).

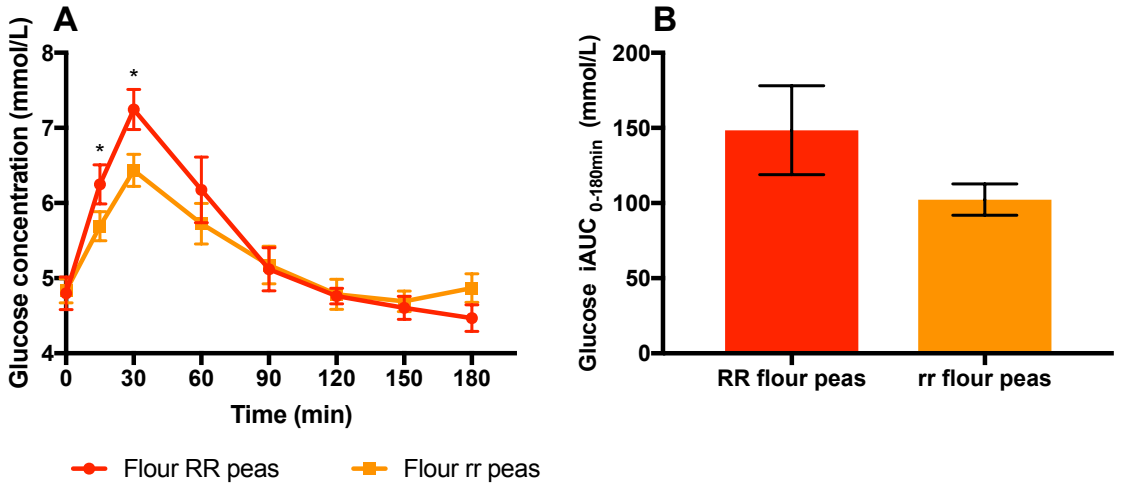

Figure 4.11 Plasma glucose concentrations following consumption of flour *RR* peas or flour *rr* peas. Data represents mean ± SEM (A) plasma glucose concentrations over time and (B) $iAUC_{0-180}$ for plasma glucose concentrations following consumption of flour *RR* peas or flour *RR* peas (*n* = 11).

4.4.4.2 Insulin concentrations

Time course data and $iAUC_{0-180}$ for serum insulin concentrations are shown in Figure 4.12. Repeated measures ANOVA analysis showed borderline significant difference in insulin concentrations (*P* = 0.09). Blood insulin $iAUC_{0-180}$ was borderline significantly different between flour *RR* peas and flour *rr* peas (*P* = 0.08).

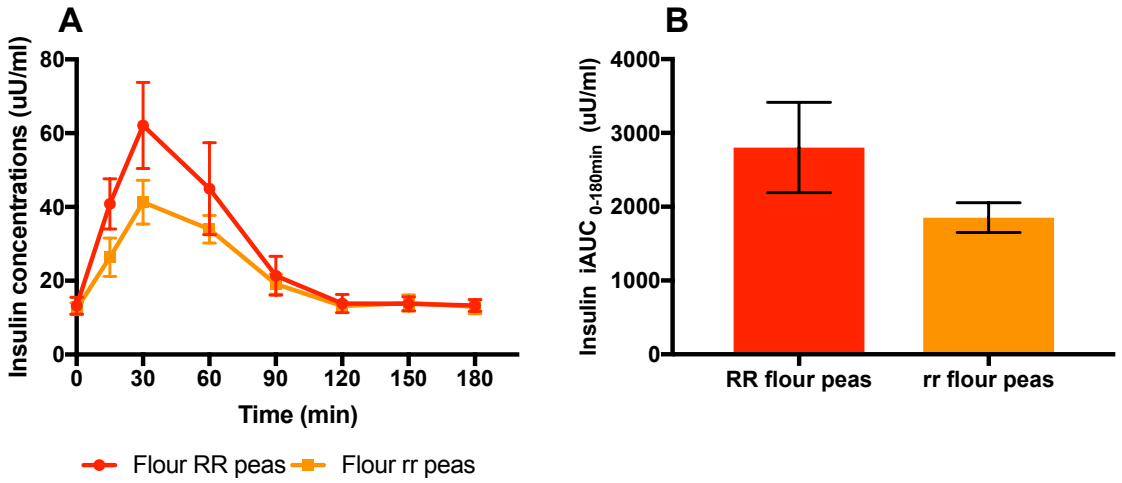

Figure 4.12 Serum insulin concentrations following consumption of flour RR peas or flour rr peas. Data represents mean ± SEM (A) serum insulin concentrations over time and (B) $iAUC_{0-180}$ for serum insulin concentrations following consumption of flour *RR* peas or flour *rr* peas (*n* = 11).

4.4.4.3 GIP concentrations

Time course data and $iAUC_{0-180}$ for plasma GIP concentrations are shown in Figure 4.13. Repeated measures ANOVA analysis showed a significant difference (*P* = 0.003). Post hoc test analysis revealed that there was a significant difference in GIP at time points 15 and 30 min (*P* = 0.005 and *P* = 0.03, respectively). Blood GIP $iAUC_{0-180}$ was not significantly different between whole *RR* peas and whole *rr* peas (*P* = 0.04).

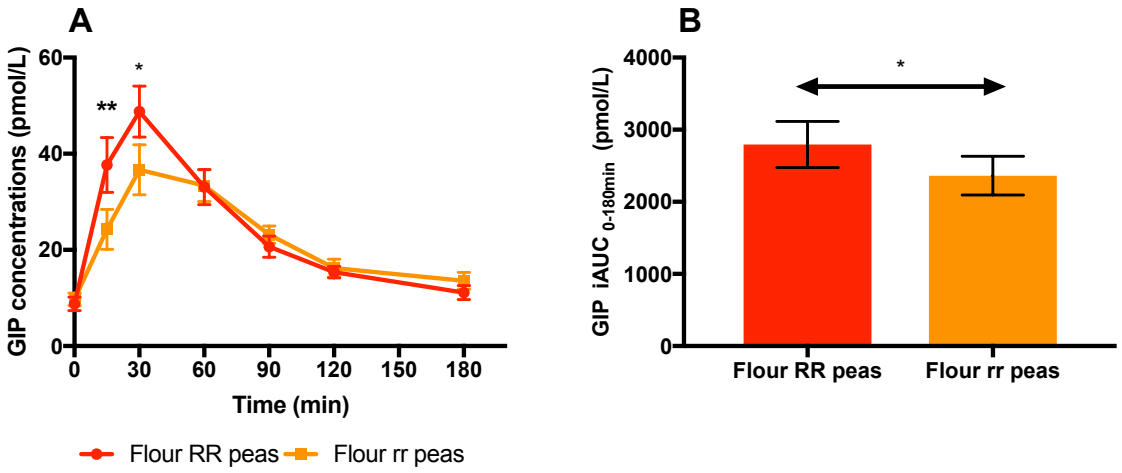

Figure 4.13 Plasma GIP concentrations following consumption of flour RR peas or flour rr-mutant peas. Data represents mean ± SEM (A) plasma GIP concentrations over time and (B) $iAUC_{0–180}$ for plasma GIP concentrations following consumption of flour *RR* peas or flour *RR* peas (*n* = 11).

4.4.4.4 GLP-1 concentrations

Time course data and $iAUC_{0–180}$ for plasma GLP-1 concentrations are shown in Figure 4.14. Repeated measures ANOVA analysis showed a significant difference (*P* = 0.002). Post hoc test analysis revealed that there was a significant difference in GLP-1 at time point 15 min (*P* = 0.001). Blood GLP-1 $iAUC_{0–180}$ was not significantly different between flour *RR* peas and flour *rr* peas (*P* = 0.5).

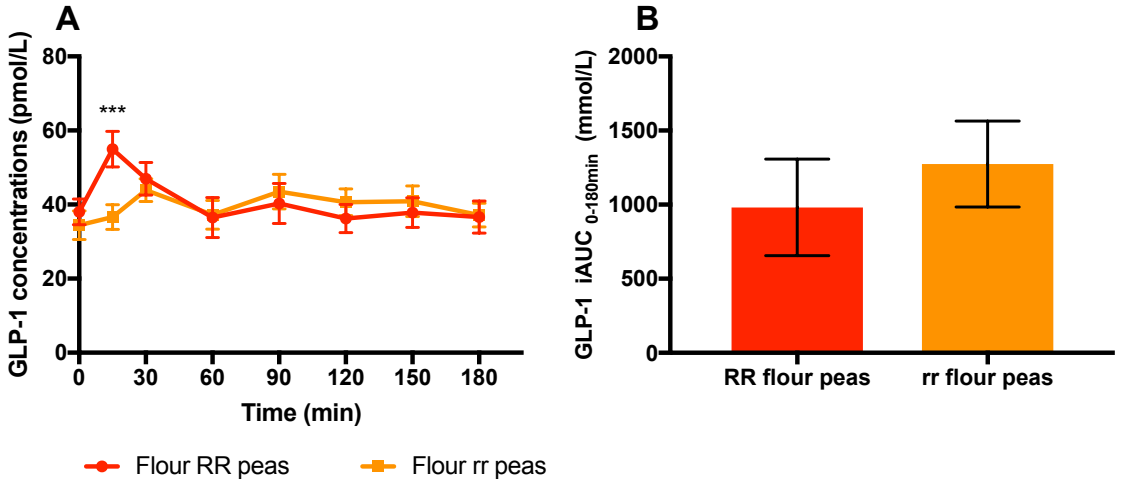

Figure 4.14 Plasma GLP-1 concentrations following consumption of flour *RR* peas or flour *rr* peas. Data represents mean ± SEM (A) plasma GLP-1 concentrations over time and

(B) $iAUC_{0-180}$ for plasma GLP-1 concentrations following consumption of flour *RR* peas or flour *RR* peas (n = 11).

