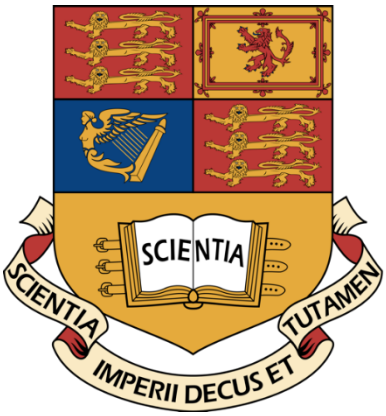


The Effects of a Natural Mutation Found in Peas and Pea-Related Products on Glycaemic Index (GI) and Postprandial Glycaemia in Healthy Humans

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ABSTRACT

Background

Low glycaemic index (GI) foods and slowly digestible carbohydrates have been shown to have an effect on the management and prevention of Type 2 Diabetes (T2D). More specifically, foods with high resistance starch (RS) content, such as the mutant (*rr*) pea of wild type (RR) peas and *rr* peas flour can potentially reduce the postprandial blood glucose and serum insulin levels. However, the GI of *rr* peas and the potential for using it to make diversified staple foods with metabolic benefits have been not studied extensively before. Therefore, this study aimed to further understand the GI of *rr* peas and prototype products made from *rr* peas such as *rr* pea pasta. Besides, comparing the postprandial glycaemia of *rr* peas and *rr* pea pasta with control (glucose drink, *RR* peas, plain pasta) to learn about how the mutation in *rr* peas and *rr* pea pasta affects the glucose metabolism in healthy humans.

Methods

This was a randomized, single-blind, controlled, cross-over, short-term human trial. Eleven healthy subjects consumed five test meals containing 25 g of available carbohydrates: glucose drink, whole wild type *RR* peas, whole variant *rr* peas, *rr* pea pasta (30% *rr* pea flour) and plain pasta. Blood samples were collected over 3h after consuming the tested foods to determine the GI, plasma glucose and serum insulin levels. Appetite and breath hydrogen were measured at the same time. The sensory characteristic scores were determined for each tested meal.

Results

The GI of *rr* pea was lower than *RR* peas, and *rr* pea pasta was lower than plain pasta but significantly higher than whole *rr* peas (GI *rr* peas:15 ± 4; *RR* peas:31 ± 10; *rr* pea pasta: 65 ±16; plain pasta:90 ± 21). *rr* pea pasta also had lower postprandial blood glucose iAUC and significantly lower insulin iAUC than plain pasta. Adding *rr* pea flour

to pasta did not affect the appetite, sensory characteristics or breath hydrogen. The glucose iAUC of *rr* pea pasta was higher than that of whole *rr* peas but the insulin iAUC was significantly lower. The whole *rr* peas showed lower post-ingestion appetite and sensory characteristics but similar breath hydrogen compared to *rr* pea pasta.

Conclusion

The *rr* pea has a low GI and *rr* pea pasta has a medium GI. Producing *rr* pea pasta from *rr* peas reduced its GI, postprandial glycaemia and insulin levels. Therefore, *rr* pea pasta has the potential to be a consumer-friendly functional staple food. Moreover, although *rr* pea pasta has a higher postprandial glycaemia and GI than whole *rr* peas, the lower insulin levels induced by *rr* pea pasta and better sensory characteristics indicate its benefits in reducing postprandial insulin levels and high consumer acceptance.

ABBREVIATIONS

AUC	Area under the Glycaemic Response Curve
BMI	Body Mass Index (BMI)
BIA	Bioelectrical Impedance Analysis
CHO	Carbohydrate
CVD	Cardiovascular Disease
CRF	Imperial Clinical Research Facility
DPP	Diabetes Prevention Program
FAO	Food and Agricultural Organization
GE	Gastric Emptying
GI	Glycaemic Index
GLP-1	Glucagon-like Peptide 1
iAUC	Incremental Area under the Glycaemic Response Curve
IDF	International Diabetes Federation
IR	Insulin Resistance
IGT	Impaired Glucose Tolerance
NINR	National Institute for Health Research
OGTT	Oral Glucose Tolerance Test
PIS	Participants Information Form
PYY	Peptide YY
RDS	Rapidly Digestible Starch
RS	Resistant Starch
SBE	Starch-branching Enzyme
SCFAs	Short-chain Fatty Acids
SLC2A2	Glucose Transporter 2 GLUT2
SDS	Slowly Digestible Starch
TDF	Total Dietary Fibre
T2D	Type 2 Diabetes
VAS	Visual Analogue Scale

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TABLE OF CONTENT

1.0 INTRODUCTION 1

1.1 Type 2 Diabetes (T2D) 1
1.2 Glucose Homoeostasis 1
1.2.1 Plasma Glucose 1
1.2.2 Serum Insulin 2
1.2.3 Glucose Homeostasis Disorders 3
1.3 Management and Prevention of T2D 4
1.3.1 Glycaemic Index (GI) and T2D 5
1.4 Starch 6
1.4.1 Resistant Starch 7
1.4.2 Pulses 8
1.4.2.1 BC1/19rr Pea 8
1.4.2.1.1 Structure and Glucose Metabolism 9
1.4.2.1.2 Processing and Glucose Metabolism 11
1.5 Hypothesis and Project Objectives 13

2.0 METHODOLOGY 14

2.1 Volunteers Recruitment 14
2.1.2 Health Screening 14
2.1.2 Inclusion and Exclusion 14
2.2 Randomization 15
2.3 Study Meals 16
2.4 Study Visit 17
2.5 Sample Collection, Processing and Analysis 20
2.5.1 Sample Collection and Processing 20
2.5.2 Sample Analysis 20
2.5.2.1 Blood Glucose 20
2.5.2.2 Serum Insulin 20
2.6 Statistical and Data Analysis 21

3.0 RESULTS 22

3.1 Baseline Charactertics 22
3.2 Glycaemic Index (GI) 23
3.3 Acute Glycaemia and Insulinaemia 24
3.3.1 Glycaemia 24
3.3.2 Insulinaemia 27
3.4 Food Rating 30
3.5 Appetite 32
3.6 Breath Hydrogen 35

4.0 DISCUSSION 36

4.1 *rr* Peas 36
4.2 *rr* Peas and Plain Pasta.....36
4.3 *rr* pea Pasta and Plain Pasta.....39
4.4 Strength and Limitations (Future Work) 44

5.0 CONCLUSION 46

6.0 REFERENCE 47

7.0 APPENDICES 63

7.1 Pre-screening Questionnaire.....63
7.2 Participants Information Sheet (PIS).....65
7.3 Informed Consent.....75
7.4 Questionnaires on Appetite 77
7.5 Food Rating Form 78
7.6 Blood Glucose Sample Analysis.....80
7.7 Serum Insulin Analysis.....82

1.0 INTRODUCTION

1.1 Type 2 Diabetes (T2D)

The dramatic global increase in the prevalence of type 2 diabetes (T2D) has made it one of the biggest public health problems in recent decades. The International Diabetes Federation (IDF) (2021) estimates that 511 million adults had T2D in 2021 worldwide, and it is expected to rise to 640 million by 2045. In UK, the number of people with T2D currently exceeds 4.9 million and is also expected to grow (Diabetes U.K, 2021). T2D reduces patients' quality of life, and it increases the risk of all-cause mortality by 2-3 times due to its long-term complications including cardiovascular disease, obesity, stroke, and chronic kidney disease (Lin et al, 2020). Additionally, is putting a huge financial strain on the economy, with the National Health Service (NHS) spending at least 10% of its budget (£10 billion) on its treatment each year (Diabetes U.K, 2019). Therefore, there is a need to identify measures to control and prevent the development of T2D and its associated chronic metabolic diseases effectively.

1.2 Glucose Homeostasis

To understand the pathophysiology of T2D, the next session will focus on explaining this mechanism. Figure 1.2.2 (Fialkowski Revilla M.K, 2018) shows the glucose homeostasis regulation. In brief, blood glucose levels in the human body are maintained within a certain range by the glucose regulatory system, which provides a stable source of fuel for organs and prevents persistent hyperglycaemia from triggering insulin resistance and leading to T2D (Röder et al, 2016 & Göke, 2008). The rate at which glucose is removed from the circulation and enters the circulation determines the plasma glucose concentrations (Aronoff et al, 2004).

1.2.1 Plasma Glucose

There are three sources of circulating glucose including intestinal absorption (fed state), glycogenolysis, and gluconeogenesis (Aronoff et al, 2004).

During the fed state, carbohydrates in food are broken down into monosaccharides mainly in the small intestine and are transported into the blood through the wall of the small intestine leading to an increase in plasma glucose (Brand-Miller et al, 2009 & Clemens et al, 2016 & Wong & Jenkins, 2007). During this process, the rate at which glucose appears in the circulation is mainly determined by the gastric emptying (GE) rate (Müller, Canfora, & Blaak, 2018), which can be influenced by diets, such as macronutrient composition, dietary fibre of the foods (Bornhorst & Paul Singh, 2014). The GE has been found to be delayed by the intake of dietary fibre in healthy subjects (Yu et al, 2014), which is thought to be related to the lower postprandial glucose response. During the fasting state, circulating glucose is primarily derived from glycogenolysis and gluconeogenesis, which are partly controlled by glucagon produced by α-cells in the pancreas. During the initial hours of fasting (8-12 hours), the glucagon promotes glycogenolysis to increase circulating glucose concentration by breaking glycogen into glucose in the hepatocytes and the myocytes. During later periods of fasting, the liver releases glucose produced by gluconeogenesis to increase the blood glucose (Aronoff et al, 2004).

1.2.2 Serum Insulin

For circulating glucose clearance, insulin, synthesized by β-cells in the pancreas, is a key regulator. Glucose is the main stimulant of human insulin release, and the level of glucose perceived by β cells will determine the amount of insulin secreted (Fu, R. Gilbert, & Liu, 2013 & Chang & Goldberg, 1978). At high blood glucose levels, glucose is taken up into the cells by the glucose transporter 2 GLUT2 (SLC2A2) on the surface of the β-cells, which eventually leads to insulin release (Henquin, 2000 & Röder et al, 2016). Insulin mainly acts on the liver, skeletal muscle and adipose tissue to increase the uptake of glucose by skeletal muscle, adipose tissue and liver to synthesize skeletal muscle, fat and glycogenesis, respectively (Vargas, Joy & Sepulveda, 2021). It also inhibits the

release of glucagon to produce glucose, finally maintaining blood glucose levels in a normal range (Röder et al, 2016).

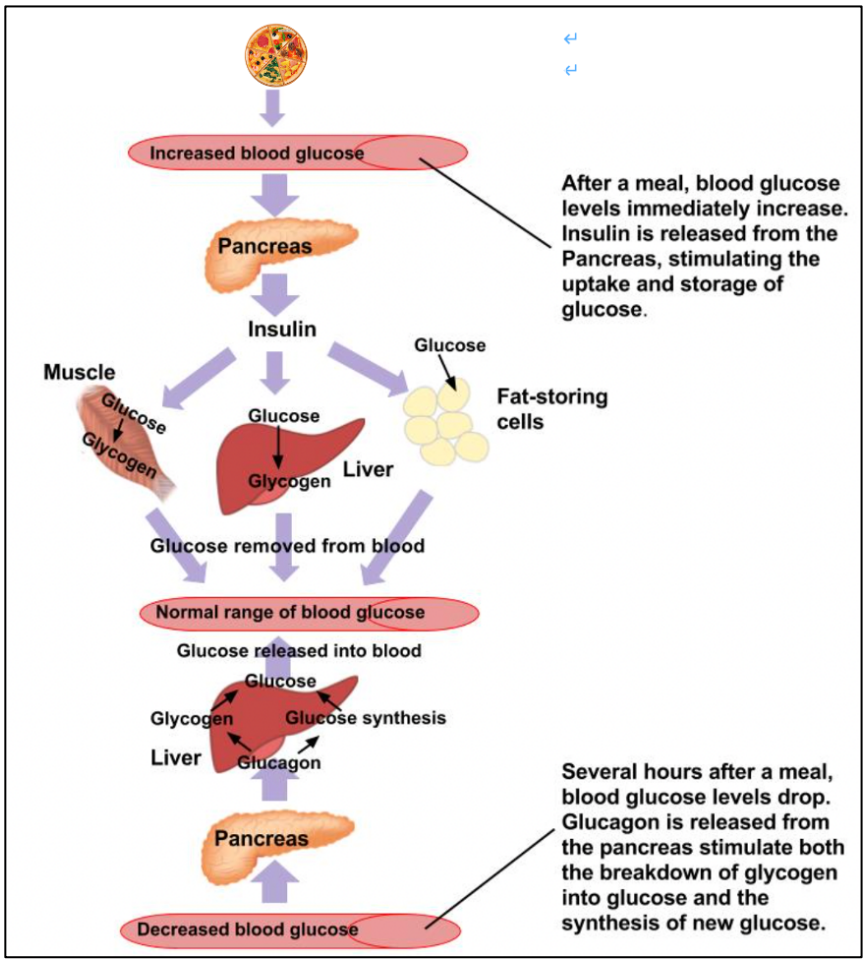


Figure 1.2.2 **Glucose homeostasis regulation.** Image taken from 'The Regulation of Glucose' (Fialkowski Revilla M.K, 2018). After food ingestion, glucose is absorbed in the small intestine and leads to an increase in blood glucose. β-cells of the pancreas can sense the high glucose concentration and then secrete insulin. Insulin promotes glucose absorption and stores glucose in the liver and reduced gluconeogenesis. In skeletal muscle, insulin stimulates glucose uptake and synthesis into skeletal muscle. In adipocytes, glucose is converted to lipids to facilitate lipogenesis synthesis. During the fasting state, glucagon is released from the pancreas to increase the breakdown of glycogen and the synthesis of glucose in the liver to increase the circulating glucose concentration.

1.2.3 Glucose Homeostasis Disorders

Glucose homeostasis disorders are mainly characterized by insulin resistance (IR) (Sapra, Bhandari & Wilhite Hughes, 2021& Kahn, 1994). Insulin resistance refers to decreased sensitivity of target organs, such as muscles and fat to insulin, which limits the uptake and utilization of glucose in the blood. It is usually caused by high insulin

requirements resulting from excessive consumption of calorie-rich foods and chronic exposure to excess glucose (Reed, Bain & Kanamarlapudi, 2021 & Hu et al, 2001). The insulin resistance triggers the compensatory response, and the setting of chronic metabolic demand for insulin finally leads to the apoptosis of the islet β cells (β cells dysfunction) leading to T2D. (Boland, Rhodes, & Grimsby, 2017). Low insulin levels excreted from β cells due to its dysfunction lead to reduced glucose uptake by adipocytes and skeletal muscle. Glucose enters the liver more and is converted into free fatty acids and subsequently leads to dyslipidemia and a series of complications including organ injury, failure and a series of metabolic syndrome including obesity, cardiovascular disease (CVD) (Ceriello et al, 2004). Therefore, the control of blood glucose levels and insulin resistance caused by high insulin requirements is the main objective of T2D prevention and management.

1.3 Management and Prevention of T2D

Current management of T2D includes basic pharmacological treatment, basal insulin injection to supplement insulin requirements (Mayfield & White, 2004), and biguanides and sulphonylureas, such as metformin, which are also commonly used in patients with T2D to increase insulin sensitivity and lower postprandial glucose (Collier et al, 2006 & Chiniwala & Jabbour, 2011). Although efforts have been made to understand the pathophysiology of T2D, the pharmacological treatment is not ideal and may have side effects, including diarrhoea and hypoglycaemia. As a chronic metabolic disease, lifestyle interventions are the focus in preventing and controlling T2D (Uusitupa et al, 2019). One such intervention might be the reduction of refined carbohydrates which have been shown to be able to act as a cornerstone of T2D prevention and management (Freeman & Pennings, 2021). Refined carbohydrates refer to foods that can be digested and absorbed rapidly and stimulate excessive blood glucose levels and insulin requirements due to low fibre content and destroyed structure of polysaccharides after industrial processing. Regular exercise, smoking cessation and limited alcohol consumption are

thought to help improve insulin sensitivity (Hu et al, 2001 & Willi et al, 2007 & Freeman & Pennings, 2021). Pan et al (1997) found the risk of T2D was reduced by 31% for subjects with impaired glucose tolerance (IGT) consuming fewer simple sugars, more dietary fibre and limited alcohol, and reduced by 46% for subjects who exercise more compared with those that did not change their lifestyle. Similar findings were observed in another clinical study, in which reducing fat and increasing fibre intake and physical exercise were found to reduce T2D risk (Penn et al, 2009). The dietary intervention also showed better effects on T2D prevention than pharmacological treatments. In Diabetes Prevention Program (DPP) study, lifestyle intervention including low-calorie diet intake and exercise was found to lower the risk of 58% of developing T2D, which was a better effect than metformin intervention (31% risk reduction) (Knowler et al, 2002). Multiple studies have shown that lifestyle is an effective way to prevent and manage T2D and its complications (Uusitupa et al, 2019). However, although people were advised to reduce food intake and increase exercise in their daily life, the incidence of T2D continued to rise, which might be related to the difficulty of changing lifestyles. Thus, the development of healthy foods has been supported currently as studies have shown that replacing refined carbohydrates with high-fibre, low-glycaemic index (GI) food can optimize glycaemic metabolism and reduce the risk of T2D (Chiniwala & Jabbour, 2011 & Olokoba, Obateru, & Olokoba, 2012).

1.3.1 Glycemic Index (GI) and T2D

Glycemic index (GI) is a value used to describe the effects of a carbohydrate-containing food on blood glucose levels after consumption (Esfahani et al, 2009). Currently, GI has been recommended as a tool to manage blood glucose in T2D patients and general population (Food and Agricultural Organization, 1998). In 1981, David Jenkins and his colleagues introduced the concept of GI (Jenkins et al, 1981). They defined the GI of food as the percentage of the incremental area under the glycaemic response curve (iAUC) 2 hours after consuming a food containing 50g carbohydrates to the iAUC after

ingesting an equivalent amount of available carbohydrates food such as glucose (Brouns et al, 2005). GI can be used to distinguish carbohydrates. High GI (>70) foods, such as refined carbohydrates including plain pasta, are rapidly broken down and absorbed into the blood, resulting in a rapid increase in glycaemia and insulin response (Brouns, 2005). Low GI (<55) foods, due to their low and slow digestibility, have a slower and smaller effect on glycaemia and insulin response. A prospective cohort study found that the dietary GI was significantly positively associated with T2D risk (Salmeron et al, 1997). Currently, low GI foods intake has been suggested as dietary prevention and management approach for glycaemic control by reducing fluctuations in blood glucose levels, reducing insulin requirements, and promoting insulin sensitivity (Fabricatore, Ebbeling, Wadden, & Ludwig, 2011 & Ojo et al, 2018 & Thomas & Elliott, 2010 & Coe, Clegg, Armengol & Ryan, 2013). In the modern diet, for better taste and flavour, the fibre is removed from food ingredients and complex mechanical and thermal processing destroys the food structure of carbohydrates, making foods, especially staple foods have a high GI and become highly digestible, ultimately leading to excessive glycaemia (Foster, 2011). Food industry is encouraged to develop widely consumed low GI foods and especially low GI starchy staples, which act as the main source of carbohydrates for humans to improve glucose metabolism in daily meals and to promote positive long-term health.

1.4 Starch

Starch is the main resource of carbohydrates and energy for humans in their daily diet. It is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on the rate and degree of digestion of starchy foods in the small intestine (Englyst, Kingman & Cummings, 1992). Rapidly digestible starch causes a rapid rise in postprandial glucose and insulin response due to the high rate of digestion and absorption, thus, its over-consumption has been thought to significantly contribute to T2D. However, slowly digestible starch results in slower starch digestion, absorption

and postprandial blood glucose, and insulin response (Birt et al 2013). RS can resist digestion in the small intestine and function as a type of fibre to reduce blood glucose and insulin levels (Sajilata, Singhal & Kulkarni, 2006).

1.4.1 Resistant Starch

Due to the resistance of its intact and compact structure to digestive enzymes, RS has shown great advantages in benefiting postprandial glucose metabolism and promoting human health (Fahmi et al, 2022). After consumption, most of the RS, escape digestion and absorption in the small intestine (Englyst, Kingman & Cummings, 1992) affecting blood glucose levels minimally (Wong & Jenkins, 2007 & Cummings & Branch, 1986). Escaped RS then enters the colon, where it can be used for bacterial fermentation (Nilsson et al, 2008) and the level of this fermentation can be measured by breathing hydrogen (Oku et al, 2009). The fermentation also can produce short-chain fatty acids (SCFAs). SCFAS triggers the release of intestinal peptides involved in glucose homeostasis and appetite regulation, such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which are thought to be able to enhance satiety and reduce energy intake (Bao, Sun & Corke, 2002 & Koch, & Jane, 2000). Previous studies also have found that RS was digested slower *in vitro* digestion (Skrabanja et al, 1999) and increased intake of RS can optimize the PPG response and ameliorate insulin resistance (IR) (Birt et al, 2013 & Ayua et al, 2021 & Boland, Golding & Singh, 2014). Dietary supplementation of RS also has been found to reduce postprandial glucose, modulate IR, and increase satiety in subjects with T2D (Bodinham et al, 2014 & Maki & Phillips, 2015), thereby reducing the risk of T2D. Therefore, traditional carbohydrates are supported to be replaced with RS-riched food ingredients, such as pulses, to increase the nutritional content of foods (e.g., fibre content). RS ingredients are also recommended to be added to staple products such as pasta, bread and baked goods providing a range of nutritional benefits (Walsh et al, 2022).

1.4.2 Pulses

Pluses are a low GI starchy food that is thought to benefit postprandial blood glucose control. Although carbohydrates account for ~50-65% of weight in pulses, they are digested slowly compared to other carbohydrate-rich foods (e.g., rice, white bread, potatoes), which contributes to their low GI (Ferreira et al, 2021). Previous studies showed the content of RS in pulse (smooth peas: 33-50%; chickpeas:36–41%) was higher than in wheat (20%–30%) (Wajira, Ratnayake & Hoovera, 2002 & Yniestra & Marure et al, 2019) and in rice (< 5%) (Ritudomphol & Luangsakul, 2019). The high protein and fibre content in pulses and legumes are thought to be able to reduce the efficiency of carbohydrate absorption and increase satiety (Ferreira et al, 2021). Currently, most studies have shown lower glucose and insulin responses after consuming pulses compared to other carbohydrate-based foods (Marinangeli, Kassis & Jones 2009 & Wong et al, 2009). Additionally, the GI of pulses is usually lower than carbohydrate-containing foods (e.g., cereals, wheat products, pasta and crackers) (McCrory et al, 2012). However, due to the sensory characteristics, the consumption of pulses as a staple food is limited. Efforts have been made to optimize the healthy function of commonly consumed staple foods by using pulses. Interestingly, adding pulses (e.g., chickpeas and yellow peas) to staple foods (e.g., bread, pasta) reduced the glycemic and insulinemic responses (Goñi & Valentín-Gamazo, 2003). Therefore, the development of RS-riched pulse and its low GI prototype products that can be widely accepted and consumed can provide an opportunity to effectively control blood glucose levels and the risk of T2D on a wide population level.

1.4.2.1 BC1/19rr Pea

Wrinkled (BC1/19) *rr* pea was found to have a specific structure that results in higher RS content, even than other common consumed pulses including wild-type (BC1/*RR*) pea (Wajira, Ratnayake & Hoovera, 2002) and therefore can act as a novel vehicle to improve glucose metabolism. Wrinkled (*rr*) pea is a natural genetic variant from smooth wild-type

(*RR*) pea where the mutation resulted in a defective starch-branching enzyme (SBE) and increased amylose and RS content. The changes in the starch structure and increased RS content caused by this mutation have been proved to reduce its digestion in the small intestine and finally benefit glucose and insulin homeostasis (Zafar & Kabir, 2017). To investigate the possibility of developing *rr* peas and prototype products of *rr* peas with glucose metabolic benefits, the following sections will focus on discussing the effects of the changes on the structure caused by mutation *rr* pea and commonly used processing on starch digestibility and glucose metabolism.

1.4.2.1.1 Starch Structure and Glucose Metabolism

Starch is formed by two structural polymers of glucose: amylose and amylopectin. The amylose/amylopectin ratio mainly depends on the activity of enzymes that are involved in the biosynthesis of branched components (Shannon, Garwood & Boyer, 2009 & Singh, Dartois & Kaur, 2010). One type of such enzyme is the SBE which is mainly responsible for the synthesis of amylopectin. SBE introduce branches in starch polymers- α-(1-6) glycosidic bonds in the glucan chain by hydrolyzing the internal α-1,4 bonds, breaking and releasing the reducing end to C-6 hydroxyls (Martin & Smith, 1995), thereby creating branches in the starch chain (Tetlow & Emes, 2014). In the *rr* peas, transposon-like insertion at r (rugosus) locus results in loss of SBE activity in developing pea embryos, thus, its amylopectin synthesis is reduced (Bhattacharyya et al, 1990). This leads to higher amylose found in *rr* peas. High amylose plays an important role in the high RS content formation and therefore in plasma glucose response after consumption (Asare et al, 2011 & Kong et al, 2015 & Shu et al, 2009). Studies show that the amylose content of *rr* pea (65-78%) is higher than wheat (20%–30%) (Cornell, 2003), *RR* peas (33-50%) (Wajira, Ratnayake & Hoovera, 2002) and chickpeas (36–41%) (Yniestra Marure et al, 2019). RS content of starch in *rr* peas (57%-71%) has been shown to be higher than *RR* peas (33%- 50%) (Bhattacharyya et al, 1990). As stated at 1.4.1, higher RS content was related to lower digestion and postprandial blood glucose. Similar results were found by

Petropoulou et al (2020), where RS-riched *rr* peas seeds resulted in lower PPG and insulin compared with the *RR* peas. Interestingly, they also found that this advantage persists even when processed into flour, which indicates the possibility of *rr* peas flour as a novel ingredient for the development of low GI, low-glycaemia functional foods.

The cells wall of *rr* peas is another factor that affects digestion and glucose response. The cell wall of pulses is mainly composed of pectin, which can act as a physical barrier that protects intact plant tissue from being digested in the small intestine (Holland, 2020 & Grundy et al, 2016 & Blackwood, 2000). Nutrients need to contact with enzymes to be digested, which may be achieved by enzyme diffusion through the permeable cell wall or physical processing (e.g., milling or chewing) resulting in cell wall rupture (Holland, 2020). The thick cell walls of pulses lead to less permeability which limits the rate and extent of nutrient release and digestion may be decreased (Grundy et al，2016 & Berry et al, 2008). *In Vitro* studies have proved that digestive enzymes (α-amylase, protease, and pancreatic lipase) cannot diffuse through the low-permeable cell walls of chickpeas and peas (Behall, Scholfield & Canary, 1988). The thicker cell walls of pulses are not easily broken and can remain intact after slight chewing, resulting in restricted contact between enzymes and nutrients and restricted swelling upon subsequent heating, thereby limiting starch gelatinization and digestibility. Compared to other plant foods, this property slows digestion (Venn & Mann, 2004). However, the cell wall may be disrupted by additional mechanical processes, such as milling, and its effect as a barrier can be reduced. Rovalino-Córdova, Fogliano & Capuano (2018) reported the importance of cell wall integrity for the kinetics of starch digestion. Petropoulou et al (2020) observed thicker cell walls for *rr* pea than *RR* peas after cooking, in the gastric samples (180 min post-ingestion) and in the small intestinal samples (60min post-ingestion). This is thought to be partly responsible for the ability of *rr* peas to reduce the magnitude and duration of postprandial blood glucose increases (Rehman & Shah, 2005). Disrupted cell walls may

also be one reason why peas are reduced effectively in controlling postprandial blood sugar and insulin when they are ground into flour in the study by Petropoulou et al (2020).