4.4.5 Rheological tests outcome

4.4.5.1 Compression test results for cooked pea seeds

For information on compression test methods, please refer to Chapter 2, section 2.6.2.2. Compression tests were performed to determine the peas' behaviour under the application of an external mechanical force.

Pea seeds were cooked as per the clinical trial (see Chapter 2, section 2.2.2).

A compression test was conducted on 20 seeds from each type at speed rates of 1 and 15 mm/s, as shown in Figure 4.15. There was a significant difference in the maximum force at 1 mm/s (P < 0.0001) between *rr* pea seeds and *RR* pea seeds, where rr pea seeds required 20 N to fracture, while RR pea seeds required 15 N. However, no difference was observed at 15 mm/s (P = 0.6), as shown in Figure 4.16. It was noted that the physical appearance of compressed peas after both speed rates were different: the *rr* pea seeds were split into larger particles, whereas *RR* pea seeds appeared to completely breakdown, as shown in Figure 4.17.

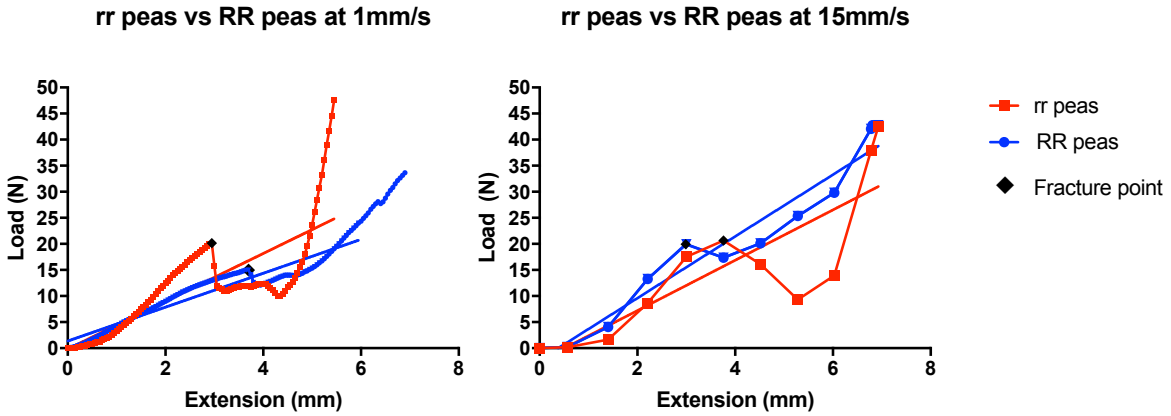

Figure 4.15 Load extension test at speed rates 1 and 15 mm/s.

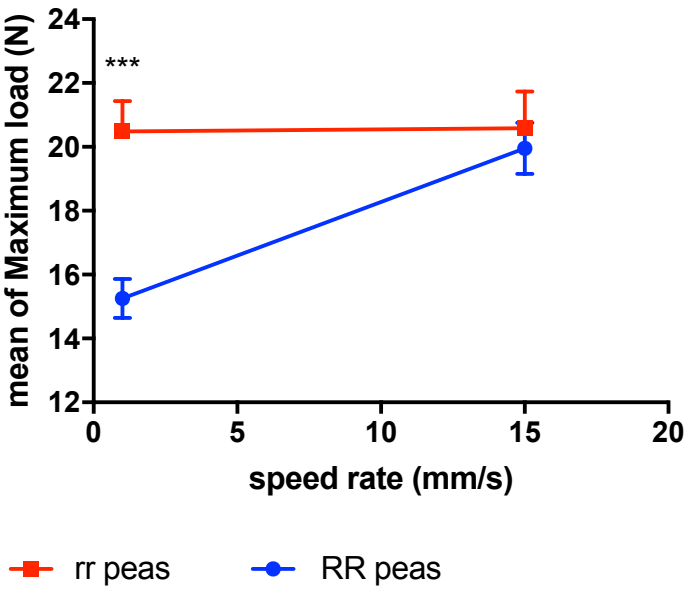

Figure 4.16 Comparison between cooked *RR* and *rr* peas at speed rates 1 and 15 mm/s.

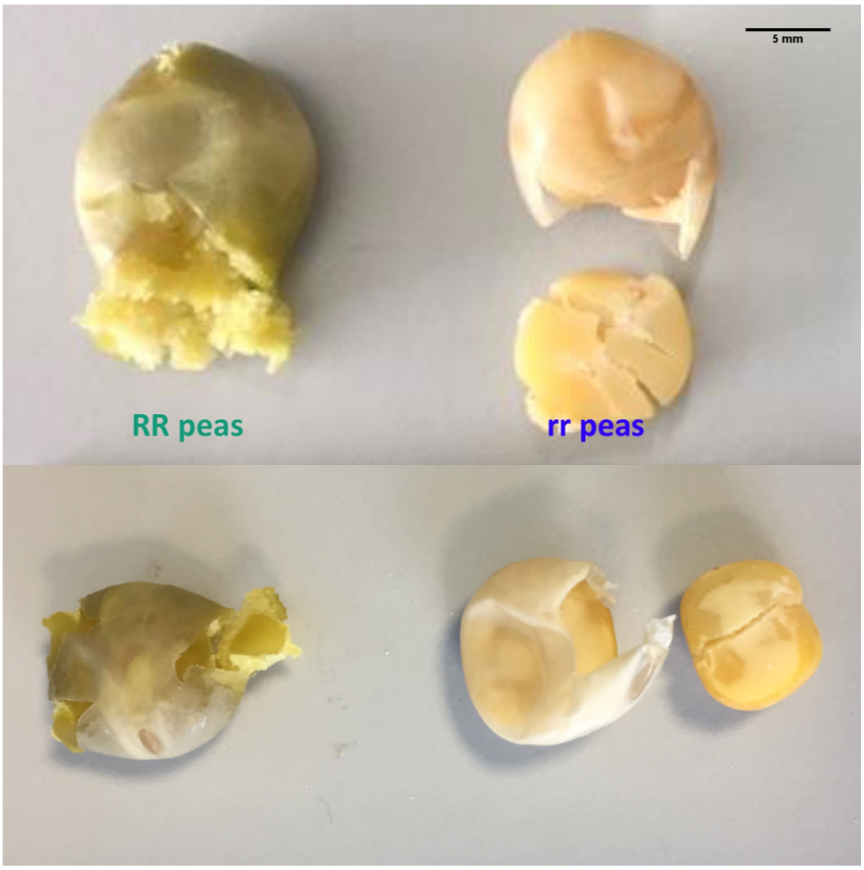

Figure 4.17 Difference in the physical appearance after compression test at speed rates 1 and 15 mm/s between cooked *RR* pea and cooked *rr* peas.

4.4.5.2 Viscosity of digesta

Results from thawed digesta samples indicated that both pea genotypes behaved as non-Newtonian fluids with shear thinning behaviour in the small intestine, as shown in Figure 4.18. Since samples were not centrifuged, solids particles were still present in the samples tested, which explains the shear thinning behaviour. This indicates that the higher shear rate will lower the average size of of particles and thus lower viscosity. Viscosity decreased with increase in shear rate from 0.1 to 0.003 Pa s. No qualitative difference was observed between time points 30 and 60 min in *RR* and *rr* pea digesta rheological properties. As for time points 120 and 180 min, results were not repeatable and hence not reliable.