1.4.2.1.2 Processing and Glucose Metabolism

To respond to consumer preferences, food industry produces widely accepted foods with good taste and flavour by using different types of processing (Birt et al, 2013). Milling is the most common mechanical process, during which the food ingredient, such as wheat is milled into flour for various food products making including white bread, plain pasta, cakes (Singh, Dartois & Kaur, 2010). When starch granules are subjected to milling, results in the loss of the natural integrity of plant foods, such as the breakdown of endosperm and the cell wall (Hallfrisch & Behall, 2000) and it reduces particle size, increased surface area, the promotion of surface pores and water penetration. These factors lead to increased exposure of carbohydrates to digestive enzymes, ultimately resulting in higher hydrolysis and higher carbohydrate digestion in the small intestine (Edwards et al, 2018 & Verkempinck et al, 2020 & Alonso, Aguirre & Marzo, 2000). Petropoulou et al (2020) observed the loss of order and structure in both *rr* and *RR* peas after milling, which was thought to be the reason for the significantly larger glucose and insulin response post- ingestion of both *RR* and *rr* peas flour than whole peas. This indicates that the integrity of the physical structure of *rr* pea plays a role in benefiting glucose metabolism. The high digestion of starch caused by reduced particle size resulting from mechanical processing is considered to increase PPG levels and insulin response (Kong et al, 2022 & Ranawana et al, 2010).

When the starch is subjected to hydrothermal treatment, the entry of water and heating leads to the disruption of the starch crystal structure and leads to swelling and increased solubility of the granules, viscosity development, loss of crystal structure, and loss of birefringence, which is starch gelatinization (Slaughter, Ellis & Butterworth, 2001 & Wang et al, 2015 & Singh, Dartois & Kaur, 2010). Gelatinization of starch ultimately increases

its susceptibility to enzymes and thus increases its digestion (Chung, Lim & Lim, 2006). Complete gelatinization requires sufficient temperature, heating time and space, if the physical structure is not well destroyed by mechanical processing, the space required for cell expansion will be limited. This will lead to incomplete gelatinization and reduced digestion in the small intestine (Fardet et al, 1997 & Champagne, Marshall & Goynes, 1990 & Marshall, 1992). On the other hand, cooling after gelatinization of starch will lead to the recrystallization of amylose starch, thereby increasing the content of RS. This is because the linear structure of amylose molecules makes them easy to form double helices, which cannot be enzymatically hydrolyzed. Therefore, foods high in amylose after heating to form more RS (Birt et al, 2013). This was supported by the study by Petropoulou et al (2020), where more birefringence and higher RS content were observed in amylose-riched *rr* peas compared to *RR* peas after cooking and digestion. This may also be the reason why postprandial blood glucose and insulin levels of *rr* peas flour are still lower than *RR* peas flour even after milling. This indicates the potential use of *rr* peas flour as functional food ingredients being added to commonly consumed food products to reduce the GI and benefit glucose metabolism.

1.5 Hypothesis and Project Objectives

There is strong evidence in the literature that *rr* peas and *rr* peas flour, can potentially benefit postprandial blood glucose, insulin levels and appetite due to their relatively high RS content and amylose ratio. However, the glycaemic index (GI) of *rr* peas was not previously fully investigated to further confirm its role as a low GI healthy food.

Due to the limited consumption of pulses widely as a staple food, a great attempt can develop functional staple foods based on *rr* peas and investigate their possibility to retain the health benefits that have been reported in the literature, even when processed. Pasta is a worldwide consumed staple food, which is an important source of carbohydrates (starch). In this study, *rr* peas were used to make a functional pasta-based food that does not lead to rapid postprandial glucose release.

Therefore, the hypothesis is:

A natural genetic variant, BC1/19*rr* peas (wrinkled peas) and *rr* pea pasta, can act as a low GI food to demonstrably improve the glucose metabolism in healthy humans.

The primary outcomes proposed to formalise this hypothesis are:

The effects of *rr* pea and *rr* pea pasta against controls (glucose drink, *RR* peas and plain pasta) on GI and postprandial glucose in healthy humans.

The secondary outcomes proposed are:

The effects of *rr* peas and *rr* pea pasta against controls (glucose drink, *RR* peas and plain pasta) on:

1. serum insulin levels
2. sensory characteristics
3. appetite measures
4. breath hydrogen measures

2.0 METHODOLOGY

This was a randomized, single-blind, controlled, cross-over human trial involving 5 study visits and was approved by the London Surrey Research Ethics Committee (REC: REC reference number 15/LO/0184).

2.1 Volunteers Recruitment

Participants of this study were recruited via Healthy Volunteer Database of the National Institute for Health Research (NIHR) Imperial Clinical Research Facility (CRF). Pre-screening questionnaires (Appendix 1) were sent via e-mail to the candidates interested in this study.

2.1.2 Health Screening

For further eligibility assessment, participants were asked to attend a healthy screening visit in which anthropometric (weight, height, body mass index (BMI)) and blood pressure measurements, health interview, and blood collection for overall metabolic health assessment were performed. A pregnancy test was performed for women of childbearing age. During the visit, the Participants Information Sheet (PIS) (Appendix 2) was provided, the nature of this study was explained and after participants fully understood this study and agreed to participate, informed consent (Appendix 3) was obtained from them.

2.1.2 Inclusion and Exclusion Criteria

Healthy males and females aged between 18 and 65 years old with a BMI between 20 and 35 kg/m^2 were suitable for this study. The detailed inclusion and exclusion criteria were as follows.

Inclusion criteria	Exclusion criteria
● Gender: male and female ● Age: 18-65 years ● BMI: 20-35 kg/m^2 (Without change more than 5% in previous 3 months)	● Weight change of ≥ 3kg in the preceding 2 months ● Current smokers (time period for not smoking less than 6 months) ● Substance abuse ● Excess alcohol intake ● Pregnancy ● Diabetes ● Cardiovascular disease ● Coronary artery disease or stroke ● Cancer ● Thyroid disease ● Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome ● 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

* Adopted from the study protocol.

14 volunteers were recruited and invited for a health screening. Two participants failed to meet the eligibility for this study, and one changed their mind and withdraw from this study before study visit 1.

2.2 Randomization

The order of treatments for each participant for this study were randomly assigned by using the Sealed Envelope system provided by Sealed Envelope Company (sealedenvelope.com). This was a single-blind trial, only researchers were aware of the intervention. Although the appearance, taste and amount were different among the food studied, the compliance of participants was ensured by the researchers and the primary outcomes were measured using standardized methods to decrease the bias from participants. There was at least a 3-day washout period between each study visit to ensure that bias has been minimized.

2.3 Study Meals

To compare the effects of different foods studied on glucose metabolism, foods used in this study contained the same content of 25 g of available carbohydrates including glucose drink (Oral Glucose Tolerance Test (OGTT) solution) (100 g), whole *RR* peas (77 g), whole *rr* peas (89 g), *rr* pea pasta (41 g) and plain pasta (69 g). The detailed nutrition information of the foods consumed by participants is shown in table 2.3. Glucose drink orange flavour was provided by Rapilose (Figure 3.3.1 A). *RR* peas and *rr* peas were sourced from John Innes Center and the preparation of peas was based on the method used by Petropoulou et al (2019). Whole peas were soaked for 24 hours overnight in 300 ml of water and were cooked for 1.5 hours in the boiling water of 2 L at medium temperature, cooked peas were served to participants after excessive water was removed (Figure 3.3.2 B). Plain pasta (100% durum wheat flour) was bought from Tesco and *rr* pea pasta (30% rr peas and 70% durum wheat flour) was provided by Hodmedods (Figure 3.3.1 C.) The *rr* pea pasta and plain pasta were cooked in boiling water at a high temperature for 17 and 12 mins, respectively, and were served to participants after excessive water was removed. Cooking temperature and time were determined by the researcher team, who tasted samples for each minute of the 8–20-minute cooking period to determine the cooking method of best-tasting samples as the standard method. For all cooked food, the temperature was measured before serving to participants, which must be over 70°C to ensure food safety. All foods were served with a bottle of water and more water was served if participants requested during the study visit.

Table 2.3 Nutrition information of glucose drink, *RR* and *rr* peas and *rr* pea pasta and plain pasta.

Analyte	Glucose drinking (100g)	RR peas (77g)	rr peas (89g)	rr peas pasta (41g)	Plain pasta (69g)
Energy value (kcal)	100	220.22	262.55	141.04	120.06
Energy value (kJ)	419	861.63	1098.26	595.73	509.22
Protein	0	16.401	21.182	6.724	3.795
Carbohydrate (available) (g)	**25**	**24.871**	**24.92**	**24.723**	**24.909**
Fructose	-	<0.1	<0.1	-	-
Galactose	-	0.077	0.267	-	-
Lactose	-	0.616	1.068	-	-
Maltose	-	<0.1	<0.1	-	-
Sucrose	-	1.771	3.56	-	-
Total Sugar	-	2.464	4.895	-	-
Total fat (g)	-	<1	1.513	0.902	0.414
Dietary Fiber (g)	-	27.72	32.307	3.485	0.828
Moisture content (g)	-	5.929	6.408	-	-

*Adopted from food labelling and Petropoulou et al (2020). – Information not traced

A B C



Figure 2.3 Experimental Intervention Meal, A—glucose drink; B—whole peas; C—pasta.

2.4 Study Visit

Participants were required to maintain their daily diet and refrain from intensive exercise regimes in-between study visits to ensure that the diet before the study visits were as similar as possible and to maintain their normal hormone levels.

Participants were asked to fast overnight before each study visit and arrive at the NIHR Imperial Clinical Research Facility (CRF) at 9:00am. Anthropometric and body composition measurements were performed on participants using bioelectrical

impedance analysis (BIA) (Tanita BC-418 analyzer; Tanita Corporation, Japan). A small intravenous cannula was inserted into the arm of the participants and was in place for the duration of study visit for blood collection. Two fasting blood sample collection, completion of questionnaires on appetite (Visual Analogue Scale) (Appendix 4) and breath hydrogen tests were performed (Baseline 1 and 2). The breath hydrogen test was performed using a Breathalyzer. Participants were required to consume the prepared breakfast within 15 min and blood sample collection, breath hydrogen test and VAS collection were done at 15, 30, 60, 90, 120, 150 and 180 min. 5 ml of blood was taken at each time point and a total of 45 ml (5×9) of blood per visit. At the end of the study, the cannula was removed, and participants were asked to complete a food rating form (Appendix 5) for the foods they consumed. Study Protocol is outlined in Figure 2.4.

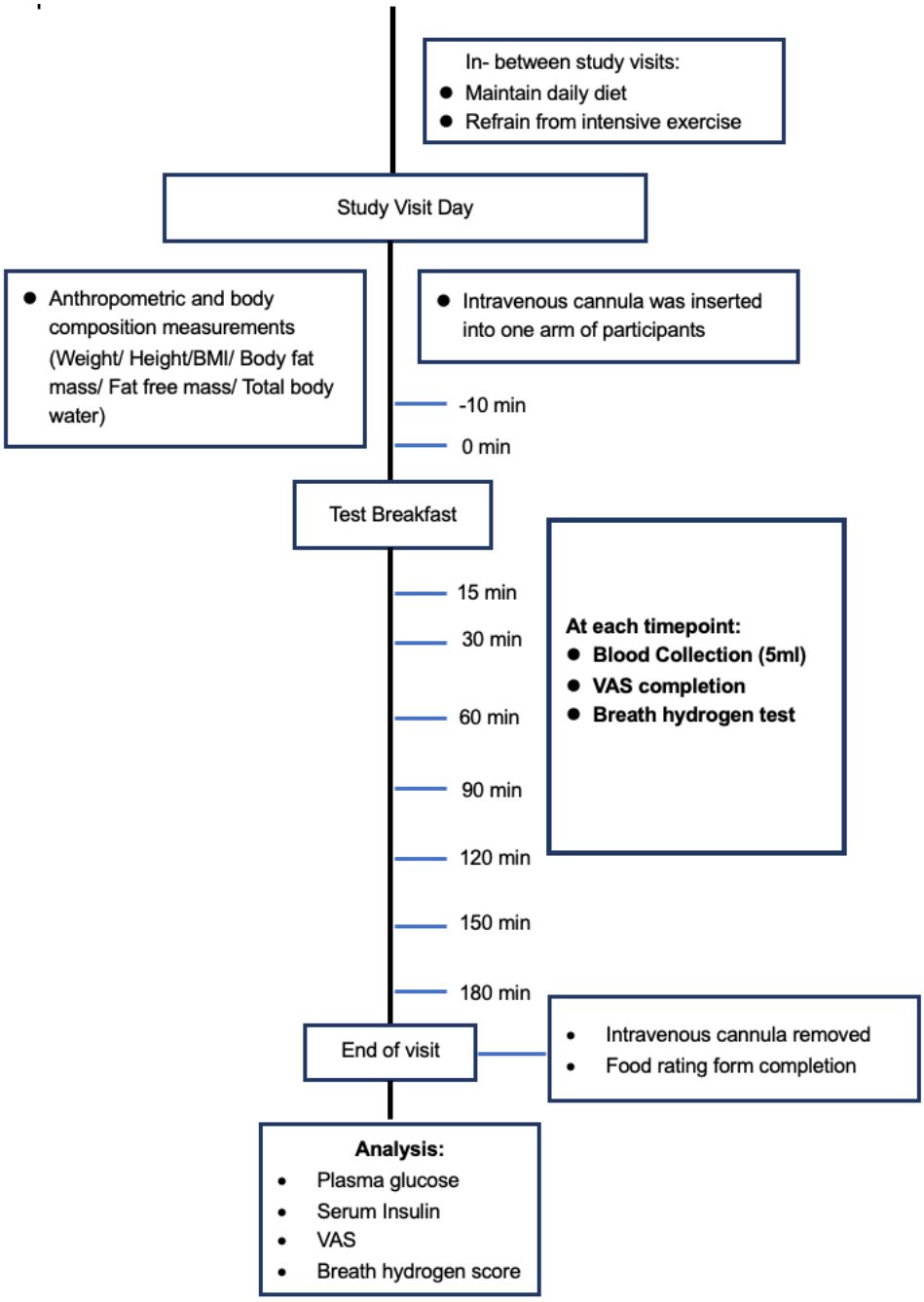


Figure 2.4 Schematic Representation of study visit

2.5 Sample Collection, Processing and Analysis

2.5.1 Sample Collection and Processing

Blood samples were separated into different vacutainers right after the collection. Table 2.5.1 shows detailed information about sample collection and processing.

Table 2.5.1 Sample Collection and Processing

Analysis	Collection	Additive	Processing
Glucose	Plasma Grey vacutainer	Sodium fluoride/ Potassium	• Collected • Centrifuged at 2500 g, at 4°C for 10 minutes • Transferred into eppindorfs • Stored at -80°C
Insulin	Serum SST vacutainer	Clot activator/ Gel for serum separation	• Collected • Clotting for at least 10mins at room temperature/ processed • Centrifuged at 2500 g, at 4°C for 10 minutes • Transferred into eppindorfs • Stored at -80°C

2.5.2 Sample Analysis

2.5.2.1 Blood Glucose

Postprandial blood glucose analysis was performed using the Randox GOD-PAP kit (Randox, GL364, 10×100ml). Experimental processes and procedures for performing glucose analysis are shown in Appendix 6.

2.5.2.2 Serum Insulin

The insulin analysis was performed using the Human/Canine/Porcine Insulin Quantikine ELISA Kit (Bio-techne, Minnesota, USA) according to the manufacturer's specifications. Experimental processes and procedures for performing the insulin analysis are shown in Appendix 7.

2.6 Statistical and Data Analysis

There was a total of 11 participants recruited for this study. Power calculations were not applicable as this was an exploratory study. Brouns et al (2005) recommended that the enrolment of at least 10 participants provide a reasonable degree of precision and power for most clinical trials measuring GI. The data from 11 participants were used for each outcome analysis, however, only 10 were used for insulin analysis, as the insulin data of one participant was out of the standard curve. IBM SPSS statistical Version 28 and GraphPad Prism Version 9 were used for statistical analysis. Normality of the data was checked using the Shapiro-Wilk test ($p \geq 0.01$). Standard transformation was performed for non-parametric data before conducting the parametric statistical tests. Five missing values were replaced by the mean value of data of timepoints adjacent to the missing time point or the average of the group value. The final baseline data was the mean of the two-baseline data (-10min; 0min). Repeated measures ANOVA with Fisher's LSD tests were used to analyse the time course data. Incremental area under the plasma glucose curves or serum insulin curves (iAUC) for the food studied was calculated using the trapezoid rule (Wolever et al, 1991) ignoring the area beneath baseline and compared using one-way ANOVA with Fisher's LSD tests between treatments.

The VAS score was determined by measuring the number of millimetres from the left end of the line to the line marked by the participants.

The GI was expressed as % average of the glucose response of tested foods divided by the glucose response of glucose drinking which has the same carbohydrate content (25g) by the same participant. The formula is shown following:

$$GI = \frac{\text{iAUC of the tested food (25g carbs)}}{\text{iAUC of the glucose drinking (25g carbs)}} \times 100$$

All results are presented as mean ± standard error of the mean (SEM). And the $p<0.05$ was set to be a significant difference.

3.0 RESULTS

3.1 Baseline Characteristics

Participants were healthy between 23 to 44 years old and with a BMI between 20.9 to 28.9 kg/m^2. They all had normal blood pressure (systolic pressure: 93-127mmHg; diastolic pressure: 58-78mmHg). Baseline characteristics of the participants are shown in Table 3.1.

Table 3.1 Baseline Characteristics of Participants

Volunteers (n=11)	Range	Mean	SEM
Gender (M:F)	5:6		
Age (years)	23-44	30.4	1.7
Height (m)	162-195.1	173.2	3.4
Body Weight (kg)	55.1-95	72.8	4.1
BMI (kg/m^2)	20.9-28.9	24.2	0.7
Systolic Pressure (mmHg)	93-127	93	3.5
Diastolic pressure (mmHg)	58-78	58	2.1
Fat Mass (%)*	14.6-32.3	24.0	1.9
Fat Mass (kg)*	11.6-22.3	17.2	1.3
FFM (kg)*	39.9-73.1	55.8	4.0
TBW (kg)*	28.8-52.1	39.8	2.7

Results are presented as range and mean values with the standard error of mean (SEM). Data were mainly obtained from screening visit. Data with a footnote* were obtained from study visit 1. FFM refers to the fat-free mass and TBW refers to the total body water.

3.2 Glycaemic Index (GI)

The GI was measured using a glucose drink as a control for all the products and is shown in Figure 3.2. Results indicated a trend for whole *rr* peas (15 ± 4) having a lower GI than whole *RR* peas (31 ± 10) (p=0.074). Whole peas either *RR* or *rr* were found to have a statistically significant lower GI compared to *rr* pea pasta (65 ±16) (p=0.0147; p=0.0303, respectively) and plain pasta (p=0.006; p=0.006, respectively). GI of *rr* pea pasta (65 ±16) was lower than plain pasta (90 ± 21) (p=0.071), indicating adding *rr* pea flour to pasta lowered its GI and *rr* peas pasta had a medium.

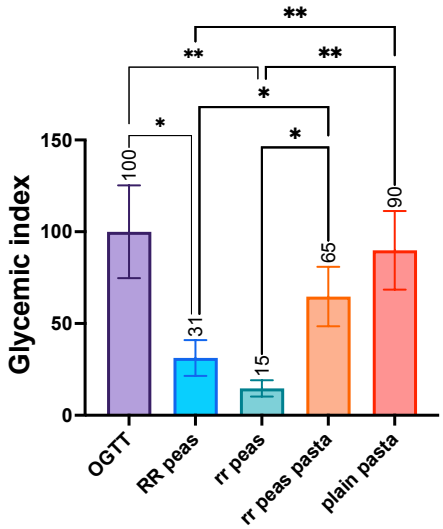


Figure 3.2 **The glycaemic index (GI) value for glucose drink, *RR* peas, *rr* peas, *rr* pea pasta and plain pasta.** Data represent mean ±SEM (n=11). GI of *RR* peas, *rr* peas, *rr* pea pasta and plain pasta were calculated based on the reference glucose drinking (GI=100) using $iAUC_{0\text{-}120min}$ shown in 3.3.1. *P<0.05, **P<0.01, ***P<0.001.

3.3 Acute Glycaemia and Insulinaemia

3.3.1 Glycaemia

Time Course Data Analysis

Glucose time course data after consumption of test products are shown in Figure 3.3.1 A. Two-way repeated measures ANOVA analysis indicated that there was a statistically significant main effect of time ($P<0.0001$) and time × food interaction ($P<0.0001$). Results did not show a statistically significant main effect of food interventions ($p=0.158$).

There was a trend that *rr* pea pasta had a lower postprandial glucose concentration compared to plain pasta. Glucose concentrations were lower in whole *rr* peas with a statistically significant difference at 30min ($p=0.009$) compared to *rr* pea pasta. The higher glucose levels in *rr* pea pasta were also observed at 30min versus *RR* peas ($p=0.003$). Between whole *RR* and *rr* peas ($p>0.05$) results did not show a statistically significant effect. The plasma glucose concentration after consuming the glucose drink had the glucose highest peak than other tested food and was statistically significantly higher between 15-30min. Interestingly, it was observed that between 90-180min the glucose concentration was under the baseline and statistically significantly lower than other tested food.

iAUC Analysis

Glucose $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ after consuming test products are shown in Figure 3.3.1 B and Figure 3.3.1 C. One-way ANOVA glucose $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ showed a statistically significant difference between food interventions ($P= 0.0004$ and $p<0.0001$ respectively) (Figure 3.3.1B & 3.3.1C).

There was a trend that glucose $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ of *rr* pea pasta was lower than plain pasta ($p=0.083$ and $p=0.070$, respectively), indicating the potential for replacing plain pasta with functional *rr* pea pasta for postprandial blood glucose management. A statistically significant reduction of $iAUC_{0\text{-}180min}$ of 65% ($p= 0.043$) and $iAUC_{0\text{-}120min}$ of 72% ($p=0.025$) after consuming whole *rr* peas compared to *rr* pea pasta

(3.3.1B, 3.3.1C) was observed. The $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ of whole *RR* peas were also found to be lower than *rr* pea pasta (p= 0.066 and 0.074, respectively). There was no statistically significant difference in $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ between whole *rr* peas and *RR* peas. Glucose drink had a statistically significant higher glucose $iAUC_{0\text{-}120min}$ and $iAUC_{0\text{-}180min}$ than whole peas either *rr* or *RR* and higher $iAUC_{0\text{-}120min}$ than *rr* pea pasta.

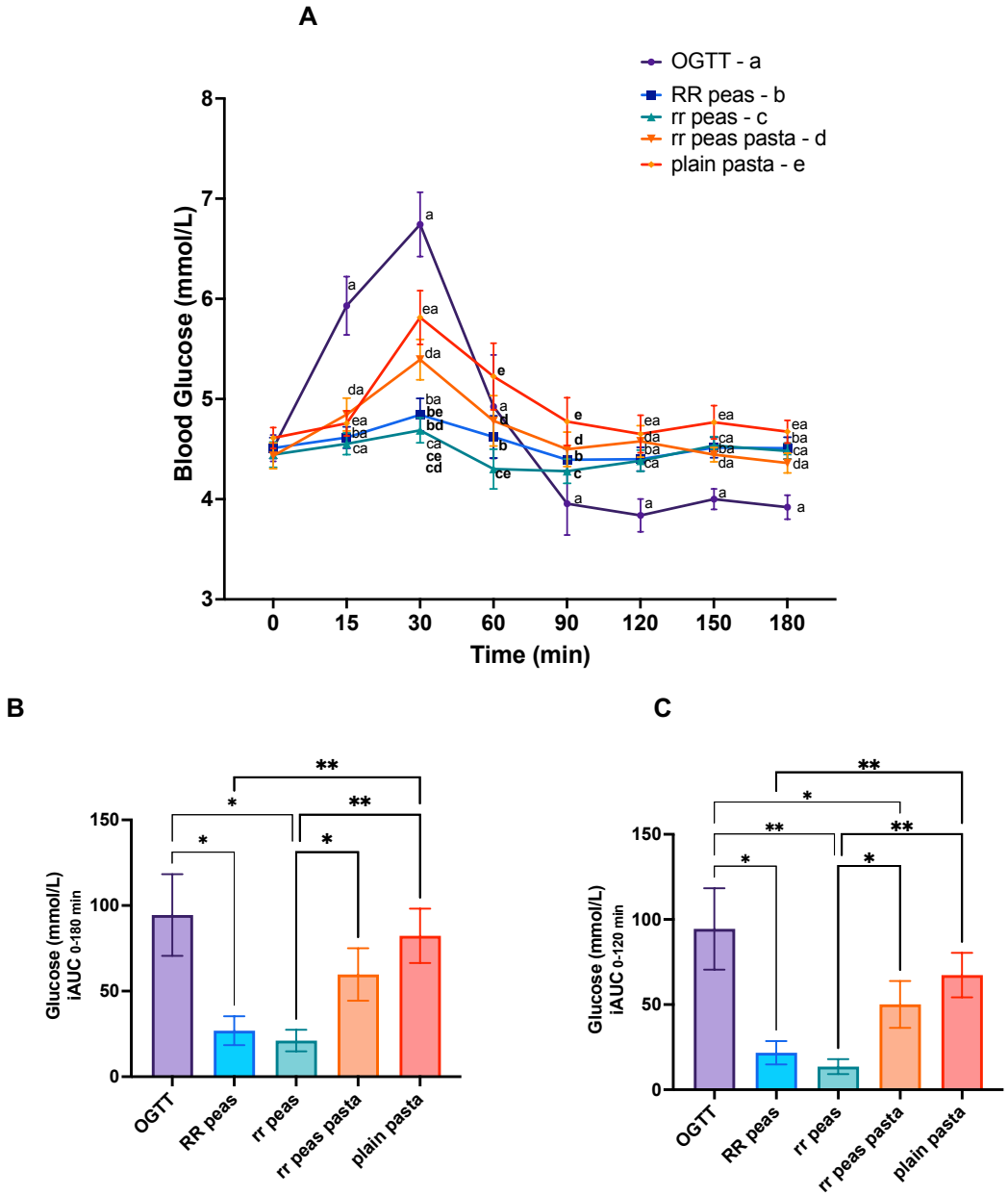


Figure 3.3.1 **(A) Plasma glucose concentration after the consumption of glucose drink (a), *RR* whole peas (b), *rr* whole peas (c), *rr* pea pasta (d) and plain pasta (e) (B) $iAUC_{0\text{-}180}$ glucose response. (C) $iAUC_{0\text{-}120min}$ Glucose response.** At each time point at (A), a single letter means this food was not significantly different from other treatments. Double letters mean these

two tested foods were significantly different. Data represent mean ±SEM (n=11). Two-way repeated measures ANOVA was used to determine the effects of treatment and time and Fisher's post hoc tests were performed between timepoints, $p<0.05$. For iAUC data, One-way ANOVA test was used to determine the overall difference between all treatments and Fisher's post hoc tests were performed between treatments, $p<0.05$. *P<0.05, **P<0.01, ***P<0.001.