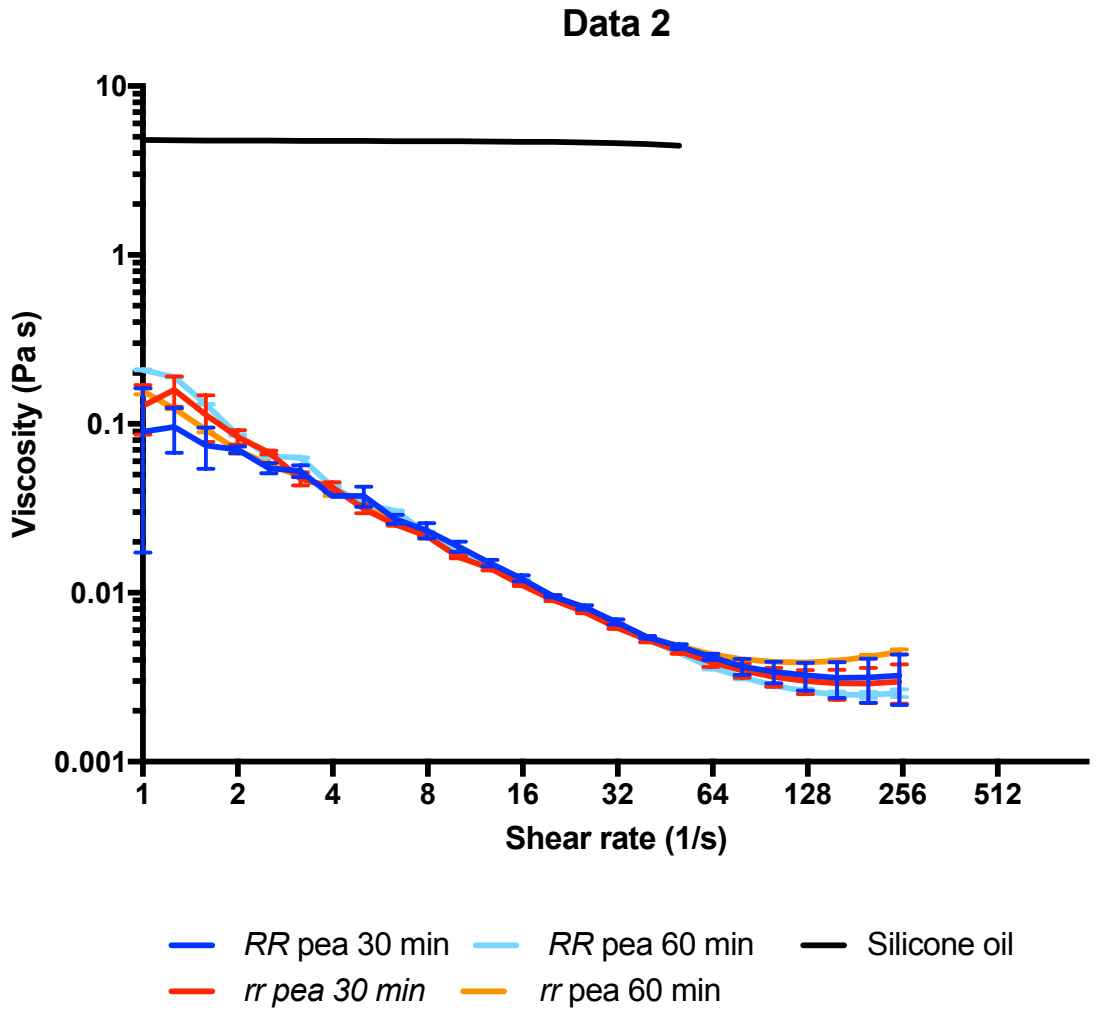

Figure 4.18 Viscosity of small intestinal digesta for whole *RR* peas and *rr* peas at 30 and 60 min compared to silicone oil as a control.

4.4.6 Microscopic images

Please refer to Chapter 2, section 2.5, for information on sample preparation.

Only whole peas were investigated here, as the focus of this experiment was to investigate the behaviour of intact cells during early digestion in the small intestine.

Images were taken from three different samples to evaluate the behaviour of cells in the two peas types. Figure 4.19 shows a representative image of all time points for both types of pea digesta. A difference in the behaviour between *rr* pea and *RR* pea digesta samples can be observed, as seen in clusters of intact cells in *rr* pea samples at time points 120 and 180 min compared to *RR* pea samples.

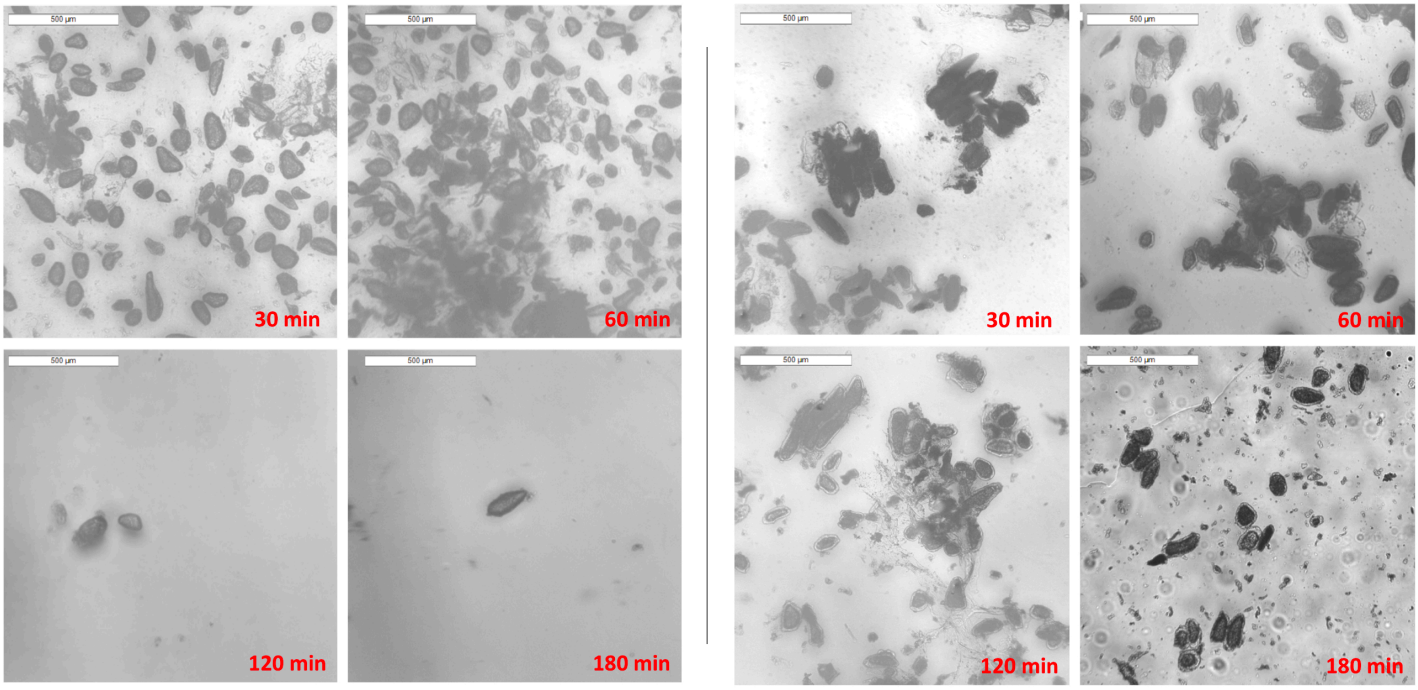

Figure 4.19 Comparison between whole *RR* peas duodenal digesta (left) and whole *rr* peas duodenal digesta (right) at different time points (30, 60,120 and 180 min).

4.5 Discussion

4.5.1 Summary of findings

- Postprandial glucose, insulin and GIP concentrations were significantly lower when intact structure was present in whole peas compared to flour peas for both *rr* and *RR* peas.
- Postprandial glucose, insulin and GIP concentrations were significantly lower after consumption of whole *rr* peas compared to whole *RR* peas.

- Despite the removal of intact structure, postprandial glucose, insulin and GIP concentrations were lower after consumption of flour *rr* peas compared to flour *RR* peas.
- GLP-1 concentration did not differ after whole *RR* and whole *rr* pea consumption.
- Compression test revealed a difference in breakage behaviour, with whole *RR* peas being completely broken into a paste-like structure, while whole *rr* peas were broken down into larger particles.
- Compression test revealed that the mean maximum force for whole *rr* peas is independent of the test speed; this is in contrast to whole *RR* peas, which showed dependency on the test speed rate.
- All aspirated pea digesta samples obtained from the small intestine at time points 30 and 60 min behaved as a non-Newtonian fluid with shear thinning behaviour, despite structural differences.
- Clusters of *rr* peas cells were observed at time points 120 and 180 min in the small intestinal digesta sample using light microscopy.
- Single cells were observed at time points 120 and 180 min in the whole *RR* pea aspirated small intestinal sample using light microscopy.

4.5.2 Detailed discussion

The aim of conducting this pilot study was to understand how the mechanisms of food structure breakdown in early digestion affects postprandial glycaemia, serum insulin and plasma gut hormone responses. The role of microstructure and starch structure on the bioavailability of glucose in the upper gastrointestinal tract was investigated by measuring postprandial blood glucose concentration. Mechanical and rheological measurements were conducted on peas and aspirated digesta to investigate the effect of food structure on peas' mechanical properties and, hence, digestion. Moreover, light microscopy was used to characterise whole pea behaviour throughout the digestion process.

Gastric emptying rate (GE)

Previous study by our group calculated the gastric emptying rate by measuring the ^{13}C octanoic acid following the consumption of *RR* peas and *rr* peas in two forms; whole and flour. Breath samples for peas and pea flour were collected immediately after the consumption of test meal which contain [^{13}C] octanoic acid. There was no statistically

significant difference between the two peas genotypes (P= 0.69, and P= 0.59) as shown in Figure 4.20.

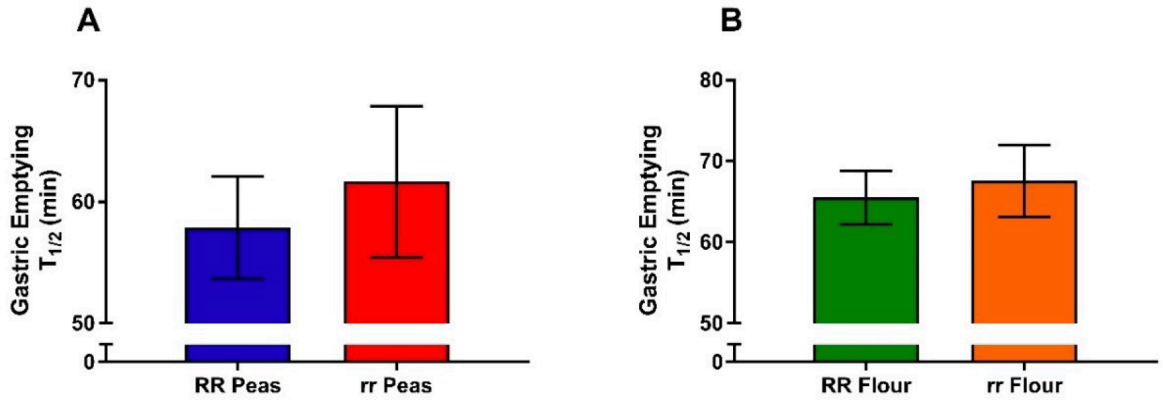

Figure 4.20 Gastric emptying rates following the consumption of whole peas and flour peas of two genotypes of peas (*RR* peas and *rr* peas). Values were compared using Paired t-test. Data represent mean ± SEM. Volunteers (n=10).

Whole peas vs flour peas

Postprandial glycaemia was lower after the consumption of whole peas compared to flour peas. Both whole peas in *rr* and *RR* had a peak value at time point 60 min, with a gradual decline afterwards when compared with flour peas. As for flour peas in *rr* and *RR* peas, an immediate release of glucose was observed, with a peak value at time point 30 min, followed by a sharp drop that was lower than baseline.

Similar behaviour patterns for insulin and GIP were observed after the consumption of flour peas *RR* and *rr* peas. This is expected, as the body secretes incretins to clear excess glucose from the bloodstream and thus a sharp drop occurs. These findings are compatible with those of our previous experimental chapter on hummus meals (whole vs flour chickpea hummus), which highlights the importance of intact cell structure. It can be noted that intact cell structure may slowed the release of starch as measured by blood glucose concentration, with a peak value at time point 60 min compared to flour, with a peak value at time point 30 min.

GLP-1 concentrations did not differ during the time course of 180 min between structures and types of peas, which could be due to similar calories ingested. It was not possible to measure the difference of the GLP-1 second phase secretion as food needs more than 180 min to reach further down the GI tract where L-cells are more dominant (Batterham and Bloom, 2003, Ørskov et al., 1996).

Whole RR peas vs whole rr peas

Plasma glucose, serum insulin and GIP concentrations in the current study were lower after the consumption of whole *rr* peas as opposed to *RR* pea intervention. Experiments on cooked whole *RR* and *rr* peas were conducted for a better understanding of peas' behaviour under mechanical forces. Compression test results showed that the behaviour of *RR* peas was dependent on test speed. Moreover, there was a difference in breakage behaviour, with *RR* peas being completely broken down and *rr* peas being split. Although the cooking process was controlled, the difference in the gelatinisation behaviour during cooking can alter the structure. A recent study on peas genotypes *rr* and *RR* peas showed that flour peas fully gelatinised compared to whole peas which were partially gelatinised. Moreover, there was a difference between whole *rr* peas and whole *RR* peas due to having limited space and water which led to structural rearrangements. Whole *rr* peas had an increase in double helical order that can be the reason of a slower digestion compared to whole *RR* peas (Petropouou et al., 2019). This led to lower post prandial glucose response. It can be assumed that *RR* peas has a weaker structure due to swelling during cooking and thus was more dependent on the GI tract motility rate and mechanical forces during digestion as opposed to *rr* peas. Other reasons can affect the mechanical properties of peas, such as higher levels of insoluble fibre in *rr* peas (Skrabanja et al., 1999) which likely contributes to a stronger structure.

Our microscopy results on aspirated small intestinal samples at time points 120 and 180 min showed that whole *rr* pea intact cells behaved differently than *RR* peas. It showed that whole *rr* peas formed small groups of cells, whereas *RR* peas cells started to separate into single cells. This may partly explain the postprandial glycaemia, as whole *rr* peas had a slower rate of disintegration of particles than whole *RR* peas.

Cell wall thickness can also play a role in the behaviour difference during digestion between both pea types. Results from a study by Petropoulou (Petropoulou, 2019) – comparing the cell walls in *rr* pea seeds and *RR* pea seeds using dry, cooked and aspirated samples – showed that the cell walls of *rr* pea seeds were thicker when cooked and during digestion compared to *RR* pea seeds. Thicker cell walls in *rr* peas can be assumed to be due to differences in cell wall composition in comparison to *RR* peas. This may affect the disintegration of cells, thus forming groups of cells during digestion, preventing enzyme actions and resisting the mechanical forces to breakdown. In contrast, *RR* pea intact cell walls are faster to disintegrate, which

increases the surface area that can bind with digestive enzymes and hence increases blood glucose concentrations.

It was noticed that there was a higher amount of glucose concentration at time point 15 min after the consumption of *rr* peas; this may be partly explained by the higher amount of sucrose compared to *RR* peas. However, glucose concentration was significantly higher at time point 60 min after *RR* pea consumption, with a sharp drop afterwards. Corresponding to postprandial glucose concentrations, insulin concentration was also higher after the consumption of *RR* peas.

GLP-1 and GIP hormones are incretins that stimulate insulin secretion post food ingestion. Although GIP and GLP-1 are stimulated by carbohydrate or fat alone (Holst and Gromada, 2004), no significant difference between *rr* pea and *RR* pea GLP-1 concentration was observed, suggesting that GLP-1 secretion is stimulated by a minimum rate of glucose absorption (Elliott et al., 1993). As for GIP concentrations, it was in line with the insulin concentration pattern having lower concentrations after the consumption of rr peas.

Results on viscosity showed that all aspirated small intestinal samples (*RR* peas and *rr* peas) behaved in a non-Newtonian manner, exhibiting a shear-thinning behaviour showing similar viscosities. This finding is in line with those of previous studies on in vivo animal digesta that showed that all digesta samples exhibited a typical shear thinning behaviour due to the highly changeable nature of rheological properties through digestion (Shelat et al., 2015). However, viscosity results from this study were lower than animal digesta results in the literature at shear rate 10 (1/s), as seen from a comparison of our results (0.017) with those for possum (2) (Lentle and Janssen, 2010) and pigs (0.1) (Shelat et al., 2015). These differences in viscosity rate largely depend on food composition and structure as well as body physiology (Takahashi, 2011). In this work, viscosities were measured for the small intestine aspirated digesta after the removal of large particles as the sample for each time point was non-homogenous and was less than 1 ml, hence was difficult to measure the whole digesta.

Similar viscosities for *RR* and *rr* peas in the aspirated small intestine digesta after the removal of large particles in this study suggest that the GI tract tends to maintain a rheological homeostasis, i.e. flow properties of digesta, by adjusting gastric motility and transit time, and increasing or decreasing gastric fluids (Lentle and Janssen, 2008b). However, results from the clinical trial showed a significant difference in

postprandial glycaemia, confirming a difference in the flow behaviour between both pea genotypes. This can be supported by the difference in the behaviour observed by light microscopy at time point 180 min, with clusters of cells in *rr* peas compared to *RR* peas. One explanation for this behaviour is the difference in the structure between both genotypes. Previous studies in literature showed that the surface of pea starch granules of *RR* peas appear smooth with no evidence of any compound granules. As for *rr* peas showed to consist of single and compound granules (Ratnayake et al., 2002)

As digesta rheology is influenced by a combination of factors such as different sizes of particles, water release and absorption, additional research is needed to further investigate parameters other than viscosity such as particle size and particle breakdown during digestion to fully understand the effect of different structure on flow and absorption of glucose.

Flour RR peas vs flour rr peas

To further investigate the effect of food structure on human digestion, the effect of pea flour (*RR* vs *rr)* on postprandial metabolic responses was investigated. Flour is prepared by milling whole dry peas to disrupt the cell wall, where starch is highly exposed to digestive enzymes; therefore, all results in this section will focus on molecular structure differences, including ratio of amylose to amylopectin content and the amount of resistant starches created by the cooking process. The *rr* peas contain higher amylose levels than amylopectin content (70% and 30%, respectively) compared to *RR* peas (30% and 70%, respectively) due to the natural mutation (Bhattacharyya et al., 1993). Postprandial glucose concentrations were lower after the consumption of flour *rr* peas compared to flour *RR* peas. This finding correlates with that of an in vitro study showing that *rr* peas had slower digestion compared to *RR* peas (Edwards et al., 2018).