3.3.2 Insulinaemia

Insulin time course data after consumption of the test products are shown in Figure 3.3.2 A. Two-way repeated measures ANOVA analysis indicated that there was a statistically significant main effect of time (P<0.0001) and time × food interaction (P<0.0001). Results did not show a statistically significant main effect of food interventions (p=0.6905).

Interestingly, there was a trend for *rr* pea pasta showing a slower and more attenuated insulin concentration increase compared to plain pasta over 0-180min. It was observed that there was no significant insulin difference between whole peas either *rr* or *RR* and *rr* pea pasta. The results of the whole *RR* and *rr* peas (p>0.05) did not show a statistically significant difference. The serum insulin concentration after consuming the glucose drink was more rapid and statistically significantly higher than other tested food between 15-30 min.

iAUC Analysis

Insulin $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ after consuming all products are shown in Figure 3.3.2 B and Figure 3.3.2 C. One-way ANOVA insulin $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ showed a statistically significant difference between food interventions (P= 0.0308 and p=0.0204 respectively) (Figure 3.3.2B & 3.3.2C).

There was a statistically significant reduction of $iAUC_{0\text{-}180min}$ of 73.6% (p= 0.0347) and $iAUC_{0\text{-}120min}$ of 77.8% (p=0.0011) after consuming *rr* peas pasta compared to plain pasta (3.3.2B, 3.3.2C), which showed similar to the glucose pattern described above. Insulin $iAUC_{0\text{-}180min}$ of *rr* pea pasta were statistically significantly lower than whole *rr* peas (p= 0.0347). The results of whole *RR* peas and *rr* pea pasta did not show a statistically significant effect. No statistically significant difference in $iAUC_{0\text{-}180min}$ and $iAUC_{0\text{-}120min}$ between whole *rr* peas and *RR* peas was observed. Glucose drink had a statistically significant higher insulin $iAUC_{0\text{-}120min}$ and $iAUC_{0\text{-}180min}$ than whole peas either *rr* or *RR* and *rr* pea pasta.

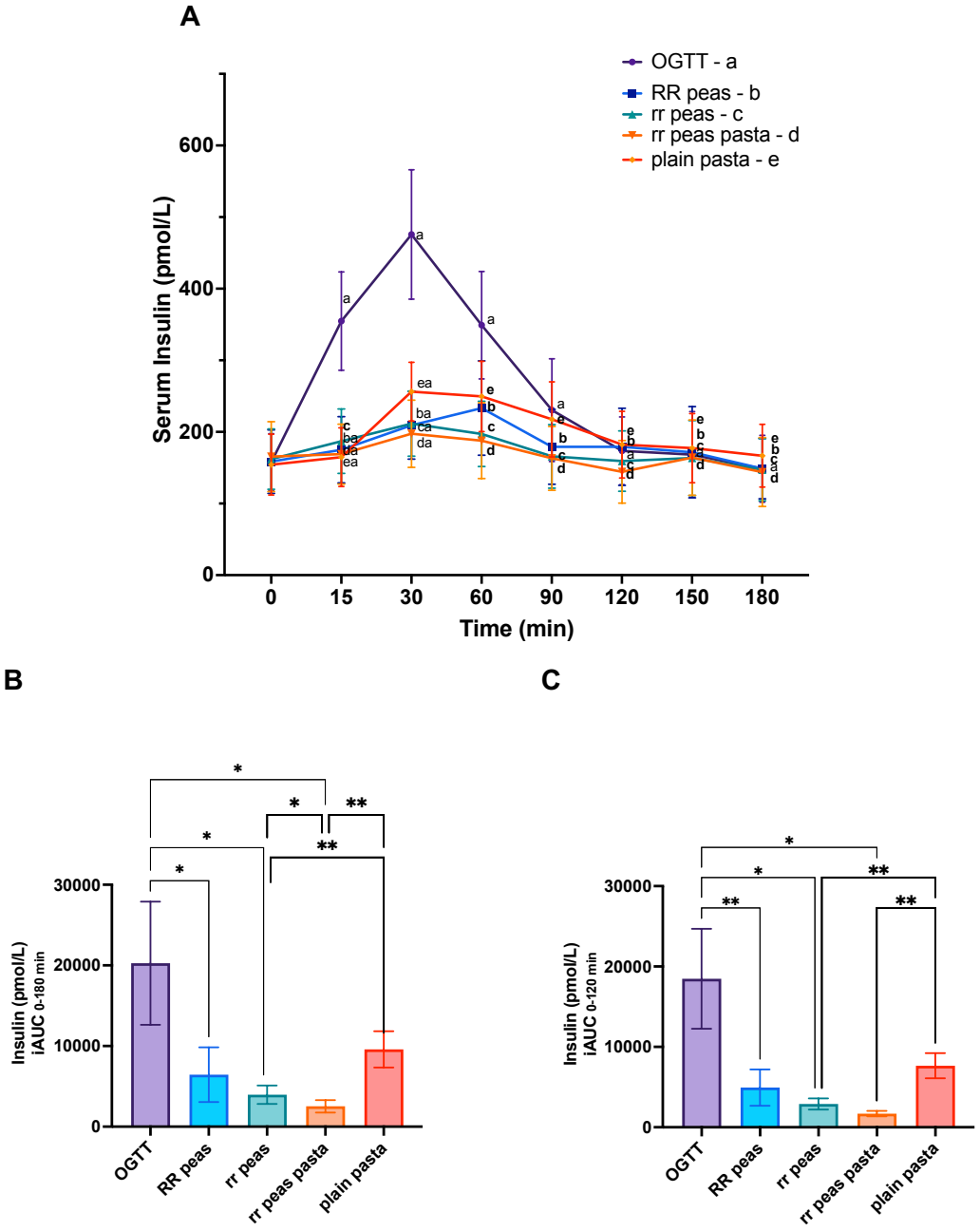


Figure 3.3.2 **(A) Serum insulin concentration after the consumption of glucose drink (a), *RR* whole peas (b), *rr* whole peas (c), *rr* pea pasta (d) and plain pasta (e) (B) iAUC$_{0\text{-}180}$ Insulin response. (C) iAUC$_{0\text{-}120min}$ Insulin response.** At each time point at (A), a single letter means this test food was not significantly different from other treatments. Double letters mean these two tested foods were significantly different. Data represent mean ±SEM (n=10). Two-way repeated measures ANOVA was used to determine the effects of treatment and time and Fisher's post hoc

tests were performed between timepoints, $p<0.05$. For (B) and (C), One-way ANOVA test was used to determine the overall difference of all treatments and Fisher's post hoc tests were performed between treatments, $p<0.05$. *P<0.05, **P<0.01, ***P<0.001.

3.4 Food Rating

Results comparing sensory characteristics of the test products are shown in figure 3.4. One-way ANOVA indicated that there was a statistically significant effect of five tested foods on overall liking ($p=0.002$), appearance ($p=0.002$), aroma ($p=0.001$), flavour ($p=0.007$) and texture ($p=0.017$).

There was no statistically significant difference between the *rr* pea pasta and the plain pasta in the overall liking, appearance, aroma, flavour or texture, indicating the high consumer acceptance of replacing existing commercial products with *rr* pea pasta with healthy benefits. Although there was no statistically significant difference between *rr* pea pasta and whole *rr* peas in aroma ($p=0.099$), *rr* pea pasta had a statistically significant better appearance ($p=0.002$), flavour ($p=0.032$), texture ($p=0.018$) and overall liking score ($p=0.003$), which indicates its potential for being a consumer-friendly functional food. However, no statistically significant difference was observed in aroma, flavour, texture and overall liking between whole *RR* peas and *rr* pea pasta ($p>0.05$) except for a statistically significant better appearance in *rr* pea pasta than whole *RR* peas ($p=0.011$). No statistical significance between the *RR* whole peas and *rr* whole peas on the overall liking, appearance, aroma, flavour and texture was observed ($p>0.05$).Glucose drink showed the highest scores on each sensory characteristic.

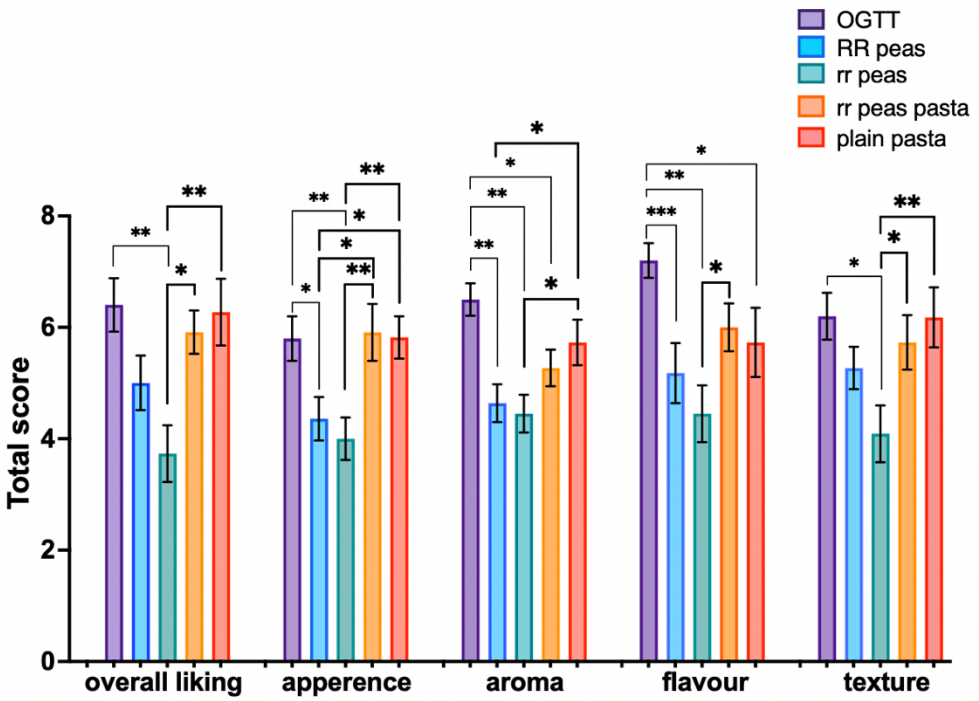


Figure 3.4 **Food rating score, measured by the food rating questionnaire**. Data represent mean ±SEM (n=11). One-way ANOVA test was used to determine the difference in total score on overall liking, appearance, aroma, flavour and texture among all treatments and Fisher's post hoc tests were performed between treatments. $p<0.05$. *P<0.05, **P<0.01, ***P<0.001.

3.5 Appetite

Time Course Data Analysis

Total appetite data after consuming the test products are shown in Figure 3.5 E. Two-way repeated measures ANOVA analysis indicated that there was a statistically significant main effect of time ($P<0.0001$) and food interventions ($p=0.005$) and time × food interaction ($P<0.010$).

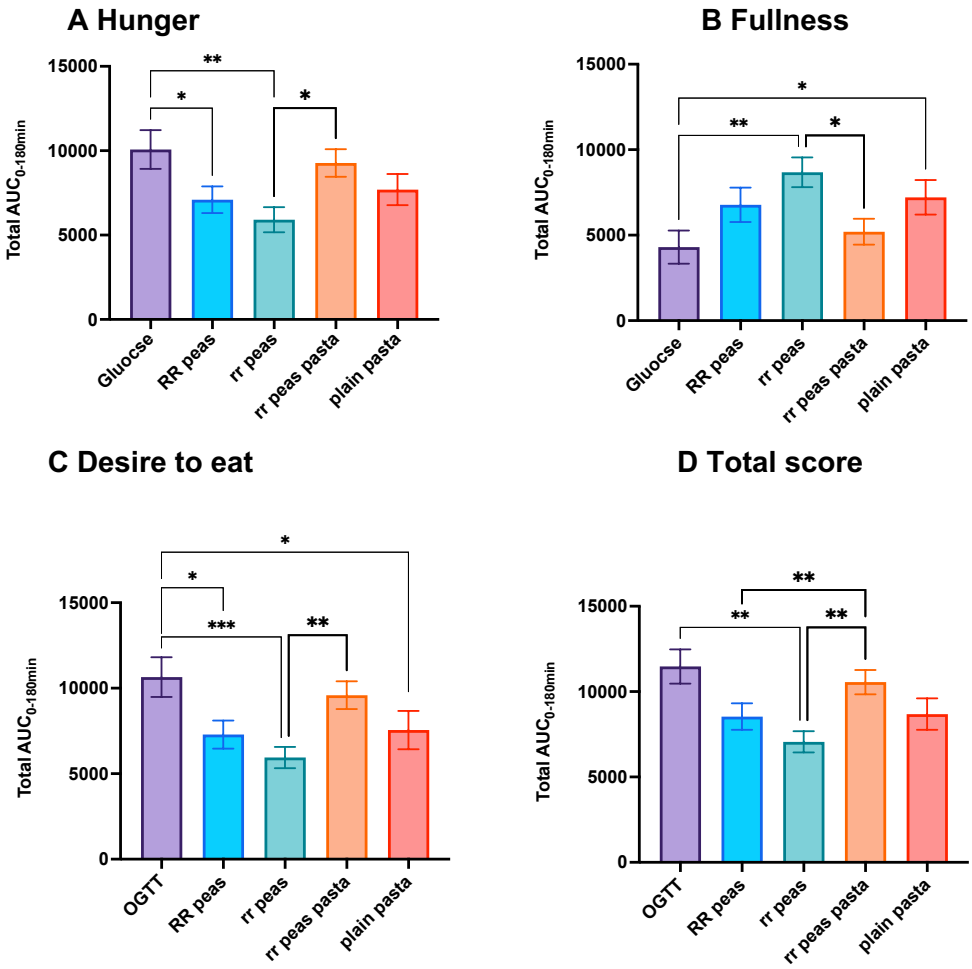
No statistically significant difference between the *rr* pea pasta and plain pasta on the appetite score (figure 3.5 E) ($p>0.05$) was observed, indicating that adding *rr* pea flour did not significantly affect the postprandial appetite. There was a statistically significant higher appetite after consuming *rr* pea pasta than whole *rr* peas ($p<0.05$) during 15-180min (figure 3.5 E) and than whole *RR* peas at 15 min($p=0.049$). No statistically significant difference between whole *rr* peas and *RR* peas in total appetite was observed ($p>0.05$). After glucose drink ingestion, participants showed a higher appetite than any other products over 180min.