One explanation is that higher amylose content made flour rr peas more resistant to digestion by forming a more rigid network that changed the rheological properties and thus led to slow starch digestibility. In contrast, *RR* peas contain more amylopectin, having a branching structure that makes them prone to amylolytic enzyme penetration due to its larger surface area compared to amylose linear structure.

Previous findings on plant-based foods with different amylose and amylopectin contents showed that amylose is more resistant to digestion compared to amylopectin (Miller et al., 1992). Also, starch physicochemical properties such as gelatinization,

temperature and volume expansion post-cooking may affect starch structure (Ramadoss et al., 2019, Panlasigui et al., 1991). We can assume that *rr* peas gelatinized differently, which resulted in new rheological properties that made rr peas more resistant to digestion (Ridout et al., 2006).

GIP concentration was in line with glucose concentrations, with a significantly lower concentration after the consumption of flour *rr* peas. Insulin concentrations also showed a borderline significant difference. Moreover, there was a difference at time point 15 min in GLP-1 with *RR* flour peas higher than flour *rr* peas. These results can be explained by the rapid increase in glucose concentrations at time point 15 min in the early phase of digestion, as no intact cells were available. Despite the quick absorption of glucose, postprandial gut hormone results are in a similar pattern to that of the whole peas.

Strengths, weaknesses and future recommendations

The major strength of this study was that we explored the mechanical properties of different types of cooked peas in relation to human digestion along with postprandial metabolic responses. We conducted a randomized crossover trial using a developed procedure to collect aspirated digesta samples without the need for X-rays.

The limitations of the current study should be noted. This was an acute study that cannot present a long-term effect on consuming peas. Moreover, we did not calculate the starch content for all interventions, as our aim is to compare 50 g dry weight in different structures in order to study its effect on digestion. Therefore, future tests are needed for the measurement of starch content, level of hydration and micronutrient content. Also, mechanical properties were not measured for flour peas due to limitations of the instrument used.

Aspirated digesta Is not representable of what is in the small intestine due to many reasons. First, particles size in the aspirated digesta was restricted to the diameter of the nasogastric tube that was used and hence no particles larger than the size of the diameter of the tube was obtained. Second, experiments were conducted on aspirated digesta after the removal of large particles within the sample as it was not possible to get any repeatable results and hence, measurement of viscosity alone was not enough to examine the behaviour of food during digestion.

Further investigation at the micro and molecular levels are needed to test the difference in the mechanical properties of cell walls and starch granules.

Future microscopic studies are needed on aspirated digesta to compare and quantify intact cells and to further investigate their behaviour during digestion.

4.6 Conclusion

Food structure and its mechanical properties play a major role in postprandial metabolic responses by modulating digestion and absorption. Our study showed that peas' mechanical properties are an important factor in altering digestion in the human small intestine; this points to the vulnerability of the *RR* peas during digestion and hence the higher level of postprandial glycaemia compared to *rr* peas. Our study also showed a difference in postprandial glycaemia when cell walls are disrupted between flour *RR* and flour *rr* peas, which confirms the importance of molecular structure in digestion. Higher amylose content and granular differences resisted digestion and lowered glycaemic levels. Understanding the mechanisms involved in lowering the postprandial glucose and insulin responses in relation to food structure and rheological and mechanical properties can help in designing foods that control the release of glucose and hence maintain the body's glucose homeostasis.

CHAPTER 5: General discussion

5.1 Summary of main findings

- A correlation was observed between mechanical properties observed in compression test results on cooked pea seeds between the two genotypes and the disintegration kinetics during digestion, as observed by light microscopy between whole *rr* peas and whole *RR* peas in the aspirated small intestine digesta.
- Significantly lower plasma glucose, serum insulin and GIP concentrations were observed when intact cell walls are present.
- Starch structural features have an impact on postprandial glycaemia if digestive enzyme access is not limited by intact cell walls, as observed between flour *rr* peas and flour *RR* peas.
- Rheological property differences exist between WH and FH; WH forms a more rigid network between its molecules compared to FH, as evidenced by the shear strain sweeps.
- Mechanical property differences were observed in the compression experiments: *RR* peas showed a dependency on speed of deformation, which resulted in a completely broken-down structure, while *rr* peas were independent from speed of deformation, resulting in the structure split.
- Aspirated pea small intestinal digesta samples of *RR* and *rr* peas with different structures behaved as a non-Newtonian fluid with shear thinning behaviour.

5.2 Introduction

Glucose homeostasis is important to human health. As glucose is a major molecule for energy supply, its movement from food into tissue is necessary for survival. Also, high levels of glucose are toxic to the body. Glucose homeostasis is complex, involving multiple organs as well as hormonal/neurohormonal and autoregulatory factors (Frayn, 2010). When glucose homeostasis is disturbed by a sedentary lifestyle or processed diet, many complications may occur that lead to many conditions and diseases, including obesity (Petersen and Shulman, 2002), insulin resistance, type 2 diabetes (Weir and Bonner-Weir, 2004), hypertension, dyslipidaemia (Zivkovic et al., 2007) and cardiovascular disease (Bonfante et al., 2015).

Empirical evidence showed that postprandial glucose and insulin sensitivity are not only governed by the content of food, but also by other factors that may play a role in the digestive process, including food structure and food breakdown (Lundin et al., 2008). Food structure can be defined as the cell structure and the spatial arrangement within the cell including cell wall. The combination of different levels of structure (from the molecular to the micro and macro levels) will develop the rheological behaviour and textural properties of food. This may have an impact on the availability and digestibility of nutrients (Aguilera, 2005, Suman Mishra, 2012).

Food rheology is the study of the deformation and flow of food under applied forces (Day and Golding, 2016). Rheological data is usually used in the food industry to determine ingredient functionality in product development, shelf life testing, evaluation of food texture or the processing of engineering calculations. In this work, I extended food rheology to relate to human digestion by bringing together nutritional physiology in order to explore the impact of peas and chickpeas on postprandial glycaemia and rheology, as I sought to understand how the physical properties change the physiological response. This effort will provide insight into the interplay between food structure, rheological properties and food breakdown during digestion, and their effect on postprandial glycaemia.

5.3 Detailed discussion of overall findings

Discussion on the work carried out in the individual chapters is presented at the end of each chapter. Here, I will bring together an overarching view of the work carried out during my PhD studies.

Postprandial blood glucose is an important determinant of metabolic risk – e.g. type 2 diabetes and coronary heart diseases (Röder et al., 2016). A major determinant of postprandial blood glucose is the balance between dietary carbohydrates and the physiological mechanism that controls blood glucose movement into the cells of the body. Food breakdown is an important part of the complex digestion process. Food is broken down into smaller particles by physical and chemical forces to release glucose to be ready for absorption (Sensoy, 2014). Moreover, gelatinisation behaviour during cooking and digestion play a role in the release of nutrient from food structure. Therefore, a combined understanding of food rheology and nutritional physiology will give a better understanding of how food is broken down during digestion. Different patterns of food breakdown results in different effect postprandial glycaemia and insulinemia (Bornhorst and Paul Singh, 2014b). It is assumed that intact food structure may change the food's rheological properties and thereby influence flow behaviour, which will slow the rate of food breakdown during digestion and hence lower postprandial glycaemic and insulinemic responses. Food rheology is important on different scales of structuring such as the macro and microstructure including the presence or absence of cell wall. Moreover, rheological properties are continuously changing during digestion depending on the physical forces including chewing and contractions.

Food is a complex system with various levels of structural heterogeneity. It can be simple liquids or solids but mostly soft condensed matter that includes a range of hierarchical nanostructures and microstructures (Fischer and Windhab, 2011). The difference in structure affects the mechanical kinetics such as in forming biopolymer chains that cross-link into networks. This will cause differences in their resistance to deformation and hence the rheological properties. A distinction can be made between strong and weak gels by applying large deformations on food materials. For example, strong gels rupture under large oscillation, whereas weak gels flow without fracture (Basu et al., 2017). In study 1, I used two isocaloric meals of hummus which differed in the microstructure; ruptured and intact cells. WH with its intact structure exhibited a strong gel behaviour: results from a small oscillatory test showed that WH formed a

rigid strong network between its molecules, evidenced by the higher storage modulus, but ruptured under large deformations. FH exhibited a weaker gel behaviour when compared to WH, as evidenced by CSSR and small deformation experiments.

Rheological properties of WH and FH are in good agreement with the in vitro digestibility test conducted on chickpea hummus that showed a significant difference in starch hydrolysis between both meals, with WH lower than FH. These observations can result in a slower breakdown rate of WH and hence a lower glycaemic response compared to FH, as shown in the clinical trial. Lower glycaemic response contributed to lower postprandial insulin and GIP responses but not GLP-1.