AUC Analysis

Results comparing $AUC_{0\text{-}180min}$ of hunger, fullness, desire to eat and total appetite after consuming food products are shown in figure 3.5 A-D. One-way ANOVA indicated that there was a statistically significant effect of food interventions on hunger ($p=0.014$), fullness ($p=0.015$), desire to eat ($p=0.006$), and total appetite score ($p=0.004$).

The *rr* pea pasta showed higher AUC on hunger, desire to eat and appetite but lower fullness, however, the difference did not reach statistical significance. Results indicated that *rr* pea pasta had a statistically significantly higher AUC on hunger ($p=0.011$), desire to eat ($p=0.008$), overall appetite ($p=0.003$) and lower fullness ($p=0.011$) versus whole *rr* peas. Whole *RR* peas also showed statistically significant higher AUC on overall appetite ($p=0.003$) than *rr* pea pasta. No significant difference between whole *RR* peas and *rr* peas on AUC of hunger, fullness, desire to eat and total appetite was observed. Glucose

drink showed statistically significant higher AUC of hunger, desire to eat, total appetite score and lower fullness than *rr* peas ($p<0.05$).



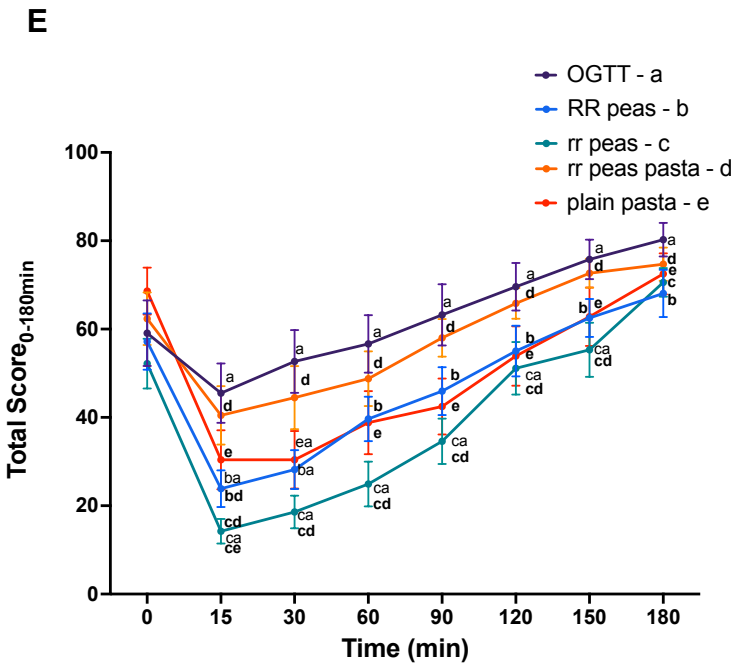


Figure 3.5 **Appetite rating score, measured by the visual analogue scales (VAS).** (A) AUC_{0-180} Hunger, (B) $AUC_{0-180min}$ Fullness, (C) $AUC_{0-180min}$ Desire to eat, (D) $AUC_{0-180min}$ Total score of appetite, (E) Time course data for the total score of appetite. At each time point at (E), a single letter means this test food was not significantly different from other treatments. Double letters mean these two tested foods were significantly different. The total score was calculated using the equation ([hunger +(100-fullness) +desire to eat]/3). Data represent mean ±SEM (n=11). One-way ANOVA test was used to determine the difference in AUC among all treatments and Fisher's post hoc tests were performed between treatments. Two-way repeated measures ANOVA was used to determine the effect of treatment and time and Fisher's post hoc tests were performed between timepoints, $p<0.05$. *P<0.05, **P<0.01, ***P<0.001.

3.6 Breath Hydrogen

Results showing the change of breath hydrogen after consuming the test products are shown in figure 3.6 (A). Two-way repeated measures ANOVA analysis indicated that there was a statistically significant main effect of time (P<0.0001). Interestingly, no statistically significant interaction effect of time × food interaction (p=0.671) (Figure 3.6 A) or main effect of treatment (p=0.872) was observed. Baseline breath hydrogen was not significantly different between groups (P > 0.05) (Figure 3.6 A).

Results comparing the breath hydrogen after the consumption of food products are shown in figure 3.6 (B). One-way ANOVA of total $AUC_{0\text{-}180min}$ on breath hydrogen score showed no statistically significant difference between all food products (p=0.907) (Figure 3.6B).

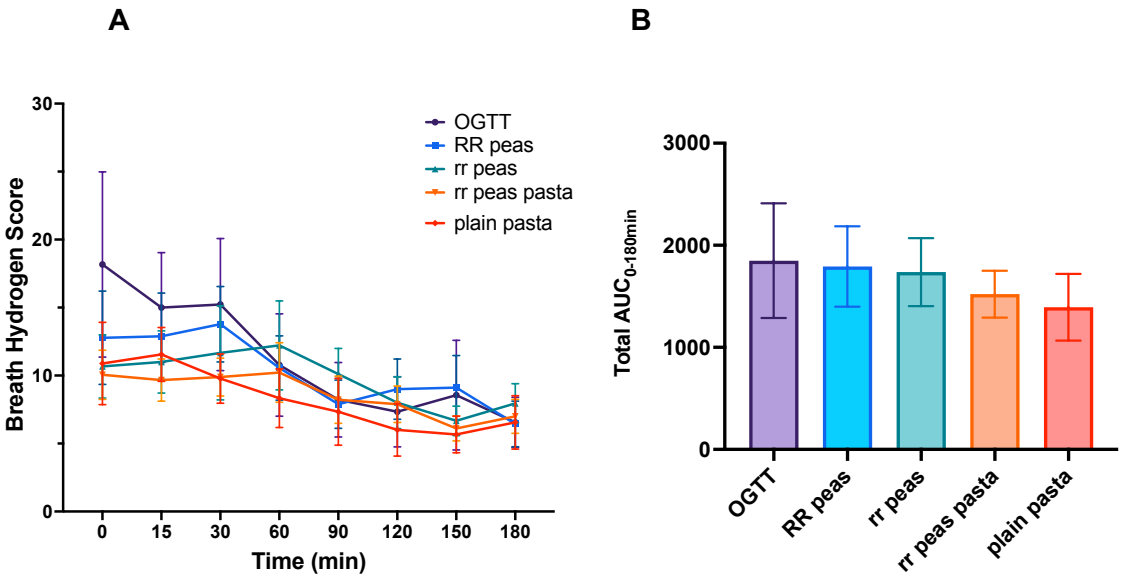


Figure 3.6 **(A) Time course data for the total score of breath hydrogen (B) Total $AUC_{0\text{-}180}$ breath hydrogen score. Data represent mean ±SEM (n=11).** Two-way repeated measures ANOVA was used to determine the effect of treatment and time and Fisher's post hoc tests were performed between time points. One-way ANOVA test was used to determine the difference in $AUC_{0\text{-}180min}$ among all treatments. No significant difference was observed between treatments.

4.0 DISCUSSION

T2D is a major health problem worldwide. To prevent and manage T2D, the consumption of low GI foods aiming at lower postprandial glucose release and insulin is encouraged. This study investigated how consumption of novel foods such as *rr* peas and *rr* peas pasta affected GI and glycemic metabolic biomarkers, including plasma glucose, serum insulin, appetite, breath hydrogen and sensory scores. These aspects are important for their subsequent application in T2D prevention and management as well as consumer acceptance.

4.1 *rr* Peas

The *rr* peas were found to have a low GI (15 ± 4) followed by low postprandial blood glucose and insulin responses compared to *RR* peas. Although the GI of *rr* peas has not been extensively studied before, the lower postprandial glycaemia compared to *RR* peas, has been observed by Petropoulou et al (2020). As discussed in 1.4.2.1.1, this effect is mainly due to higher RS content found in *rr* peas (57%-71%) compared to *RR* peas (33%-50%).

Thus, *rr* peas, as a low GI food, have the potential to be used for the production of commonly consumed foods aiming at lowering postprandial glycemia and insulinaemia and for optimizing blood glucose metabolism and potentially preventing T2D.

4.2 rr Peas Pasta and Plain Pasta

Results showed that the *rr* pea pasta had a medium GI (65 ±16), resulted in lower postprandial blood glucose and significantly lower insulin response than plain pasta. This suggested that *rr* peas flour used to replace 30% durum wheat flour in pasta played a major role in lowering GI, postprandial blood glucose and insulin levels. As mentioned in 1.3, the consumption of foods that cause significant blood glucose increase and high insulin requirements are the main causes of insulin resistance and T2D. Thus, the reduction in glycaemia and a significant reduction in insulin response after consuming *rr*

peas pasta showed advantages for its further possible use in the prevention and management of T2D.

In line with the results of the study by Goñi & Valentín-Gamazo (2003), where replacing 25% durum wheat flour with chickpeas flour significantly reduced the GI, glucose $AUC_{0\text{-}120min}$ of 12 females (GI: chickpea pasta 58±6; plain pasta 73±5). This may be due to the high amylose and RS content in *rr* peas and chickpeas. Compared with wheat starch (18% to 33%) (Buleon at al, 1998), pulses including chickpeas (28% -40%) and *rr* peas (60-76%) have higher amylose (Singh, Sandhu & Kaur 2004 & Polesi et al, 2011). Amylose molecules usually have a smaller volume and surface area than amylopectin, making it less available to amylase (Singh, Dartois & Kaur, 2010 & Parada & Aguilera, 2011). Furthermore, the RS content is higher in chickpeas (31.9%) and *rr* peas (39.8%) compared to 3% RS in wheat (Nguyen, Drawbridge & Beta, 2022 & Moongngarm et al, 2014 & Polesi, Sarmento & Anjos, 2011). As mentioned in 1.4.1, the low degree of hydrolysis of RS can lead to lower postprandial blood glucose and serum insulin (Tiboonbun, Moongngarm & Preparation, 2011 & Osorio-Díaz et al, 2014 & Afandi et al, 2018 & Wong & Jenkins, 2007). Interestingly, no statistically significant difference in GI and glycaemia was observed between plain pasta and *rr* pea pasta in this study, although *rr* pea has a high RS content. Conversely, replacing 25% of wheat flour with chickpeas flour in pasta showed a statistically significant effect on GI and glycaemia (Goñi & Valentín-Gamazo, 2003). It may be due to that only females were involved in the chickpea pasta study. Sicree et al (2008) have reported that glycemic responses to food are more pronounced in women, which are possibly related to height, muscle mass and gonadal hormones. Except for RS mentioned above, after examining the nutritional information of the tested foods, it found that the protein content in *rr* peas pasta (6.724g) was higher than that in plain pasta (3.795g). Studies have shown the inverse correlation between protein levels and GI (Senavirathna et al, 2014). Protein can potentially promote protein-starch interactions, which ultimately reduces the availability of starch granules to

digestive enzymes (Laleg et al, 2016). Finally, as discussed in the introduction, the thicker starch cell wall of pulses than wheat has been proved to reduce the permeability and effect of barrier limiting nutrition efflux and starch gelatinization, which can contribute to lower postprandial blood glucose and insulin response.

Most interestingly, Greffeuille et al (2015) reported that replacing 35% durum wheat flour with faba beans flour for pasta reduced the blood glucose response but led to a more intense and rapid insulin response of 15 healthy subjects. Protein content may be the reason for the different findings in this study. In addition to blood glucose (Wolever, 1990), protein, amino acids arginine and phenylalanine are possible hyperinsulinemia agents (van Haeften et al, 1989 & Calbet & MacLean, 2002). Greffeuille et al (2015) & Yoshimoto et al (2020) reported that high protein content, including insulinotropic amino acids in faba bean pasta and arginine and phenylalanine in peas, could promote insulin release. In this study, the low blood glucose induced by *rr* pea pasta played the main role in the limitation of insulin release. However, the higher protein content in faba bean pasta than *rr* pea pasta might be the reason why there was an increase in insulin response (protein: faba bean pasta: 12.57g; *rr* pea pasta: 6.72g).

Results showed that the appetite after consuming *rr* pea pasta was higher than plain pasta. However, the difference did not reach statistical significance, indicating that *rr* pea pasta could not result in overconsumption of subsequent meals that may further lead to drastic changes in blood glucose and insulin levels. Pulses have been thought to be able to increase satiety and reduce appetite due to the high content of RS and protein (Johnson, Thomas & Hall, 2005). However, it was not observed that *rr* pea pasta reduced postprandial appetite, possibly because the difference in RS and protein content between the *rr* pea pasta and plain pasta failed to produce a significant difference in appetite. On the other hand, the volume of food has been thought to influence satiety after consumption (Żurakowski et al, 2006). Compared to 69g consumed plain pasta, *rr*

pea pasta had smaller amounts (41g), which may potentially reduce postprandial satiety. Further research is warranted to specifically investigate how adding *rr* peas flour affected satiety. Such studies should consider higher incorporation rates of *rr* peas flour the control of amount and volume of between control and tested foods.