Further investigation was done on different food structures to explore their rheological and mechanical properties during initial digestion and their effect on postprandial glycaemia. In study 2, *RR* peas and rr peas were used as models. It has been shown in the literature that there are clear structural differences between *RR* and *rr* peas (Ratnayake et al., 2002). These are the amylose:amylopectin ratio, the starch granule characteristics, and the resistant starch content between the two pea genotypes (Bhattacharyya et al., 1993). Specifically, *rr* peas have higher amounts of amylose than amylopectin. Highly branched amylopectin results in higher digestibility rates due to higher penetration of digestive enzymes as opposed to linear amylose. Also, a recent microscopic analysis showed that *rr* peas have a thicker cell wall compared to *RR* peas (Petropoulou, 2019).

The food's flow behaviour during digestion can be used as an indicator of food breakdown rate. Many factors affect food flow behaviour such as cell-cell adhesion, mechanical properties, viscosity, and particle size. In study 2, viscosity and mechanical properties were investigated. Higher viscosity meals tend to resist forces during digestion and hence result in a lower breakdown rate. Here, viscosity was measured for the aspirated whole *rr* pea and whole *RR* pea samples after the removal of large particles within the sample at time points 30 and 60 min. Results showed that both genotypes exhibited a shear thinning behaviour, which suggests an increased rate of food breakdown with time. However, no differences in viscosity between both peas genotypes was observed.

Mechanical properties of both cooked peas were investigated to explore the behaviour of peas under the application of an external mechanical force. Results from the compression tests showed that the mechanical response of *rr* peas was independent from speed of deformation and resulted in a split fracture. In contrast, the mechanical

response of *RR* peas was dependent on speed of deformation and resulted in a complete breakdown of the pea. Interestingly, in this work, microscopic images of both aspirated whole pea samples showed that there was a difference in the behaviour between *rr* peas and *RR* peas during small intestine digestion at time point 180 min, with *rr* peas forming groups of cells, whereas *RR* peas separated.

Several assumptions can be made here. One is that the larger cell wall thickness in *rr* peas led to changes in the adhesive forces between cells that changed the rheological behaviour of cells, leading to clusters of cells instead of single cells. This prevented digestive enzymes to access inner starch of the large cluster of cells. Another assumption is the higher amylose content in *rr* peas as opposed to *RR* peas. It was shown previously that higher amylose content can increase viscosity by forming entanglements between molecules to produce a more rigid network than amylopectin (Annison and Topping, 1994, Ma et al., 2017). Results from the clinical trial showed significantly lower postprandial glycaemia after *rr* flour peas compared to *RR* flour peas, which confirmed the impact of amylose content on glucose absorption.

In this study, it can be assumed that as food continues to break down during digestion, *rr* peas resisted forces which changed the rheological behaviour during digestion. This is due to the presence of mutation that resulted in a different cell wall composition and thicker cell wall; thus, a new entanglement was formed, creating a more resistant network that affected the peas mechanical properties. As a result, *rr* pea cells formed clusters of cells, as observed in the small intestine digesta sample at time point 180 min. In contrast, *RR* peas broke down due to their weak mechanical properties compared to *rr* peas, resulting in a higher significant glucose concentration as observed from the clinical trial compared to *rr* peas.

In summary, this thesis aimed to improve our understanding of how food breakdown rate is affected by food structure and its rheological properties, and hence the impact on blood glucose concentrations. Food structure, including intact cell wall and starch structure, play a crucial role in limiting access of digestive enzymes to the cell. They also affect the rheological and mechanical properties that contribute to the rate of food breakdown during digestion and hence postprandial glycaemia and insulinemia.

5.4 Future recommendations

Future work is needed in order to understand the mechanism by which food structure breakdown effect the glucose absorption and related metabolic responses. It was hypothesized that food structure and its rheological properties have an effect on the post prandial metabolic responses. This section highlights areas for further research built on the conclusions developed from this thesis.

Extend understanding of food structure effect on the rheological properties in relation to food breakdown during digestion

Examining the rheological properties of hummus meals in study 1 was beneficial as we were able to indirectly relate post prandial glycemia concentrations with hummus rheological properties. In study 1, a difference in the rheological properties were observed between WH and FH before ingestion showing WH to have a more rigid network compared to FH suggesting a difference in the flow behaviour between both meals during digestion that resulted in a difference in the peak of post prandial glucose concentrations. One of the major challenges was that we do not know whether the difference in the rheological properties observed can be directly translatable to humans' samples. Future work is needed to examine the rheological properties of both meals starting from the bolus and throughout digestion in stomach, small intestine, and colon. This may give a better understanding on how GI tract motility and fluids affect food structure, thus leading to changes in the food break down and rheological properties of foods. Although preliminary attempts to test flow behaviour of digesta peas using viscosity as a parameter, were carried out as part of study 2, further investigations are needed. It was shown that there was a trend of a difference in viscosity at time point 60 min between *rr* and RR peas small intestinal digesta suggesting that there may be a difference in viscosity at 120 min and 180 min which may cause a difference in the post prandial glucose concentrations. However, experiments on these samples were challenging due to the small quantity in each sample for each time point. This resulted in data that were not repeatable for time point 120 min and 180 min and hence not reliable. The collection of intestinal samples in large quantities is a real possibility for future studies, despite proving challenging.

Mechanical properties of cooked peas were also measured in an attempt to explain the difference in post prandial glucose concentrations. Future research is needed to develop a technique to test rheological properties and mechanical properties on a

smaller scale such as digesta particulates and cell wall properties. Although a small number of studies investigated the digesta rheological properties, using in vitro samples and samples obtained from animals, understanding in this area is still extremely limited. The findings regarding flow behaviour and the rheological testing on digesta in study 2 requires significant development. One of the limitations of study 2 is the inability to test the mechanical properties of the small particles within digesta due to the Instron instrument limitation to detect forces less than 0.03N. Therefore, future research can be applied using micro and nano-indentation methods where a thin probe indents the cell surface while applied load and probe displacements are monitored. This will allow the evaluation of the elastic modulus of cell walls which can give an indication of cell wall rigidity and mechanical properties before and after indentation. Changes in cell wall rheological properties may play a role in the food's breakdown during digestion by resisting forces and hence limiting glucose absorption. The microscopic findings from study 2 showed a difference between the behaviour of *RR* and *rr* peas digesta at time point 180 min where rr peas digesta formed a cluster of cells compared to RR pea digesta suggesting that this behaviour may play a role in increasing the viscosity for rr peas compared to RR peas, and hence a difference in the post prandial glucose concentrations. However, the exact underlying mechanism for this behaviour is unclear. It would be ideal to examine the cell wall composition in vitro in relation to the mechanical properties for both types of peas to further understand pectin role in keeping cells glued together and resisting small intestine physical forces. Moreover, it would be also interesting to further investigate the microscopic images by extracting quantitative information from these images to correlate mechanical properties with the cell microstructure. Moreover, particle size measurements are needed to explore the effect of physical forces during digestion on food structure. This will allow for a better understanding of the rheological changes that occur on intact cells as digestion progresses.

Application of findings other types of food products to further understanding and development

As results from this thesis have shown the importance of food structure and its rheological properties in lowering postprandial glyceamia and insuluinemia, such tests should be applied to other types of legumes to test their rheological behaviour of samples before, during, and after digestion. Understanding how rheological properties effect food breakdown may help in controlling the flow of food and hence control the

release of glucose. this may help in designing foods with specific rheological properties with specific disintegration behaviour under GI tract chemical and physical forces. Moreover, investigating the different enzyme diffusion through the cell wall of different legumes is needed to fully understand the whole picture on the breakdown. Furthermore, more complex food structures, such as mixed meals, would need significant further development and validation to ensure that the results are still reflected in the complex meals foods.

5.5 Conclusion

In summary, the data presented in this thesis demonstrated the interplay between food structure and its rheological properties, early digestion and the effect on postprandial glucose release. The importance of food's rheological properties in relation to food breakdown was established. Different rates of breakdown during digestion resulted in different levels of postprandial glycaemia, providing insight into the structure–function relationship of food.

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Appendices

Appendix 1: Hummus Recipe for WH

Meal 1: WHOLE CHICKPEAS checklist

	Prepare Ingredients:	
		235 grams of chickpeas (drained by hand)
		40 grams of water
		30 grams of olive oil
		20 grams of tahini (mix really well to remove all limbs)
		20 grams of lemon juice
		6 grams of spice mix
		3 grams of salt
		5 grams of coriander (washed, dried, and cut in small pieces)
	Add **235.0 grams drained chickpea** in the **food processor**	
	Start **blending** the chickpea	
	Add 40 **grams water** halfway through then continue blending	
	Pause food processor and **add the rest of ingredients**	
	Start blending again until you get thick almost creamy texture	
	Add hummus (250.0 grams) + cucumber sticks+ half of bell pepper	

Total Prepared weight: 359.0 grams of hummus
Serving size: 250 grams of hummus

Appendix 1: Hummus Recipe for FH

Meal 2: CHICKPEA FLOUR Checklist

	Pre-heat hub on setting 3-4	
	Prepare Ingredients:	
		117 grams of chickpea flour
		440 grams of water
		30.8 grams of olive oil
		20.5 grams of tahini (mix really well to remove all limbs)
		20.5 grams of lemon juice
		6.2 grams of spice mix
		3.1 grams of salt
		5.1 grams of coriander (washed, dried, and cut in small pieces)
	Add 117.0 **grams chickpea flour** in a non-stick cooking pan	
	Add 440.0 **grams water** and mix it with a small whisk (**around 5 minutes**)	
	Place the pan on the hob and continue mixing **for 2.5 minutes after feeling the steam** (you should get a **thick, no lumps mixture**)	
	Place the mix into the food processor **and add all ingredients as fast as you can** and mix until you get the hummus texture	
	Add hummus (438.0 grams) + cucumber sticks+ Bell pepper	

Total prepared weight: 646.2 grams of hummus
Serving size: 438.0 grams of hummus

Appendix 2: Volunteer information sheet study 1, version 4 (16/03/2016)

Information Sheet for Research Participants

The Acute Effects of Food Structure on Appetite Regulation

You will be given a copy of this Information Sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

You are being invited to take part in a research study investigating Effects of Food Structure on Appetite Regulation. Before you decide if you would like to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation.

Thank you for reading this

Part1

What is the purpose of this study?

The continuing rise of the obesity epidemic has a great effect on individuals' health and continues to present a challenge globally on a scientific, medical, and governmental level. There are different approaches to treat obesity such as diet, drugs and surgical interventions, several studies have also focused on identifying the determining factors leading to obesity which could lead to effective preventative strategies.

The aim of this study is to test the effect of three structurally-different meals, with the same nutritional content and volume, on appetite regulation. This may be important in terms of controlling body weight and therefore preventing obesity

Who is suitable to participate?

- Male and female healthy adults (aged 18 to 65 years)
- Normal to overweight individuals (body mass index (BMI) 20-29.9 kg/m2)
  - BMI is equal to body weight (kg) divided by height squared (m2)

Who is NOT suitable to participate:

- Has thyroid defects
- Is under hormone or steroids therapy
- Is pregnant or lactating (female)
- Had given birth within the past year (female)
- Is taking drugs that could affect appetite or plasma glucose levels.
- Is taking natural remedies that modulate appetite or plasma glucose levels.
- Has excessive alcohol intake
- Had blood donation within 12wks prior to start date
- is a smoker
- Has history of any disease with unknown outcome
- Has Diabetes
- Has nut allergy

Do I have to take part?

It is entirely up to you whether or not to take part. If you do we will ask you to sign a consent form. You are free to withdraw at any time and you do not have to give a reason. A decision either not to take part or to withdraw from the study will not affect the standard of care you receive.

What will happen to me if I take part?

If you do decide to participate, you will first be asked to attend the Clinical Investigations Unit at Hammersmith Hospital where you will be interviewed and examined by one of the research doctors. You will have a blood test (to check your liver, kidneys, cholesterol and ensure that you are not anaemic or diabetic) and height, weight will also be taken. You will also have an electrocardiogram (ECG). This is a non-invasive test to look at the health of your heart. Your blood pressure will also be recorded. All women of child bearing age will be asked about past and current pregnancy status as well as breast feeding. No Paracetamol or any other medications will be given.

If you do decide to participate, you will be enrolled in the study for 4 weeks:

Visits 1, 2, 3 and 4: Study Days:

Initially we will provide you with a 24hr food diary (which will be mailed to your address) to record your dietary routine for the week prior to each study visit. On the day before the study we will ask you to refrain from strenuous exercise, caffeine and alcohol on the day prior to each study day. You will then be requested to fast overnight (you are allowed to drink water).
On the study day, you will be asked to arrive at the Clinical Investigations Unit at Hammersmith Hospital at approximately 9:30am. For each of the 4 visits, we will record your height, weight, and body fat. A small plastic tube (cannula) will be inserted into your arm. This will be in place for the duration of the study day and will be used to take blood samples without causing you any further discomfort.
At approximately 10:00 am you will consume a breakfast introduced in a one of the four different structures; standard, solid, semi solid, or liquidized, in randomly selected order. You also be given a total of 1.5g of soluble Paracetamol to measure your gastric emptying rate (i.e. how quick you digest your food). During the study you will have blood samples taken at certain time points (9 time-points). A total of 8 ml of blood will be taken (about ½ tablespoon of blood). These will be taken through the plastic cannula. You will be asked to fill in questionnaires (visual analogue scales) to measure your feelings of hunger, fullness, and food acceptability during the study.
After 3 hours the cannula will be removed and we will then serve you a meal and you should eat this until you are comfortably full. A final questionnaire should be filled once you have finished eating and you will be able to go home.

We request that you do not start any other new diets or intensive exercise regimes in-between the four study days as this may give us conflicting results. Also as you will be taking Paracetamol on the study day, you will be instructed to avoid taking St John's wort (herbal remedy), and additional dose of Paracetamol on the same day, unless directed by your GP/physician.

What is the dietary intervention being investigated?

All subjects will receive four different meals that contain the same components served in different forms. These will be commercially available food items.

Will I get paid for participating?

All participants will be offered £100 plus transportation to and from the research unit; £25 for each study visit plus transportation to and from the research unit.

What are the possible disadvantages and risks of taking part?

National Institute for Health Research/Wellcome Trust Imperial CRF provides a safe environment for all studies that take place in the unit. The safety of all participating subjects is ensured by a well-trained staff and qualified researchers. This study will only include healthy volunteers, so the risk is limited to bruising from cannula insertion, and the possibility of blood loss, which will be taken into consideration in the screening phase.

What are the possible benefits of taking part?

You may not benefit directly from this study but the results may help scientists and doctors in the future treat patients with obesity.

What happens when the research study stops?

Once the study has finished, the results of the study can be made available to you and/or your GP if you wish. Your samples will be stored for one year after the study completion.
If you have any problems immediately following the study, then you should contact one of the research doctors on the numbers provided.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. We will also ask for your permission to inform your GP of your participation in this study.

This completes part 1.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part2

What if new information becomes available?

Sometimes during the course of a research study, new information becomes available about the treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time and you do not need to give a reason. Information collected may still be used. Any stored blood that can still be identified as yours will be destroyed if you wish.

What if something goes wrong?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation. If you are harmed due to someone's negligence, then you may have grounds for a legal action.

What can I do if I have any complaints or concerns?

If you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Principal Investigator, Professor Gary Frost, through his secretary on 020 8383 3242 or by email at g.frost@imperial.ac.uk

What will happen to the results of the research study?

The results are likely to be published six months following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results of the study can be made available to you and/or your GP should you wish.

Who is organising and funding the research?

This study is organised by the research team at the National Institute for Health Research/Wellcome Trust Imperial clinical research facility, and is being funded by the Saudi Cultural Bureau in London as a part of educational grant.

Who has reviewed the study?

This study has been reviewed by Stanmore Research Ethics Committee.

Contact for Further Information

The researchers and doctors involved in the study, Professor Gary Frost and Dr Edward Chambers will be available by telephone.

During working hours through Professor Gary Frost's secretary	020 8383 3242
At all other times through Hammersmith Hospital switchboard	020 8383 1000

Appendix 2: Volunteer information sheet study 2, version 2 (25/02/2015)

Information Sheet for Research Participants

Dietary resistant starch from peas for healthy glucose homeostasis

STUDY 2 - Evaluating the Impact of Resistant Starch from Peas on Small Intestinal Digestion

You will be given a copy of this Information Sheet and a signed copy of your consent form to keep, should you decide to participate in the study.

You are being invited to take part in a research study investigating the effect of dietary fibre on the diet. Before you decide if you would like to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year. You are free to withdraw at any time without explanation.

Thank you for reading this

What is the purpose of this study?

Diabetes mellitus type 2 is a disease that is caused by insulin resistance, whereby the body fails to respond or produce insulin, a hormone that regulates carbohydrate metabolism. Without regulated carbohydrate metabolism glucose remains within the bloodstream; having disadvantageous effects. Insulin is secreted by β-cells that are found within a specific region of the pancreas, known as the Islet of Langerhans. These β-cells can deteriorate and fail to release insulin due to lifestyle factors such as age and obesity, as well as genetic factors.

Resistant starches are found within certain food products, particularly fruits, vegetables and whole grains, and are believed to be beneficial to β-cells. This is because the resistant starch is not digested within the gut and is instead used by bacteria within the gut. The bacteria ferment the resistant starch to produce short chain fatty acids (SCFAs), which are believed to improve β-cell function.

We are investigating the effects of different food products containing resistant starches found naturally in peas. The aim of this study is to see how resistant starches influence the digestion of food in the stomach and gastrointestinal (GI) tract.

This study was reviewed and received favourable opinion by South East-Coast Surrey Research Ethics Committee.