There was no significant difference in the levels of breath hydrogen between the *rr* pea pasta and plain pasta. Breath hydrogen can act as an indicator of the amount of carbohydrates that are inhibited for digestion in the small intestine as it measures the carbohydrate level that is fermented by *coliform* bacteria (Oku et al, 2009). Lack of significant differences may be due to that it takes longer for the fermentation of undigested complex carbohydrates. Compared to rapid changes in postprandial concentrations of blood glucose and insulin, H_2 is produced and increased for a longer time (Nakamura, Oku & Ichinose, 2004). Thus, the observed period (180min) in this study was not enough to observe a difference in H_2 levels. Nakamura, Oku & Ichinose (2004) found that the blood glucose response curve stabilized from 90min post-ingestion, however, the breath hydrogen levels began to increase at 120min, reached a peak at 180min, and maintained the peak until 360min. Future studies need to observe postprandial H_2 levels for a longer period to determine whether adding *rr* peas flour to pasta promotes large intestinal fermentation.

Importantly, similar sensory characteristics between the *rr* pea pasta and the plain pasta suggested the potential for its widespread consumption as a staple food. In general, foods with sensory scores that are too low may be difficult to be widely accepted, but too high may lead to overeating. Therefore, it is achievable for *rr* pea pasta to replace the existing commercial staple food to optimize blood glucose metabolism due to good sensory as a commercial product.

4.3 rr pea pasta and plain pasta

Results indicated that *rr* peas pasta had a statistically significant higher GI and postprandial glucose response than whole *rr* peas. Most interestingly, compared to whole *rr* peas, the insulin $iAUC_{0\text{-}120min}$ response after consuming *rr* pea pasta was significantly lower. This suggested that although the postprandial blood glucose caused by *rr* pea pasta was higher, its insulin-lowering effect exhibited advantages in preventing insulin resistance and protecting the normal function of β-cells. Therefore, *rr* pea pasta can be considered to be effective for T2D and its related metabolic diseases prevention.

In line with results reported by Marinangeli, Kassis & Jones (2009), a statistically significant higher level in the GI and glucose response of yellow pea pasta (30% yellow pea flour) than whole boiled yellow peas (GI: pea pasta: 59.9±11.9; plain pasta: 93.3±9.4). This may be related to the nutrients mentioned above. The higher RS, amylose, fibre and protein of pulses than wheat results in lower postprandial glucose response and GI (Jenkins et al, 2000 & Frid et al, 2000). After checking the nutrient composition, the content of protein (21.18g and 6.74g, respectively) and fibre (32.30 and 3.49, respectively) in *rr* peas was much higher than that in *rr* pea pasta. The industrial processing of pasta also contributes to the increased glycemic effect. Petropoulou et al (2020) and Oboh & Agu (2010) reported that the milling or grounding of pulses could reduce its beneficial effects on glycaemia. As discussed in the introduction, milling leads to the rupture of starch cell intactness, reduced granules size, and increased surface area, which promotes its contact with digestive enzymes and gelatinization, thereby causing an increase in postprandial blood glucose (Holm et al, 1988 & Holm et al,1989). This is supported by a clinical study involving ileostomy patients, coarse porridge endosperm (2mm particles) was digested more slowly than smooth porridge endosperm (<0.2mm particles), and AUC of postprandial glucose and insulin were 30% and 40% higher than those of the former (Edwards et al, 2015). Milling is also a high shear process that reduces RS content (Devi et al, 2009 & Birt et al, 2013), which may be a possible reason for the higher glycemic response of *rr* pea pasta. Nayak, Berrios & Tang (2014)

found that mashing processing of potatoes significantly reduced the RS content from 69.05% to 2.08%. In addition, extrusion, exposing large surface area of pasta to boiling water and industrial drying processes used for pasta production may destroy anti-nutritional factors including polyphenols and phytates (Marinangeli, Kassis & Jones, 2009), which are able to limit glucose absorption (Thompson et al, 1984 & Alonso et al, 2000).

To our knowledge, no study compared the insulin response between pulses and pulses pasta before. However, lots of studies have reported that compared with wheat-based foods, pulses elicit lower glycemic responses, and insulin responses have shown a similar trend to glycemic responses, with lower insulin responses following ingestion of pulses than wheat food products (Olmedilla-Alonso et al, 2013 & Schäfer et al, 2003). This suggests a difference from the results of this study, which may be related to the presence of high protein content in whole *rr* peas. The study of Schäfer et al (2003) was designed to have approximately the same nutritional content, thus, protein did not affect the results. While checking the nutritional information of the tested food in this study, compared with *rr* peas pasta containing 6.72g protein, the protein content in whole *rr* peas was much higher (21.18g). As mentioned above, insulinotropic amino acids in the protein of peas can promote insulin release (Yoshimoto et al, 2020). Nuttall et al (1984) also found that insulin responses were significantly increased with an increased protein content of consumed foods. Thus, although glucose is the main stimulant of insulin release (Olmedilla-Alonso et al, 2013 & Schäfer et al, 2003), the large difference in protein content of the two tested foods dominated the difference in insulin release, which ultimately led to the high protein whole *rr* peas having higher insulin secretion than *rr* pea pasta.

The appetite after consuming *rr* pea pasta was significantly higher than whole *rr* peas, indicating the risk of increased energy intake at the meal after pasta consumption. In line

with the report that pulse consumption has the potential to improve satiety and reduce food intake (Ramdath, Renwick & Duncan, 2016). This may be associated with higher fibre in pulse, especially RS, as *rr* pea pasta only contains 30% RS-riched peas and this amount might be reduced after milling. As stated in 1.4, consumption of RS has been considered to improve satiety and reduces appetite and food intake through promoting producing gut hormones (PYY and GLP-1) (Puertollano et al, 2014). Al-Mana & Robertson (2018) found that 10 overweight males had a significantly lower dinner energy intake after eating breakfast and lunch containing 48 g of RS. Secondly, compared with whole *rr* peas, *rr* pea pasta may have a shorter chewing time due to reduced firmness, which were thought to be able to lower the appetite score (de Graaf, 2012 & Zijlstra et al, 2009). This study did not investigate RS content and gut hormone content after consuming tested foods, therefore, cannot provide reasons for the difference within the scope of this study.

No significant difference on breath hydrogen was observed between whole *rr* peas and *rr* peas pasta, however, it cannot be explained as a similar fermentation in the large intestine after consuming these two foods. Muir et al (1995) showed increased breath hydrogen after the ingestion of higher RS during a period of 8h. However, in this study, the observed period might be not enough to determine whether there is a difference. Further study needs to measure breath hydrogen levels for a longer period after meals to determine the rate and level of fermentation of tested foods in the large intestine.

It should be noted that *rr* pea pasta had significantly higher scores for most of the sensory characteristics than whole *rr* peas. Even though the health benefits of pulses have been recognized, one of the reasons pulses are limited as a widely consumed staple food is the sensory shortcomings (Bresciani & Marti, 2019). Thus, the better sensory of *rr* pea pasta suggested its potential for daily consumption. However, the aroma of rr pea pasta was not significantly higher than whole *rr* peas, this may be related to the several

molecules in peas including 1-pentanol and fatty acid oxidation (Trindler, Kopf-Bolanz & Denkel, 2021). In line with the results that adding pea flour to food products induced unpleasant odours (Marinangeli, Kassis & Jones 2009 & Saint-Eve et al, 2019). There is a need for new methods to reduce the undesirable odours, such as reducing the ratio of peas flour added to the products or adding other products which can formask the odours. In any case, the superiority shown by *rr* pea pasta in most sensory aspects indicated its wider consumption potential as a derivative of *rr* peas than whole *rr* peas.

4.4 Strength and Limitations (Future Work)

The main advantage of this study was the investigation of multiple outcomes, allowing a comprehensive understanding of the effects of *rr* peas and *rr* pea pasta on glucose metabolism. Most of the current studies in the literature only discuss 1-2 aspects of GI, glucose response, insulin, sensory, appetite or breath hydrogen. This does not allow for an in-depth analysis and explanation of the strengths and weaknesses of the food products in terms of glucose metabolism and health benefits. Another advantage of this study was that the design of the intervention and control supported a more comprehensive interpretation of the effects of *rr* pea pasta on blood glucose. Using plain pasta as a control, it was possible to analyse the effect of *rr* pea flour on lowering blood glucose and insulin and to analyse the potential commercial use based on sensory testing. Using whole *rr* pea as control can gain insight into the effect of processing and the proportion of pea flour added on the health benefits of *rr* pea pasta and provide ideas for the production and optimisation of future prototype products of *rr* pea.

However, this study had some limitations. First, this study measured GI, postprandial blood glucose and serum insulin in healthy men and women but did not verify whether *rr* peas and *rr* pea pasta can provide health benefits to patients with T2D or prediabetes. To further investigate and expand its health benefits, its effects need to be validated in subjects including patients with T2D.

Second, this study used an acute study design, but it is unclear what effect long-term continuous intake of *rr* peas and *rr* pea pasta has on glycemia, insulin (insulin resistance). Therefore, there is a need for long-term intervention studies.

Third, modifications in pasta production conditions may further increase its advantages in glucose metabolism. For example, studies can increase the proportion of *rr* pea flour in pasta or change the production processing of *rr* pea flour, such as replacing milling with cutting or chopping that is less damaging to plant tissue.

Additionally, further research is needed on the principle of the effects of *rr* pea pasta and *rr* peas on blood glucose and serum insulin. An in *vitro* trial and intestinal sample analysis may help researchers to observe starch in different stages including RS content and starch morphology, etc explaining the rationale for its dominant role in blood glucose metabolism. Moreover, further research needs to further investigate the effects of *rr* peas and *rr* pea pasta on appetite, breath hydrogen and gut hormone levels, such as GLP-1 and PYY, which could help explain the relationship between changes in appetite, RS and gut fermentation. Finally, glycemia, insulinaemia, sensory and appetite of *rr* pea pasta should be tested in larger samples (more ethnic groups) to validate their effectiveness and incorporate them into diets around the world.

5.0 CONCLUSION

In conclusion, the present study demonstrated that *rr* pea has a low GI, and lower glycaemic and insulinaemic response than *RR* peas. Adding *rr* pea flour as the partly ingredient to pasta could provide a consumer-friendly food product that showed positive results in reducing GI, glycaemic and insulinaemic responses. Moreover, although *rr* pea pasta has a higher glycemic response compared to whole *rr* peas, this study shows its advantages in reducing insulin levels and has better sensory characteristics.

Thus, we conclude that *rr* peas can act as a low GI food and pasta, partly made from *rr* pea, can potentially act as a novel staple food. If the further study can have a larger number of participants and involve T2D patients and longer follow-up, *rr* pea pasta might be able to act as a substitute for current commercial products and could help reduce the risk of T2D and related diseases caused by high blood glucose and insulin.

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7.0 APPENDIX

7.1 Appendix 1: Pre-screening Questionnaire

Peas Study

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?	
Do you have any allergy or food intolerance?	
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	

GP name and address	

7.2 Appendix 2: Participants Information Sheet (PIS)

Information Sheet for Research Participants

Dietary resistant starch from plant-based foods for healthy glucose homeostasis

STUDY 6 - Evaluating the Impact of pea products, high in resistant starch content, on blood glucose.

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 resistant starches on glucose. 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 commonly consumed food products containing resistant starches found naturally in peas. The aim of this study is to see if resistant starch improves glucose metabolism by lowering plasma glucose and insulin in the blood.

This study was reviewed and received favourable opinion by London 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 £25 for each visit plus up to £15 for travel expenses for each visit.

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, and ensure that you are not anaemic or diabetic) and height, weight and blood pressure measurements will also be taken. All women of child bearing age will have a pregnancy test.

The health screening should last about 1 hour.

Study Days 1, 2, 3 and 4:

On the day before each study day we will ask you to complete a 24 hour food diary in which you write down all the food and drink that you have consumed during those 24 hours. You will also be asked to refrain from strenuous exercise, caffeine and alcohol. You will then be requested to fast overnight (you are allowed to drink water).

On each study day, you will be asked to arrive at the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital at approximately 9:00am. 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 9:30am you will consume a standard breakfast containing bread or glucose once and for the rest of the visits a breakfast that will contain different food products high in resistant starches made from peas.

You will also be asked to breathe into a breath analyser every 30 min after consuming the different type of breakfast meal tests (control and intervention) so that we can measure your breath hydrogen levels. This will provide us with another measurement of how quickly the different starches eaten at breakfast have moved through your body.

During the study you will have blood samples taken 8 times at certain time points for plasma glucose and insulin measurements. For each time point, we will collect about 4 ml, which is about a teaspoon. A total of 34ml of blood will be taken. These will be taken through the plastic cannula.

During the study you will be asked to fill in questionnaires (visual analogue scales) to measure your feelings of hunger and nausea.

We will also ask you to collect urine each time you go to the toilet during the study visit. You will be provided with an appropriate measurement container to collect urine and will be asked to note the total volume following each collection.

Measurements will be taken for 3 hours after you eat the breakfast. At the end of the study, we will remove the cannula and you will be able to go home. During the course of the study day you will be able to read or watch DVDs if you wish.

We request that you do not start any other new diets or intensive exercise regimes in-between the four study visits as this may give us conflicting results. There would also need to be a period of at least 3 days between study visits.

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.

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

COVID-19 Precautions

Due to the current situation we are taking every precaution to ensure the safety of our participants. Researchers will be wearing masks throughout the visit and request that you do the same unless you are medically exempt. Researchers will wear gloves and aprons when performing measurements where it is necessary to have direct contact with you. There will be hand sanitiser available in every room in the research facility. Additionally, we will need to complete a COVID-19 risk questionnaire before entering the facility.

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 harm or injury as a result of taking part in this study, you will 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;). 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 Research Governance and Integrity Team.

Study Investigators Contact details

Name: Gary Frost

Role: Principle Investigator

Email: g.frost@imperial.ac.uk

Phone number: +44 20 3313 8037

Research Governance and Integrity Team details

Name: Becky Ward

Role: Research and Governance and Integrity Manager

Email: becky.ward@imperial.ac.uk / RGIT@imperial.ac.uk

Telephone: +44 207 594 9459

What if you discover something about my health?

With your consent, we will inform your General Practitioner (GP) or Doctors' Surgery that you are planning on participating in this study. If there are any abnormal test results, we will ensure you are informed as soon as possible, and the information will be passed on to your GP if further action is necessary. The study doctor will be available to discuss any concerns you may have about your health in relation to the study.

What will happen to the results of the research study?

When the study has been completed, we will produce a report detailing the results. We would also be happy to send a copy of your results to yourself and your GP if you so wish. None of the results will hold information allowing for the personal identification of individual participants.

Imperial College London is the sponsor for this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Imperial College London will keep your personal data for 10 years after the study has finished in relation to data subject consent forms and primary research data.

We will need to use information your information in order to conduct this research project.

This information will include your: initials, NHS number, name, contact details.

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

LEGAL BASIS

As a university we use personally-identifiable information to conduct research to improve health, care and services. As a publicly-funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study.

Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research.

SHARING YOUR INFORMATION WITH OTHERS

For the purposes referred to in this privacy notice and relying on the bases for processing as set out above, we will share your personal data with certain third parties.

- Other College employees, agents, contractors and service providers (for example, suppliers of printing and mailing services, email communication services or web services, or suppliers who help us carry out any of the activities described above). Our third party service providers are required to enter into data processing agreements with us. We only permit them to process your personal data for specified purposes and in accordance with our policies.

WHAT ARE YOUR CHOICES ABOUT HOW YOUR INFORMATION IS USED?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

- We need to manage your records in specific ways for the research to be reliable. This means that we will not be able to let you see or change the data we hold about you.
- If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study.

WHERE CAN YOU FIND OUT MORE ABOUT HOW YOUR INFORMATION IS USED

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- by asking one of the research team
- by sending an email to Katerina.petropoulou12@imperial.ac.uk, or
- by ringing us on 07428732523

COMPLAINT

If you wish to raise a complaint on how we have handled your personal data, please contact Imperial College London's Data Protection Officer via email at dpo@imperial.ac.uk, via telephone on 020 7594 3502 and/or via post at Imperial College London, Data Protection Officer, Faculty Building Level 4, London SW7 2AZ.

If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO). The ICO does recommend that you seek to resolve matters with the data controller (us) first before involving the regulator.

Contact for Further Information The researchers and doctors involved in the study, Professor Gary Frost and Dr Katerina Petropoulou (Katerina.petropoulou@imperial.ac.uk, **tel:** 07428732523), 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

Thank you for interest in this study!

7.3. Appendix 3: Informed Consent

Participant Consent Form

Dietary resistant starch from peas for healthy glucose homeostasis

STUDY 6 - Evaluating the Impact of pea products, high in resistant starch content, on blood glucose.

Study Protocol Number:

Name of Principal Investigator: Prof. Gary Frost

Please initial the box if you agree with each statement.

1. I confirm that I have read and understand the subject information sheet dated..... version ..... for the above study and have had the opportunity to ask questions which have been answered fully. ☐

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

3. I understand that my participation in this study in confidential and that any data collected will not be personally identifiable ☐

4. I give permission for sections of any of my medical notes to be looked at by responsible individuals fro Imperial College London, from Imperial College Healthcare NHS Trust or from regulatory authorities where it is relevant to my taking part in this research ☐

5. I give permission for my GP to be informed of my participation in the study and for my GP to be informed of my health if something is discovered that I am not aware of. ☐

6. I give / do not give (delete as applicable) consent for information collected about me to be used to sup ☐ other research in the future, including those outside of the European Economic Area (EEA).

7. I give / do not give (delete as applicable) consent for samples collected during this study to be used in future ethically approved studies. I give permission for my samples to be sent to other organizations, including those outside of the European Economic Area (EEA). ☐

8. I agree to have my bloods taken.

9. I give consent that if I withdraw from the study after blood samples have already been taken, these sa ☐ s can be used for future analysis given that they will not be personally identifiable to me.

10. I agree to take part in this study

11. I give/do not give (delete as applicable) consent to being contacted to potentially take part in other research studies. ☐

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

7.4. Appendix 4: Questionnaires on Appetite (Visual Analogue Scale)

Subject:..... Visit: Time:

HOW HUNGRY DO YOU FEEL RIGHT NOW?

NOT AT ALL EXTREMELY

HOW FULL DO YOU FEEL RIGHT NOW?

NOT AT ALL EXTREMELY

HOW THIRSTY DO YOU FEEL RIGHT NOW?

NOT AT ALL EXTREMELY

HOW STRONG IS YOUR DESIRE TO EAT?

NOT AT ALL EXTREMELY

HOW SICK DO YOU FEEL RIGHT NOW?

NOT AT ALL EXTREMELY

7.5. Appendix 5: Food Rating Form

Subject ID:	**Initials:**
Visit 1: ☐ **Visit 2:** ☐ **Visit 3:** ☐ **Visit 4:** ☐	**Date:**

1) Please tell us how much you like the soup **OVERALL.**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

2) Please tell us how much you like the **APPEARANCE** of the soup.

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

3) Please tell us how much you like the **AROMA** of sample the soup.

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

1	2	3	4	5	6	7	8	9

4) Please tell us how much you like the **FLAVOUR** of the soup.

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

5) Please tell us how much you like the **TEXTURE** of the soup.

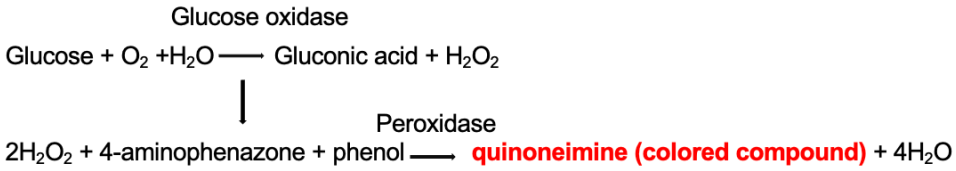
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

7.6 Appendix 6: Blood glucose Samples Analysis

Postprandial blood glucose analysis was performed using the Randox GOD-PAP kit (Randox, GL364, 10×100ml). The experimental process is shown in Figure 2.5.2. Plasma glucose blood sample (20 µl) and standard (20 µl) were mixed with 1000 µl solution (mixture of buffer and GOD-PAD reagent), respectively, and incubated for 10 min at 37°C. Then a mixture of 200 µl was transferred to a microplate and a spectrophotometer (FLUOstar Omega Multi-Mode Microplate Reader with CCD-based Spectrometer, BMG LABTECH, Germany) was used to measure its absorbance at 540nm. Glucose concentration was calculated based on the equation below using Microsoft Excel version 16.52. The experimental principle is that glucose in the sample is catalyzed by glucose oxidase into gluconate and hydrogen peroxide. Then the peroxidase catalyzes the hydrogen peroxide to produce colored products phenol and 4-aminophenylhydrazone, forming the indicator - red purple quinonimine dye. The spectrophotometer mentioned above was used to measure the absorbance of quinonimine, which is proportional to the concentration of glucose in the sample and standard (Barham & Trinder, 1972).

$$\text{Glucose concentration (mmol/l)} = \frac{\text{A sample}}{\text{A standard}} \times \text{Standard Conc (mmol/l)}$$

*A=absorbance
standard concentration= 5.47 mmol/L *(ranged for each kit)



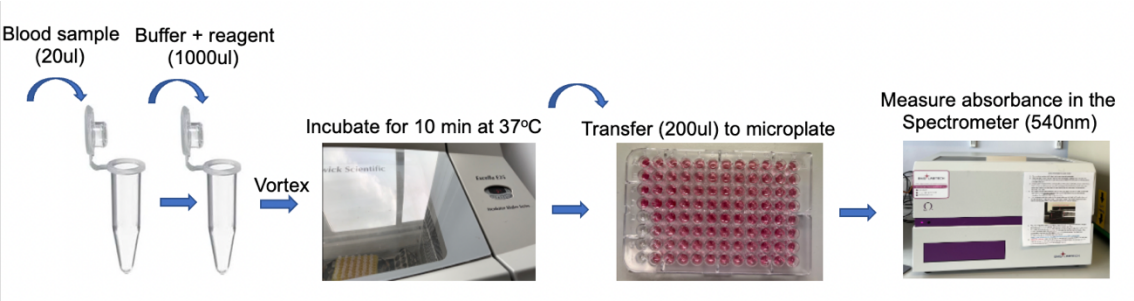


Figure 2.5.2 **The Procedures of Glucose Analysis using Randox GOD-PAP Kit**.

All assays were performed in duplicate to improve the accuracy of the results. Samples from five visits of one participant were performed in one microplate to avoid variability within the analysis of participants' samples. The coefficient of variation (CV) for this study was 1.9%, which is a measure of relative variability used for Randox GOD-PAP kit (CVs below 5% were accepted). This study performed 11 separate glucose measurements and the CV given above was the mean of all assays performed.

7.7. Appendix 7: Insulin samples analysis

The insulin analysis was performed using the Human/Canine/Porcine Insulin Quantikine ELISA Kit (Bio-techne, Minnesota, USA) according to the manufacturer's specifications. For this technique, a 96-well plate is used and coated with the capture antibody which binds to the insulin in the serum sample. The detection antibody is then added, and it binds to insulin molecules. Thus, the insulin is sandwiched between these two antibodies. Following this, the streptavidin- horseradish peroxidase (HRP-B) is added which binds to the detection antibody and a colouring agent is following added which reacts with streptavidin-HRP-B to produce the coloured products. Finally, the optical density is measured using a spectrophotometer which indirectly indicates the insulin concentrations.

The procedure of this assay is shown in Figure 2.5.3. The protocol was divided into two main steps: plate preparation and assay procedures. To prepare the plate, the capture antibody (100 µl 2ug/ml) was added to the microplate and incubated overnight (24h) at room temperature. Then the plate was washed using the provided washing buffer and the reagent diluent (300µl) was added to the plates which were incubated at room temperature for at least 1 hour. After that, the microplate was washed with wash buffer. To perform the assay, serum samples or standards (100 µl) were added to the prepared microplate and incubated for 2 hours at room temperature. After washing the microplates, the detection antibody (100 µl 200 ng/ml) was added and incubated for another 2 hours. Then streptavidin- horseradish peroxidase (HRP) (100 µl) was added and incubated for 20mins, and the plates were washed again followed by adding a colouring agent (100 µl) incubating for 20mins. Finally, the 0.16M sulfuric acid (50 µl) was added the plate and the optical density of the plates was determined using a spectrophotometer at 450nm and 540nm (FLUOstar Omega Multi-Mode Microplate Reader with CCD-based Spectrometer, BMG LABTECH, Germany) later.

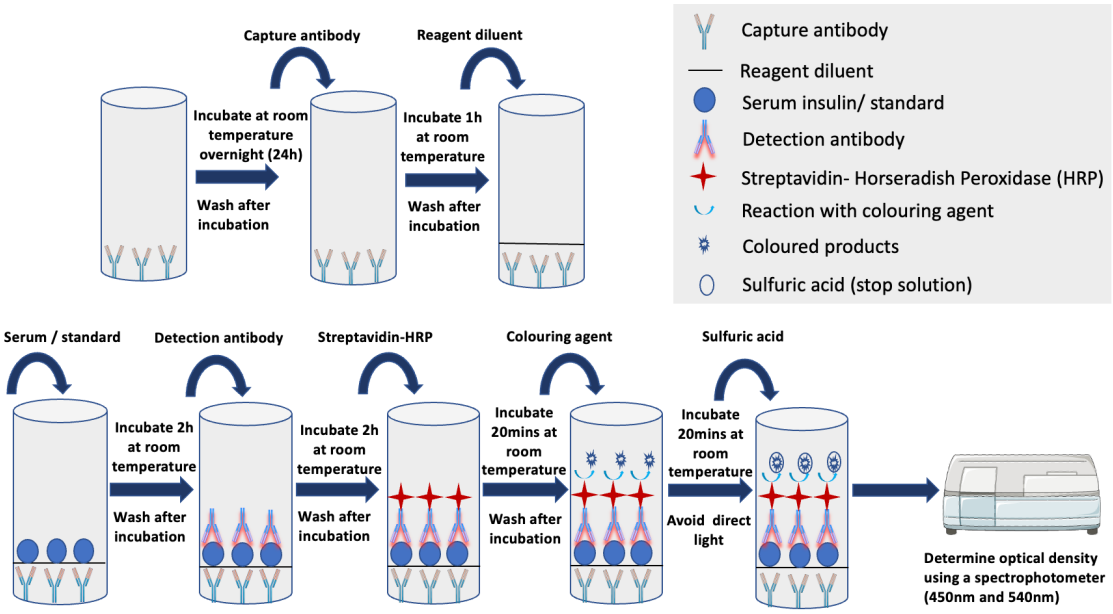


Figure 2.5.3 **The Procedures of Insulin Analysis using Human/Canine/Porcine Insulin Quantikine ELISA Kit**

The standard curve was prepared by serially diluting the insulin standard in seven vials containing 1000 µl, 500 µl, 250 µl, 125 µl, 62.5 µl, 31.25 µl and 15.63 µl standard concentrations. All assays were performed in duplicate to improve the accuracy of the results. Samples from five visits of one participant were performed in one microplate to avoid variability within the analysis of participants' samples. The coefficient of variation (CV) for this study is 5%. This study performed 10 separate glucose measurements and the CV given above was the mean of all assays performed.