Who is suitable to participate?

- Male and female healthy volunteers (aged 18 to 65 years)
- Normal to overweight individuals (body mass index (BMI) 20-35 kg/m^2)
    - BMI is equal to body weight (kg) divided by height squared (m^2)

You are NOT suitable to participate if you have:

- Gained or lost ≥ 3kg weight in the past two months

- Current smokers
- History of substance abuse
- Excessive alcohol intake
- Pregnancy
- Diabetes
- Cardiovascular disease
- Cancer
- Any gastrointestinal disease e.g. irritable bowel syndrome or inflammatory bowel disease
- Kidney disease
- Liver disease
- Pancreatitis
- Use of medications likely to interfere with energy metabolism, appetite regulation and hormonal balance, including: anti-inflammatory drugs or steroids, antibiotics, androgens, phenytoin, erythromycin or thyroid hormones.

Do I have to take part?

It is entirely up to you whether or not to take part. If you do we will ask you to sign a consent form. You are free to withdraw at any time and you do not have to give a reason. A decision either not to take part or to withdraw from the study will not affect the standard of care you receive.

Will I get paid for participating?

You will be reimbursed £400 for completing the study.

What will I have to do?

Visit 1 – Health Screening:

If you do decide to participate, you will initially be invited to attend a health screening to confirm your eligibility for the study. You will be asked to attend the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital where you will be interviewed and examined by one of the researchers. You will have a blood test (to check your liver, kidneys, cholesterol and ensure that you are not anaemic or diabetic) and height, weight and blood pressure measurements will also be taken. You will also have an electrocardiogram (ECG). This is a non-invasive test to look at the health of your heart. All women of child bearing age will have a pregnancy test.

The health screening should last about 1 hour.

Visit 2 – Study Visit

On the day before the study visit we will ask you to refrain from strenuous exercise, caffeine and alcohol. You will then be requested to fast overnight from 10 pm (you are allowed to drink water).

On the study day, you will be asked to arrive at the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital at approximately 8:00am. You will stay at the Clinical Research Facility for a 4 day visit (three nights).

A small plastic tube (cannula) will be inserted into your arm. This will be in place for the duration of the study visit and will be used to take blood samples without causing you any further discomfort.

Two thin, bendy plastic tubes will be placed through your nose and into your stomach and small intestine. The tubes will be placed by a Gastroenterologist. They will put some lubricant on the end of each tube to make it slippery and place them through your nose and down into your stomach and small intestine. You will be sat upright as the tubes are placed and asked to drink water through a straw as this will help the tubes to go down. It will feel a bit uncomfortable whilst the tubes are being put in, however, any discomfort should subside once the tubes have been positioned. These tubes will allow us to take samples from your stomach and small intestines after you have eaten to see how different foods are digested. These tubes will remain in place for the duration of the 4 day visit.

On each of the 4 study mornings you will consume a standard breakfast that will contain different resistant starches each morning. Blood samples will be taken at certain time points for 3 hours after you eat the breakfast. A total of 80ml of blood will be taken each morning (about 16 teaspoons of blood). These will be taken through the plastic cannula. Following each test breakfast, samples from your stomach and small intestines will be taken every 15 minutes for 3 hours.

Following the 3 hour measurement period, you will be able to read or watch DVDs at the Clinical Research Facility. We will provide all of the food you require for the duration of the 4 day study visit. You will be requested to fast every evening of the visit from 10 pm (you are allowed to drink water).

Following the final samples on the fourth study day, the tubes in your stomach and small intestines and the plastic cannula will be removed and you will be able to go home.

What are the possible disadvantages and risks of taking part?

In the event that we discover something about your health that you were unaware of, for example if your kidney tests are abnormal or if you have diabetes, we would immediately inform you of this and with your consent inform your GP so that you can be referred to an appropriate specialist. If you require more urgent assessment we would arrange this for you immediately within the hospital.

Some of the procedures in this study, such as the recording of your weight, height and blood pressure present no risk to you. Other procedures, such as taking blood samples, can cause mild discomfort. The risks of taking a blood sample include: slight discomfort when the needle is inserted and possible bruising and a localised infection. These procedures will only be carried out by experienced doctors under aseptic conditions to minimise all these risks.

The potential risks of the stomach and small intestines tubes include blockage of a tube, a misplaced tube or infection. This procedure will only be undertaken by a Gastroenterologist and care will be taken to minimise any discomfort from the procedure. The tubes will be placed using a system that provides ‘real-time’ information about the position of the tube during placement to minimise discomfort and all of these risks.

There are no major side effects associated with eating foods containing resistant starch; however, some people may experience mild abdominal bloating.

What happens when the research study stops?

Once the study has finished, the results of the study can be made available to you and/or your GP if you wish. If you have any problems immediately following the study, then you should contact one of the research doctors on the numbers provided.

What if new information becomes available?

Sometimes during the course of a research study, new information becomes available about the treatment that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time and you do not need to give a reason. Any stored blood or urine samples that can still be identified as yours will be destroyed.

What if something goes wrong?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator (Professor Gary Frost; g.frost@imperial.ac.uk; 020 8383 3242). The normal National Health Service complaint complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

What can I do if I have any complaints or concerns?

If you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Principal Investigator, Professor Gary Frost, through his secretary on 020 8383 3242 or by email at g.frost@imperial.ac.uk

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it.

All electronic data about you will be stored on Imperial College departmental database. This is a confidential computer system which requires a specific password for access and can only be viewed by authorised persons. It is a requirement that your GP is informed of your participation in this study.

What will happen to the results of the research study?

The results are likely to be published six months following the study. Your confidentiality will be ensured at all times and you will not be identified in any publication. At the end of the study, the results of the study can be made available to you and/or your GP.

Contact for Further Information

The researchers and doctors involved in the study, Professor Gary Frost and Dr Ed Chambers, will be available by telephone

During working hours through Professor Gary Frost's secretary	020 8383 3242
At all other times through Hammersmith Hospital switchboard	020 8383 1000

Appendix 3: Pre-screening questionnaire

Pre-screening Questionnaire

Age	
Weight	
Height	
BMI	
Have you gained or lost any weight in the last 3 months? If yes, approximately how much weight did you gain/lose and in what length of time? *Eg: I lost 3kg in 2 months.*	
Are you a smoker or ex-smoker?	
Do you take any regular medication?	
Are you allergic to Paracetamol?	
Do you any food allergies or intolerance?	
The main ingredient in the test meals you will be receiving in the study is **chickpea**. Would that be acceptable to you?	
Do you currently suffer from any particular illness?	
Have you taken part in any research studies in the last 3 months?	
Have you donated any blood in the last 3 months?	
Database details:	
Full name	

Date of birth	
NHS number	
Contact address	
Phone number	
Email address	

Appendix 4: Consent Form study 1, version 2 (01/10/2015)

Imperial College London

Department of Investigative Medicine,
HammersmithHospital Campus,
ImperialCollegeLondon
6th Floor, CommonwealthBuilding,
Ducane Road,
W120NN
Tel 020 838 33242
Fax 020 838 33142

The Acute Effect of Food Structure on Appetite Regulation

Please initial the box if you agree with each statement.

1. I have been given the opportunity to ask questions and discuss the study ☐

2. I have received satisfactory answers to all my questions ☐

3. I have received enough information about the study ☐

4. I confirm that I have read and understand the information sheet (version 4, 1.03.2016) *"The Acute Effect of Food Structure on Appetite Regulation"* for the above study. ☐

5. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

6. I understand that sections of any of my research notes may be looked at by responsible individuals from Imperial College and/ or Imperial College NHS Healthcare Trust where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

7. I understand that my GP will be informed about my participation in the study ☐

8. I understand that my blood samples may be stored and used in the future for ethically approved studies ☐

9. I agree to take part in this study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 4: Consent Form study 2, version 1 (15/01/2015)

Imperial College London

Department of Investigative Medicine,
HammersmithHospital Campus,
ImperialCollegeLondon
6th Floor, CommonwealthBuilding,
Ducane Road,
W120NN
Tel 020 838 33242
Fax 020 838 33142

Dietary resistant starch from peas for healthy glucose homeostasis

STUDY 2: Evaluating the impact of resistant starch from peas on small intestinal digestion

Please initial the box if you agree with each statement.

1. I have been given the opportunity to ask questions and discuss the study ☐
2. I have received satisfactory answers to all my questions ☐
3. I have received enough information about the study ☐
4. I confirm that I have read and understand the information sheet: *"Evaluating the impact of resistant starch from peas on small intestinal digestion– Version 2 – 250215" for* the above study. ☐
5. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
6. I understand that sections of any of my research notes may be looked at by responsible individuals from Imperial College, Imperial College NHS Healthcare Trust, and regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
7. I agree that that my Identifiable information can be stored on Imperial College London computer systems ☐
8. I agree to have my GP informed about participating in this study. ☐
9. I agree to have my blood taken. ☐
10. I agree to have my collected tissue samples stored and used in future ethically approved studies. ☐
11. I agree to take part in this study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes