The effect of pulse consumption on carbohydrate digestion, markers of glycaemia and satiety

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Submitted in accordance with the requirements for the degree of
Doctor of Philosophy
The University of Leeds

School of Food Science and Nutrition

2022

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others. Details of the jointly-authored publications and contribution of each authors are outlined on the next page.

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Chapter 2 is the publication**:**

<u>Hafiz MS</u>, Campbell MD, O'Mahoney LL, Holmes M, Orfila C, Boesch C. Pulse consumption improves indices of glycemic control in adults with and without type 2 diabetes: a systematic review and meta-analysis of acute and long-term randomized controlled trials. *European Journal of Nutrition*, **61**, 809–824 (2021). doi.org/10.1007/s00394-021-02685-y.

The candidate was responsible for conducting the systematic review by searching the databases, screening of the trials, bias assessment of the trial, and data extraction. The candidate also was responsible for conducting the meta-analyses, subgroup analyses, and writing all drafts of the manuscript. Lauren O'Mahoney had conducted the duplication for screening of the trials and bias assessment. Melvin Holmes has conducted the meta-regression and provided support in the meta-analysis. Matthew Campbell, Caroline Orfila, and Christine Bosch provided supervision, feedback on designs of the systematic review and meta-analysis and contributed to proofreading and editing of the manuscript.

Chapter 3 is the publication:

<u>Hafiz MS</u>, Campbell MD, Orsi N, Mappa G, Orfila C, Boesch C. Impact of food processing on postprandial glycaemic and appetite responses in healthy adults: a randomized, controlled trial. *Food & Function*, **13**, 1280-1290 (2022). doi.org/10.1039/D1FO02304G.

The candidate was responsible for designing the protocol of the trial, conducting the human study, analysis of the data, and writing all drafts of the manuscript. Nicolas Orsi and Georgia Mappa had conducted the multiplex measurements and analysis of the biomarkers related to the human study by using Luminex 200. Matthew Campbell, Caroline Orfila, and Christine Bosch provided supervision, feedback on design of the human study, ethical approval, and contributed to proofreading and editing of the manuscript.

Chapter 4 is the manuscript in preparation:

Hafiz MS, Holmes M, Orfila C, Boesch C. Impact of chickpea processing on *in vitro* starch digestion, and correlation between *in vitro* and *in vivo* data. *Plant food for human nutrition*.

The candidate was responsible for conducting all the experiments, analysing the data, and writing all drafts of the manuscript. Melvin Holmes has provided support in the statistical analyses of the data. Caroline Orfila and Christine Bosch provided supervision, feedback on design of the experiments and contributed to proofreading and editing of the manuscript.

List of accepted conference abstracts

<u>Hafiz, M.,</u> Campbell, M., Orfila, C., Bosch, C. Impact of pulse consumption on indices of glycaemic control – a systematic review and meta-analysis. *In: 6th International Conference on Food Digestion, Granada, Spain.* 2019.

<u>Hafiz, M.,</u> Campbell, M., Orfila, C., & Boesch, C. Chickpea processing does not impact postprandial glycaemic response. *Proceedings of the Nutrition Society*, 2020, **79**(OCE3), E786.
DOI: https://doi.org/10.1017/S0029665120007727

<u>Hafiz, M.,</u> Campbell, M., Mappa, G., Orsi, N., Orfila, C., & Boesch, C. Influence of glycaemic index on subjective appetite responses in healthy adults. *Proceedings of the Nutrition Society*, 2021, 80(OCE2), E60.
DOI: https://doi.org/10.1017/S0029665121000720

<u>Hafiz, M.,</u> Campbell, M., Orfila, C., & Boesch, C. the impact of pulse consumption on indices of glycaemic control - a systematic review and meta-analysis. *In: 3rd International Conference on food bioactives & health, Parma, Italy.* June 2022

As shown above, several publications have been attained therefore, this thesis will be presented using the thesis by publication format. The incorporation of published papers will inevitably lead to overlapping with other sections in the thesis. The thesis will be constructed by including a general introduction (Chapter 1), three chapters of results (chapters 2-4) and the final chapter will include an overall discussion and conclusion (Chapter 5).

Acknowledgement

First and foremost, I owe my deepest gratitude to Allah for his endless blessings on me and for providing me the strength, patience, and the courage to complete this PhD.

I would like to express my sincere gratitude to my supervisors Christine Bosch, Caroline Orfila, and Matthew Campbell (previous co-supervisor) for their invaluable guidance, continuous support, and endless motivation throughout this journey. I feel extremely privileged and honoured to have a chance to work under their supervision. It was impossible to complete this PhD journey without their advice, they were very understanding and encouraging during difficult times. I also would like to thank Dr. Melvin Holmes for providing me the statistical support whenever I needed.

I would like to acknowledge King Abdulaziz University and Saudi Arabian Cultural Bureau for awarding me this scholarship in order to pursue my PhD studies abroad.

I am thankful to the staff of the School of Food Science and Nutrition including all the lab technicians for their help and advice whenever I needed it. I am also very grateful to all my colleagues at the University of Leeds. In particular, I would like to thank Fatin Al-Harbi, Ganwarige Fernando (Sumali), Sadia Zulfiqar, Opeyemi Adekolurejo, and Yunqing Wang (Shirley) and many more for their invaluable friendship, and I appreciate the time we spent together that made my journey enjoyable.

A massive gratitude goes to my parents Mr. Saeed and Mrs. Asmaa: thank you for showering me with never ending love and prayers that always gave me the strength and made me achieve my dream. Thank you Dad for your being such an amazing person and father, and for the infinite ways in which you have supported me. Thank you Mum for your endless advises, prayers and guidance throughout my life. I don't think I would be able to accomplish this journey and achieve my dreams without you. I also want to thank my siblings Abdulmohsin, Abeer, Hassan, and Hamza for always believing in me, encouraging me, and supporting in every possible way.

And last but not least, I would like to express my deepest appreciation to people that are the joy of my life. My life companion, my husband Mishal, thank you for believing in me when I doubt my abilities, coping with my emotions when I was near breaking and always pushing me. I can’t capture the endless support you provided me in a few sentences. Without you, it was impossible to take a single step. My children Linah and Anas, thank you for always being there to enlighten my life. You were there through the journey, through tears and laughter, thanks for your patience with me. May this journey enlighten your life now and always.

Abstract

Pulses are important components of healthy diets, known for their low glycaemic index. However, the outcomes of the trials regarding the impact of pulse consumption on markers of glycaemic control are considerable variable. A systematic review and meta-analysis was conducted to comprehensively review the evidence from intervention studies on the effect of pulse consumption on acute glucose response and long-term glycaemic indices. The meta-analysis revealed significant reduction of -2.90 mmol/L and -1.38 mmol/L in postprandial glucose in adults with and without type 2 diabetes respectively. Long-term pulse consumption significantly attenuated fasting blood glucose in normoglycaemic populations by -0.06 mmol/L; and fasting blood glucose, glycated haemoglobin, and homeostatic model assessment of insulin resistance in type 2 diabetes adults by -0.54 mmol/L, -0.17%, and -0.47 respectively. However, the effect size (ES) varied considerably across the trials with high degree of heterogeneity with no significant effect of pulse type, dose and duration of the trial. In addition, variations in the physical form of the pulses significantly impacted postprandial glycaemic response, although this aspect has so far received little attention. Therefore, an acute postprandial study was designed in order to demonstrate the impact of food processing on postprandial glycaemic and satiety responses. The cross-over trial investigated the effect of three different physical forms of chickpeas i.e. whole chickpeas (ChW), pureed chickpeas (ChPu), and chickpea pasta (ChF) against instant mashed potatoes (Con) as a carbohydrate-matched control group. Baseline and postprandial interstitial glucose responses, captured by continuous glucose monitoring, revealed lower postprandial glycaemic incremental area under the curve (iAUC) for 3 hours after chickpea intake in comparison with the control by 57.7%, 68.8% and 59.4% for ChW, ChPu, and ChF respectively. Postprandial subjective satiety and appetite responses were determined using visual analogue scale (VAS), covering hunger, fullness, and prospective food intake, which indicated significant hunger reduction and fullness increase after intake of all three forms of chickpeas compared to the control.

Further, an *in vitro* digestion experiment was conducted using the static INFOGEST protocol, involving the food samples that were used in the human study, to compare and correlate carbohydrate digestibility in these samples with the outcomes of the *in vivo* glycaemic response study. *In vitro* digestion results revealed limited bioaccessiblity of chickpea carbohydrates compared to potato control irrespective of chickpeas being processed differently. The *in vitro* digestibility outcomes significantly correlated with the mean postprandial glycaemic responses *in vivo*.

In conclusion, the systematic analysis of pulse based intervention studies and the in vivo trial revealed that pulse consumption improve the markers of glycaemic control regardless of their physical forms. Pearson correlations indicated strong associations between the outcomes of the degree of carbohydrate digestibility of various pulses *in vitro* and their postprandial glycaemic index *in vivo* and hence simulated *in vitro* digestion techniques could be utilised to prior conducting glycaemic human studies to predict physiological response. Different processing methods have minimal effect on carbohydrate digestibility from pulses and hence do not eliminate their low glycaemic index benefits. Therefore, pulse consumption should be included in dietary guidelines as a distinctive group for regular consumption.

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Chapter 1 General introduction

Pulses are dry edible non-oil seeds that belong to the leguminoseae family and include beans (*Phaseolus vulgaris*), peas (*Pisum sativum L.*), lentils (*Lens culinaris*), and chickpeas (*Cicer arietinum L.*). Pulses are considered an excellent source of plant proteins comprising 20% to 25% of their dry weight, with good levels of essential amino acids such as cysteine, methionine, and tryptophan (Singh, 2017). Carbohydrates are the major constituent of pulses with up to 65 % of their weight, comprising of starch, oligosaccharides and soluble and insoluble dietary fibre. Pulse starch has been known to have limited bioavailability attributed to the ability of encapsulating the starch granules by intact plant cell walls, higher amylose to amylopectin ratio in comparison to other carbohydrate sources, and presence of several enzyme inhibitors such as phenolic compounds and lectins (Chung et al., 2008). Collectively, these properties have been directly linked to reduced digestibility and lower postprandial glycaemic responses after pulse intake (Hoover and Zhou, 2003). Recently, there is increased attention on promoting pulse consumption particularly due to the unique nutrient profile that provides considerable amount of plant proteins and hence considered a sustainable part of diet. Moreover, the lipid content of the pulses is considered as low and healthy attributing to the monounsaturated and polyunsaturated fatty acids along with sterols. Collectively, these nutritional properties of pulses were linked with improvement of many chronic conditions such as type 2 diabetes and cardiovascular disease. In addition, consumption of pulses has been suggested to improve lipid profile, blood pressure, platelet activity, and cardiometabolic health (Ferreira et al., 2021). There have been several studies that investigated the effect of various processing techniques on attenuating the physicochemical properties of pulses such as gelatinisation, water holding capacity and crosslinking of starch (Aparna et al., 2000, Kutoš et al., 2003, Rehman et al., 2001, Rosin et al., 2002, Siddhuraju and Becker, 2001, Negi et al., 2001, Alonso et al., 2000a). However, there is a dearth of information on the link between physical and structural characteristics of pulses and carbohydrate digestibility and postprandial glycaemia *in vivo*. Furthermore, the systematic analysis is lacking regarding the impact of pulse

intake on acute glucose responses from previously published studies. Therefore this project was designed to investigate the potential effects of pulse intake on glycaemic regulation and carbohydrate digestibility.

1.1 Thesis overall hypothesis, aim and objectives

1.1.1 Overall hypothesis

Pulse intake improves indices of acute and chronic glycaemia, and regulates satiety beneficially irrespective of the physical forms of consumed pulses.

1.1.2 Thesis aim

This PhD project was aiming to investigate the effect of pulse consumption on carbohydrate digestion, markers of glycaemia and satiety.

1.1.3 Thesis objectives

1- To systematically review acute and long-term randomised clinical trials investigating the effect of pulse intake on indices of glycaemic control among adults with and without type 2 diabetes mellitus and quantify the effect size on glycaemic biomarkers.

2- To select a suitable pulse source as well as processing methods based on the outcomes of the systematic review to design the human study.

3- To comprehensively assess the postprandial responses in measures of glycaemic and satiety control after ingestion of differently processed chickpeas in a randomised controlled crossover study in normoglycaemic adults.

4- To investigate *in vitro* the rate and extent of carbohydrate digestion in differently processed chickpeas, which were used as intervention in the human study conducted for this PhD project.

5- To correlate the outcomes of the *in vitro* digestion with the results from the *in vivo* study.

1.2 General insight on dietary carbohydrates

Dietary carbohydrates are a diverse group of biomolecules that are generally the most abundant component of the human diet, comprising a major nutrient source for energy metabolism (Cummings and Stephen, 2007). Carbohydrates are defined as "polyhydroxy aldehydes and polyhydroxy ketones and compounds resulting from their oxidation, reduction, substitution, and polymerization" (Clemens et al., 2016). As a result, the carbohydrate group comprises a wide range of compounds with a variety of chemical and physiological properties and hence there are various approaches to their classification. Carbohydrates are chemically classified according to the degree of polymerization i.e. number of sugar molecules linked by glycosidic bonds, into monosaccharides, disaccharides, oligosaccharides, and polysaccharides (Cummings et al., 1997, Cummings and Stephen, 2007, Mann et al., 2007). Monosaccharides are simple sugar units such as glucose, galactose, and fructose which are found in foods as free sugars or as constituent units of larger molecules such as disaccharides, oligosaccharides, and polysaccharides (Cummings and Stephen, 2007, Qi and Tester, 2019). Fructose, a monosaccharide sugar that can be found in honey and ripened fruits, is considered as the sweetest sugar compared to other mono- and disaccharides such as glucose and galactose (Qi and Tester, 2019). Disaccharides such as sucrose, lactose, maltose, and trehalose are molecules with two single sugar molecules linked by either α- or β-glycosidic bonds (Scapin et al., 2017). Oligosaccharides are carbohydrates consisting of three to nine monosaccharides connected by α- and β-glycosidic bonds, forming compounds such as raffinose and stachyose. These oligosaccharides can be found in many plant foods such as legumes, onions artichokes. Dietary polysaccharides consist of ten or more monosaccharide units and include starch and non-starch polysaccharides (Englyst and Englyst, 2005). Starch is the most important dietary carbohydrate consisting mainly of two types of α-glucans: amylose and amylopectin representing up to 99% of its dry weight (Tester et al., 2004). Amylose is a long linear chain of glucose monomers linked by α-1-4 glycosidic linkages with variable size (200 to 2000 linked glucose units) according to botanical origin such as wheat, corn, and pulses. On the other hand, amylopectin is a relatively large (2000 to 2 million glucose

units), highly branched molecule consisting of a short amylose backbone (20 to 30 linked glucose units) that is heavily branched through α-1-6 glycosidic linkages (Figure 1-1). The size of the amylopectin molecule and the associated branches vary considerably depending on the source. Both amylose and amylopectin chains can form double helix structures which can assemble into crystalline domains (Tester et al., 2004). The ratio of amylose to amylopectin varies among starches according to the plant origin from less than 2% amylose in waxy starches to up to 70% in high amylose starches (Van Hung et al., 2006). The amorphous and highly branched structure of amylopectin is considered more susceptible to gelatinisation and hence hydrolysis by enzymes such as α-amylase in comparison to the linear and compact structure of amylose (Li et al., 2019a). Amylopectin branches can give rise to different diffraction patterns such as type-A in which is found in cereals, type-B which is found in tubers, and type-C which is a combination of both A and B polymorphism and is found in legumes (Rodriguez-Garcia et al., 2021). Resistant starch (RS) is defined as the fraction of starch that escapes digestion in the small intestine and is therefore susceptible for fermentation in the colon. There are many food sources of RS such as legumes and green bananas, and are classified into several subtypes according to factors that affect their resistance to enzymatic degradation (Table 1-1). RS type I which is found in grains and legumes mainly resists digestion due to the presence of intact cell walls which make the starch physically inaccessible. RS type II has crystalline structure and is present in raw foods. RS type III is retrograded starch and found in cooked and cooled starchy foods. RS type IV which is a chemically modified starch that resists enzymatic hydrolysis. RS type V is a type of starch present in high amylose food which resists digestion due to interaction with lipid compounds and formation of amylose-lipid complexes (Birt et al., 2013).

Non-starch polysaccharides, also known generally as dietary fibre, are structural carbohydrates that represents the main constituent of primary or secondary plant cell walls. They are non-digestible by the human gastrointestinal system and include a group of compounds such as cellulose, hemicellulose, lignin, and pectin (Lattimer and Haub, 2010). However, as per definition of the American Association of Cereal Chemists, dietary fibre is a

generic term that includes all carbohydrates that are not digestible by the human gastrointestinal tract in addition to non-starch polysaccharides including oligosaccharides and resistant starch (American Association of Cereal Chemists, 2001). Dietary fibre, in particular non-starch polysaccharides, can be categorised according to their functional properties into water soluble or insoluble fibre. Soluble dietary fibre are compounds that bind water molecules and swell forming a viscous gel that escapes digestion in the small intestine and are largely fermented by the colonic microflora. Soluble fibres include inulin type fructans, gums, and pectins. In contrast, insoluble fibre such as lignans, cellulose, and some hemicelluloses are largely resistant to colonic fermentation due to their limited solubility in water and hence play a role in faecal bulking (Wong and Jenkins, 2007).

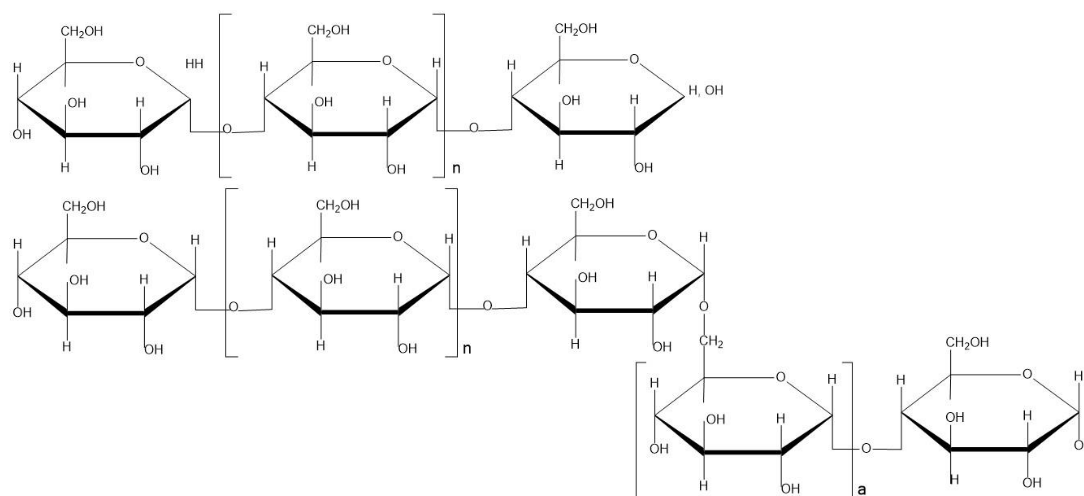

Figure 1-2 Structure of amylose and amylopectin chains. n = number of molecules in amylose and amylopectin backbone chain. b = number of branching points

Table 1-1 Type of resistant starches and their food sources

Resistant starch type	Description	Food source
RS type I	Physically inaccessible starch	Whole or coarsely grounded grain kernels or legume seeds
RS type II	Granular starch	Green bananas and high amylose maize starch
RS type III	Retrograded starch	Cooked and cooled starchy foods
RS type IV	Chemically modified starch	Cross-linked starch and octenyl succinate starch
RS type V	Amylose-lipid complex	High amylose starch

1.3 Physiology of carbohydrate metabolism

A complex set of physiological factors control carbohydrate metabolism, including hormonal and neural regulation that affect digestion, absorption and uptake of nutrients by different organs (Röder et al., 2016). Although there are many sources of continuous glucose supply to the blood stream for delivering to the organs such as carbohydrate breakdown, glycogenolysis, and gluconeogenesis; intake of dietary carbohydrates are considered as the major source (Jequier, 1994). The digestion of carbohydrates starts in the mouth mechanically by the chewing process that involves reduction of the particle size, mastication to lubricate the food with the saliva and form a bolus to prepare it for further digestion (Peyrot des Gachons and Breslin, 2016). Human saliva also contains α-amylase produced by the salivary gland which initiate the hydrolysis of carbohydrates in the mouth by cleavage of α-glyosidic bonds and the digestion can continue in the stomach until α-amylase is deactivated due to the decrease in pH (Freitas et al., 2018). The optimal conditions for the activity of the salivary α-amylase is pH 7 and 37 ˚C. When carbohydrates reach the small intestine, the degradation continues by the action of pancreatic enzymes release into duodenum and includes α-amylase into maltose, maltotriose and dextrin. Those digestible fractions are hydrolysed further into monosaccharides such as glucose by the action of brush border enzymes such as disaccharidases and α-glucosidase. The rate and extent of that process, however, varies considerably depending on the structure of the food particles, surface area, and degree of crystallinity of the starch. Several studies have shown that reducing the particle size and increasing the surface area and the crystallinity of starch content of the food particles has resulted in accelerated digestion time and increased the total digestibility (Farooq et al., 2018, Alpos et al., 2021, Martens et al., 2018). However, not all carbohydrates are susceptible to digestion by the human gastrointestinal system. Some oligosaccharides such as raffinose and polysaccharides as well as resistant starch and non-starch polysaccharides are not hydrolysed by human digestive enzymes either due to lack of the required enzymes in the human gut or due to the preservative structure that prevents enzymatic diffusion through the cells and hence these polysaccharides remain undigested. Therefore, those food particles enter the

colon undigested where some of these carbohydrates are degraded by the microflora. The microbiome consists of a wide range of microbial species with the majority being the Bacteroidetes, Firmicutes and Actinobacteria, strongly influenced, however, by short term and long term carbohydrate intake (Walker et al., 2011). These microbes encode most enzymes needed for the cleavage of the dietary carbohydrates linkages, and the enzyme families potentially associated with microbiome activity include glycoside hydrolases, polysaccharide lyases, and carbohydrate esterases (Flint et al., 2012). Degradation of the undigested structural polysaccharides and resistant starch by the gut microorganisms is suggested to account for up to 10% of dietary energy depending on the carbohydrate content of the ingested diet (Walker et al., 2011).

Following carbohydrate hydrolysis in the small intestine and glucose liberation, glucose is then absorbed and transported actively by sodium-dependent glucose transporter SGLT-1 and glucose transporters GLUT-2 located in the brush border membrane of intestinal epithelial enterocytes, followed by facilitated diffusion through glucose transporters GLUT-2 located on the basolateral membrane of the enterocytes into capillary blood (Thorsen et al., 2014). In contrast to SGLT-1, GLUT-2 is a passive transporter that permits the movement of glucose along the concentration gradient (Figure 1-2). GLUT-2 is a member of glucose transporter family that transport monosaccharides passively across the plasma membrane following concentration gradient. Those members of glucose transporters are expressed in different tissues (Table 1-2) (Pujol-Giménez et al., 2013). Moreover, SGLT-1 also plays a pivotal role in glucose induced secretion of regulatory hormones such as insulin, GIP, and GLP-1 functioning as a glucose sensor in the small intestine (Röder et al., 2014).

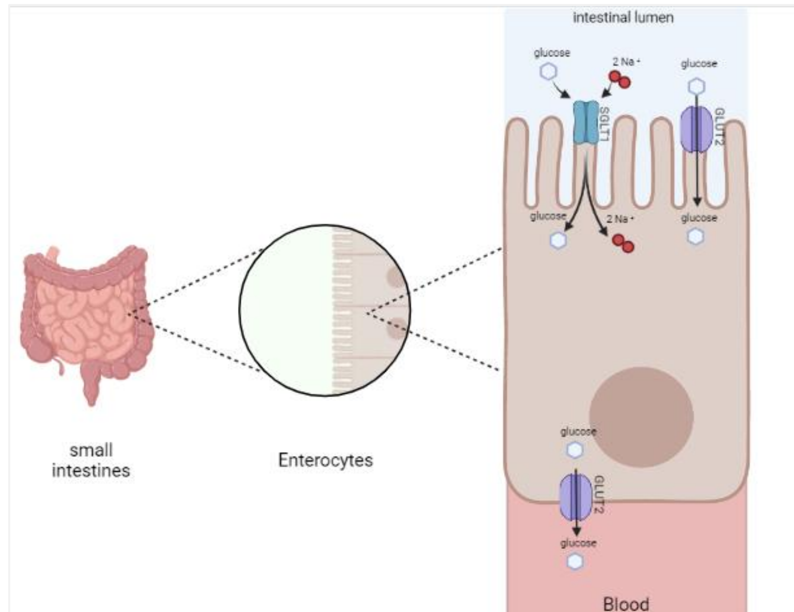

Figure 1-3 Glucose transporters across the intestinal epithelium

Table 1-2 Summary of the main features of human facilitative glucose transporters

Glucose transporter	Main tissue location	Main substrate	Insulin sensitivity
GLUT-1	Erythrocytes, brain, liver, β-cells	Glucose	No
GLUT-2	Liver, pancreas, intestine, kidneys, β-cells	Glucose	No
GLUT-3	Brain	Glucose	No
GLUT-4	Heart, muscles, adipose tissue,	Glucose	Yes
GLUT-5	Intestines, testes, kidney	Fructose	No
GLUT-6	Brain, spleen, leukocytes	Glucose	No
GLUT-7	Small intestine, colon	Glucose	No
GLUT-8	Testes, brain	Glucose	No
GLUT-9	Liver, kidney	Urate	No
GLUT-10	Liver, pancreas	Glucose	No
GLUT-11	Muscles, heart, adipose tissue	Glucose, fructose	No
GLUT-12	Muscles, adipose tissues, heart, small intestine	Glucose	Yes

The table is adapted from (Pujol-Giménez et al., 2013, Wood and Trayhurn, 2003)

1.3.1 Hormonal regulation of carbohydrate metabolism

Glucose homeostasis is determined by the balance between the rate at which glucose enters the circulation from the intestine along with the liver's production of glucose, and the rate of glucose disposal whether by uptake by various body organs such as muscles or adipose tissue or via excretion. The human gastrointestinal system is the largest endocrine organ in the body secreting many hormones such as glucagon like peptide1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP), peptide YY (PYY) and cholecystokinin (CCK) (Ahlman and Nilsson, 2001). These hormones play a crucial role in modulation of various GI functions such as gastric and intestinal motility and emptying rate, regulation of the blood flow to the GI, and regulation of secretion of other hormones, all of which have essential functions in the digestion and absorption of nutrients (Sternini et al., 2008).

1.3.1.1 Insulin

Insulin is the primary regulatory hormone of blood glucose homeostasis, that assists in regulating blood glucose levels between 3.5-5.5 mmol/L along with glucagon in humans. The synthesis of insulin is carried out by the β-cells of the islets of Langerhans of the pancreas (Guthrie and Guthrie, 2004). Elevated postprandial glucose levels in the blood (exceeding 5 mmol/L) enhance glucose uptake via the glucose transporter GLUT-2 which in turn stimulates insulin secretion (Thorens, 2015). The process involves a complex cascade of actions initiated by the uptake of glucose. Briefly, uptake of glucose by β-cells of pancreas causes escalation in the ratio of ATP/ADP through glycolysis which in turn leads to depolarisation of the cell membrane, closure of K channels, and increase in intracellular calcium by opening of calcium channels. The increment of intracellular calcium stimulates the exocytosis of insulin into the blood stream (Jouvet and Estall, 2017, Komatsu et al., 2013). The release of insulin by the pancreatic cells is also influenced by the secretion of glucagon like peptide1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) by the enteroendocrine cells in the intestinal epithelium stimulated by the ingestion of food (Drucker, 2013). Moreover, the modulation of insulin secretion was also suggested to be impacted by presence of other nutrients such as monosaccharides other than glucose

(mannose and fructose), certain amino acids such as L-leucine, isoleucine, and valine, and free fatty acids in particular non-esterified fatty acids (Jouvet and Estall, 2017, Kyriazis et al., 2012, King et al., 2018, Newsholme et al., 2006). Acute exposure of β- cells to these nutrients stimulates insulin secretion via the metabolism of those nutrients in β- cells that simulate the effect of glucose metabolism and therefore induce secretion of insulin (Newsholme and Krause, 2012).

Insulin is a blood glucose regulatory, anabolic protein hormone composed of 51 amino acids divided into two chains that are linked together by disulphide bonds (Qaid and Abdelrahman, 2016). Insulin regulates blood glucose concentration by binding to its receptors located on the cells especially liver, muscles and adipose tissue to allow uptake of glucose from the blood and thereby stimulates synthesis and storage of lipids, proteins and carbohydrates (Chang et al., 2004, Mayer et al., 2007). This occurs as a result of binding of insulin to its receptor which initiates insulin receptor signalling that is leading to the translocation of GLUT-4 to cell membrane and thereby enhancing the uptake of glucose into cells (Saltiel and Kahn, 2001). GLUT-4 is an insulin dependent glucose transporter that is located mainly in muscles and adipose tissue where it facilitates the uptake of glucose into cells of those insulin responsive organs (Bryant et al., 2002). The insulin dependent glucose uptake by these tissues stimulates synthesis of storage fat and glycogen by promoting lipogenesis and glycogen synthesis, and inhibits lipolysis and glycogenolysis as well as gluconeogenesis (Qaid and Abdelrahman, 2016, Wilcox, 2005).

1.3.1.2 Glucagon

Glucagon is a polypeptide hormone composed of 29 amino acids and produced and secreted by α-cells of pancreas. The secretion is mainly stimulated by low levels of glucose in the blood (glycaemic threshold estimated as 3.7 to 4.0 mmol/L) (Jiang and Zhang, 2003). Glucagon is a counter-regulatory hormone to insulin that maintains glucose levels by promoting hepatic glucose output through glycogenolysis and gluconeogenesis to elevate glucose levels in the blood, and prevent storage of glucose by inhibiting glycogen synthesis in liver and muscles. Glucagon

also triggers amino acid uptake and breakdown of protein by the liver and stimulate the formulation of ketone bodies from protein. Moreover, glucagon stimulates lipolysis and inhibits lipogenesis (Qaid and Abdelrahman, 2016).

1.3.1.3 Incretins

Incretins are gut derived peptide hormones produced and secreted by enteroendocrine L cells located in the small intestine and central nervous system (Campbell and Drucker, 2013). The two most widely known incretins are glucagon like peptide1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP). The secretion of incretins is primarily stimulated by food intake and in turn potentiates insulin secretion in a glucose-dependent manner accounting from 20 to 70 % of insulin response after food ingestion and is largely dependent on glycaemic load of the ingested meal (Diakogiannaki et al., 2012, Drucker, 2013, Kuhre et al., 2015). GLP-1 is a polypeptide hormone composed of 30 amino acids, and is considered a cleavage product of the pre proglucagon gene that is expressed on enteroendocrine L cells found in the distal small and large intestine sections, α cells of pancreas, and in neurons found in brainstem and hypothalamus (Kuhre et al., 2015, Shah and Vella, 2014). The postprandial response of GLP-1 occurs in two phases in response to nutrient load. The first phase occurs immediately after starting food ingestion and lasts up to 30 minutes. However, since the receptors responsible for GLP-1 are located in distal intestine, the first phase of GLP-1 release cannot be attributed to direct contact with nutrients from the gut. Therefore, it is suggested that the major deriver of the first phase is the central nervous system. The second phase of the GLP-1 release occurs later and is suggested due to direct contact of the food with receptors located in intestine (Shah and Vella, 2014).

GIP is a 24 amino acid peptide hormone secreted by enteroendocrine L cells in the small intestine along with their secretion from central nervous system (Campbell and Drucker, 2013). Both GLP-1 and GIP exert similar responses on pancreatic β- cells by potentiating insulin production and secretion and α-cells by inhibiting glucagon release. In addition to their insulinotropic action, GLP-1 hormone is shown to enhance β- cell health by conferring glucose sensitivity to glucose resistant cells, promoting proliferation, and prevent

apoptosis. Moreover, GLP-1 is suggested to downregulate hepatic glucose production, slow down gastric emptying, and reduce gut motility which in turn together affect gastric distension and hence control satiety (Drucker, 2006, Shah and Vella, 2014). In addition, GLP-1 promote satiety centrally by promoting the sense of satiety and regulating the feeding. However, it is worth to note that the insulinotropic effect of incretins is often transitory hence both hormones are rapidly degraded by the action of dipeptidyl peptidase-4 (DPP-4) and cleared by the kidney (Drucker, 2013).

1.4 Physiology of postprandial glycaemic response

1.4.1 Definition of postprandial glycaemia

Postprandial glycaemia refers to the transient surge in blood glucose levels after intake of carbohydrate containing food, that is determined by rate of blood glucose elevation caused by carbohydrate digestion and absorption; followed by gradual decline which is largely regulated by the action of insulin-dependent glucose metabolism (Sheard et al., 2004). Fasting blood glucose in normoglycaemic individuals tends to be regulated between 3.9 to 5.4 mmol/L, the values however vary depending to factors such as gender, age, and lifestyle factors (diet and physical activity). After ingestion of meals, usually around 10 to 20 minutes after consumption, blood glucose values tend to increase gradually peaking usually around 45 to 60 minutes, and return to baseline usually within 120 to 150 minutes post meal (Slama et al., 2006). Nevertheless, postprandial glycaemia is usually interpreted as a 4 hour period following meal intake (Monnier and Colette, 2006). The peak time and magnitude is largely dependent on several intrinsic human factors relating to the health status of the individuals, their lifestyle factors (i.e. physical activity, dietary habits and sleep patterns), rate of food digestion and absorption, secretion of regulatory hormones such as GLP-1, GIP and insulin; and extrinsic food related factors such as quantity of the ingested food, macro- and micronutrient composition of the meal, structural properties and digestibility of the carbohydrates ingested, and presence of compounds that inhibit activity of certain digestive enzymes such as polyphenols (Prpa et al., 2020). Carbohydrates are a major driver for the postprandial glycaemic

excursion, and therefore their digestion and absorption along with insulin and glucagon secretion, and glucose metabolism in the liver and peripheral tissues are determinants of postprandial glucose levels (American Diabetes Association, 2001). In individuals with impaired glucose tolerance, the ingestion of carbohydrate results in exaggerated and prolonged postprandial glucose excursion.

1.4.2 Contribution of postprandial glucose levels to the progression of diabetes and related complications

Clinical diagnosis of diabetes often relies on an individual's fasting glucose values, despite the fact that most people spend the vast majority of their time in a postprandial state (Berry et al., 2020). Postprandial glucose concentrations play a vital role in management of overall glycaemic control and are positively correlated with glycated haemoglobin ($HbA1_c$). In fact, it was shown that postprandial glycaemia was a better predictor of overall glucose control compared to fasting glucose (Monnier and Colette, 2006). $HbA1_c$ is a diagnostic marker that reflects the average plasma glucose over the past 2 to 3 months, by measuring the percentage of haemoglobin that has been irreversibly glycosylated. The concentrations of postprandial blood glucose were suggested to be more accurate in predicting diabetes and related complications as they were shown to be strongly associated with all-cause mortality and cardiovascular disease compared to fasting blood glucose and $HbA1_c$ in individuals with and without type 2 diabetes mellitus (Berry et al., 2020). According to The Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study group, postprandial glucose (measured at 2 hour following meal) was a stronger predictor of coronary heart disease and all-cause mortality compared to fasting plasma glucose from the data collected of 10 prospective cohort studies (Meigs et al., 2002, Group and Group, 2001, Levitan et al., 2004). The management of postprandial glucose is crucial in improvement of glucose homeostasis and prevention of its related complications (Madsbad, 2016).

1.4.3 Dietary factors that affect carbohydrate digestion and postprandial glycaemic response

There are several extrinsic factors (i.e. related to the food ingested) and intrinsic factors (i.e. related to the person eating the food) that can affect the digestibility of carbohydrates present in the food. The intrinsic factors relate to starch hydrolysis and are largely dependent on the cell wall properties, crystallinity of the starch granules, interaction with other compounds present, as well as ratio of amylose and amylopectin. These factors will be discussed in more detail below.

1.4.3.1 Effect of plant cell wall properties on carbohydrate digestion and postprandial glycaemia

The cell wall is a distinctive component of plant cells that provides additional protection to the cells by maintaining structural integrity, as well as acting as a barrier against external stress. The walls of plant cells are sophisticated supramolecular assemblies composed of cellulose embedded in a hydrated matrix of hemicellulose, pectin and protein forming primary and secondary cell wall (Houston et al., 2016). Lignin can also be found in secondary cell walls. The proportional composition and organisation of those compounds is substantially variable across different species of plants along with tissue type (Zhang et al., 2021). The variation in these composition result in disparity of cell wall properties such as porosity, permeability, viscosity, cell separation and rupturing, which in turn significantly affect the rate and degree of starch digestion, absorption and bioaccessibility of the encapsulated nutrient (Grundy et al., 2016). To digest starch granules, the enzyme should be able to access intracellular starch granules. This can be done either by diffusion of enzymes through the pores of the cell, or by rupturing if the cell walls that have limited permeability which is the case in legumes (Dhital et al., 2017). The cell wall of cereals (monocotyledonous) is known as a type II primary cell wall which is rich in arabinoxylans and low in pectins, while legumes (dicotyledonous) have type I primary cell wall which is low in arabinoxylans and rich in pectic polysaccharides. These differences were shown to result in marked disparity in digestibility profiles despite being treated under the same conditions (Edwards et al., 2021).

1.4.3.2 Morphological properties

Starch granules are composed of highly organised molecules of amylose and amylopectin chains. The ratio and the arrangement of amylose and amylopectin chains within starch influence their crystalline structure and physiochemical properties (Tester et al., 2004). The ratio of both polysaccharides varies according to the botanical origin of the plant. However, most starches comprise 20-30% amylose and 70-80% amylopectin (Lovegrove et al., 2017). They are deposited in specialised plastids called amyloplasts forming highly organised granules varying in their shapes and sizes across different species (Tester et al., 2004). Amylopectin with highly amorphous structure is prone to gelatinisation and hydrolysis in comparison to amylose compact structure. The higher susceptibility of amylopectin to gelatinisation results in protracted enzymatic hydrolysis and hence increased digestibility compared to amylose. Therefore, it has been shown that starches with naturally high amylose content such as legumes, or intentionally modified such as high amylose rice, tend to have limited gelatinisation under normal cooking temperatures and therefore demonstrate lower availability for digestion and reduced postprandial glucose levels (Haralampu, 2000). The organisation of amylose and amylopectin chains is another important factor that determines the crystallinity of the starch and the digestibility profile. There are three known polymorphs of starch crystals, type A, B, and C. Type-A polymorph which is mainly found in cereals, is composed of a relatively shorter chain of amylopectin (23-29 glucose units) compared to type-B, linked to each other by hydrogen bonds forming outer double helical structure with linear chains of amylose between them (Rodriguez-Garcia et al., 2021). Type-B polymorph structure is composed of longer amylopectin chains (30 to 44 glucose units) with water molecules inter-spread. This type of structure is common in raw potatoes and green bananas and known for limited digestibility when native. However, the structure can be disrupted by gelatinisation (Sajilata et al., 2006). Type-C polymorph is common in legumes and is a combination of both A and B, with B polymorph is located centrally and a polymorph is located peripherally. This unique characteristics of this polymorph allow it to resist immediate disruption of the crystallinity when susceptible to hydrothermal processing (Sarko and Wu, 1978, Guo et al., 2017)

1.4.3.3 Food processing methods

Different processing methods have been shown to affect digestibility of carbohydrates and amount of resistant starch and hence may impact on postprandial glycaemic response (Mahadevamma and Tharanathan, 2004). Hydrothermal processing in particular can cause dramatic irreversible changes in starch granules by gelatinisation resulting in loss of molecular order. The process involves high temperatures with excess water content that cause swelling of amorphous regions and collapse of the crystalline structure, leading to increased susceptibility to enzymatic breakdown (Matignon and Tecante, 2017). The process, however, is largely affected by various factors such as amylose/amylopectin ratio and permeability of the cell wall to water, and extrinsic factors such as water content and temperature of the cooking (Matignon and Tecante, 2017). The gelatinisation of starch is often followed up by gradual retrogradation when the temperature is decreased which involves re-association of the chains to produce a form that is more resistant to digestion. However, the degree of retrogradation varies according to the amylose and amylopectin ratio. This is due to the differences in tendencies of both chains to retrogradation. Amylose chains retrograde to a greater rate and extent compared to the branched amylopectin chains (Matignon and Tecante, 2017). On the other hand, mechanical processing methods such as milling can affect the digestibility profile by disrupting the integral properties of the cells leading to loss of protective barrier properties of the cell wall and hence increased susceptibility of the intracellular starch to enzymatic hydrolysis (Edwards et al., 2021). The process involves reduction of the particle size by applying physical forces that result in increased surface area and therefore enhances susceptibility to amylolysis and digestion (Li et al., 2014).

1.5 Disrupted metabolic balance

1.5.1 Insulin resistance

Insulin resistance is defined as "the reduced sensitivity of tissues to insulin-mediated biologic activity" and now is being identified as an independent risk factor for CVD (Sinaiko and Caprio, 2012, Spence et al., 2010). Insulin resistance occurs when the β- cells of the pancreas produce sufficient insulin, however, insulin receptors or intermediates in insulin signal pathways gradually become insensitive and thus the glucose molecules are not able to enter the target cells (Sinaiko and Caprio, 2012). Persisting high blood glucose leads to increased production of insulin in an attempt of the body to control the hyperglycaemia. The process usually happens gradually and therefore patients often remain unaware of their condition. Whilst there are several genetic and environmental causes of insulin resistance, obesity remains the major known cause of insulin resistance. In particular, central obesity, which is characterized by a higher proportion of visceral fat and relatively lower subcutaneous fat, has been linked with insulin resistance and negative impact on metabolic health (Cirulli et al., 2018). Increased adiposity due to the obesity results in greater amounts of non-esterified fatty acids, glycerol, some hormones such as leptin and adiponectin, and pro inflammatory cytokines such as interleukin-6 and tumour necrosis factor α released by the adipocytes (Kahn et al., 2006). These obese individuals are at a significantly higher risk to develop insulin resistance as a result of an accumulation of fat in the liver and muscles, while fatty pancreas is considered a cause of insulin deficiency. This condition might be reversible in some aspects if detected early and accompanied by weight loss or muscle build up (Skyler et al., 2017).

1.5.2 Prediabetes

Prediabetes is a state that is considered an intermediate stage between normoglycaemia and diabetes. This state carries an increased risk for developing type 2 diabetes. Almost 10% of prediabetic individuals progress to diabetes every year. The same percentage of the population, however, shows improvement by reversing their condition. As developing diabetes is a process

that happens gradually over the years, almost all diabetic patients go through the prediabetes stage before progressing into manifest diabetes (Tabák et al., 2012). Several studies have shown that there is an increased risk in developing diabetic complications such as nephropathy (kidney damage), retinopathy (eye damage), neuropathy (nerve damage) and cardiovascular disease even with the intermediate stage. The pathogenesis of microvascular complication as a result of prolonged hyperglycaemic events is complex. Briefly, chronic hyperglycaemia result in increased production of advanced glycation end products associated with acceleration of polyol pathway exerting pro inflammatory activity. Collectively, these stimulate oxidative stress and increase production of cytokines, resulting in microvascular damage along with insufficient oxygen due to impaired blood flow (Papanas et al., 2011). Although the term prediabetes has been explained by numerous organizations, the diagnostic criteria vary considerably (Bansal, 2015). According to American Diabetes Association, an individual is considered as a prediabetic if they have impaired fasting glucose in the range from 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) or impaired glucose tolerance evident by elevated 2-h plasma glucose after oral glucose tolerance test 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) or $HbA1_c$ 5.7–6.4% (39–47 mmol/mol) (American Diabetes Association, 2018). On the other hand, the WHO defined prediabetes with a higher threshold of fasting glucose concentration (> 6·1 and <7.0 mmol/L) (World Health Organization, 2006).

1.5.2.1 Role of diet in prevention of diabetes

Although there is no cure once diabetes has developed, the medical and lifestyle interventions aim to control blood glucose levels and therefore reduce the complications and improve quality of life (American Diabetes Association, 2018). Prediabetes, on the other hand, is a reversible condition and lifestyle interventions have been shown to positively affect insulin sensitivity and secretion and delay the onset of the disease. The Finnish Diabetes Prevention Study and the United States Diabetes Prevention Program have reported a decreased risk of diabetes by 58% after 3 years of lifestyle modification intervention (Lindström et al., 2003, Diabetes Prevention Program Research Group, 2002). Other trials have reported similar reductions in diabetes risk in

individuals with prediabetes after lifestyle modifications for 3 years (Tuomilehto et al., 2001). These interventions were primarily based on improving the quality of the diet and increasing physical activity levels to achieve optimal body weight and hence better control of glucose parameters (Tabák et al., 2012).

To better understand the effect of specific foods on increasing the risk of type 2 diabetes, a meta-analysis of prospective studies using modifications to different food groups has been carried out to articulate a non-linear dose-response relation (Schwingshackl et al., 2017). The outcome of the analyses suggested inverse associations through decreased relative risk (RR) of developing diabetes with intake of whole grains (RR: 0.77), vegetables (RR: 0.95), fruits (RR: 0.96), and legumes (RR: 0.96). On the other hand, increased refined grains and sugar sweetened beverages consumptions were positively associated with the risk of diabetes with RR 1.01 and 1.30 respectively. Likewise, a review of meta-analyses investigated the role of several food groups in prevention of diabetes, and concluded that intake of food high in fibre such as whole grains and pulses was associated with reduction of risk for diabetes of up to 21% (Psaltopoulou et al., 2010). An observational study in 84,941 nurses over 16 years reported that adherence to high fibre low glycaemic index diet along with other lifestyle modification can play a role in prevention of the majority of type 2 diabetes cases (Hu et al., 2001). The potential mechanistic evidence for risk reduction is mainly based on reduced postprandial glucose levels (i.e. low glycaemic index) after intake of those food groups compared to refined grains and sweetened beverages that elicit high postprandial glucose which has been implicated in the aetiology of metabolic diseases such as type 2 diabetes (Blaak et al., 2012). Several epidemiological studies have reported reduction in the risk many chronic conditions such as diabetes with consumption of low glycaemic index diet (Wong and Jenkins, 2007). A meta-analysis on 37 prospective cohort studies has reported a significant positive association of glycaemic index and glycaemic load with type 2 diabetes (RR of 1.40 and I.27 respectively) (Barclay et al., 2008).

Although carbohydrates in the diet play a crucial role toward regulation of glycaemic profile, the relative proportion of carbohydrate in a diet does not

have a significant impact on risk of diabetes as observed in epidemiological studies (Ley et al., 2014). It is the quality of carbohydrate determined by glycaemic index that has the potential impact on raising blood glucose after meal particularly on those that already have impaired glucose values (Rizkalla et al., 2002). Slowly or partially digested carbohydrates (due to presence of non-digestible components such as resistant starch) such as those found in wholegrains and legumes are considered to have a significant impact on slowing the progression of many diseases including diabetes attributed to lower glycaemic index of those foods. Delayed absorption of the carbohydrate results in lower postprandial glucose levels and consequently reduces the amount of insulin secreted (Rizkalla et al., 2002). The consumption of a diet high in fibre has been linked to improvement in the metabolism of carbohydrates and fats, enhanced insulin sensitivity and weight reduction and thus reducing the risk of diabetes primarily due to lower postprandial glycaemia associated with intake of dietary fibre (Kaline et al., 2007). In several epidemiological studies, total fibre intake was strongly correlated with the reduction of diabetes risk. Results of a prospective cohort study on 3428 men found an inverse association between fibre intake (both soluble and insoluble) and diabetes risk (Wannamethee et al., 2009). A recent review of meta-analyses regarding the effect of fibre intake on diabetes development has illustrated that intake of soluble fibre had resulted in significant reduction of insulin and $HbA1_c$ (McRae, 2018).

1.6 Physiology of appetite regulation

Regulation of food intake is a sophisticated physiological process comprising afferent and efferent signals to balance energy intake and expenditure. This involves provocation of sense of hunger in order to induce eating, and a sense of fullness subsequent to the meal ingestion (Druce and Bloom, 2006). This homeostasis is regulated centrally through hypothalamus and brain stem, and peripherally through gastrointestinal tract with various hormones involved that are discussed below.

1.6.1 Ghrelin

Ghrelin is a 28 amino acids, orexigenic hormone produced mainly by the stomach and duodenum, acting as endogenous ligand for growth hormone secretagogue receptor located in hypothalamus and brain stem with a half-life of 30-40 minutes (Tong et al., 2013). Ghrelin is a potent stimulator of meal initiation by activating hunger response and contributes towards both short-term food intake and long-term energy balance. Ghrelin concentrations in plasma rise during fasting status and are significantly restrained immediately following meal ingestion. Central and peripheral administration of ghrelin has been shown to increase food intake, inhibit insulin secretion, stimulate lipogenesis and decrease lipolysis (Pradhan et al., 2013). However, it has been suggested that intake of different macronutrients exerts variable effect on ghrelin suppression. While carbohydrates are suggested to be most potent ghrelin suppressors in the acute phase (2-3 hours postprandially), proteins appear to be stronger long-term regulators of ghrelin, contributing towards enhanced satiety. On the other hand, fat intake has been shown to have only a mild effect on ghrelin suppression (Koliaki et al., 2010). Nevertheless, this effect has been seen only in individuals with normal body weights. Ghrelin levels are significantly affected by the weight status as lower circulating ghrelin levels are present in most obese adults with blunted postprandial fluctuations. However, this condition appears to be reversible, as weight loss was shown to upregulate ghrelin levels (Zigman et al., 2016).

1.6.2 Polypeptide tyrosine tyrosine (PYY)

PYY is a 36 amino acid anorexigenic gut hormone derived mainly from enteroendocrine L-cells located in distal gastrointestinal tract. The secretion of PYY from gastrointestinal tract is largely nutrient dependent. The plasma concentration of PYY is reported to be low under fasting conditions and starts to increase within 15 minutes following meal initiation directly proportional to caloric load. PYY remains elevated for up to six hours with peak time around one to two hours (Karra et al., 2009). Elevated levels of PYY have been linked to reduced food intake by stimulating satiety signals into hypothalamus receptors, delaying gastric emptying, and increasing gastrointestinal transit time. A previous study reported significantly reduced appetite and food intake after intraperitoneal infusion of PYY in a dose-dependent manner with no effect of body weight on the reported outcomes (Batterham et al., 2003, Batterham et al., 2002). Although postprandial PYY levels are largely correlated with the total caloric intake, varying macronutrient composition had substantial impact on PYY release from the gut in response to meal intake. In fact, higher fat intake was shown to be a stronger stimulator of PYY secretion in comparison with proteins and carbohydrates (Essah et al., 2007).

1.6.3 Leptin

Leptin is a 167 amino acid hormone that is synthesised and released mainly from white adipose tissue cells and hence the circulating levels of leptin in plasma are correlated positively with the body fat reflecting energy stores in adipocytes. Although leptin is considered a long-term regulator of energy balance, recent research has shown that acute food intake, in particular overfeeding, can also impact plasma concentrations of leptin (Kelesidis et al., 2010). Leptin levels are a direct indicator of energy reserves and hence signal hypothalamus to regulate appetite. Moreover, leptin may also play role in increasing energy expenditure via stimulating sympathetic nerve activity and promoting thermogenesis from brown adipose tissue (Scarpace et al., 1997, Haynes et al., 1997).

1.7 Methodological outline of the thesis

1.7.1 Systematic review and meta-analysis

Systematic reviews are type of review that involve detailed identification and critical assessment, of the previously published literature related to a specific topic and meeting the predetermined eligibility criteria, in a systematic way according to a comprehensive protocol derived *a priori*. Systematic reviews aim to provide meticulous summaries of the existing evidence under scope of explicit pre-implemented methodological guidelines. Systematic reviews are considered the highest level of evidence among the evidence hierarchy in research particularly when accompanied by meta-analysis. Meta-analysis is the process of integrating the findings of individual studies and quantifying the concluded results by applying statistical methods. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) protocol was followed to conduct the systematic review and meta-analysis (Liberati et al., 2009). it is an evidence based set of information aimed to provide the researchers with the necessary guidance in order to minimise the bias and ensure reporting the systematic reviews in accurate, reliable, and transparent manner allowing the readers to assess the complete review process including strengths and weaknesses. The pre-planned written protocol of the systematic review was prospectively registered on International Prospective Register of the systematic review (PROSPERO) to ensure complete transparency of the protocol.

To our knowledge, there hasn’t been any systematic review and meta-analysis that has been published assessing the effect of pulse intake on acute postprandial glycaemic measures in adult populations. In addition, the available evidence on the influence of long term pulse consumption on biomarkers of glycaemic control was reviewed only once by Sievenpiper et al. in 2009. Therefore, the purpose of this part was to systematically review the published acute and long-term randomised controlled trials related to the topic.

Research questions behind this chapter were as follows:

1. What is the effect of acute and chronic pulse consumption on measures of glycaemic control in normoglycaemic and type 2 diabetic populations from previously published literature?
2. What are the factors contributing to the reported heterogeneity in reported outcomes?

1.7.2 Randomised controlled trial investigating impact of processing methods of chickpeas on postprandial glycaemia

Randomised controlled trials are prospective studies in which subjects are randomly assigned to intervention arms and/or control arm to measure the effectiveness of certain intervention/treatment on a proposed condition. This can be done either in parallel design in which subjects are divided randomly between study arms, or crossover in which all subjects receive all interventions in a random order to minimise the variations derived by interpersonal variabilities. Randomised controlled trials are considered on second level of evidence right after the systematic reviews among evidence hierarchy in researches. In this project, a randomized controlled trial was designed to investigate the difference in postprandial responses of glycaemia, satiety, and their related hormones after intake of chickpeas that had been processed using different methods which affected structural integrity, such as pureeing and milling. The hypothesis tested was that different processing methods in particular mechanical does not impact on the glycemic properties of pulses. There were some studies published that were investigating *in vitro* the effect of mechanical processing of chickpeas, particularly methods that result in cell wall disruption, and showed a significant increase in the rate of starch digestion and starch release following processing compared to non-processed chickpeas. However, little was known regarding the impact of processing methods on postprandial glucose. In particular, the effect of extrusion process on pulses was never been studied weather *in vitro* or *in vivo*. In addition, only one trial has been published, to our knowledge, that measured *in vivo* the postprandial responsiveness of pulse intake on objective satiety indices such as incretins and ghrelin hormones in comparison to non-pulse control food with matched carbohydrate content. Therefore, the purpose

of this study was to assess the acute postprandial glycaemic and satiety measures following chickpea ingestion that was prepared following different processing methods on adults with normal glucose metabolism. Postprandial glucose responses were measured in the study using continuous glucose monitoring system (Abbott, Free Style Libre Pro), that measures the glucose concentration in interstitial tissues, which allowed to measure the glucose responsiveness of the participants beyond the study sessions. Therefore, it was capable to assess the effect of intervention on standardised subsequent meal's glucose response. In addition, the subsequent meal was standardised to provide simple and rapidly digestible carbohydrates, in order to assess the effect of low glycaemic index breakfast on a relatively high glycaemic index lunch. Moreover, the trial involved blood collection at baseline and every 30 minutes after meal intake for 3 hours. The purpose of blood collection was to determine the differences in postprandial responses of various hormones related to glycaemia and satiety such as insulin, GLP-1, ghrelin and leptin, after intake of differently processed chickpeas and compared to mashed potatoes. The blood collection was performed periodically (baseline, 30, 60, 90, 120, 150, and 180 minutes) by inserting a cannula in the antecubital fossa upon arrival in each session. The participants remain cannulated during the session for repeat blood collections, and the cannula then removed after 3 hours. Before starting the trial, the required course of venepuncture and cannulation was undertaken that was provided by VeinTrain Ltd., and accomplished by supervised training for the cannulation following standard operating procedures guidelines set by the University of Leeds. The study protocol was ethically approved by the MaPS and Engineering joint Faculty Research Ethics Committee (MEEC FREC) at the University of Leeds in July 2019, please find the ethical approval in the appendix.

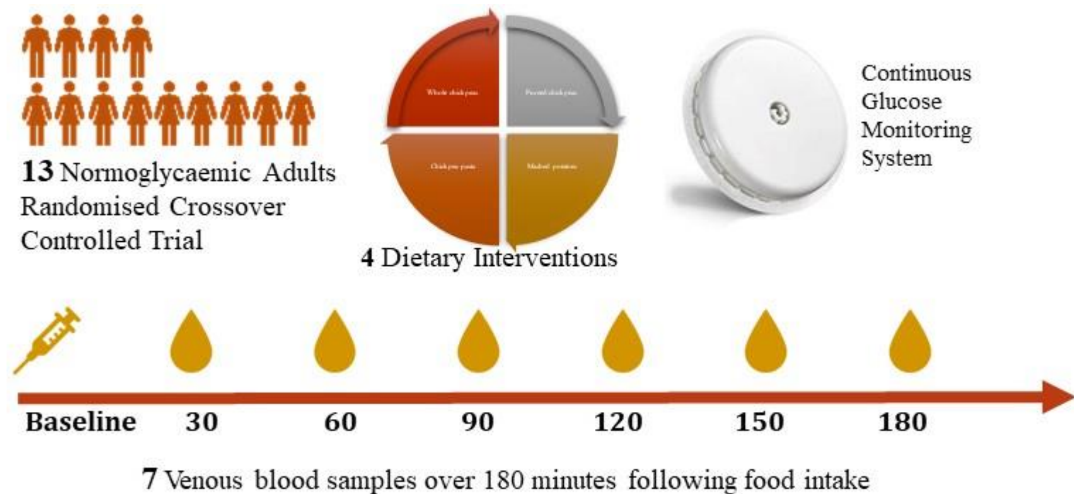

Figure 1-4 Schematic workflow of the human study

1.7.3 *In vitro* carbohydrate digestibility of differently processed chickpeas

There are numerous factors that contribute to the digestion and absorption of carbohydrates and consequently postprandial blood glucose responsiveness after ingestion of these foods. This includes the structure of the food and its rheological properties that affect the susceptibility of the nutrients to digestive enzymes and hence their availability. Therefore, the purpose of this part of the project was to investigate and gain insights on the cell wall structure of the food items that were used in the human study, assess their rate and extent of digestibility *in vitro* by using simulated digestion, and to correlate the degree of digestibility with the estimated glycaemic index of the food items. The study was designed based on the hypothesis that differently processed chickpea foods would have significant differences in degree and rate of carbohydrate digestibility *in vitro* in line with the outcomes of the human study.

To investigate the cell wall structure of the chickpea samples, the food samples were dispersed in 1.0% w/v sodium phosphate buffer carefully to avoid any breakage of the cell walls. The samples were stained by 2.5% iodine to visualize starch granules and 5 mM calcofluor white stain to visualize the cell walls. The microstructure was then investigated under light microscopy.

Before initiating digestion experiments, the enzyme activity was precisely determined for salivary amylase (Sigma Aldrich), porcine pancreatic amylase (Megazyme), and pancreatin (Sigma Aldrich) at 37 ˚C, a temperature that reflects the digestion environment in the human gut. To assess *in vitro* digestibility of the food samples, static *in vitro* digestion was performed following the INFOGEST protocol (Brodkorb et al., 2019). The static method was preferred over dynamic considering it is a potential and widely applicable method in evaluating the influence of various conditions such as food structure, food composition, and food processing digestibility.

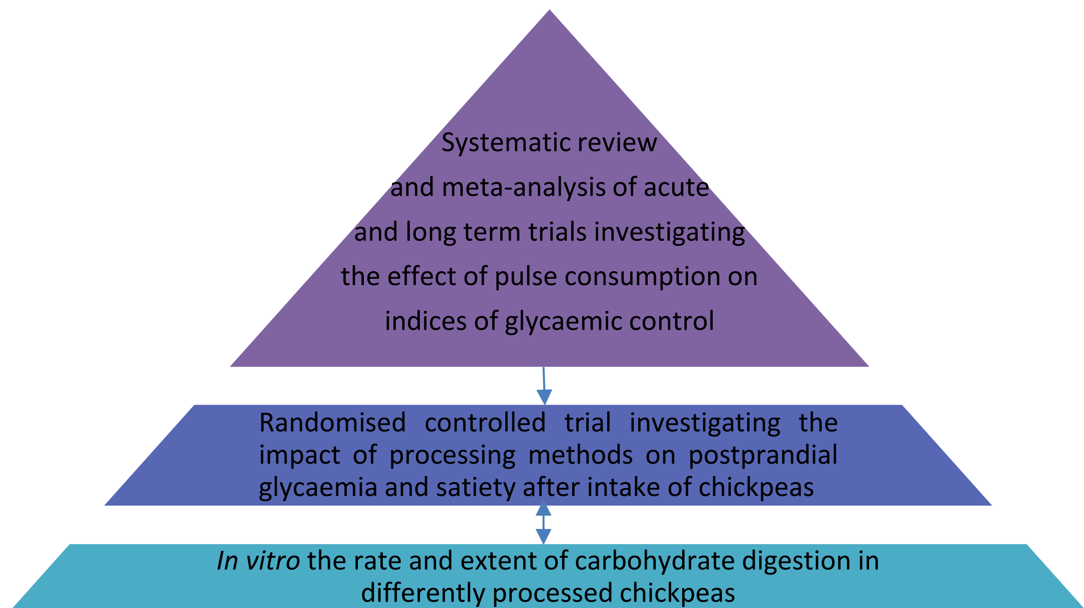

Figure 1-5 Schematic framework of the thesis

1.8 Outline of the thesis

Chapter 1: This thesis starts with an overview on the classification of dietary carbohydrates and provides detailed mechanisms of human physiological response after intake of carbohydrates in particular with lower glycaemic index such as pulses.

Chapter 2: This chapter consists of a systematic review and meta-analysis on the effects of pulse intake on indices of glycaemic control. The review was conducted on randomised controlled trials to investigate the acute impact on postprandial glycaemia and insulinaemia, and long-term effects on measures of glycaemia such as fasting blood glucose and insulin. The outcomes of each included trials were extracted and the mean difference across the trial was calculated by conducting the meta-analysis. Furthermore the effect of different variables such as processing method, dose of the pulses, and duration of the trials was assessed by subgroup analysis and meta regression. The systematic review and meta-analysis forming this chapter was published in the peer reviewed journal 'European Journal of Nutrition'.

Chapter 3: In this chapter the influence of different processing methods applied to pulses was investigated, on postprandial glycaemic and satiety responses in healthy adults. The processing methods assessed in the study were in particular that might impact the physical and structural properties of pulse cells such as pureeing and extrusion. The glucose response was assessed interstitially by continuous glucose monitoring system and satiety responses were obtained by using visual analogue scale. Chickpeas were chosen for this purpose due to the wider range of products available that are made of chickpeas. This chapter has been published in the peer reviewed journal 'Food & Function'.

Chapter 4: This chapter present data on the *in vitro* digestion of differently processed chickpea samples that were used in the human study as intervention, and correlates the outcomes of the *in vitro* analysis with the results from the *in vivo* study. The experiment and the outcomes forming this chapter have been prepared as manuscript which has been submitted for publication to the journal 'Plant food for human nutrition'.

Chapter 5: The final chapter includes a general summary and overall discussion of the main results of this PhD project and highlights areas for future studies on this topic.

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Chapter 2 Pulse consumption improves indices of glycemic control in adults with and without type 2 diabetes: a systematic review and meta-analysis of acute and long-term randomized controlled trials

2.1 Abstract

Purpose: Findings from randomized controlled trials (RCTs) evaluating the effect of pulse intake on glycemic control are inconsistent and conclusive evidence is lacking. The aim of this study was to systematically review the impact of pulse consumption on post-prandial and long-term glycemic control in adults with and without type 2 diabetes (T2D).

Methods: Databases were searched for RCTs, reporting outcomes of post-prandial and long-term interventions with different pulse types on parameters of glycemic control in normoglycaemic and T2D adults. Effect size (ES) was calculated using random-effect model and meta-regression was conducted to assess the impact of various moderator variables such as pulse type, form, dose, and study duration on ES.

Results: From 3334 RCTs identified, 65 studies were eligible for inclusion involving 2102 individuals. In acute RCTs, pulse intake significantly reduced peak post-prandial glucose concentration in participants with T2D (ES: -2.90; 95% CI: -4.60, -1.21; $p \leq 0.001$; $I^2 = 93\%$) and without T2D (ES: -1.38; 95% CI: -1.78, -0.99; $p \leq 0.001$; $I^2 = 86\%$). Incorporating pulse consumption into long-term eating patterns significantly attenuated fasting glucose in normoglycaemic adults (ES: -0.06; 95% CI: -0.12, 0.00; $p \leq 0.05$; $I^2 = 30\%$). Whereas, in T2D participants, pulse intake significantly lowered fasting glucose (ES: -0.54; 95% CI: -0.83, -0.24; $p \leq 0.001$; $I^2 = 78\%$), glycated hemoglobin A_{1c} (HbA_{1c}) (ES: -0.17; 95% CI: -0.33, 0.00; $p \leq 0.05$; $I^2 = 78$) and homeostatic model assessment of insulin resistance (HOMA-IR) (ES: -0.47; 95% CI: -1.25, -0.31; $p \leq 0.05$; $I^2 = 79\%$).

Conclusion: Pulse consumption significantly reduced acute post-prandial glucose concentration > 1 mmol/L in normoglycaemic adults and > 2.5 mmol/L in those with T2D, and improved a range of long-term glycemic control parameters in adults with and without T2D**.**

2.2 Introduction

The European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA) advocate increasing fiber intake, specifically through the consumption of pulses as a means to improve blood glucose control in adults with and without T2D (Rydén et al., 2013, Evert et al., 2019). Several epidemiological studies have reported inverse associations between pulse intake and incidence of T2D (Agrawal and Ebrahim, 2013, Villegas et al., 2008). In addition, RCTs suggest that pulse consumption may improve acute post-prandial glucose control, and lower fasting blood glucose, insulin and HbA_{1c} levels when incorporated into long-term eating patterns (Kim et al., 2014, Ramdath et al., 2018).

Pulses are rich sources of low glycemic index (GI) carbohydrates (CHO, up to 65%), and protein with up to 25% (dry weight) (Singh, 2017). Low GI, fiber rich foods have been shown to reduce post-prandial glycemic responses (PPGR) compared to foods with similar CHO content (Brummer et al., 2015, Fabbri et al., 2016), as well as protein addition to breakfast is suggested to improve PPGR (King et al., 2018). In addition, pulses contain phytochemicals such as catechins and procyanidins which have been demonstrated to suppress the enzymatic activity of CHO digestive enzymes including α-amylase and α-glucosidase thereby contributing towards improved post-prandial glycemic control (Padhi and Ramdath, 2017, Prasad et al., 2018, Perez-Hernandez et al., 2020).

A number of randomized controlled trials have assessed the effect of pulse intake on acute post-prandial and long-term glucose response (Abete et al., 2008, Abete et al., 2009, Abeysekara et al., 2012, Alizadeh et al., 2014, Anderson et al., 2014, Anderson et al., 1984, Anguah et al., 2014, Augustin et al., 2016, Barnard et al., 2006, Boers et al., 2017). The studies differed in the type of pulses used, processing, doses and control group, and in different volunteer profiles (Bornet et al., 1987, Bornet et al., 1989, Cryne et al., 2012, Dandachy et al., 2018, De Natale et al., 2009, Dilawari et al., 1981, Jenkins et al., 1982a, Jenkins et al., 1980a, Jenkins et al., 1982a, Jenkins et al., 1980b, Ramdath et al., 2018). The study outcomes vary considerably with low quality of evidence and therefore the true effect size of pulse intake on

measures of glycemic handling remains unclear (Viguiliouk et al., 2017). A previous systematic review and meta-analysis by Sievenpiper et al. (2009) concluded a significant reduction in fasting blood glucose and insulin after long-term consumption of pulses alone, as part of low GI or high fiber diets (Sievenpiper et al., 2009). However, the review was published in 2009 and only long-term trials were included in their review. Considering that there are more than 20 long-term trials published since 2009 and given the lack of summarized evidence on post-prandial glucose response after intake of pulses, the aim of the current systematic review is to update the evidence on long-term effects of pulse consumption on glycemic indices as well as integrate the acute glucose response along from RCTs on individuals with and without T2D.

2.3 Methods

The guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Liberati et al., 2009) were followed for conducting this systematic review and meta-analysis. The systematic review was prospectively registered with PROSPERO (CRD42019162322).

2.3.1 Search strategy and study selection

We searched Pubmed and Cochrane library databases to identify all randomized clinical trials (RCTs) conducted and relevant to the topic until 28th of January 2021. Full search terms are illustrated in Supplemental Table 1. No filters for language, date of publication, or design of the study were applied when searching the databases. An additional manual search was conducted through reviewing reference lists of selected articles and reviews.

The study selection process was performed in duplicate independently by two reviewers by initially reviewing the titles and abstracts and finally reviewing the full texts to identify all eligible RCTs. Included studies were randomized controlled trials either acute (assessing single meal response) or long-term (assessing intake > 2 weeks) (Grunberger et al., 2016), including all adults except type 1 diabetes mellitus and gestational diabetes, investigating the effect of intake of pulses in comparison to control diet, on

parameters of glycemic control measured using capillary or venous blood. Studies were excluded if they investigated legumes other than pulses such as soya beans or green peas, failed to use a matched available carbohydrate control in acute glucose response trials; the pattern of pulse consumption was not specified; used pulse fractions such as their extracts; protein isolates or husk only; reported subsequent second meal effect rather than immediate response; did not exclude or account for confounding factors whether in participants or intervention diets that might impact glucose metabolism; or outcome measures of glycemic control were not reported. In studies where different interventions were used in different arms, only data from arms that met the eligibility criteria were included in the analysis. Included trials were limited to published and peer reviewed RCTs available as full texts in English. Corresponding authors were contacted to request the full text in cases where the full text was not available online before deciding on exclusion.

2.3.2 Data extraction and quality assessment

Data were extracted by single author and included: first author and year of publication; publishing journal; design of the study; intervention arms; number of visits in acute studies; study duration in long-term studies; sample size and participant characteristics (gender, health status, age group and body mass index); intervention design and control (type, dose and format); pulse characteristics (type, dose and physical form). Although data extraction was conducted by single author, it was randomly reviewed in duplicate to avoid possible errors. The outcome measures of acute trials were extracted for means and standard deviations of baseline and post-prandial glucose (mmol/L) and insulin (mIU/L) values and their area under the curves (AUCs). In the long-term trials, baseline and post-intervention mean and standard deviation values were extracted for fasting blood glucose (mmol/L), insulin (mIU/L), glycated hemoglobin (%) and insulin resistance expressed as HOMA-IR. Where data were presented in non-standard units, they were converted to standard reporting units. If data was available in figure format only, values were digitized using Graph Digitizer. In trials not

reporting the standard deviation, the values were derived from standard errors or confidence intervals (CI).

Bias assessment of individual trials was performed independently by two reviewers following the updated Cochrane Collaboration's tool for assessing risk of bias (RoB2) (Sterne et al., 2019). The trials were classified into three categories "high risk, low risk, or some concerns raised" in 5 domains which are as follows: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. The proposed algorithm was followed in signaling questions to judge risk of bias of each domain as well as overall risk of bias. Publication bias was visually assessed by inspection of funnel plots and quantitatively using Egger's test for each outcome (Egger et al., 1997).

2.3.3 Data analysis

Data were analyzed using Review Manager (RevMan) 5.3.5 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014; and R Core Team (2020), R: A language and environment for statistical computing, R foundation for statistical computing, Vienna, Austria. The random effects model was chosen assuming that the RCTs included in the analysis were functionally inequivalent. Weighted averages were calculated in trials using more than one arm for intervention to avoid errors in analyses (Borenstein et al., 2011). RCTs not reporting the amount of pulses administered were excluded from the meta-analysis. Pooled random effects analyses were performed to estimate the effect size in acute and long-term RCTs on normoglycemic and T2D participants. The entered data included sample size, reported means and standard deviations for intervention arms and their matched carbohydrate controls of each trial. Effect size was estimated for post-prandial glucose and insulin response in acute RCTs and for the difference between pre- and post-intervention in fasting blood glucose, insulin, glycated hemoglobin, and HOMA-IR values as raw mean differences and 95% CIs. A negative ES was interpreted as favoring pulse intake, while a positive ES favored control. The inter-study variance was assessed using tau^2 and I^2 along with calculation of prediction intervals (PI). Sensitivity

analysis was performed to explore the impact of removing one RCT on outcomes, as well as investigate removal of studies with high risk of bias on ES (Higgins et al., 2019).

Subgroup analysis and meta-regression were performed if ≥ 10 RCTs could be included in the meta-analysis to explore the variations in ES, considering pulse type or processing method used in intervention arms, control food used for comparison, and dose or duration of the study as variables (Higgins et al., 2019).

2.3.4 Grading the evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool was conducted by single author for interpreting outcome data to evaluate the certainty of evidence (McMasterUniversity, 2020). Evidences on the ES can be graded to 'very low', 'low', 'moderate', or 'high' based on evaluation outcomes in 5 domains. The domains are as follow: overall risk of bias, inconsistency, indirectness, imprecision, and other considerations.

2.4 Results

A total of 3334 studies were identified through database searches and additional sources, of which 2966 were screened based on title and abstract only. Of these, 150 studies were reviewed as full text and subsequently 85 studies were excluded for not meeting the inclusion criteria, as detailed in the study selection flowchart (Figure 2-1). In total, 65 RCTs were included in the final systematic review and 59 RCTs in the meta-analysis, involving a total of 2102 individuals (905 with and 1197 without T2D). The RCTs were classified according to the design of the study as acute post-prandial (n=37, Table 2-1, 2-2) or long-term (n = 28, Table 2-3, 2-4) trials and separated into normoglycaemic (Table 2-1, 2-3) and T2DM (Table 2-2, 2-4).

Assessment of risk of bias across the studies indicated concerns for the majority of RCTs due to lack of information on randomization concealment as well as selection of the reported results (Supplemental Table A-2). There were 10 RCTs that fell into the 'high risk' category due to concerns in three

or more domains. These were mainly the trials that were published more than twenty years ago; in appreciation of the fact that the standards on reporting RCTs were substantially different then, we have not removed these studies from the meta-analysis.

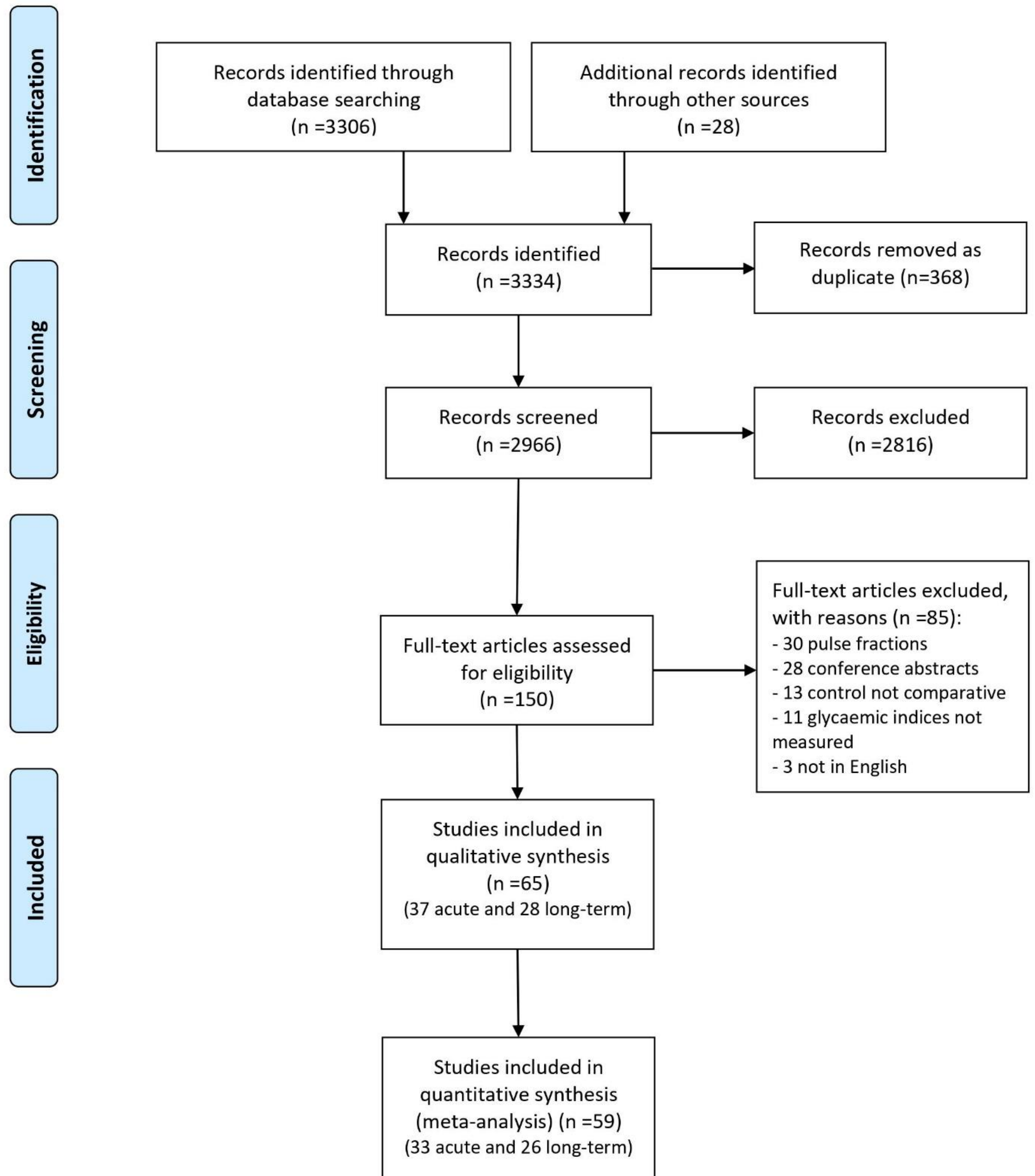


Figure 2-1 Flow diagram of trial selection

Table 2-1 Summary of acute RCTs investigating the effect of pulse intake on glycaemic indices in normoglycaemic adults

Reference	Country of study	Design	N	Age, y[1]	BMI[1]	Pulse type	Format	Other CHO source	Total CHO (pulse only), g	Control	Outcomes
(Agustia et al., 2019)	Indonesia	NR	11	20.1 ± 1.3	20.9 ± 1.9	Beans	Flour	Rice	50	Glucose	Glucose
(Akhtar et al., 2019)	Pakistan	C	24	22.5 ± 2.4	21.8 ± 1.7	Beans	Flour	Wheat flour	50 (20)	Wheat flour	Glucose, insulin
(Anderson et al., 2014)	Canada	C	17	22.1 ± 3.0	22.9 ± 1.2	Beans, lentils, chickpeas	Whole, pureed and Flour	Tomato sauce	38.7 (25)	Whole wheat flour	Glucose
(Anguah et al., 2014)	US	C	12	28.0 ± 10.0	23.3 ± 3.1	Lentil	Whole, pureed	Rice, wheat	NR	Rice and egg burritos	Glucose
(Augustin et al., 2016)	Canada	C	10	53.0 ± 7.0	29.4 ± 3.8	Chickpeas	Pureed	-	25	White bread	Glucose, insulin
(Boers et al., 2017)	UK	C	12	37 ± 9	22.8 ± 1.6	Chickpeas	Flour	Wheat	57 (8.5)	High fiber flat bread	Glucose
(Bornet et al., 1989)	France	C	6	23.9 ± 1.7	20.6 ± 1.7	Beans	Flour	-	35	Extruded wheat	Glucose, insulin
(Dandachy et al., 2018)	Lebanon	C	16	22.9 ± 12	22.7 ± 10.6	Chickpeas	Flour	Wheat	NR	Wheat flour	Glucose
(Dilawari et al., 1981)	India	C	6	36.3 ± 9.7	NR	Lentils, beans	Whole	-	50	Rice	Glucose
(Greffeuille et al., 2015)	France	C	15	24 ± 11.2	22.4 ± 7.0	Beans	Flour	Wheat	50 (17.5)	Wheat pasta	Glucose, insulin
(Jenkins et al., 1980)	UK	C	10	NR	NR	Beans; peas; chickpeas; lentils	Whole	-	50	White bread	Glucose
(Jenkins et al., 1982)	UK	C	9	29.0 ± 8.0	NR	Lentils	Whole	-	50	White bread	Glucose
(Johnson et al., 2005)	Australia	C	11	32.0 ± 6.6	24.7 ± 2.7	Chickpeas	Flour	Jam, milk	50 (NR)	White bread	Glucose, insulin
(Marinangeli et al., 2009)	Canada	C	22	NR	NR	Peas	Flour, whole	Wheat	50 (NR)	White bread	Glucose
(Mehio et al., 1997)	Lebanon	C	12	24.0 ± 3.4	22.8 ± 2.1	Chickpeas	Pureed	NR	50	White bread	Glucose, insulin
(Mollard et al., 2011)	Canada	C	25	21.3 ± 2.5	21.6 ± 1.5	Chickpeas, lentils, peas	Whole	Macaroni	98.7 (40)	Macaroni and cheese	Glucose
(Moravek et al., 2018)	Canada	C	24	27.4 ± 1.2	24.3 ± 0.5	Lentils	Whole	Rice/potato	50 (NR)	Rice or potatoes	Glucose, insulin

Reference	Country of study	Design	N	Age, y[1]	BMI[1]	Pulse type	Format	Other CHO source	Total CHO (pulse only), g	Control	Outcomes
(Nestel et al., 2004)	Australia	C	19	61.5 ± 6.4	26.5 ± 3.8	Chickpeas	Pureed	Milk	50 (33)	White bread and jam	Glucose
(Potter et al., 1981)	US	C	8	NR	NR	Beans	Pureed	-	75	Brown rice	Glucose
(Ramdath et al., 2017)	Canada	C	10	45.1 ± 11.0	27.7 ± 6.1	Lentils	Whole	-	25, 25	White bread	Glucose
(Ramdath et al., 2018)	Canada	C	10	40.0 ± 10.0	25.0 ± 4.1	Lentils	Whole, pureed and Flour	-	50	Potatoes	Glucose
(Reverri et al., 2015)	US	C	12	49.0 ± 14.0	32.2 ± 5.7	Beans	Pureed	-	NR	Couscous	Glucose
(Tappy et al., 1986)	Switzerland	C	6	NR	NR	Beans	Flakes	-	50	Potatoes	Glucose
(Torsdottir et al., 1989)	Sweden	C	6	24.0 ± 6.0	22.2 ± 1.1	Beans	Pureed	-	43	Potatoes	Glucose, insulin
(Traianedes and O'Dea, 1986)	Australia	C	6	30.0 ± 10.0	24.3 ± 1.7	Beans	Whole	-	50	Glucose	Glucose, insulin
(Winham et al., 2017)	US	C	12	36.0 ± 15.0	23.3 ± 5.4	Black beans, chickpeas	Whole	Rice	50 (15)	Rice	Glucose, insulin
(Wong et al., 2009)	Canada	C	14	NR	NR	Beans, chickpeas, lentils, peas	Whole	-	50	White bread	Glucose
(Yoshimoto et al., 2020)	Japan	C	12	37.8 ± 9.5	22.9 ± 3.5	Peas	Flour	-	50	Rice	Glucose, insulin
(Zafar et al., 2015)	Kuwait	C	13	21.4 ± 2.3	23.6 ± 2.4	Chickpeas	Flour	Wheat, milk	NR	White bread	Glucose
(Zhu et al., 2019)	China	C	10	20.7 ± 2.3	22.0 ± 2.1	Beans	Whole	-	50	White rice	Glucose
(Zurbau et al., 2018)	Canada	C	21	26.7 ± 12.3	22.2 ± 2.8	Chickpeas	Whole	Tomatoes	50 (NR)	Potatoes	Glucose

[1]Age and BMI are reported as mean ± SD; BMI, body mass index; CHO, available carbohydrates; C, crossover; N, number of participants; NR, not reported

Table 2-2 Summary of acute RCTs investigating the effect of pulse intake on glycaemic indices in T2D adults

Reference	Country of study	Design	N	Age, y[1]	BMI[1]	Pulse type	Format	Other CHO source	Total CHO (pulse only), g	Control	Outcomes
(Bornet et al., 1987)	France	C	18	57 ± 8.5	27.9 ± 4.7	Lentils, beans	Whole	-	50	Glucose	Glucose, insulin
(Jenkins et al., 1980)	UK	C	6	43 ± 5	NR	Lentils	Whole	Soya	50 (23)	Whole meal bread	Glucose
(Mani et al., 1992)	India	C	6	58 ± 9	NR	Lentils	Whole	Semolina	50 (16)	Semolina	Glucose
(Olmedilla-Alonso et al., 2013)	Spain	C	12	66.4 ± 6.2	30.1 ± 3.6	Beans	Whole	-	57.8	White bread	Glucose, insulin
(Schafer et al., 2003)	Germany	C	9	61 ± 14	29.9 ± 8.7	Peas	Whole	Carrots	40 (37)	Potato	Glucose, insulin
(Thompson et al., 2012)	US	C	17	58.6 ± 20	31.9 ± 7.9	Beans	Whole	Rice	50 (15)	Rice only	Glucose

[1]Age and BMI are reported as mean ± SD; BMI, body mass index; CHO, available carbohydrates; C, crossover; N, number of participants; NR, not reported

Table 2-3 Summary of long-term RCTs investigating the effect of pulse intake on glycaemic indices in normoglycaemic adults

Reference	Country of study	Design	Duration, weeks	N	Age, y[1]	BMI[1]	Intervention	Dose, g/day	Control	Outcomes
(Abete et al., 2008)	Spain	P	8	32	NR	32.5 ± 4.3	Low GI diet with pulse intake	130	Energy restricted high GI diet	Glucose, insulin, HOMA-IR
(Abete et al., 2009)	Spain	P	8	35	38.0 ± 7.0	31.8 ± 3.0	High pulse diet	100	Energy restricted diet	Glucose
(Abeysekara et al., 2012)	Canada	C	8	87	59.7 ± 6.3	27.5 ± 4.5	Pulse-based diet	250	Regular diet	Glucose, insulin
(Alizadeh et al., 2014)	Iran	P	6	34	36.1 ± 8.2	NR	hypocaloric diet enriched in pulses	190	Hypocaloric diet	Glucose, insulin, HOMA-IR
(Anderson et al., 1984)	US	P	3	10	53.9 ± 8.5	NR	Beans supplemented diet	115	Oat bran diet.	Glucose
(Cryne et al., 2012)	Canada	C	4	21	28.1 ± 5.9	25.2 ± 3.5	Spray dried chickpeas, lentils, peas	100	Dehydrated potato flakes	Glucose, insulin, HOMA-IR
(Gravel et al., 2010)	Canada	P	16	132	51.7 ± 8.6	29.8 ± 5.1	Pulse based meals	110	Isocaloric control meals	Glucose, insulin
(Kim et al., 2017)	Australia	C	4	51	35.1 ± 15.6	27.7 ± 6.9	Diet high in dairy, whole grains, nuts and pulses	150-225	Diet high in red and meat and refined grains	Glucose
(Marinangeli and Jones, 2011)	Canada	C	4	23	52.0 ± 11.2	30.5 ± 4.4	whole pea flour muffin	50	White wheat flour muffin	Glucose
(Nestel et al., 2004)	Australia	C	6	20	56.6 ± 7.6	25.6 ± 3.2	Chickpea based diet	200	Wheat-based diet	Glucose, insulin, HOMA-IR
(Pittaway et al., 2007)	Australia	C	5	27	50.6 ± 10.5	28.8 ± 4.4	Chickpeas based diet	200	Low fiber wheat-based diet	Glucose, insulin, HOMA-IR
(Saraf-Bank et al., 2016)	Iran	C	6	26	50.0 ± 6.6	28.9 ± 4.3	Habitual diet enriched with pulses	65	Habitual diet without pulses	Glucose, HbA_{1c}
(Tonstad et al., 2014)	US	P	16	123	48.4 ± 10.7	36.4 ± 3.5	High-fiber bean-rich diet	125	low-carbohydrate diet	Glucose, HbA_{1c}
(Tovar et al., 2014)	Sweden	C	4	46	61.6 ± 5.4	28.8 ± 8.1	Whole grain, barley and pulse rich diet	168	Low pulse diet	Glucose, insulin, HbA_{1c}, HOMA-IR
(Venn et al., 2010)	New Zealand	P	72	113	42.0 ± 10.7	35.4 ± 5.5	High pulse diet	180	Low pulse diet	
(Winham et al., 2007)	US	C	8	16	43.0 ± 20.0	27.8 ± 5.6	Beans/peas enriched diet	120	Carrot enriched diet	Glucose, insulin, HbA_{1c}, HOMA-IR

[1]Age and BMI are reported as mean ± SD; BMI, body mass index; C, crossover; N, number of participants; NR, not reported, P, parallel study design

Table 2-4 Summary of long-term RCTs investigating the effect of pulse intake on glycaemic indices in T2D adults

Reference	Country of study	Design	Duration, weeks	N	Age, y[1]	BMI[1]	Intervention	Dose, g/day	Control	Outcomes
(Hassanzadeh-Rostami et al., 2019)	Iran	P	8	64	59.6 ± 5.9	27.3 ± 3.4	Pulses	100	Red meat	Glucose, insulin, HbA_{1c}
(Hosseinpour-Niazi et al., 2015)	Iran	C	8	31	58.1 ± 6.0	27.7 ± 3.3	Pulse-based TLC diet	190	Pulse-free TLC diet	Glucose, insulin
(Islam et al., 2015)	Bangladesh	P	4	30	52.4 ± 5.6	25.1 ± 2.2	Mixed pulse and wheat bread	NR	Wheat bread	Glucose
(Jang et al., 2001)	Republic of Korea	P	16	76	56.6 ± 8.6	24.6 ± 2.2	Black bean powder mixed with wholegrains powder	15	Cooked refined rice	Glucose, insulin, HOMA-IR
(Jenkins et al., 2012)	Canada	P	12	121	53.0 ± 10.0	29.9 ± 5.5	Low GI pulse diet	190	High wheat fiber diet	Glucose, HbA_{1c}
(Jimenez-Cruz et al., 2003)	US	C	6	14	53.0 ± 9.0	32.3 ± 5.9	Low GI Mexican style diet with pulses	35	High GI Mexican style diet.	
(Jiménez-Cruz et al., 2004)	US	C	3	8	51.0 ± 3.0	30.7 ± 7.9	Low GI high fiber diet with pulse	NR	High GI low fiber diet	Glucose, HbA_{1c}
(Kang et al., 2014)	Republic of Korea	P	12	185	50.4 ± 9.9	25.5 ± 3.2	Whole grains and pulses	30-70	Refined rice diet	Glucose, insulin, HOMA-IR
(Kim et al., 2014)	Republic of Korea	P	12	99	55.4 ± 11.9	24.1 ± 3.4	Whole grains and pulses	30-70	Refined rice diet	Glucose, insulin, HbA_{1c}, HOMA-IR
(Kim et al., 2016)	Republic of Korea	P	12	80	NR	NR	Whole grains and pulses	30-70	Refined rice diet	Glucose, insulin, HbA_{1c}, HOMA-IR
(Liu et al., 2018)	China	P	4	106	57.4 ± 8.8	26.6 ± 1.0	Extruded adzuki bean convenient food	170	Low GI diet	Glucose, insulin, HbA_{1c}
(Winham and Hutchins, 2007)	US	C	8	23	45.9 ± 21	27.4 ± 5.1	Canned baked navy beans	130	Canned carrots	Glucose, insulin, HbA_{1c}, HOMA-IR

[1]Age and BMI are reported as mean ± SD; BMI, body mass index; C, crossover; N, number of participants; NR, not reported

2.4.1 Parameters of post-prandial glycemic control

The meta-analysis showed that pulse intake significantly improved parameters of post-prandial glycemic handling. Post-prandial plasma glucose was overall significantly reduced in normoglycaemic adults (n = 27 RCTs, ES -1.38; 95% CI -1.78, -0.99; $p \leq 0.001$; $I^2 = 86\%$, PI -3.33, 0.57) and in adults with T2D (n = 6 RCTs, ES -2.90; 95% CI -4.60, -1.21; $p \leq 0.001$; $I^2 = 93\%$, PI -8.97, 3.17) (Figure 2-2, 2-3), with high heterogeneity between studies. Egger's test of publication bias did not indicate presence of funnel plot asymmetry ($p > 0.05$) (Figure A 1). Subgroup analysis of pulse type revealed that lentils (n = 9 RCTs) were most effective in reducing PPGR (ES -1.60; 95% CI -2.23, -0.97; $p \leq 0.0001$, $I^2 = 84\%$), followed by dried peas (n = 5 RCTs; ES -1.32; 95% CI -2.07, -0.56; $p \leq 0.005$, $I^2 = 81\%$), beans (n=14 RCTs; ES -1.18; 95% CI -1.74, -0.62; $p < 0.0001$, $I^2 = 82\%$), and chickpeas (n = 11 RCTs; ES -0.97; 95% CI -1.48, -0.47; $p < 0.001$, $I^2 = 78\%$). However, the differences in ES were not significant between types of pulses ($p = 0.49$) (Figure A 2). Further, analysis and meta-regression of processing method revealed that ES was significantly lower when pulse flour was used as intervention (n = 10 RCTs; ES -0.81; 95% CI -1.33, -0.29; $p \leq 0.005$, $I^2 = 83\%$) compared to whole (n = 14 RCTs; ES -1.84; 95% CI -2.32, -1.37; $p \leq 0.0001$, $I^2 = 80\%$) and pureed pulse (n = 7 RCTs; ES -1.65; 95% CI -2.33, -0.98; $p \leq 0.0001$, $I^2 = 70\%$) with ($p < 0.05$) for subgroup differences (Figure A 3). Moreover, subgroup analysis by grouping control foods used in the post-prandial trials suggested that the ES was greater when potatoes were used as control and pasta was the lowest (Figure A 4). Sensitivity analysis by removal of studies with high risk of bias did not change the ES.

ES of post-prandial insulin responses were also significantly lower in both adults with and without T2DM (n = 3 RCTs; ES -19.43; 95% CI -24.01, -14.85; $p \leq 0.0001$, $I^2 = 0\%$) and (n = 11 RCTs; ES -11.26; 95% CI -22.11, -0.41; $p \leq 0.05$, $I^2 = 90\%$), respectively.

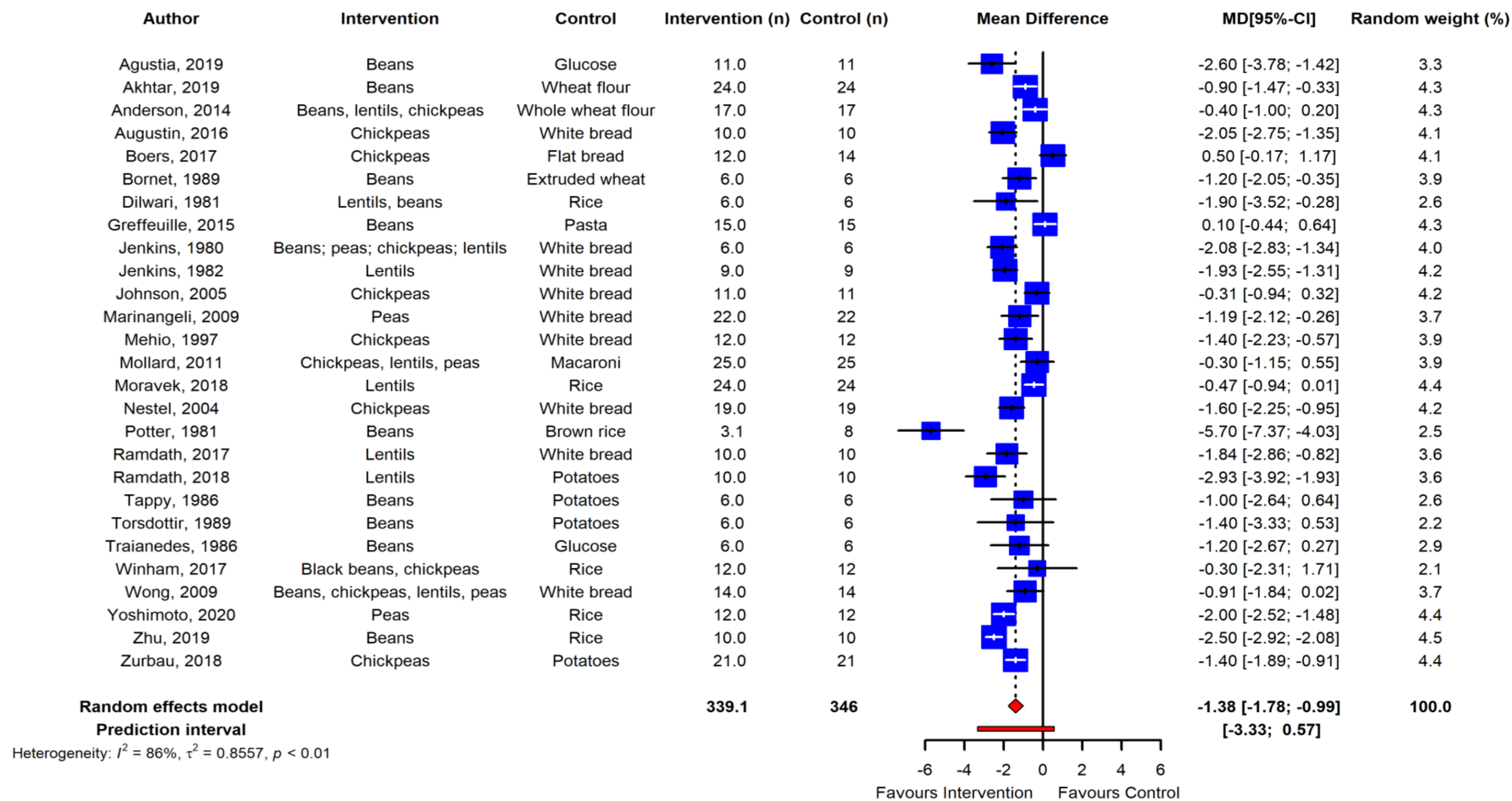


Figure 2-2 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of acute trials investigating pulse intake on postprandial glucose response among healthy individuals. The effect size was statistically significant for normoglycaemic adults

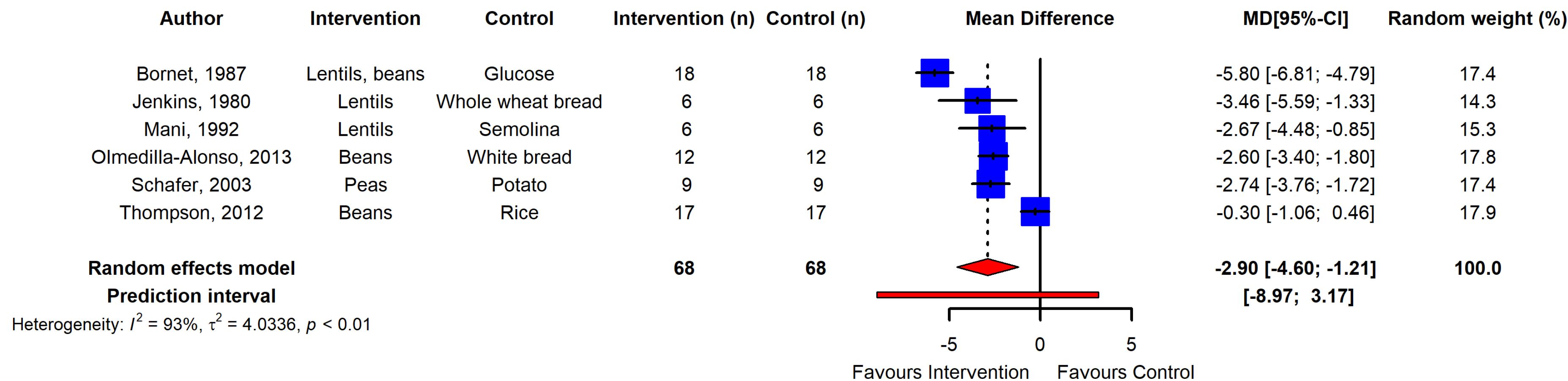


Figure 2-3 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of acute trials investigating pulse intake on postprandial glucose response among T2D individuals. The effect size was statistically significant for adults with T2D

2.4.2 Long-term parameters of glycemic control

The meta-analysis revealed that long-term pulse intake has a small reducing effect on fasting blood glucose levels in normoglycaemic adults (n = 16 RCTs) with low heterogeneity between studies (ES -0.06; 95% CI -0.12, 0.00; $p \leq 0.05$; $I^2 = 30\%$; PI -0.21, 0.09) (Figure 2-4). Sensitivity analysis showed that independent removal of one trial changed the ES interpretation from significant to non-significant. Pulse consumption in normoglycaemic adults had no significant effect on fasting insulin, HbA_{1c} and HOMA-IR, although the effect direction was toward reduction (n = 9 RCTs; ES -0.11; 95% CI -0.76, 0.55; $p = 0.75$); (n = 4 RCTs; ES -0.03; 95% CI -0.11, 0.06; $p = 0.54$); (n = 7 RCTs; ES -0.02; 95% CI -0.18, 0.14; $p = 0.78$), respectively.

Long-term pulse intake resulted in a significant reduction of fasting blood glucose in adults with T2D as estimated from data of 10 RCTs (ES -0.54; 95% CI -0.83, -0.24; $p \leq 0.005$; I^2=78%; PI -1.44, 0.37), albeit with high heterogeneity among studies (Figure 2-5). HbA_{1c} and HOMA-IR were also significantly reduced in adults with T2DM with high heterogeneity between studies (n = 6 RCTs; ES -0.17; 95% CI -0.33, -0.00; $p \leq 0.05$; $I^2 = 78$; PI -0.69, 0.36) and (n = 4 RCTs; ES -0.47; 95% CI -1.25, -0.31; $p \leq 0.05$; $I^2 = 79\%$; PI -3.63, 2.69) (Figure 2-6). Sensitivity analysis revealed that independent removal of one trial in estimation of ES of HbA_{1c} reduced the heterogeneity significantly (Hassanzadeh-Rostami et al., 2019), and removal of two RCTs changed the interpretation from significant to non-significant when estimating the ES of HOMA-IR (Kim et al., 2016, Kim et al., 2014). However, reduction in fasting blood insulin in T2DM adults was not significant (n = 8 RCTs, ES -1.18; 95% CI -2.54, -0.08; $p > 0.05$; $I^2 = 63\%$). Egger's test did not indicate funnel plot asymmetry in long-term trials ($p > 0.05$) (Figure A 5 and A 6).

The GRADE assessment for each outcome, summarized in Supplemental Table A3, revealed 'low' grades for acute PPGR in normoglycaemic and T2DM, mainly downgraded due to inconsistency and indirectness of these outcomes. Evidence on long-term parameters fasting glucose, HbA_{1c} were

graded as ‘very low’ due to low ratings for consistency, directness, and precision that led to decrease in the level of certainty

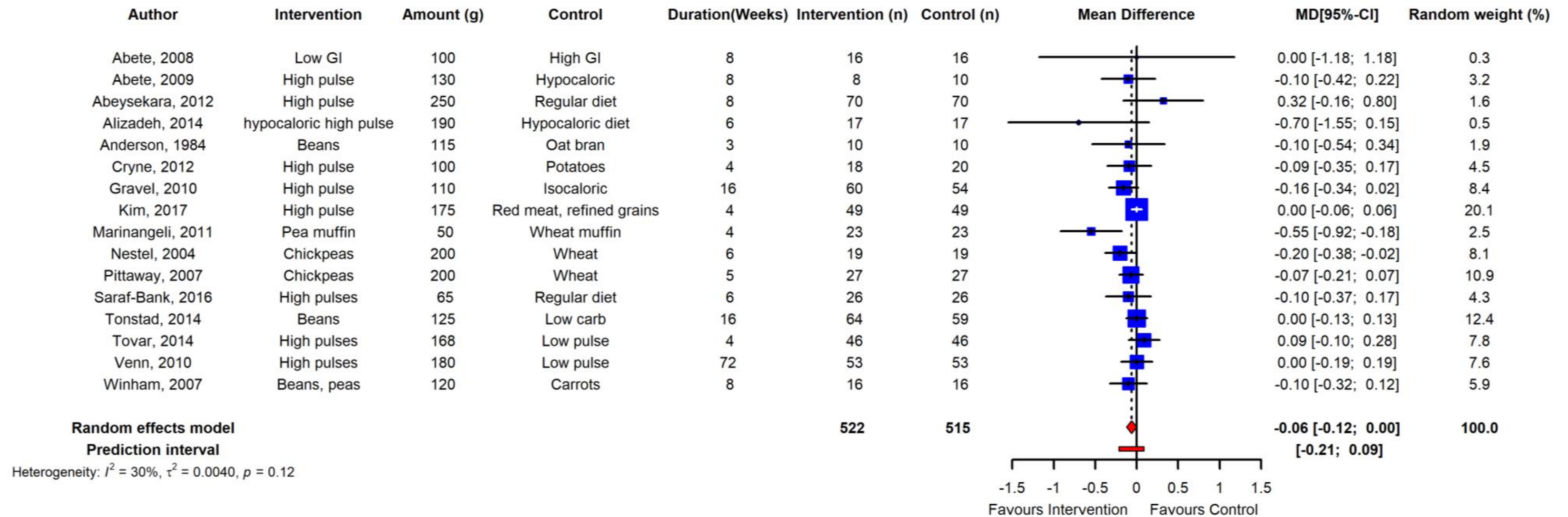


Figure 2-4 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of long-term trials investigating pulse intake on fasting glucose among healthy individuals. The meta-analysis concluded that long-term pulse intake has small but significant effect on reducing fasting blood glucose levels in normoglycaemic adults

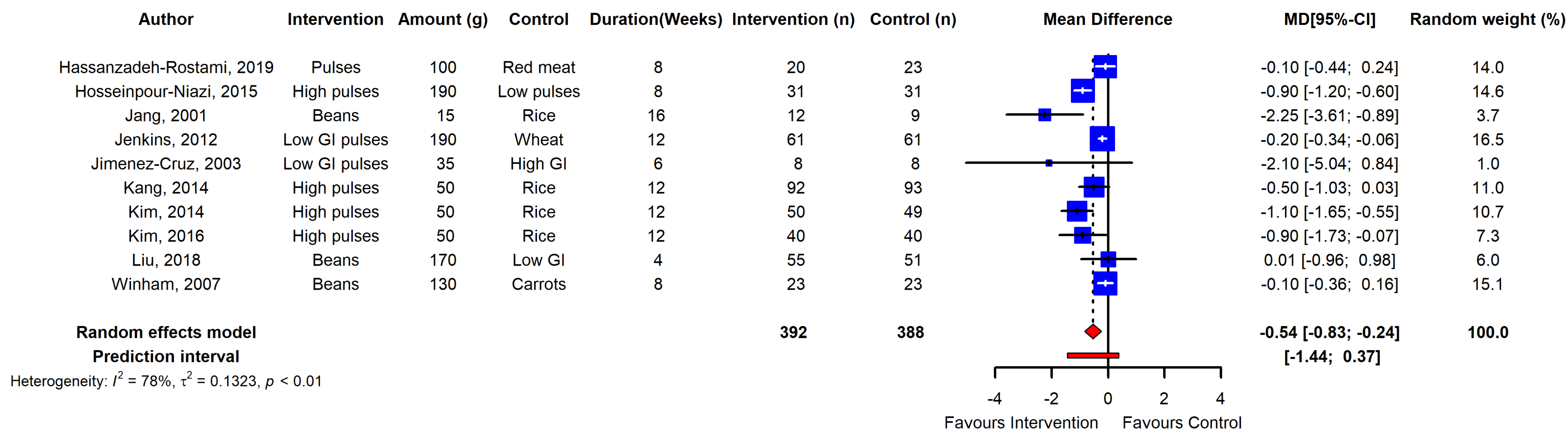
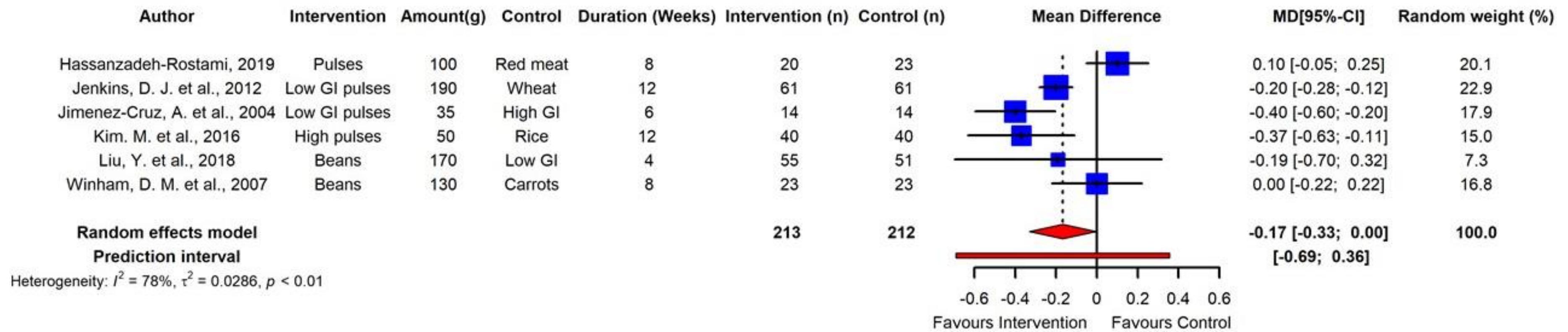


Figure 2-5 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of long-term trials investigating pulse intake on fasting glucose among T2D individuals. Long-term pulse intake resulted in a significant reduction of fasting blood glucose in adults with T2D

A



B

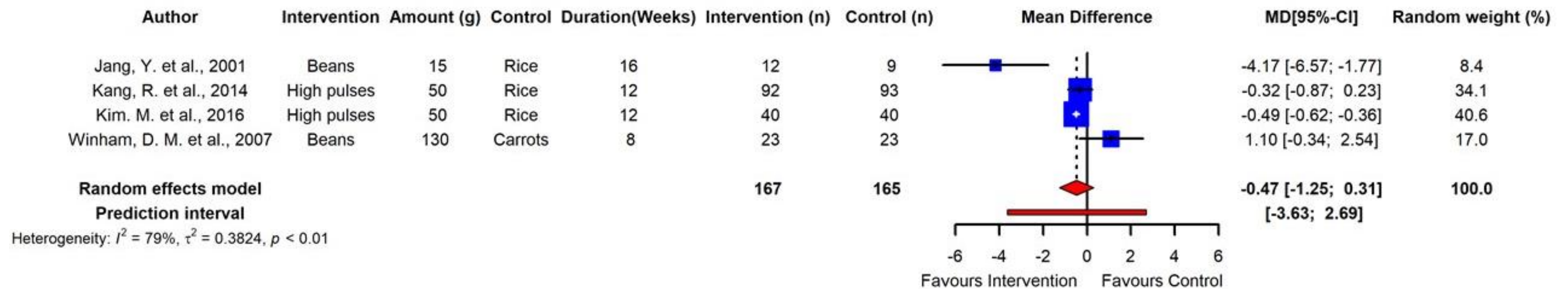


Figure 2-6 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of long-term trials investigating pulse intake on fasting glycated haemoglobin (a); and HOMA-IR (b) among T2D individuals

2.5 Discussion

In this systematic review and meta-analysis, we found that pulse intake enhances glycemic regulation on both acute post-prandial responses and long-term glycemic indices. We demonstrate that pulse intake leads to clinically significant reductions in PPGRs, with a mean reduction of PPGR > 1 mmol/L in normoglycaemic individuals, and > 2.5 mmol/L in those with T2D, and consequently significantly reduced insulin was observed ≥ 20 mIU/L. Long-term pulse intake was reported to reduce fasting glucose, HbA_{1c} and HOMA-IR with more pronounced effect in adults with T2DM.

Post-prandial glycemic control plays a crucial role in prevention of chronic diseases such as cardiovascular disease, in both normoglycaemic and T2D individuals (Berry et al., 2020). The estimated magnitude of the reduction in PPGR is similar to the reported effect of some glucose lowering therapies such as DPP-4 inhibitors (Hollander and Kushner, 2010, Riddle et al., 2011). However, the certainty of evidence is impaired due to substantial inter-study variances. Possible modifiers were identified in acute RCTs, such as differences in pulse type, processing methods, and the control used as a comparison, which were explored by subgroup analysis. Although lentils are suggested by subgroup analysis to be the most potent type in controlling PPGR, other types of pulses still show a clinically significant impact (range -1.60 to -0.95 mmol/L) in normoglycaemic adults with substantial inter-study heterogeneity. There are only a few trials that have assessed the impact of processing on post-prandial glycemic responses and the results are mixed with some RCTs finding no significant impact of processing in attenuating PPGR, while others suggest that pulse flour resulted in significantly higher PPGR in comparison to other physical forms (Anderson et al., 2014, Anguah et al., 2014, Ramdath et al., 2018). Our meta-analysis supports the finding that intervention foods using pulse flour were found to be 50% less effective in attenuating PPGR when comparing to other physical forms. However, pulse flour used as intervention in the RCTs was incorporated into bakery products or pasta, with the flour being only 25-35% of the composition of final product; the incorporation of legume flour with cereal flours resulted in a lower effect when compared to whole pulses which were mostly consumed

alone. Nevertheless, the lower efficacy of pulse flour could also be explained by breakage of the cell walls during the milling process, resulting in increased exposure of the starch to digestive enzymes whilst wet pureeing may result in cell separation, keeping more cells intact (Ramdath et al., 2018). However, due to the high heterogeneity within subgroups, possibly due to the presence of different pulse types within a subgroup and lack of standardized protocol for food processing, definitive outcomes cannot be concluded and therefore more studies are needed to investigate effects of processing on post-prandial glycemic handling.

In alignment with blood glucose, pulse intake favorably affected post-prandial insulin levels with a larger effect in T2D population where reduction in PPGR was greater. There were large variations between RCTs with regards to characteristics of participants such as mean age (22-66 y) and BMI (20-31), that might influence insulin secretion and sensitivity.

Long-term RCTs show that pulse intake leads to a favorable impact on fasting blood glucose in adults with and without T2D, and improved HbA_{1c} and HOMA-IR in those with T2D. The attenuation of fasting blood glucose was small in normoglycaemic individuals (mean difference of ~0.06 mmol/L over median duration of 6 weeks), and greater in with T2D (mean difference of ~0.5 mmol/L over median duration of 8 weeks). We conducted a comparison of ES considering presence of diabetes as a modifier, and found significant differences between both conditions ($p < 0.05$) (Supplemental Figure A7).

Post hoc meta-regression was performed to investigate the effect of pulse dose and study duration, and found low doses of pulses were more effective in reducing fasting blood glucose in adults without T2DM. However, there was no significant effect of study duration in modifying the ES (Supplemental Figure A8 and A9). Our findings are in agreement with Sievenpiper et al. reporting inverse association between pulse dose in interventions and ES (Sievenpiper et al., 2009).

The reduction of HbA_{1c} (mean reduction of ~ 0.3%) is also considered to be clinically significant as the effect is comparable to low doses of some oral anti-diabetic agents such as α-glucosidase inhibitors (Sherifali et al., 2010). Considering that HbA_{1c} reflects average glucose levels over the 8-12 weeks life span of erythrocytes (Derr et al., 2003), it is not surprising that some studies with an intervention duration shorter than this did not report an improvement in this measure. This together with subgroup analysis of study duration emphasizes the importance of conducting long-term RCTs of > 8 weeks in duration to report the outcomes of pulse intake and other dietary interventions on measures of glycemic control.

The beneficial effect of pulse intake on regulation of glucose metabolism could be related to several mechanisms. The bioavailability of carbohydrates from pulses can be reduced by factors such as low free sugar content and high levels of resistant starch (Perez-Hernandez et al., 2020). In cooked whole or blended pulses, the presence of thick cell walls is likely to prevent access of amylolytic enzymes to the starch substrate (Edwards et al., 2021). Thermal processing increases fiber solubility, but the impact of this on glycemic effects is not known (Aldwairji et al., 2014). Furthermore, the crystalline nature of pulse starch and presence of fiber polysaccharides (both soluble and insoluble) as well as protein and lipids, contribute to delaying the gastric transit thereby slowing the arrival of food into the small intestine and hence lower the glycemic response (Perez-Hernandez et al., 2020, Edwards et al., 2015).

Other systemic effects may be via the microbial fermentation of fiber and resistant starch in the colon to short chain fatty acids (SCFA) such as propionate, butyrate and acetate (Prasad et al., 2018). These SCFA reduce glucose release from the liver and thus promote muscle glycolysis, improved insulin secretion and glucose homeostasis via gut-brain axis and suppression of free fatty acid synthesis (Mandaliya et al., 2018). The soluble fiber is suggested to have beneficial impact on reduction of post-prandial glycemic effects attributing to the viscosity and gel-forming properties (Meyer et al., 2000, Weickert and Pfeiffer, 2018). Presence of fiber along with slowly digestible starch in pulses has been linked to improved blood glucose profile,

insulin sensitivity and urinary C-peptide, and tends to normalize insulin levels in individuals with hyperinsulinemia (Rizkalla et al., 2002).

To our knowledge, this is the first meta-analysis summarizing the impact of pulse intake on acute PPGR reported after pulse intake, and the most comprehensively assessing long-term impact of pulse consumption on glycemic handling indices. Post-prandial glycemic biomarkers are highly correlated with long-term indices and are considered as independent risk factors in progression of several health conditions such as diabetes and coronary heart diseases (American Diabetes, 2001). Therefore, including acute post-prandial trials in this review, and adopting raw mean difference over standardized mean difference beside employment of meta-regression allow better understanding over previous meta-analysis regarding the role of pulses in controlling glycemic indices (Sievenpiper et al., 2009). Furthermore, we have assessed the certainty of the evidence by following GRADE method, and calculated the prediction intervals to estimate clinical consequences of the heterogeneity and to provide a range into which we can predict the outcome of future studies to fall based on current evidence. Our prediction intervals are broad including both positive and negative intervals, reducing the confidence in predicting that results of a future trial would favor pulse intake, although broad prediction intervals are common in RCTs. However, there are several limitations in our analysis that should be considered. First, the risk of bias ranged from 'some concerns' to 'high risk', and the quality of evidence was graded from 'low to 'very low'. This is largely due to substantial inter-study heterogeneity that remained unexplained despite subgroup analysis. Additional variables such as ethnic background, genetic predisposition, physiological factors such as age, gender and BMI, lifestyle of the participants might contribute toward observed heterogeneity in reported outcomes and thus affecting the grading of the evidence. The quality of the RCTs was downgraded mostly due to inappropriate way in conducting or reporting of randomization process, or due to unavailability of trial protocol or register information. These factors collectively reinforce importance of high quality RCTs to support the beneficial effect of pulse intake on glycemic handling (Viguiliouk et al., 2019). Second, we have

included only RCTs with defined pulse consumption in the meta-analysis while excluding those that included pulses in selective eating patterns such as low GI or high fiber diets. While this may have reduced the number of studies included, it also increased knowledge about particular types and forms of pulses. Thirdly, there were 28 studies excluded due to inability of accessing the full text, and 3 papers were excluded as they were not available in English language. These collectively might have resulted in publication bias. Finally, the data extraction procedure was performed by single author which might introduced some biases.

Overall, pulse intake significantly reduced PPGR in both normoglycemic and individuals with T2D, and therefore are recommended for consumption as a low GI food. Long-term pulse consumption resulted in favorable effects on measures of glycemic control especially in those with T2D. Although whole or pureed lentils showed more promising effects, due to high heterogeneity between studies, it is not possible to give a specific recommendation with regards to pulse type, dose, form (i.e. processing method) and duration of intake. Carefully controlled acute studies are required to study the impact of differently processed pulses on glycemic parameters. Furthermore, well-designed long-term RCTs are needed to establish effectiveness of pulse rich diets and dose-response relationships in order to refine dietary recommendations for pulse intake.

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Chapter 3 Impact of food processing on postprandial glycaemic and appetite responses in healthy adults: a randomized, controlled trial.

3.1 Abstract

Chickpeas are among the lowest glycaemic index carbohydrate food eliciting protracted digestion and enhanced satiety responses. *In vitro* studies suggest that mechanical processing of chickpeas significantly increases starch digestion. However, there is little evidence regarding the impact of processing on postprandial glycaemic response in response to chickpea intake *in vivo*. Therefore, the aim of this study was to determine the effect of mechanical processing on postprandial interstitial glycaemic and satiety responses in humans. In a randomised crossover design, thirteen normoglycaemic adults attended 4 separate laboratory visits following an overnight fast. On each occasion, one of four test meals, matched for available carbohydrate content and consisting of different physical forms of chickpeas (whole, puree, and pasta) or control (mashed potato), was administered and followed by a subsequent standardised lunch meal. Continuous glucose monitoring captured interstitial glucose responses, accompanied by periodic venous blood samples for retrospective analysis of C-peptide, glucagon like peptide-1 (GLP-1), ghrelin, leptin, resistin, and cortisol. Subjective appetite responses were measured by Visual Analogue Scale (VAS). Postprandial glycaemic responses were comparable between chickpea treatments albeit significantly lower than the control ($p < 0.001$). Similarly, all chickpea treatments elicited significantly lower C-peptide and GLP-1 responses than the control ($p < 0.05$), accompanied by enhanced subjective satiety responses ($p < 0.05$), whilst no significant differences in satiety hormones were detected among different intervention groups ($p > 0.05$). Chickpea consumption elicits low postprandial glycaemic responses and enhanced subjective satiety responses irrespective of processing methods.

3.2 Introduction

Specific dietary habits, including the regular consumption of ultra-processed food, have been proposed as causative factors of non-communicable diseases (NCDs) such as obesity and type 2 diabetes (T2D) (Forouzanfar et al., 2016, Nardocci et al., 2020, Poti et al., 2017, Yardley and Campbell, 2020, Campbell et al., 2020). Ultra-processed foods, which are typically high in refined carbohydrates and low in fibre content, induce substantial glucose dysregulation and have been shown to increase appetite and prospective food intake (Fardet, 2016, Holt and Miller, 1994, Pan and Hu, 2011, Slavin and Green, 2007, Hall et al., 2019, Hafiz et al., 2021). However, emerging evidence suggests that other factors inherent to food, including the type, physical integrity, and viscosity of starch and carbohydrate source, as well as presence of protein also significantly impact postprandial glucose elevation (Brand-Miller et al., 2009, Allerton et al., 2016, Howard et al.). For example, high fibre foods are reported to elicit reduced postprandial glycaemic responses compared to similar carbohydrates with lower fibre content (Livesey and Tagami, 2009), and, the co-ingestion of protein with carbohydrate rich foods has, in some studies, been shown to attenuate postprandial glucose excursions and enhance insulin secretion especially in the presence of secretagogue amino acids such as arginine and leucine (King et al., 2018). As such, complex carbohydrate rich foods which preserve plant structure, are high in fibre and protein content may result in more favourable postprandial glucose.

Chickpeas *(Cicer arietinum L.)* are pulses rich in slowly digestible carbohydrates, soluble and insoluble dietary fibre, and high quality proteins including bioactive peptides. As a result, chickpeas are widely characterised as having a very low glycaemic index (GI) (reported between 25 to 45) and energy density (Wood and Grusak, 2007, Jukanti et al., 2012). Findings of interventional studies suggest a significant attenuation in postprandial glycaemic responses (PPGRs) and suppressed subjective appetite and prospective food intake after chickpea intake when compared to other carbohydrate rich foods with similar amounts of available carbohydrates (McCrory et al., 2010, Zafar and Kabir, 2017). Greater intraluminal viscosity,

reduced gastric emptying and promotion of incretin secretion are considered as proposed mechanisms by which chickpeas can enhance satiety along with reduction of postprandial glycaemia (Becerra-Tomás et al., 2019).

Importantly, some *in vitro* studies investigating the effect of mechanical processing of chickpeas, particularly methods that result in cell wall disruption, show a significant increase in the rate of starch digestion and starch release following processing compared to non-processed chickpeas (Dhital et al., 2016, Edwards et al., 2021). However, little is known regarding the impact of processing methods on postprandial glucose, and little research has investigated the impact of pulse intake on satiety hormones such as incretins and ghrelin *in vivo* (Anderson et al., 2014, Binou et al., 2020).

Therefore, this study aimed to assess the acute postprandial interstitial glycaemic and satiety responses to chickpea ingestion following different processing methods in healthy adults. We used a continuous glucose monitoring (CGM) as a less invasive method to collect glycaemic information over the intervention period, including post-meal effects.

3.3 Methodology

3.3.1 Study design

This study followed a randomised, crossover, controlled design to assess the postprandial glucose response to chickpeas that were differently processed in normoglycaemic adults. Experimental procedures consisted of four visits; and randomisation was conducted using an online programme (http://www.randomization.com).

Participants were screened for eligibility and recruited for the trial at the human study facility in the School of Food Science and Nutrition at the University of Leeds. The included participants were healthy adults aged 18-65 years, presenting with fasting blood glucose < 5.6 mmol/L and body mass index (BMI) 18-29.9 kg/m^2. The exclusion criteria for the study were BMI ≥ 30 kg/m^2 (obese), fasting blood glucose > 5.5 mmol/L, the presence of

disease, allergies, or medication use known to impact food digestion, appetite, food sensory, or glucose metabolism. Written informed consent was obtained from all participants prior to participation and the study procedures were conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures were approved by the Mathematics and Physical Sciences and Engineering Joint Faculty Research Ethics Committee at the University of Leeds (Ethics reference MEEC 18-035). The study was prospectively registered at www.isrctn.com as ISRCTN14869733.

3.3.2 Study procedure

Nineteen participants were recruited between 15 August to 20 December 2019. Participants attended four sessions to assess the postprandial responses to four different meals (three different chickpea meals and one control meal). The sessions were conducted over a two week period with a minimum of two days between visits allowing for washout (Madrid-Gambin et al., 2018). The order of the interventions was random as per pre-generated sequences (Supplemental Table B1). Each session commenced on the morning at 9:00, after an overnight fast (10-12 hours). One day prior to the first experimental visit, participants were fitted with a Continuous Glucose Monitor (FreeStyle LibrePro, Abbott, Wiesbaden, Germany), which was placed on the upper arm as previously described (Mahadevamma and Tharanathan, 2004). The monitor remained in place for the duration of the two week intervention period. Interstitial glucose values were obtained by reading the CGM glucose sensors that recorded values every 15 minutes over the two week period. The participants were blinded from the data collection.

Participants were requested to avoid legume and alcohol intake, and limit vigorous exercise for a minimum of 24 h before each experimental visit, and to otherwise maintain their dietary habits and physical activity constant throughout their visits to minimise variations due to these factors. Participants were asked to record dietary intake in the 24 h period before each visit.

Upon arrival, participants assumed a seated rested position whilst an intravenous cannula was inserted in the forearm for the periodic collection of venous blood samples. Stylets were used to keep the vein patent for during the 3 h observation window. Following a resting blood sampling, test meals were provided along with one cup of water, and volunteers were asked to consume their meals (see below) within 15 minutes. Participants remained seated throughout the three hour observation window, and intravenous blood samples were obtained every 30 minutes from the inserted cannulas. Subjective appetite levels were also recorded at baseline and over three hours after meal intake using a visual analogue scale (VAS) on 100 mm line with intervals describing individual's perception of hunger fullness and prospective food intake (Flint et al., 2000). After 3 h, cannulas were removed, and participants were given a standardised lunch meal to be consumed within one hour following discharge.
Blood samples were collected in serum separator tubes (SST, BD Vacutainer) for serum isolation and in ethylenediamine tetraacetic acid (EDTA, BD Vacutainer) tubes for plasma collection. Plasma samples were treated with the addition of two protease inhibitors: di-peptidyl peptidase-4 (DPP-IV) and aprotinin at a final concentration of 1 mg/mL to preserve GLP-1, ghrelin, and leptin (Bielohuby et al., 2012). Blood samples were kept on ice and centrifuged within 30 minutes at 2000 rpm for 10 minutes at 4° C for plasma separation and 2000 rpm for 15 minutes at 25° C for serum, and subsequently stored in aliquots at -80° C until analysis.

3.3.3 Study food

The experimental test meals comprised of three differently processed chickpea foods: whole chickpeas (250 g), pureed chickpeas (250 g), and fusilli made out of chickpea flour (217 g), each providing 50 g available carbohydrates, mainly as starch. The control intervention was Smash® instant mashed potatoes (425 g, providing 50 g available carbohydrates). All experimental foods were matched in total available carbohydrates, which was analytically estimated by using an Available Carbohydrate kit (KACHDF), Megazyme International (Bray, Ireland). Fat and salt contents were equalized by addition of olive oil and table salt. The nutrition information

of all intervention foods is shown in Table 3-1. Whole chickpeas were obtained from ready to eat tins of chickpeas (Sainsbury's, UK), which were rinsed with tap water and drained for 5 minutes, before weighing. Pureed chickpeas were also prepared using the same canned chickpeas (Sainsbury's, UK), pureed using an electric blender for 5 minutes to obtain an incorporated texture. Chickpea fusilli (Ugo) was cooked freshly on the day; the pasta was boiled for 3 minutes in water and drained for 5 minutes. Smash® instant mashed potatoes was freshly prepared by mixing with boiling water according to instructions on the packaging. All test meals were served at room temperature.

The lunch meals consisted of a cheddar cheese sandwich (Morrison's, UK), salted crisps (Sainsbury's, UK), and 150 mL of carbonated soft drink (Coca-Cola, UK). The nutritional content of lunch food is described in Table B 2.

Table 3-1 Macronutrient composition of the intervention and control food

Nutrition information	*ChW*	*ChPu*	*ChF*	*Con*
Weight, g	250.0	250.0	217.0	425.0
CHO, g[1]	50.0 (57%)	50.0 (57%)	50.0 (56%)	50.0 (68%)
Fibre, g	15.3	15.3	12.4	4.7
Fat, g[2]	8.0 (20%)	8.0 (20%)	8.0 (20%)	8.0 (24%)
Protein, g[3]	19.3 (23%)	19.3 (23%)	21.3 (24%)	6.2 (8%)
Salt, g	0.8	0.8	0.8	0.8
Energy, kJ	1460.6	1447.6	1497.4	1241.9

ChW, chickpeas whole; ChPu, chickpeas pureed; ChF, pasta made of chickpea flour; Con, mashed potatoes
1 values in the brackets present the percentage contribution of the carbohydrate toward total energy of the meal
2 values in the brackets present the percentage contribution of the fat toward total energy of the meal
3 values in the brackets present the percentage contribution of the protein toward total energy of the meal

3.3.4 Biochemical analysis of blood markers

Plasma C-peptide, ghrelin, leptin, resistin, cortisol, and GLP-1 were measured using a commercially available fluid phase multiplex immunoassay kit as per manufacturer's instructions (Invitrogen ProcartaPlex Human metabolism/obesity panel, Fisher Scientific, Leicestershire, UK) using a Luminex 200 ™, Houston, Texas. The intra-assay variation was < 15% for each analyte.

3.3.5 Statistical analysis

The primary objective of the trial was to compare differences in postprandial interstitial glycaemic responses determined by continuous glucose monitoring system, after consuming pulses with different processing in comparison to a high GI control food. Secondary outcomes were serum C-peptide, incretin, appetite hormones, as well as subjective appetite response and the subsequent meal's glycaemic response. Plasma c-peptide levels were analysed to reflect levels of insulin considering the longer half-life of c-peptide (20-30 minutes) compared to insulin (3-5 minutes), which allows a more stable test window. The sample size was calculated to detect differences of at least one standard deviation of PPGR between intervention arms. Sample size calculations were based on data from previous studies conducted in our laboratories (not-published), with estimated average peak glucose response (±SD) of 6.4 ± 0.9 mmol/L after consumption of pulses. According to the calculation, a total of 18 participants would be required for this crossover study for a significance level of 0.05 and a probability of 80%. However, previous acute studies have shown that ten participants on average are sufficient to detect a minimum difference of 1 mmol/L of postprandial glucose peak response (Brouns et al., 2005, Mollard et al., 2014).

The effect of intervention food on peak postprandial interstitial glycaemic and blood insulinaemic rise (c-max) along with other biomarkers was assessed using a two factors repeated measure ANOVA and comparisons were conducted using Bonferroni's test, where a significant difference was observed. Postprandial interstitial glycaemic and blood insulinaemic incremental area under the curves (iAUCs) were calculated using the

trapezoidal rule, omitting values below the baseline, over 120 and 180 minutes after consuming intervention and control foods, and the data were analysed using one-way ANOVA. In outcomes where values below the baseline were of interest such as satiety responses, total area under the curves (tAUCs) was calculated in place of iAUC (Wolever, 2004).

Subjective hunger, fullness, and prospective food intake scores were analysed for differences using one-way ANOVA along with their tAUCs, and post hoc analysis using Bonferroni's test where a significant difference was detected.

All statistical analyses were performed using SPSS (version 26, IBM), with a statistical difference of $p < 0.05$ considered as significant.

3.4 Results

In total, 30 volunteers were initially screened for participation in the trial, 19 volunteers initiated their visits out of which 13 completed all four study visits (Figure 3-1), 4 males and 9 females. Baseline characteristics of study participants are shown in Table 3-2.

Table 3-2 Participant characteristics

	Mean	SD
Age (y)	28.7	6.6
females (n)	9	-
Smoking, yes (n)	3	-
Height (cm)	164.5	10.6
Weight (kg)	63.6	11.1
Body mass index (kg/m2)	23.2	2.5
Fasting glucose (mmol/L)[1]	4.1	0.5
Glycated haemoglobin A1c (%)[1]	4.48	0.22

1 measured by continuous glucose monitors

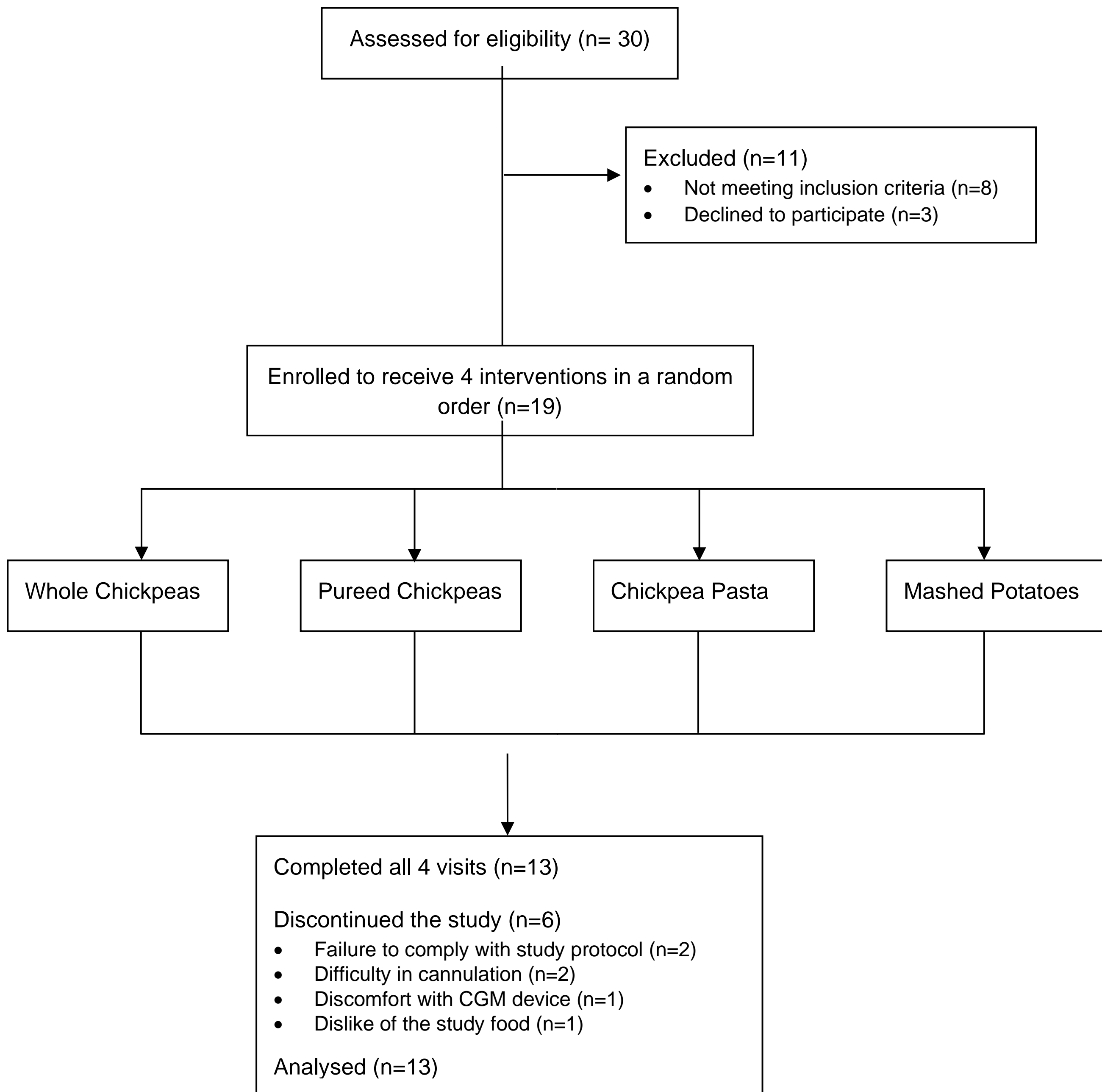

Figure 3-1 Flow diagram of participant selection

3.4.1 Postprandial interstitial glycaemic responses

Interstitial glucose values were obtained for all participants throughout study period of 14 days via LibreView software which allows to interpretation of the glucose profiles in various manners. Those values were obtained by directly connecting each CGM devise with the software and hence a complete glucose profile was downloaded for each participant. However, in this trial we have studied only the values related to the study design (fasting, after breakfast, and after lunch intake).

All participants on all study visits presented with fasting interstitial glucose values below 5.5 mmol/L, with no significant differences between the intervention arms in baseline values of interstitial glucose, and there was no effect of gender, age, or BMI on the fasting interstitial glucose status of volunteers. A significant time x intervention interaction effect was observed when assessing postprandial interstitial glucose concentration in response to test meals ($p < 0.001$). Interstitial glucose increased after breakfast consumption in all groups (time $p < 0.001$) (Figure 3-2), with the greatest temporal rise observed after ingestion of *Con* (intervention $p < 0.001$) when assessed as absolute concentrations and iAUC ($p < 0.001$) (Figure 3-3). Postprandial interstitial glucose peak (c-max) was comparable across chickpea conditions, and significantly lower compared to *Con* ($p < 0.001$); no differences were observed in time to peak with peak glucose occurring at 45-minutes post-consumption under all conditions (Figure 3-2).

Interstitial glucose levels were significantly higher after intake of *Con* compared to all treatments from 30 to 90 minutes ($p < 0.05$). Following intake of *ChF*, glucose values were gradually lowered back to baseline values at 75 minutes after following peak at 45 minutes, before rising to a second peak at 90 minutes, while other chickpea treatments (*ChW* and *ChPu*) showed a slower reduction in glucose concentrations with no significant differences among chickpea treatments. Mean glucose iAUCs (0-3 h) were significantly lower after intake of all forms of chickpea breakfasts in comparison to *Con* ($p < 0.001$), however there were no significant differences among chickpea processing methods (Figure 3-3).

3.4.2 Subsequent meals' glycaemic response

Following the standardised lunch, glucose peak (c-max) occurred at 45 minutes under all conditions. Peak glucose was significantly attenuated under both *ChW* and *ChPu* (p = 0.049), as compared to *Con* condition, but not *ChF* (p = 0.156). Total glucose exposure expressed as average iAUCs of interstitial glucose during this period was comparable between *ChW*, *ChPu*, and *ChF* and was lower than *Con* (p = 0.01) (Figure 3-2 and 3-3).

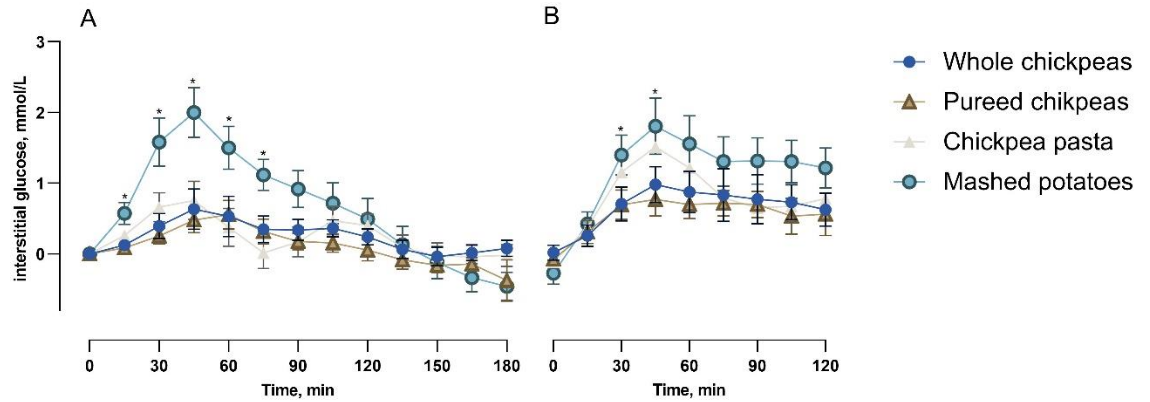

Figure 3-2 Time-course changes in interstitial glucose profile following breakfast (A) and lunch (B). Values are incremental means ± SEM for n = 13 participants after consuming intervention meals. *Indicates significant differences between Con and all three forms of chickpeas by Bonferroni's test ($p < 0.05$). † Indicates significant differences between Con and ChW and ChPu by Bonferroni's test ($p < 0.05$).

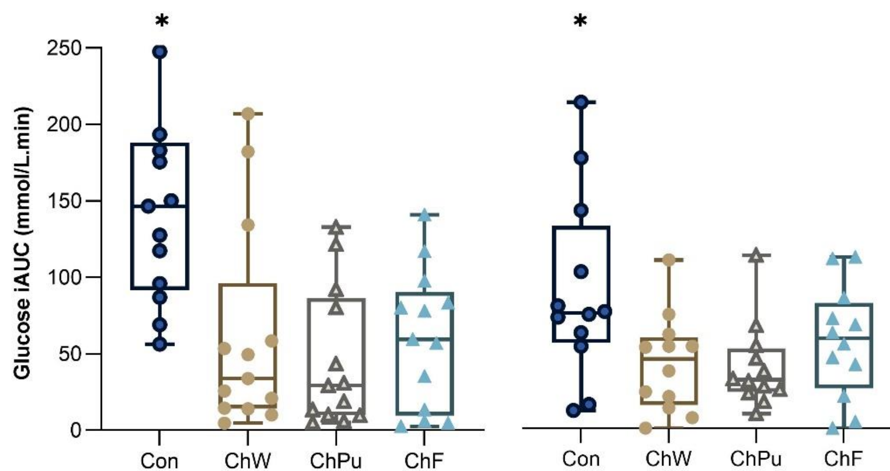

Figure 3-3 Incremental areas under the curve (iAUC) of the changes in interstitial glucose after breakfast (A), lunch (B). ChW, chickpeas whole; ChPu, chickpeas pureed; ChF, pasta made of chickpea flour; Con, control mashed potatoes. Values are means ± SEM for n = 13 participants after consuming intervention meals. *Indicates significant differences between Con and all three forms of chickpeas by Bonferroni's test ($p < 0.05$).

3.4.3 Subjective appetite responses

Average subjective appetite responses of all participants are shown in Table 3-3, with no significant differences between the interventions arms in baseline values of hunger, fullness, and prospective food intake. There were high interpersonal variabilities observed in reporting the subjective responses, however, results remained robust following adjustment for potential confounders. Subjective responses of hunger at the end of the visit and total (AUC 0-3 h) were significantly greater for *Con* compared to all forms of chickpeas ($p < 0.05$); and responses of fullness (AUC 0-3 h) after ingesting *Con* were significantly lower compared to all chickpea meals ($p < 0.05$). There was no significant difference between conditions observed for prospective food intake. However, we observed significantly lower hunger ratings in normal weight individuals at 60 min after *ChF* ($p = 0.04$), and at 180 min after *ChW* ($p = 0.03$) in comparison to overweight participants. There was no significant gender x intervention interaction for any related to hunger, fullness, or prospective food intake.

Table 3-3 Incremental subjective appetite responses as measured by visual analogue scale over 3 hours after intervention [1]

	ChW		*ChPu*		*ChF*		*Con*		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Hunger score 60 min	23.8	13.6	20	8.2	22.3	11.5	28.5	11.2	0.255
Hunger score 180 min	36.2 [a]	17.7	33.8 [a]	13.5	36.2 [a]	15.3	48.5 [b]	11.4	0.045
Hunger total AUC0–3h, mm × h	91.2 [a]	37.7	85.4 [a]	23.4	89.6 [a]	30.9	113.5 [b]	26.8	0.035
Fullness score 60 min	43.1	15.3	43.1	11.7	40.8	8.6	33.8	9.6	0.137
Fullness score 180 min	31.5	15.9	30.8	11.9	26.9	12.9	20	12.7	0.095
Fullness total AUC0–3h, mm × h	107 [a]	37.2	107 [a]	26.0	101 [a]	25.2	80 [b]	25.2	0.012
Prospective food intake score 60 min	26.9	17.6	26.9	16.4	26.2	12.7	36.2	8.7	0.208
Prospective food intake score 180 min	41.5	19.8	39.2	11.3	38.5	11.1	50	11.2	0.123
Prospective food intake total AUC0–3h, mm × h	104	41.1	102	34.0	98.1	32.9	126	25.6	0.165

ChW, chickpeas whole; ChPu, chickpeas pureed; ChF, pasta made of chickpea flour; Con, mashed potatoes.

[1]n = 13.

Different superscript letters indicate significant differences within means in a row (Bonferroni's post hoc test, p<0.05)

3.4.4 Plasma hormonal responses

There was a trend for mean postprandial GLP-1 responses to be lower after *ChW* intake compared to all other conditions, although these results were not statistically significant (Figure 3-4 A). When comparing postprandial iAUCs of GLP-1, significantly higher iAUCs were observed after intake of *Con* compared to all other treatments ($p = 0.041$). A similar pattern was noted in postprandial plasma C-peptide levels that were significantly lower following intake of all chickpea interventions compared to *Con* after both 30 ($p = 0.05$) and 60 minutes ($p < 0.001$) (Figure 3-4 B). Similarly, iAUC 0-3h postprandial C-peptides levels were also significantly lower for all chickpea treatments ($p < 0.001$).

Postprandial plasma resistin levels in *Con* were significantly higher at 30 minutes compared to *ChW* ($p = 0.05$), and at 60 minutes compared to *ChW* and *ChF* ($p = 0.02$). However, this could be due to unexplained slightly higher baseline values in the *Con* group, although the difference was not statically significant when comparing baseline values of all treatments ($p = 0.061$) (Figure 3-4 D).

No significant differences were observed in postprandial leptin, ghrelin, and cortisol values between all conditions ($p > 0.05$) (Figure 3-4).

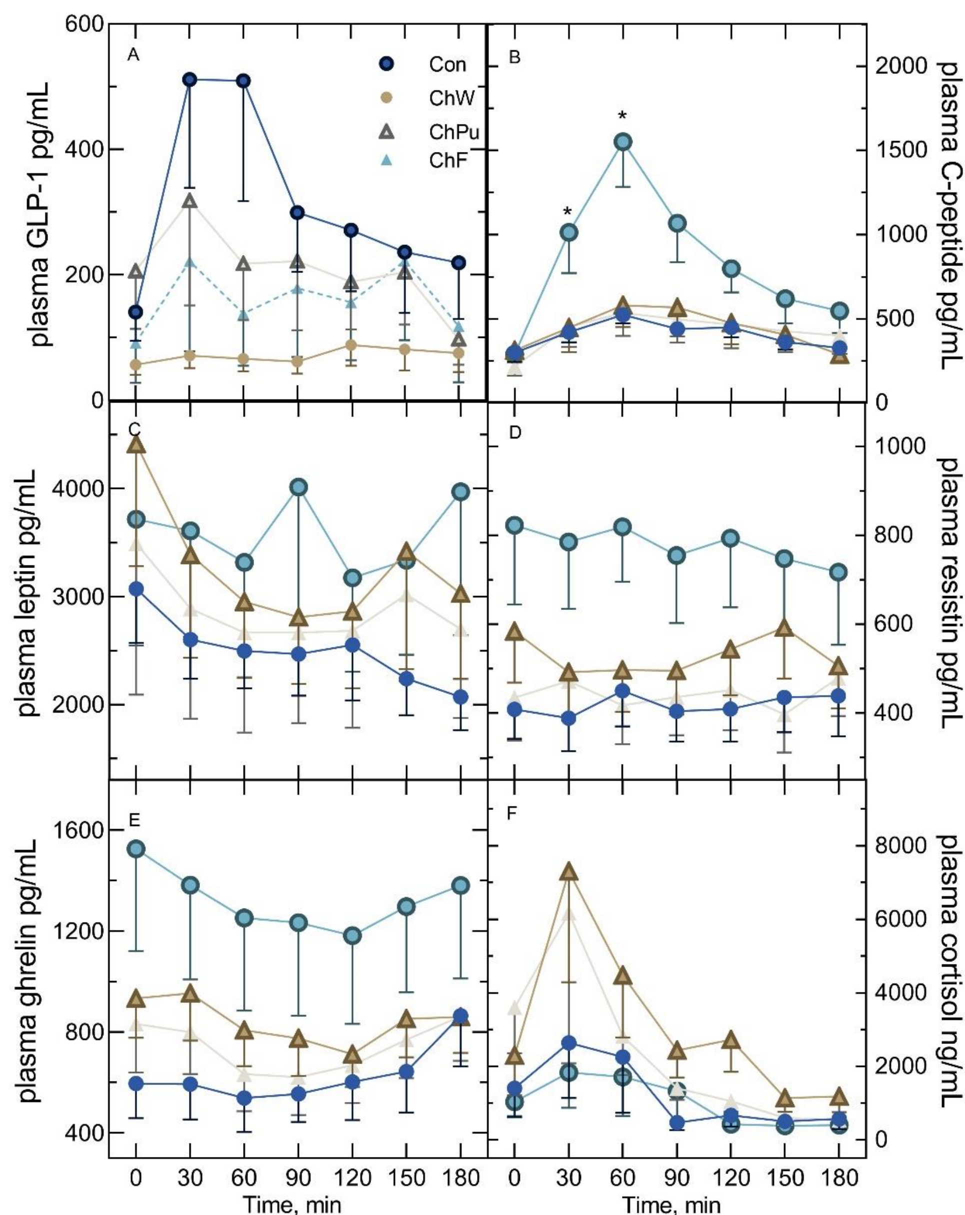

Figure 3-4 Postprandial responses of plasma GLP-1, C-peptide, leptin, resistin, ghrelin, and cortisol following intake of breakfast in all conditions. (A) GLP-1, (B) C-peptide, (C) leptin concentrations, (D) resistin concentrations, (E) ghrelin concentrations, and (F) cortisol. ChW, chickpeas whole; ChPu, chickpeas pureed; ChF, pasta made of chickpea flour; Con, mashed potatoes. Values are means ± SEM for n = 13 participants after consuming intervention meals. *Indicates significant differences between Con and all three forms of chickpeas by Bonferroni's test ($p < 0.05$).

3.5 Discussion

The present study was designed to determine the effects of different chickpea processing methods on subsequent postprandial interstitial glycaemic and appetite responses. The outcomes of the study indicate a comparable attenuation in postprandial interstitial glycaemic and appetite responses after chickpea intake irrespective of their physical form compared to the reconstituted mashed potato control. Average peak glucose was numerically higher after *ChF* compared to *ChW* (mean difference of ~ 0.12 mmol/L in maximum glucose rise), although differences failed to reach statistical significance and the magnitude of the difference is largely negligible. Likewise, peak glucose levels were higher after lunch intake in the *ChF* group, but the difference was not statistically significant owing to substantial variations within the group. Our outcomes are in contrast with some previous findings showing that ingestion of pulse flour based meals led to significantly higher postprandial glycaemic responses compared with whole pulses (Jenkins et al., 1982a, O'Dea and Wong, 1983, Tovar et al., 1992). This discrepancy is likely to be due to divergent test meals, specifically the use of pulse flour based pasta in the present study as opposed to other test meals made from pulse flour such as bread. White pasta is generally considered to elicit a lower glycaemic response compared to white bread, despite both being produced from refined wheat flour (Chiavaroli et al., 2018). Commercial dried pasta is manufactured industrially using an extrusion process that results in a dense product which reduces the digestive enzyme accessibility and thus elicits substantially lower postprandial glucose responses (Brennan and Tudorica, 2008). The structure of pulse pasta was described as quite a compact protein/starch network which may limit access to digestive enzymes (Bresciani et al., 2021). Moreover, different varieties within a given pulse type have demonstrated compositional differences that lead to significantly different glycaemic responses when given the same amount of carbohydrates (Ramdath et al., 2017). It was not possible, as part of our trial, to keep the variety of chickpea seeds constant since we used commercial products. Our findings are consistent with another study reporting that pureeing pulses or grinding them

to flour does not impact on immediate blood glucose levels (Anderson et al., 2014). Above mentioned discrepant findings are likely to be due to differences in the degree of processing applied in flour preparations, which may have resulted in differences in cell wall integrity and hence starch bioaccessibility (Edwards et al., 2021, Dhital et al., 2016). The extent of intracellular starch digestion from chickpeas is largely dependent on cell wall integrity that act as a barrier regulating hydration and controlling the permeability to α-amylase. Consequently, the starch granules in intact chickpea cells are generally less susceptible to gelatinization and amylolysis highlighting the underpinning mechanism to their lower postprandial glucose response (Edwards et al., 2021). We observed intact chickpea cells in *ChW* and *ChPu* samples hence explaining the lower glycaemic response. In the case of *ChF*, we did not observe intact cells, but a dense network of what appeared to be starch, protein and cell wall material. This dense structure appears to compensate for the lack of intact cells, since this sample also showed an attenuated postprandial glycaemic response. On the other hand, *Con* consisted of rehydrated potato flakes which form a hydrated, easily accessible starch matrix lacking in cellular or native starch structures. We have found this food to be a good control in glycaemic studies since it is easy to prepare consistently prior to consumption, is well accepted by participants and leads to consistent glycaemic responses between participants.

We have also shown that the beneficial effect of chickpeas on glycaemic responses was extended to the subsequent meal as made evident by lower glycaemic responses following intake of the standardised lunch. Interestingly, the attenuated postprandial glucose effect following subsequent feeding was limited to *ChW* and *ChPu* only, which might be attributed to the larger pulse particle size and the presence of intact cells in those treatments (Howard et al., 2021). This finding is consistent with a study showing that only whole pulses are effective in reducing glucose concentrations in response to subsequent feeding in normoglycaemic adults (Anderson et al., 2014). The exact mechanisms behind the beneficial effect of pulses on reduced glycaemic response following a second meal are yet to be elucidated. The effect of short chain fatty acids resulting from the

fermentation of indigestible carbohydrates in suppressing glucose metabolism is a proposed mechanism (Nilsson et al., 2008, Verbeke et al., 2010). Furthermore, intact cells have been demonstrated to promote different microbes compared to isolated resistant starches (Huang et al., 2021b). These short chain fatty acids can be detected in blood as early as three hours following food ingestion, and might therefore affect glucose metabolism (Mollard et al., 2014). Another proposed mechanism is slow, albeit sustained, release of glucose through the slowly digestible starch present in less processed chickpeas (Vinoy et al., 2016, Wolever et al., 1988). Food items containing high amounts of slowly digestible starch ingested at breakfast are suggested to induce slow glucose appearance throughout the day (Jenkins et al., 1982b, Wolever et al., 1988, Liljeberg and Björck, 2000, Vinoy et al., 2016). The slow digestion of these starches is proposed to induce a delayed and prolonged response of incretin (180 to 300 minutes following slowly digestible starch intake), which in turn affect the digestion rate and glucose appearance following intake of a subsequent meal (Wachters-Hagedoorn et al., 2006).

In line with postprandial glycaemic responses, insulin (as represented by C-peptide) and incretin responses (as represented by GLP-1) were significantly lower after ingestion of all chickpea treatments compared to *Con,* with no significant differences between different processing methods. We noted peak glucose and GLP-1 responses at 45 minutes following breakfast ingestion, followed by a c-peptide peak at 60 minutes, reinforcing the insulinotropic activity that is mediated by incretin, in agreement with previous findings correlating blood insulin levels with GLP-1 (Juntunen et al., 2002).

The results of the study also show a significant increase in postprandial satiety as represented by significantly higher subjective fullness scores, and significantly lower hunger and prospective food consumption scores after ingestion of chickpea foods compared to *Con*. However, the effect on satiety was not paralleled by appetite hormone response. We found higher secretion of the anorexic hormone GLP-1 after *Con* ingestion compared to other groups, however, no differences were detected in postprandial leptin and in

the orexigenic gut hormone, ghrelin. A previous trial investigating the impact of incorporating chickpea flour in flat breads reported no effects on GLP-1 levels although significantly higher levels of ghrelin were measured as a result of the intervention (Dandachy et al., 2018). However, the incorporated chickpea flour only amounted to 30% in the intervention meals, accounting for consistency in both glucose and insulin responses (Dandachy et al., 2018).

To the best of our knowledge, no other studies have assessed the acute postprandial responses of GLP-1, ghrelin, and leptin after pulse intake. The effect of protein intake on postprandial ghrelin secretion is still controversial, with some studies suggesting enhanced secretion while others reported reduced levels after protein inclusion in meals (Erdmann et al., 2004, Blom et al., 2006). However, findings of previous trials showed that the administration of high fibre and/or high protein diets trigger the secretion of incretin hormones in both acute and long-term settings (Bodnaruc et al., 2016, Ma et al., 2009, Nauck and Meier, 2018). The proposed mechanism is that fibre can lead to increases in incretin secretion, principally through short chain fatty acid production after fermentation of non-digestible carbohydrates in the colon (Bodnaruc et al., 2016). This can explain the lower responses observed in our trial as we only investigated 3-hour responses following a meal intake.

A major strength of our trial lies with quantifying the amount of available carbohydrates in our laboratories rather than relying on food labels in which carbohydrates are often calculated by difference. Also, use of a standardised CGM system allowed us to comprehensively profile individual glucose responses throughout the course of a protracted observation period. Moreover, we assessed the hormonal responses following intervention in order to clarify the mechanism(s) underpinning the regulation of glucose levels. However, caution is warranted when comparing the present outcomes with the literature. Firstly, as CGM systems do not measure glucose in blood but in interstitial fluid, a delay of 4.5 minutes relative to circulatory levels has been estimated. Further, interstitial glucose levels

could be up to 11.4% lower mean absolute relative difference compared to reported capillary blood glucose values and 12% in comparison to venous blood glucose analysed by Yellow Springs Instrument (Bailey et al., 2015). Secondly, the test foods used in the trial are not made from the same chickpea variety. While the use of store brand products is more realistic, it does introduce variation due to potential varietal and therefore compositional differences (e.g. carbohydrate and protein content), which in turn might affect postprandial responses. This was partially mitigated by measuring carbohydrate content experimentally. Thirdly, it cannot be excluded that the two day washout period as part of the crossover design, despite randomisation, might have introduced carryover effects and hence influenced the subsequent sessions' responses, although it has been shown in literature that no carryover effects were detected in glucose values after 48 hours of chickpea consumption (Madrid-Gambin et al., 2018). Finally, although our sample size was sufficient to detect clinical significant differences in our primary outcome, a larger sample may be necessary to detect differences in our secondary outcomes.

In conclusion, this study showed that postprandial interstitial glucose levels and incretin hormones are unaffected by chickpea processing methods. However, the presence of intact cells appear to have effects on the glycaemic response to the subsequent meal. The use of CGM provides more information on subsequent meal effects that would be impractical to obtain otherwise.

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Chapter 4 Impact of chickpea processing on *in vitro* starch digestion, and correlation between *in vitro* and *in vivo* data.

4.1 Abstract

Chickpeas are pulses characterized by a high content of protein, fat, vitamins, fibre, and a lower digestible carbohydrate. Several processing methods were suggested to impact the cell wall integrity of chickpeas and significantly increase proportion of starch available for digestion. However, the effect of extrusion to produce products such as pasta, on altering these properties of chickpeas have not been previously investigated. Extrusion is the process of food production by application of high pressure, shear and temperature which significantly alter food properties. Therefore, the aim of this study was to compare the degree of starch digestibility of extruded chickpea pasta with whole and puree chickpeas along with highly digestible instant mashed potatoes. The available carbohydrate content of all food samples was quantified, and the process of digestion was simulated *in vitro* following the INFOGEST static model. The estimated available carbohydrate content was 23.38 g, 18.75 g, and 9.90 g per 100 g of food sample for chickpea pasta, canned chickpeas, and mashed potatoes respectively. *In vitro* digestibility revealed up to 31% carbohydrate digestion in whole canned chickpeas; up to 33% carbohydrate digestion in puree chickpeas and chickpea pasta; and 87% carbohydrate digestion in control mashed potatoes after 3 hours of intestinal digestion. Moreover, the percentage digestibility of the tested chickpea samples was well correlated with their respective glycaemic index values from a previous *in vivo* trial. In conclusion, *in vitro* digestion revealed limited starch bioaccessibility of chickpea foods in comparison to control mashed potatoes despite mechanical processing methods which demonstrated limited effect on starch digestibility from chickpea.

4.2 Introduction

Chickpeas are grain legumes widely consumed around the world. They contain high content of protein and carbohydrate with low amounts of lipids (Jeong et al., 2019). They are also considered as an important source of many micronutrients such as folate, niacin, thiamine, iron, zinc and magnesium as well as substantial amounts of many phytochemicals (Mudryj et al., 2014, Vishwakarma et al., 2018). Recently, there has been growing interest in pulses particularly because of their health benefits in improving glycaemic control and therefore contributing toward prevention and management of chronic diseases such as type 2 diabetes mellitus (Curran, 2012, Tharanathan and Mahadevamma, 2003, Hafiz et al., 2021). In addition, pulses are now used as ingredients in many products to promote them as 'healthier' alternatives to traditional preparations of food items such as breads, crisps, soups, and pasta considering their lower glycaemic index along with higher protein contents (Vaz Patto et al., 2015). However, it is well known that different processing methods can alter both physical and functional characteristics of food constituents particularly starch by various degrees (Drulyte and Orlien, 2019). Milling in particular is among the most widely used application in the food processing industry to produce numerous food products. It involves reducing the particle size of cereals or legumes resulting in breakage of cell wall and differentiation particularly when accompanied with dehulling which involves removal of some parts of external layers (Oghbaei and Prakash, 2016). These external layers are usually rich in nutrients such as dietary fibre, minerals and vitamins along with phenolic compounds, tannins and phytic acid that act as enzyme inhibitors in the gastrointestinal system reducing digestibility and bioaccessibility of the nutrients accompanied with (Vasishtha et al., 2014). Moreover, the milling process involves reduction in particle size and hence increased surface area which in turn increase susceptibility to the enzymatic hydrolysis (Edwards et al., 2021). Extrusion of leguminous seeds, such as peas and kidney beans, have been suggested to result in up to 60% increase in rapidly digestible starch and 10 to 50% reduction in slowly digestible and resistant starch as evident from previous work on *in vitro* digestion (Alonso

et al., 2000b, Lintas and Cappelloni, 1992, Sharma et al., 2015). These changes in starch digestibility might affect the glycaemic index of pulses and therefore reduce their ability to contribute toward enhanced glycaemic control. Therefore, the aim of this study was to investigate the effect of mechanical processing methods such as pureeing and milling on cell wall integrity and *in vitro* digestibility of starch from chickpeas *(Cicer arietinum L.)* by following INFOGEST model of static simulation of gastrointestinal digestion. Further, the outcomes of these experiments were correlated with data from a previous *in vivo* trial, which determined the effect of differently processed chickpea meals on postprandial glycaemic responses in normoglycaemic adults.

4.3 Methodology

4.3.1 Materials

Food samples used in this experiment were the intervention meals that were used to investigate the effect of processing methods on postprandial glycaemia (Hafiz et al., 2022). These include three different forms of chickpeas (whole chickpeas, pureed chickpeas, and pasta made of chickpea flour) along with the control mashed potatoes. Whole and pureed chickpeas used in the analyses were obtained from ready to eat canned chickpeas (Sainsbury's®), and the chickpea pasta was obtained by boiling fresh fusilli made of chickpea flour (Ugo®) for 3 minutes in unsalted water. All chickpea samples were drained before homogenisation with IKA® T10 Ultra-Turrax on low speed for 3 minutes prior to digestion. The purpose of this was to produce a texture that is similar to food mastication by humans, although there are significant variations in food mastication among population. Mashed potatoes were prepared by mixing instant mashed potato flakes (Smash®) with boiling water in a 1:5 (w/v) ratio. All chemicals used in the experiment were analytical grade and were bought from Sigma-Aldrich unless otherwise stated.

4.3.2 Determination of available carbohydrates in the food samples

The available carbohydrate content was determined using Megazyme® assay K-ACHDF according to the manufacturer's instructions. After hydrolysis of the available carbohydrates into simple sugars by enzymatic digestion, the sugars in were quantified. Briefly, 1 gram of the sample was accurately weighed in a Duran bottle and homogenised with MES/TRIS buffer until dispersed by using a IKA® T10 Ultra-Turrax for 3 minutes. The starch was digested using thermostable Megazyme® α-amylase (1 μL/ml) supplied with the kit, along with incubation at 95 ˚C below boiling temperatures with continuous agitation. The hydrolysis was then completed by addition of Megazyme® protease (2 μL/ml) and amyloglucosidase (4 μL/ml) following the protocol. After completion of the digestion, one mL of the supernatant was collected from each bottle for sugar quantification and the remaining mixture was used for determining fibre content. The quantity of total fibre including both soluble and insoluble was identified by precipitation with 95% ethanol after digestion of carbohydrate followed by filtration using Ankom® filters to quantify the undigested particles.

4.3.3 Simulated static *in vitro* digestion

The INFOGEST protocol for standardised static *in vitro* simulation of gastrointestinal food digestion was followed for analysis of the samples (Brodkorb et al., 2019). Electrolyte simulated salivary fluid (eSSF), electrolyte simulated gastric fluid (eSGF) and electrolyte simulated intestinal fluid (eSIF) were prepared at 1.25 concentration to account for the addition of enzyme solutions and calcium chloride ($CaCl_2$) as well as adjustment of the pH without affecting the electrolyte balance. Detailed ingredients of electrolyte simulated fluids are available in the INFOGEST protocol (Brodkorb et al., 2019). The final volume of the digesta mixture (test food + simulated fluid + enzymes + pH adjustment solutions) in each phase was adjusted by addition purified water after calculating the requirement of acid or base and water by running a trial experiment. All electrolyte solutions were warmed up and kept in a water bath at 37 °C before starting the digestion

procedure, and the enzyme solutions were kept on ice. Moreover, a blank test tube was run in parallel that had water instead of test food but contained all other fluids and enzymes to account for possible presence of any sugars in enzymes. All food samples were homogenised using IKA® T10 Ultra-Turrax as above without significant distruption of the structure. To start the digestion, 5 g of minced test foods were added into pre-warmed eSSF (1:1, wt/wt). Further, to start the digestion process salivary α-amylase (75 U/ml) was added to all samples with pre-estimated volume calculated previously by determination of enzyme activity. Purified water was added to to achieve a final volume of 10 mL. The digesta mixture was then incubated in a shaking water bath at 37 °C for two minutes to simulate the oral digestion phase.

The mixture from the oral digestion was then adjusted to gastric phase condition by adding the previously warmed eSGF (0.8:1, vol/vol), porcine pepsin (2000 U/ml), $CaCl_2$ (1.5 mM), 5 M HCl to adjust the pH to 3.0 , and purified water with formerly determined values to adjust final volume of the digesta mixture at 20 mL and 50:50 (vol/vol) final ratio of oral chyme to eSGF. The test tube was vortexed for 30 seconds, and the pH was adjusted to 3 by titration with HCL before incubation in shaking water bath set at 100 rpm at 37 °C for two hours. One test tube was taken for each sample at the end of the oral and gastric digestion period to measure the extent of starch digestion at the end of each phase.

After completing the two hour incubation at 37 °C, the test tubes from the gastric phase were exposed to intestinal conditions by addition of eSIF (0.4:1, vol/vol), suspension of porcine pancreatin dissolved in eSIF (100 U/mL), $CaCl_2$ (0.6 mM), bile salts (10 mM), 5 M NaOH (titration for adjustment of pH to 7) and purified water to achieve a final volume of 40 mL and 50:50 (v/v) final ratio of gastric chyme to eSIF. The test tubes were then incubated again at 37 °C in the shaking water bath for 3 hours. However, multiple test tubes were taken at several time points (15, 20, 30, 60, 90, 120, 180 minutes) during the three hour time period to measure the rate of starch digestion in each sample. At each sampling point, all test tubes were immediately incubated at 100 °C for 5 minutes upon removal from 37 °C

incubator to deactivate the enzymes and stop the digestion. The mixture was then centrifuged at the supernatant was used for sugar content analyses.

4.3.4 Quantification of sugar content

The sugar content of the digested test food was quantified using the 3-amino-5-dinitrosalicylic acid (DNSA) assay as explained previously by Perez-Hernandez et al, and calculated using a standard curve with maltose (Perez-Hernandez et al., 2020). DNS agent reacts with the carbonyl groups of the reducing sugars such as glucose, fructose, and maltose under alkaline pH. Boiling temperature conditions generate DNSA with deep yellow to orange colour which intensity is directly proportionate to the concentration of the reducing sugar content. The intensity of the yellow-orange dye was measured by Biotek plate reader at 540 nm. The amount of carbohydrate digested in the first 20 minutes of the intestinal phase was considered as rapidly digestible starch, and the rest of digested carbohydrate after 180 minutes was considered as slowly digestible starch.

4.3.5 Microstructural characterisation

Microstructural characteristics of chickpea samples were determined using light microscopy diffraction. Samples for light microscopy were collected from cooked food and dispersed in 1.0% w/v sodium phosphate buffer using IKA® T10 Ultra-Turrax for 3 minutes on each sample as explained above. After that, 0.05 ml of the suspension was dropped on the glass slide for visualisation. Starch granules of the samples were visualised using 2.5% iodine to stain the samples before looking under microscope. To stain cell walls, 5 mM calcofluor white stain was used along with 10% sodium hydroxide to determine the intactness of the cell wall among all food samples.

4.3.6 *In vivo* glycaemic response and glycaemic index

The effect of processing method on carbohydrate digestibility *in vivo* was determined by measurement of postprandial glycaemic response to different chickpea food items in comparison to mashed potatoes. Postprandial glucose concentrations were measured interstitially using continuous

glucose monitors (Libre Pro, Abbott) as described by Hafiz et al previously (Hafiz et al., 2022), and the glycaemic index of each chickpea food item was estimated from the incremental area under the curve of postprandial glucose values, considering the control mashed potatoes as the reference food with glycaemic index of 100. The estimated glycaemic index data from *in vivo* experiment was correlated with the results of *in vitro* digestibility of carbohydrate from the food items used in the experiment.

4.3.7 Statistical analyses

Each experimental analysis was performed in triplicates and all the results were expressed as mean values along with their standard deviations. The results were analysed using t-test and one way ANOVA with significance are set as $p < 0.05$. All statistics were performed using SPSS. Results of the *in vitro* experiments were correlated with the data from *in vivo* using Pearson correlation (SNEDECOR, 1957).

4.4 Results

4.4.1 Available carbohydrates and fibre content in food samples

Available carbohydrate content of the food samples was determined by hexokinase assay as indicated in Megazyme protocol. The calculated results for available carbohydrate in canned chickpeas were slightly higher when comparing to the values provided by the manufacturers on the labels and McCance and Widdowson's datasets (18.75 g, 16.50 g, and 18.30 g respectively) as shown in (Table 4-1). Similarly, the estimated available carbohydrate values were higher in control mashed potatoes to what was reported in the McCance and Widdowson's datasets and the values provided by the manufacturers on the labels (8.29 g, 6.10 g, and 7.10 g respectively) (Table 4-1). However, as the total dietary fibre content of the food samples was estimated by measurement of the weight of the undigested food particles, there is a chance of overestimation by interpreting the undigested proteins as fibre, which could be the reason behind higher interpreted values by analysis, although the percentage of proteins that remain undigested after the analyses remains residual (Greenfield and Southgate, 2003).

Nevertheless, it would be recommended to quantify the residual undigested protein in future analyses to account for the overestimation in fibre content.

Table 4-1 Dietary Carbohydrate and fibre content of the food samples analysed by Megazyme available carbohydrate kit in comparison to food labels and databases

Food sample	Available CHO g/100g			Total fibre content g/100g		
	Megazyme	***Food label***	***McCance and Widdowson's***	***Megazyme***	***Food label***	***McCance and Widdowson's***
ChW	18.75 ± 2.08	16.50	18.3	8.1 ± 0.41	6.1	7.1
ChPu	19.8 ± 2.78	NR	NR	8.2 ± 0.64	NR	NR
ChF	23.38 ± 1.10	22.40	NR	7 ± 0.36	5.7	NR
Con	11.90 ± 1.75	10.90	13.5	1.28 ± 0.1	1.1	1.4

ChW: whole chickpeas, ChPu: pureed chickpeas, ChF: pasta made of chickpea flour, Con: control mashed potatoes

4.4.2 Simulated static *in vitro* digestion

Different form of chickpeas were investigated to understand the influence on the extent of *in vitro* starch digestibility following INFOGEST static model. The outcomes of the analyses showed very limited starch digestion from all tested chickpea foods (up to 30% of predetermined available carbohydrate) after 3 hours of intestinal digestion compared to control mashed potatoes which showed greater digestibility (up to 90%) as illustrated in Table 4-2 and Figure 4-1, and there was no significant differences between chickpea foods in degree of carbohydrate digestibility ($p > 0.05$), despite observed differences in cell wall structure (Figure 4-2). There was a significant difference in the rate of digestion between the chickpeas and control mashed potatoes, as the majority of digestion took place in first 30 minutes in mashed potato samples, while it gradually increased until 180 minutes in chickpea foods (Figure 4-1).

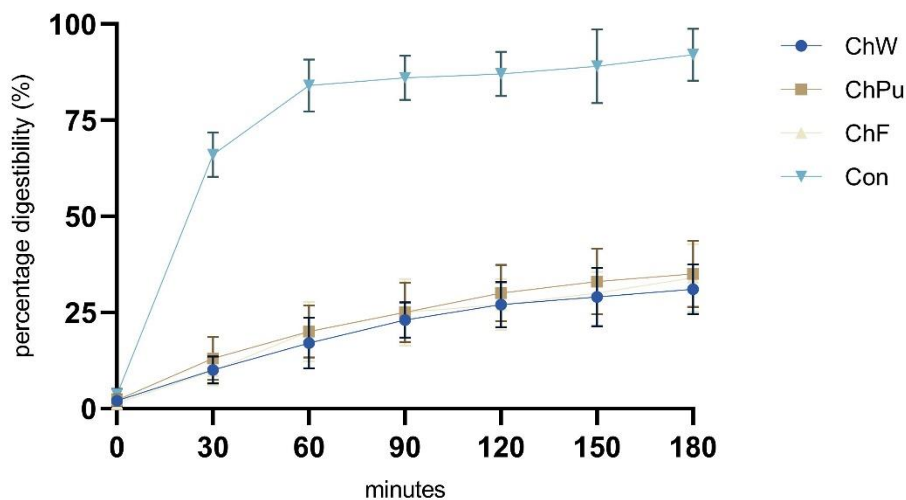

Figure 4-1 Percentage digestibility of carbohydrate from different chickpea samples and control

ChW: whole chickpeas, ChPu: pureed chickpeas, ChF: pasta made of chickpea flour, Con: control mashed potatoes, RDS: Rapidly digestible starch, SDS: Slowly digestible starch

Table 4-2 Cumulative amount of digested starch during each phase of the *in vitro* digestion

Phase	ChW	ChPu	ChF	Con
Oral, g	0.36 ± 0.14	0.31 ± 0.24	0.37 ± 0.19	0.23 ± 0.21
Gastric, g	0.42 ± 0.22	0.53 ± 0.34	0.34 ± 0.15	0.36 ± 0.16
Intestinal 30 min, g	1.89 ± 0.47	1.97 ± 0.89	2.30 ± 1.03	6.56 ± 1.45
Intestinal 60 min, g	3.24 ± 0.85	2.99 ± 0.94	4.76 ± 1.34	8.76 ± 2.76
Intestinal 90 min, g	4.43 ± 1.65	4.97 ± 1.50	4.95 ± 1.90	8.36 ± 2.87
Intestinal 120 min, g	5.12 ± 1.64	5.36 ± 1.98	5.37 ± 1.87	8.82 ± 2.65
Intestinal 150 min, g	5.68 ± 2.45	5.75 ± 1.87	6.94 ± 1.68	7.99 ± 3.57
Intestinal 180 min, g	5.80 ± 2.15	5. 99 ± 2.47	7.52 ± 2.49	8.65 ± 2.47
RDS, g	1.35 ± 0.76	1.64 ± 0.85	2.12 ± 0.36	5.95 ± 1.98
SDS, g	4.5 ± 1.74	4.35 ± 1.87	5.4 ± 2.78	2.7 ± 0.37

Values are presented as mean ± SD

Values are calculated from digestion of 5g cooked food sample and interpreted as g/100g of cooked food sample.

ChW: whole chickpeas, ChPu: pureed chickpeas, ChF: pasta made of chickpea flour, Con: control mashed potatoes, RDS: Rapidly digestible starch, SDS: Slowly digestible starch

4.4.3 Light microscopy

Food samples were observed under microscope to investigate the internal cell wall integrity among canned chickpeas and chickpea pasta (Figure 4-2). Micrographs show intact but mostly separated cells of chickpea cotyledon with starch granules encapsulated with very few ruptured cells, which might be during dispersing the food samples in the buffer. On the other hand, micrograph images of the chickpea pasta showed ruptured cell wall particles and starch granules spread evident of structural damage of chickpea cotyledon, with very few cells that remained intact encapsulating the starch granules.

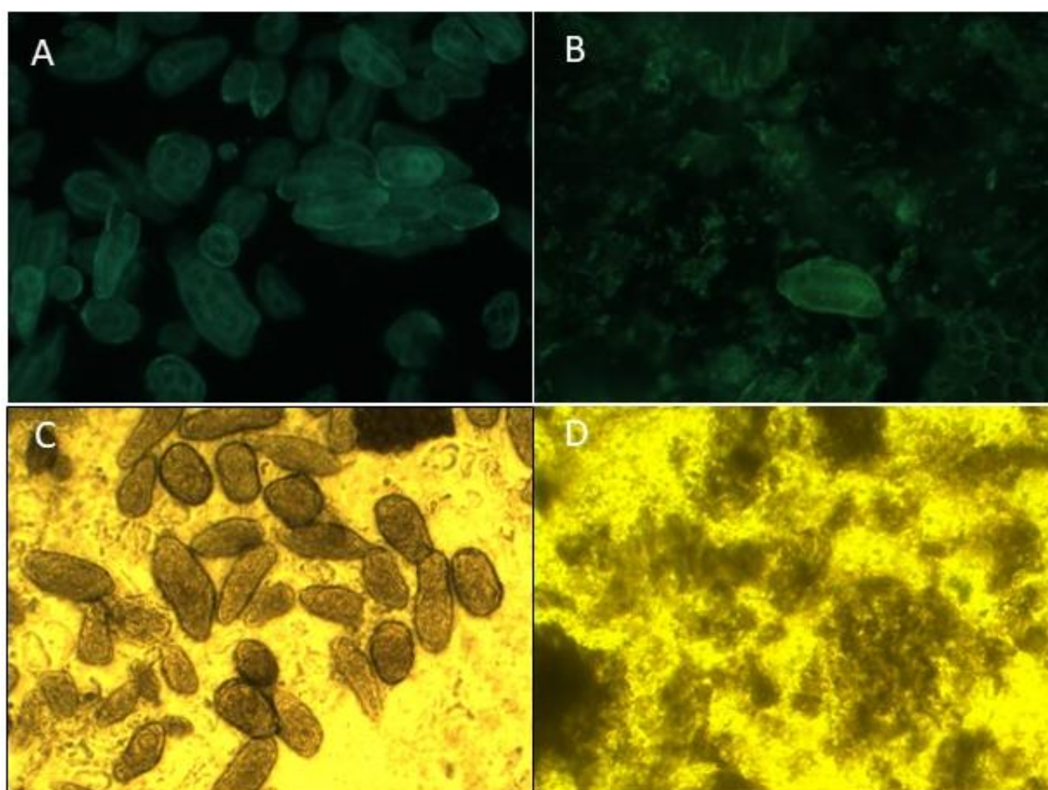

Figure 4-2 Micrographs of chickpea cells obtained from canned chickpeas (A, C) and chickpea pasta (B, D) after homogenisation and staining of cellulose with calcofluor white stain (A, B) and starch with iodine (C, D).

4.4.4 Correlation between *in vivo* glycaemic response and *in vitro* starch digestibility

Estimated glycaemic index values were 38.9 ± 37.7, 27.9 ± 23.8, and 36.5 ± 35.8 for whole chickpeas, pureed chickpeas, and chickpea pasta respectively. The glycaemic index values for chickpea food obtained from *in vivo* experiment are considered low according to International Organisation for Standardisation-recommended classification (Atkinson et al., 2021). The Pearson correlation revealed significantly positive correlation when the glycaemic index values of each food item were correlated with their percentage carbohydrate digestibility (r = 0.989, p = 0.011). Moreover, a significant positive association was observed when correlating the glycaemic index with the ratio of rapidly digestible starch to slowly digestible starch (r = 0.986, p = 0.014).

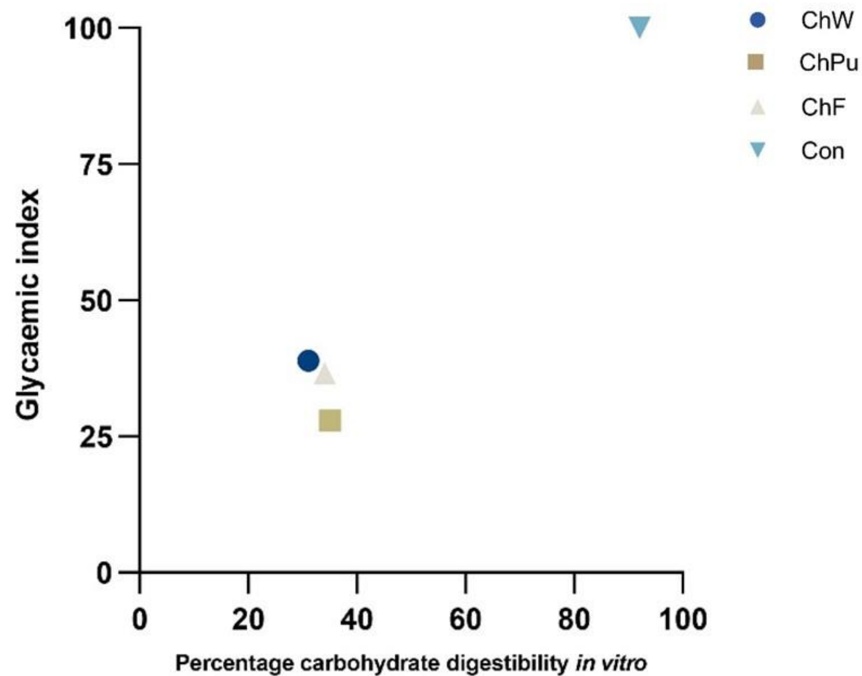

Figure 4-3 Pearson correlation of *in vivo* glycaemic index and *in vitro* percentage starch digestibility

4.5 Discussion

The purpose of this study was to enhance our understanding of the effect of mechanical processing methods on postprandial glycaemia, in particular chickpea cotyledon, and to further investigate the effect on *in vitro* digestion and available carbohydrate content in chickpeas. The light microscopy imaging indicate significant disruption of cell wall integrity in chickpea pasta as evident by scattered pieces of broken cell walls in the sample in comparison to whole and pureed chickpeas where intact cells were captured (Figure 4-2). However, these structural differences did not impact carbohydrate digestibility from chickpeas as suggested by the results of the *in vitro* digestion. Furthermore, no differences in speed of digestion were observed among chickpea samples, which indicates no variations in the ratio of slowly digestible starch to rapidly digestible starch. However, the rate of digestion was substantially different between chickpeas and potatoes. In the intestinal phase, starch amylolysis from mashed potato progressed faster within the first hour, whereas the digestion of starch from chickpeas progressed slowly and to a less extent.

The data of *in vitro* analyses indicates higher proportion of rapidly digestible starch in potatoes as evident by starch digestion in first 20 minutes during intestinal phase, while in chickpeas there is higher proportion of slowly digestible starch which gets digested in later phase of intestinal digestion, and resistant starch which escape the digestion in the small intestine and therefore susceptible for fermentation in the colon (Frost et al., 2016). These findings are in agreement with another study that reported significantly lower total starch digestion from chickpeas *in vitro* by following static or dynamic models compared to wheat (Edwards et al., 2021). Overall, lower digestibility of carbohydrates features all types of pulses along with their tendency to preserve cell wall structure and resist rupture when mashing after cooking when comparing to other cereal grains (Singh, 2017, Silva-Cristobal et al., 2010, Jood et al., 1988). The lower digestibility of starch from pulses might be due to several factors. First, the thick cell wall structure properties of the pulses maintain their integrity and resist swelling along with reduced the permeability to amylase dispersion and therefore diminished susceptibility of

starch to gelatinization and amylolysis, and hence contributed to limited digestion of the encapsulated starch, which explain the lack of differences in postprandial responses between whole and puree chickpeas despite pureeing the chickpeas (Edwards et al., 2021, Singh, 2017). On the other hand, pasta is generally made in the food industry using extrusion methods to obtain unique nutritional features of slower starch digestion regardless of the cell wall structural properties. This process result in a compact texture with limited gelatinisation restricting starch availability to amylolysis and thus lower glycaemic response (Granfeldt and Björck, 1991, Chiavaroli et al., 2018, Huang et al., 2021a). Collectively, these processing conditions are thought to downregulate the overall carbohydrate susceptibility to enzymatic hydrolysis. Second, the presence of higher amount of protein in pulses might have an effect on the degree of starch digestion compared to potatoes and other cereal grains which have substantially lower protein content (King and Slavin, 2013, Juliano, 1999). Endogenous protein in food sources is suggested to interact with starch molecules to form starch protein complexes limiting the amylolysis and thus leading to supressed starch digestion (López-Barón et al., 2018, Lu et al., 2016, Ye et al., 2018). Furthermore, pulses contains several anti-nutritional factors such as saponin, tannin, trypsin inhibitor, hemagglutinin, and phytic acids along with other molecules exhibiting antioxidant properties such as flavonoids, (flavonols, flavanones and isoflavones), and non flavonoids, such as hydroxybenzoic as well as hydroxycinnamic acids (Fratianni et al., 2014, El-Adawy, 2002). Presence of these compounds in the foods retard starch digestion by interacting with starch granules as well as inhibiting enzymes involved in digestion such as α-amylase and α-glucosidase although food processing techniques significantly reduce the amount of those molecules (THOMPSON and YOON, 1984, Sun and Miao, 2020). The findings of *in vitro* digestibility of carbohydrates from chickpeas are highly correlated with data from a human study, conducted in our facilities, reported very low glucose excursion in the blood stream after ingestion of chickpeas, with no difference between processing types, indicating limited digestion of starch from those meals. Moreover, the ratio of rapidly digestible starch present in the food samples was positively correlated with estimated glycaemic index

values. The proportion of rapidly digestible starch in food is thought to be directly correlated to the glycaemic index of the same food according to the literature (Englyst et al., 1999, Brouns et al., 2005). This strong correlation of the outcomes emphasises the competence of the *in vitro* digestion techniques in predicting the *in vivo* responses.

The outcomes of the analyses indicate limited digestion of the starch during oral phase (less than 2%) possibly due to the short incubation period, reinforcing that the majority of the starch digestion takes place in intestinal phase.

There are several drawbacks of these analyses that should be noted. The use of a simple static model for *in vitro* digestion of food samples does not fully reflect the dynamic human digestion process. However, static models showed moderate to high correlation with *in vivo* glycaemic response of various food items (Bohn et al., 2018, Monro et al., 2010), and therefore can be used as simplified alternative to the dynamic digestion models.

In conclusion, no differences were observed in the degree of starch digestion between chickpea samples processed in three different ways, suggesting that wet pureeing or processing to make pastas does not alter low *in vitro* digestibility properties of starch from chickpeas despite losing the structural integrity of chickpea cotyledons and cells, and therefore did not eliminate lower glycaemic index properties of chickpeas *in vivo*.

4.6 References

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Chapter 5 General discussion, directions for future work and conclusions

Increasing prevalence of obesity and type 2 diabetes is a major concern worldwide, with postprandial glucose being an independent risk factors in the aetiology of type2 diabetes and its related complications (Blaak et al., 2012, Cavalot et al., 2011, Lebovitz, 1998). The glucose excursion following a meal intake is mainly determined by the presence of carbohydrates in the diet and the rate and extent of their digestion and absorption. However, empirical evidence has suggested that there are several factors that influence the process of carbohydrate metabolism and related outcomes including the structure of the food and processing method applied (Grundy et al., 2016). Pulses are known to be an excellent source of carbohydrates due to their lower glycaemic index in comparison other carbohydrate sources, attributing to the presence of slowly digestible starch and protein. Consumption of pulses has been linked to reduced risk of chronic metabolic diseases such as type 2 diabetes primarily implying to their lower glycaemic index properties. Carbohydrate content of pulses (up to 60% of their dry weight) is consisting mainly of starch along with fraction of oligosaccharides such as raffinose, stachyose, and verbascose, and non-starch polysaccharides primarily as cell wall constituent (Singh et al., 2017). These oligosaccharides in pulses are predominantly α-galactosides that are known to have α 1-6 bond between galactose molecules. These linkages in α- galactosides are resistant to hydrolysis by human digestive enzymes due to unavailability of α-galactosidases and hence escape digestion in the small intestine and therefore do not contribute to postprandial glucose excursion (Berrios et al., 2010). The starch content of pulses is suggested to have limited digestibility and low glycaemic index properties. The encapsulated starch granules in cotyledons are surrounded by intact cell walls and protein matrices that impose a barrier against gelatinisation and amylolysis (Li et al., 2019b). Several processing mechanism were linked to alterations of α-galactosides content as well as impact on cell wall permeability *in vitro*, including mechanical processing methods such as extrusion. The slower rate and extent of starch digestion from pulses is a determinant by several factors in

which cell wall integrity plays a crucial rule (Bhattarai et al., 2017). However, the barrier role of the cell wall in pulses is suggested to be altered by various processing conditions resulting in alteration of starch digestion kinetics *in vitro* (Pallares Pallares et al., 2018, Xiong et al., 2019, Dhital et al., 2016). The implication of different processing methods on the postprandial glycaemic and satiety responses of pulses was lacking evidence. Therefore, this thesis aimed to investigate the impact of processing methods on the indices of glycaemia and satiety *in vivo*.

5.1 Summary of overall project findings

The systematic review and meta-analysis on acute and long-term studies investigating the effect of pulse intake on indices of glycaemic control **(chapter 2)**

- The review on acute studies revealed a significant reduction in postprandial glycaemia after intake of pulses compared to control in adults with normoglycaemia (ES -1.38; 95% CI - 1.78, – 0.99; $p \leq 0.001$; $I^2 = 86\%$) and with type 2 diabetes (ES -2.90; 95% CI -4.60, -1.21; $p \leq 0.001$; $I^2 = 93\%$).
- The heterogeneity in the reported effect size was explored by subgroup analysis of pulses type, physical form, and control group used for comparison. The analysis revealed that there were no significant differences between different pulse types in their postprandial glycaemic responses. However, variations in the physical form of the pulses had a significant impact on attenuating the postprandial glycaemic response.
- The analysis of long-term trials (3-72 weeks) indicated that regular pulse consumption had a beneficial effect on management of glycaemia in particular in individuals with type 2 diabetes, by reducing fasting glucose, insulin, glycated haemoglobin, and measures of insulin resistance (HOMA-IR).
- Meta-regression revealed no significant effect of the intervention dose or study duration on the biomarkers of interest among long-term trials

The impact of different mechanical processing methods of chickpeas on postprandial glycaemic and satiety responses in normoglycaemic adults in a randomised controlled trial **(chapter 3)**

- The outcomes of the human study revealed significantly lowered postprandial glycaemic responses after ingestion of chickpeas (all three types) compared to control mashed potatoes as measured by continuous glucose monitoring system.
- The findings suggest that altering the physical form of the chickpeas had not resulted in significant differences in postprandial glucose concentrations.
- All forms of chickpeas were comparable in subjective satiety control evident by postprandial hunger, fullness and prospective food intake as measured by visual analogue scale.
- Hormonal regulation of digestion, glycaemia and satiety also had not impacted by variation in the physical form of chickpeas as evident by plasma levels of c-peptides, ghrelin, leptin, and GLP-1.
- Subsequent meal's glycaemic response was significantly lowered after intake of chickpea and pureed in comparison to control, indicating extended effect of pulses on glucose homeostasis.

The impact of variation in physical form of chickpeas on rate and extent of *in vitro* digestion **(chapter 4)**

- This chapter has demonstrated that the rate and degree of *in vitro* starch digestion of chickpeas was significantly lowered than potatoes and was not affected by processing methods (whole, pureed, flour).
- The results of *in vitro* digestion were in line with the outcomes of the *in vivo* study indicating the beneficial effect of pulses was not altered by alteration in the mechanical processing methods.

5.2 General discussion of overall PhD project

In the following chapter an overarching analysis and in depth discussion are provided on different aspects of the project.

5.2.1 Interplay between structural properties, digestibility and glycaemia.

Food has a sophisticated matrix with various levels of microstructural heterogeneities that affect their physical, biochemical and functional properties and hence their health effects. In this research project, the systematic review of the literature indicated that different processing methods applied to pulses had resulted in attenuation in postprandial glycaemic excursion (Hafiz et al., 2021). However, this impact was not observed when conduction of *in vivo* study investigating the impact of processing methods on postprandial glucose, as well as, *in vitro* digestion did not resulted in any difference in line with the human study (Hafiz et al., 2022). The microstructural imaging revealed significant disruption of cell wall structure in chickpea pasta samples and abundant release of starch granules into extracellular matrix. It is generally assumed that intact food structure possess slower digestion properties and that disruption of the cell wall integrity results in accelerated digestion and greater glycaemic excursion. However, this disruption of the cellular structure and cell wall integrity did not result in significant alteration in carbohydrate digestibility nor postprandial glycaemia as evident by the outcomes in chapter 3 and chapter 4. This discrepancy in the outcomes could be explained by several mechanisms. First, the detailed comparison of the studies included in the systematic review, revealed the substantial differences in the pulse provided as intervention across the trials. Most of the studies that used whole or pureed pulses as intervention compose their carbohydrates composition solely from pulses (Augustin et al., 2016, Greffeuille et al., 2015, Reverri et al., 2015, Ramdath et al., 2017). On the other hand, pulse flour based interventions were prepared mostly by fortification of the traditional recipes with relatively small fraction of pulse flour into other carbohydrates such as wheat flour (Akhtar et al., 2019, Boers et al., 2017, Greffeuille et al., 2015). The large

differences in pulse proportions used in intervention meals would have a significant impact on the outcomes reported in previous trials and hence the difference when comparing to the results of this project (Viguiliouk et al., 2019). Second, the pulse flour based intervention used in this project was in form of pasta that was made of chickpea flour. Pasta is made in food industry using extrusion methods to obtain unique nutritional features of slower starch digestion regardless of the cell wall structural properties. This process result in a compact texture with limited gelatinisation restricting starch availability to amylolysis and thus lower glycaemic response (Granfeldt and Björck, 1991, Chiavaroli et al., 2018, Huang et al., 2021a). In addition, milled seeds are hydrated by mixing with water to produce the dough followed by extrusion. This process results in an amorphous protein network that entraps starch granules protecting from hydrolysis (Bruneel et al., 2010, Zou et al., 2015). Although pulse flour lacks gluten, a major protein constituent in wheat that is responsible for forming the starch protein matrix, the protein network was still found but to a lesser extent (Petitot and Micard, 2010, Petitot et al., 2010, Rosa-Sibakov et al., 2016, Laleg et al., 2016).

5.2.2 The effect of pulse intake on appetite regulation

The dietary composition of pulses is unique and comprises several nutritional and anti- nutritional factors that are believed to contribute toward appetite regulation. These include oligosaccharides, resistant starch, and non-starch polysaccharide such as celluloses, hemicelluloses, lignin, pectin content that slow gastric emptying rate and therefore contribute toward greater fullness (Acheson, 2010). Moreover, food high in dietary fibre are suggested to slow down the eating rate attributing to the prolonged chewing time, which in turn transmits orosensory satiety signals to the brain suppressing the appetite (Dagbasi et al., 2020). Prolonged oral exposure to food has also shown to increase satiety by stimulating the secretion of GLP-1 and PYY hormones that play role in gastric emptying (Reichardt et al., 2014). In addition higher protein content of pulses in comparison to other grains is suggested to increase satiety by stimulating gut hormone secretion as well as by formation of a network with starch that delays digestions (McCrory et al., 2010).

However, very little knowledge exists on the effect of alteration of structural properties in pulses and its effect on postprandial appetite regulation.

In this project, it was demonstrated that pulse intake has resulted in greater levels of satiety as evident by subjective hunger and fullness scores when comparing to the control mashed potatoes in chapter 3 (Hafiz et al., 2022). The difference, however, was failed to reflect in satiety hormones such as ghrelin and GLP-1. As explained earlier, GLP-1 is an incretin hormone that is secreted from enteroendocrine L cells located in the small intestine and colon as well as centrally by the central nervous system and hence the secretion augmented by multiple factors (Campbell and Drucker, 2013). It is suggested to coordinate multiple prandial and postprandial effects on metabolism including incretin effect, gastric emptying, and satiety. However, the impact of GLP-1 levels on controlling food intake and the underpinning mechanism is still not conclusive. While some studies report aneroxegenic effect after administration of GLP-1 evident by suppressed food intake, other report that blockade of GLP-1 receptors was not sufficient to supress the satiety, suggesting a synergistic effect with other satiety signals (Melhorn et al., 2014, Müller et al., 2019b, Krieger, 2020). Similarly, the effect of orexigenic gut hormone ghrelin, which is suggested to influence short term hunger sensation and accelerate gastric emptying, is still controversial with several sympathetic and parasympathetic mechanisms involved (Klok et al., 2007, Moran et al., 2004, Müller et al., 2015). Collectively, the hormonal regulation of meal induced satiety is a complex system and hence absence of significant difference can be expected especially when small sample size is involved. Moreover, there were no differences detected in measures of subjective appetite nor hormonal regulation among differently processed chickpea samples in agreement with the outcomes of the *in vitro* digestibility from chapter 4. In addition, these findings were in parallel with the outcomes of glycaemic regulations indicating no impact of alteration of the physical form on postprandial hunger, fullness, and prospective food intake.

5.2.3 Long term effect of pulse intake on glycaemic indices

Pulse consumption over long term period is thought to beneficially attenuate the markers related to glycaemic control such as fasting glucose, glycated haemoglobin, and measures of insulin resistance (HOMA-IR) considering their content of dietary fibre and lower glycaemic index (Sievenpiper et al., 2009). The effect can be directly mediated through continuous regulation of glucose tolerance post meal ingestion leading to improvement of overall glucose homeostasis (Ketema and Kibret, 2015). In addition, pulse intake is associated with increased production of short chain fatty acids (SCFA) due to anaerobic fermentation of dietary fibre (oligosaccharides, resistant starch, and non-starch polysaccharides) by gut microbiota (Salamone et al., 2021). These SCFAs, such as acetate, propionate and butyrate, are suggested to attenuate glucose metabolism directly by enhancing the expression of glucose transporters (GLUT-4) and translocation to the cell membrane, and indirectly through affecting the hormonal regulation of glucose metabolism (Müller et al., 2019a, den Besten et al., 2013). Several studies have shown that increasing concentrations of SCFA can stimulate secretion of GLP-1 hormone which in turn regulate blood glucose by stimulating insulin release from β-cells and supressing secretion of glucagon along with their role in inducing satiety sensation as explained previously (He et al., 2020, Barrera et al., 2011). In addition, SCFA also stimulate the secretion intestinal hormone peptide YY (PYY) which also induce satiety and promote glucose uptake by muscles and adipose tissue (Batterham et al., 2002, van den Hoek et al., 2004). Furthermore, SCFAs were also suggested to enhance the secretion of hormone leptin from the adipocyte which in turn increase glucose absorption by muscles and adipose tissue and also play role in regulation of long term satiety (D'Souza et al., 2017, McDuffie et al., 2004, Fernández-Formoso et al., 2015).

Despite the beneficial effects explained above, the evidence on the impact of long term pulse consumption on improvement of glycaemic indices is still not conclusive. A significant knowledge gap is the inadequacy of high quality clinical trials that are conducted to assess the long-term effects of pulse intake whether in normoglycaemic or type 2 diabetes population. A

systematic review identified the long term studies that were relevant to the topic systematically in chapter 2 and found significant impact on glucose homeostasis as evident by downregulation of fasting glucose and glycated haemoglobin. The effect was more profound in trials investigating individuals with type 2 diabetes where greater variations in the markers are. However, large variations were recorded across the trials downgrading the quality of existing evidence on long term benefit of pulse intake. The observed variations across the trials were among composition of the intervention meals and the comparison control groups used in the studies. While some trials assessed specifically the effect of pulse intake alone, most of the studies investigated the impact of pulse ingestion in context of high fibre diet or low glycaemic index diet, with or without specifying the proportion of pulses within the dietary intervention. Moreover, very large heterogeneity was observed among the control diets used for comparison in long term trials which have resulted in large variations in the quantified mean differences ranging from negative to positive impact. In addition, the recommended daily servings of pulses were lacking harmonisation, ranging from two servings per day to few servings per week.

5.3 Implications of the current findings

Pulses are defined by the American Food Agriculture Organisation as the edible non-oil seeds of crops from Leguminosae family that are harvested dry including various types of beans, peas, chickpeas and lentils (FAO, 1994). As per definition, it excludes from the pulse group the seeds that are used for oil production such as soy and peanuts, and the seeds that are harvested fresh such as green beans and green peas. Both terms (pulses and legumes) are often used interchangeably by health care specialists and the general population despite being distinct in compositional and functional characteristics (Mitchell et al., 2022). There is little consensus about the terminology used in reference to pulses whether in dietary guidelines released by health organisations or nutritional research publications entangling the evidence on health benefits of pulses. Moreover, there are no

harmonisations of the dietary recommendations regarding the optimal amount of pulse intake, their serving size or the food groups associated with (Marinangeli et al., 2017). Some countries, such as Australia and Nordic Countries and United States, include recommendations about pulse intake within the vegetable groups; some other countries, such as India, incorporate pulses within starchy carbohydrates; while others, such as Canada and the Netherlands, promote pulse consumption to be part of meat and alternatives food group as a source dietary proteins (National Health and Medical Research Council, 2013, Norden, 2014, NIN, 2011, Brink et al., 2019, Health Canada, 2019, USDA, 2020). Interestingly, dietary guidelines of the United Kingdom include pulses in vegetable food group with recommendation of one serving per day, and dietary proteins food group but without specifying the recommended amount of intake on daily basis (Public Health England, 2016). However, there are few countries that have specific dietary guidelines for pulses standing them alone. In Brazil, there is a food group named beans that includes all types of pulses as well as other legumes, with the nomenclature reflecting the higher proportion of bean consumption in the country (Brazil, 2015). In South Africa, a separate food group was developed named legumes including pulses and soy beans, and recommended to be consumed regularly (Vorster et al., 2013, Venter et al., 2013). The serving size also varies considerably ranging from 75 g to 300 g with recommended frequency of consumption ranging from two serving per week to twice daily (Marinangeli et al., 2017). Considering that dietary guidelines for pulse consumption differ considerably across different countries, health organisations should establish specific quantity of intake that provides the nutritional values based on the evidence, and hence that amount should be promoted globally for regular consumption and to be used in future researches. Moreover, since outcomes of this project indicate no significant effect of processing method on reducing the beneficial properties of pulses, health organisations should urge food industries to escalate the production of pulse based food choices with various types of pulses to promote the consumption across the nation.

5.4 Project limitations

- The conducted systematic review of the published literature included both acute and long term pulse consumption studies and their impact only on glycaemic indices. However, it would have been beneficial if other biomarkers of cardiometabolic health such as, lipid profile, blood pressure, and inflammatory markers, included in our review as the impact of glucose homeostasis is directly related to individual's cardiometabolic health. Moreover, the studies on the influence of pulse intake on acute and long term satiety management and weight control were not assessed in this project.
- Chickpea pasta was used in the human study and in the *in vitro* digestion as food sample representing chickpea flour. However, as mentioned earlier, the compact structure of pasta that is produced during extrusion substantially limits enzyme accessibility and hence is thought to had a significantly reduce carbohydrate digestion and postprandial glycaemia in comparison to flour based products other than pasta. Therefore, it is difficult to draw a conclusion regarding the effect of milling on digestibility and postprandial glycaemia.
- The small number of participants used in the trial is a major drawback in assessing the significant difference among the secondary outcomes. although, the current number of participants was considered as sufficient to estimate the differences in primary outcome postprandial glycaemic responses, the sample size was not sufficient to draw a significant conclusion in other biomarkers such as GLP-1 and ghrelin due to higher individual variations among those biomarkers.
- The open label design of the study is considered as a limitation as it holds chance to introduce bias. However, it was impossible to conduct single or double blinded trial due to the nature of the food products which is considered as a drawback of the study.
- Furthermore, although the participants were instructed to avoid pulse intake the day before their scheduled visits, other food items can still have carryover effect on glucose homeostasis and therefore might impact the outcome of interest. It would be beneficial in future work to standardise

the meals on the day before their first visit as well as the day of the visit and the day after the visit. This is particularly important as the pulses can have carryover effect up to 48 hours after intake attributing to the short chain fatty acid produced by fermentation of indigestible carbohydrates, which in turn impact glucose metabolism as explained previously.

- With regards to the *in vitro* digestion, the INFOGEST static digestion model was used when digesting the samples. Although static methods are considered potential and are widely applicable in evaluating the influence of various conditions such as food structure, food composition, and food processing digestibility (Alegría et al., 2015), they do not accurately reflect the dynamic gut environment. Therefore, is considered a major drawback in concluding the outcomes. It would be beneficial in future studies to assess the digestibility using the dynamic model, which simulate gastrointestinal model accurately to assess the digestibility in each phase of the gut.

5.5 Future directions

The work presented in thesis has demonstrated that consumption of pulses has a significant effect on improving acute postprandial and long-term glycaemic indices despite their physical structure. The main recommendations future work that could be undertaken can be summarised as below.

The role of pulse intake in regulating measures of cardiometabolic health other than glycaemia is a place of interest. Pulses contain considerable amount of oligosaccharides, non-starch polysaccharides and resistant starch. These compounds escapes digestion from the small intestine and are fermented in the colon. The production of short chain fatty acids from the process is suggested to play role in improving biomarkers related to cardiometabolic health such as lipid profile, inflammatory markers, and blood pressure. Therefore, the future work on pulses should incorporate these aspects whether conducting a systematic review or clinical trial. Moreover, the unique composition of pulses attributing to high protein content along with slowly digestible carbohydrate comprise them as a great component of

diet to improve satiety control and promote weight management. Current perspective of research in the systematic review was limited to the glycaemic indices. However, the reciprocate relation between weight management and glucose homeostasis is well documented. In addition, since it has been shown in chapter 3 that pulse intake can improve short term subjective appetite control but not the hormones related to satiety, it will be interesting in the future to conduct a systematic review and meta-analysis that include those aspects. Furthermore, a clinical trial (especially a long-term trial) that involves evaluation of satiety and the implication on weight should be considered in future research.

The impact of mechanical processing on carbohydrate digestibility and measures of glycaemic indices was investigated using pureed chickpeas and chickpea pasta. However, it is thought that the structure of pasta had an influence on resisting hydrolysis despite the ruptured cell walls. Thus it would be interesting in the future to assess some type of products that are synthesised solely from pulse flour other than pasta, to conclude the true impact of mechanical processing that disrupt the cell wall integrity on pulse digestibility and postprandial glycaemia and satiety. Furthermore, in future studies larger sample size should be considered to allow better understanding of the impact on the secondary outcomes.

In the current research project, the impact of mechanical processing methods was investigated using chickpeas as a model due to wide applications and product availability among the food industry. However, there are several types of pulses other than chickpeas, that have considerable differences in nutrition composition in comparison to chickpeas. Furthermore, it has shown in the systematic review that varying types of pulses have different effect in the postprandial glycaemic measures although the difference was not significant. Therefore, the impact of mechanical processing techniques on digestibility, glycaemia and satiety should be investigated using different varieties of pulses in future. This will allow to investigate whether current findings are specific to chickpeas or can be expanded to include all type of pulses.

5.6 Conclusion

The substantial attribution of pulse intake in improving glucose homeostasis and controlling satiety is reinforced by the work carried out during this PhD project. There were no differences observed in carbohydrate digestibility nor postprandial glycaemia among differently processed chickpea samples. Thus it can be concluded that the attenuation of favourable effects of pulses by processing is not evident and that pulse consumption is beneficial in reduction of postprandial glucose excursion attributing to the lower digestibility regardless of the physical form. In addition, pulse consumption may contribute toward reducing the risk of chronic diseases such as type 2 diabetes attributing to enhanced postprandial glycaemic regulation. Therefore, dietary recommendations should integrate pulses as a distinctive food group for regular consumption among the general population.

5.7 References

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Appendix A: supplementary information of Chapter 2

Table A 1. Predetermined search terms

Search category	Search terms used
1. Population	(normoglycaemic) OR (normoglycaemia) OR (normoglycemia) OR (healthy) OR (normal) OR (diabetic) OR (diabetes) OR (T2DM) OR (T2D) OR (NIDDM) OR (adults)
2. Intervention	(pulses) OR (pulse) OR (legumes) OR (leguminous) OR (legume) OR (beans) OR (bean) OR (peas) OR (pea) OR (chickpeas) OR (chickpea) OR (lentils) OR (lentil) OR (gram)
3. Outcomes	(glucose) OR (FPG) OR (PPG) OR (glycemic) OR (glycaemia) OR (glycemia) OR (insulin) OR (post-prandial) OR (postprandial) OR (PPGR) OR (OGTT) OR (insulinemic) OR (insulinaemic) OR (HOMA) OR (Homeostatic Model Assessment of Insulin Resistance) OR insulin resistance) OR (IR) OR (glycated hemoglobin) OR (HbA1c) OR (A1C) OR (A1c) OR (glycated haemoglobin) OR (glycosylated haemoglobin)
4. Combined search	(1) AND (2) AND (3)

Table A 2. Risk of bias assessment

Study	Risk of bias assessment					
	Domain					Overall
	Randomization process	*Deviation from intended intervention*	*Missing outcome data*	*Measurement of the outcome*	*Selection of the reported results*	
abete, 2008	some concerns	low risk	low risk	low risk	some concerns	some concerns
Abete, 2009	some concerns	low risk	low risk	low risk	some concerns	some concerns
Abeysekara, 2012	low risk	low risk	some concerns	low risk	low risk	some concerns
Agustia, 2019	some concerns	low risk	some concerns	low risk	some concerns	some concerns
Akhtar, 2019	some concerns	low risk	some concerns	low risk	some concerns	some concerns
Alizadeh, 2014	some concerns	low risk	low risk	low risk	some concerns	some concerns
Anderson, 1984	some concerns	low risk	some concerns	low risk	some concerns	high risk
Anderson, 2014	some concerns	low risk	low risk	low risk	some concerns	some concerns
Anguah, 2014	some concerns	low risk	low risk	low risk	low risk	some concerns
Augustin, 2016	some concerns	low risk	low risk	low risk	some concerns	some concerns
Boers, 2017	low risk	low risk	low risk	low risk	low risk	low risk
Bornet, 1987	low risk	low risk	low risk	low risk	some concerns	some concerns
Bornet, 1989	some concerns	low risk	some concerns	low risk	some concerns	high risk
Cryne, 2012	some concerns	low risk	low risk	low risk	some concerns	some concerns
Dandachy, 2018	some concerns	low risk	low risk	low risk	some concerns	some concerns
Dilwari, 1981	some concerns	low risk	some concerns	low risk	some concerns	high risk

Gravel, 2010	low risk	low risk	low risk	low risk	some concerns	some concerns
Greffeuil, 2015	some concerns	low risk	low risk	low risk	some concerns	some concerns
Hassanzadeh-Rostami, 2019	low risk	low risk	low risk	low risk	some concerns	some concerns
Hosseinpour-Niazi, 2015	low risk	low risk	low risk	low risk	some concerns	some concerns
Islam, 2015	some concerns	low risk	low risk	low risk	some concerns	some concerns
Jang, 2001	some concerns	low risk	low risk	low risk	some concerns	some concerns
Jenkins, 1980	some concerns	low risk	some concerns	low risk	some concerns	high risk
Jenkins, 1980	some concerns	low risk	some concerns	low risk	some concerns	high risk
Jenkins, 1982	some concerns	low risk	low risk	low risk	some concerns	high risk
Jenkins, 2012	low risk	low risk	low risk	low risk	some concerns	some concerns
Jimenez-Cruz, 2003	some concerns	low risk	low risk	low risk	some concerns	some concerns
Jimenez-Cruz, 2004	some concerns	low risk	low risk	low risk	some concerns	some concerns
Johnson, 2005	some concerns	low risk	low risk	low risk	some concerns	some concerns
Kang, 2014	some concerns	low risk	low risk	low risk	some concerns	some concerns
Kim, 2014	low risk	low risk	low risk	low risk	some concerns	some concerns
Kim, 2016	low risk	low risk	low risk	low risk	some concerns	some concerns
Kim, 2017	some concerns	low risk	low risk	low risk	some concerns	some concerns
Liu, 2018	low risk	low risk	low risk	low risk	some concerns	some concerns
Mani, 1992	some concerns	low risk	some concerns	low risk	some concerns	high risk

Marinangeli, 2009	some concerns	low risk	low risk	low risk	some concerns	some concerns
Marinangeli, 2011	some concerns	low risk	low risk	low risk	some concerns	some concerns
Mehio, 1997	some concerns	low risk	low risk	low risk	some concerns	some concerns
Mollard, 2011	low risk	low risk	low risk	low risk	some concerns	some concerns
Moravek, 2018	some concerns	low risk	low risk	low risk	some concerns	some concerns
Nestel, 2004	some concerns	low risk	low risk	low risk	some concerns	some concerns
Olmedilla-Alonso, 2013	some concerns	low risk	low risk	low risk	some concerns	some concerns
Onyechi, 1998	some concerns	low risk	some concerns	low risk	some concerns	high risk
Pittaway, 2007	some concerns	low risk	low risk	low risk	some concerns	some concerns
Potter, 1981	low risk	low risk	some concerns	low risk	low risk	some concerns
Ramdath, 2017	some concerns	low risk	low risk	low risk	some concerns	some concerns
Ramdath, 2018	some concerns	low risk	low risk	low risk	some concerns	some concerns
Reverri, 2015	some concerns	low risk	low risk	low risk	some concerns	some concerns
Saraf-Bank, 2016	some concerns	low risk	low risk	low risk	some concerns	some concerns
Schafer, 2003	some concerns	low risk	low risk	low risk	some concerns	some concerns
Tappy, 1986	some concerns	low risk	some concerns	low risk	some concerns	high risk
Thompson, 2012	low risk	low risk	low risk	low risk	some concerns	some concerns
Tonstad, 2014	some concerns	low risk	low risk	low risk	some concerns	some concerns
Torsdottir, 1989	some concerns	low risk	some concerns	low risk	some concerns	high risk

Tovar, 2014	low risk	low risk	low risk	low risk	some concerns	some concerns
Traianedes, 1986	some concerns	low risk	some concerns	low risk	some concerns	high risk
Venn, 2010	some concerns	low risk	some concerns	low risk	some concerns	high risk
Winham, 2007	low risk	low risk	low risk	low risk	some concerns	some concerns
Winham, 2007	low risk	low risk	low risk	low risk	some concerns	some concerns
Winham, 2017	some concerns	low risk	low risk	low risk	some concerns	some concerns
Wong, 2009	low risk	low risk	low risk	low risk	some concerns	some concerns
Yoshimoto, 2020	low risk	low risk	low risk	low risk	some concerns	some concerns
Zafar, 2015	some concerns	low risk	low risk	low risk	some concerns	some concerns
Zhu, 2019	low risk	low risk	low risk	low risk	some concerns	some concerns
Zurbau, 2019	low risk	low risk	low risk	low risk	some concerns	some concerns

Table A 3. GRADE assessment

Outcome	No. of trials	No. of participants	Certainty assessment					Effect estimate	Grade
			Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations		
PPGR in normoglycemia	27	690	Not serious	Serious [a]	Serious [b]	Not serious	none	-1.23 [-1.59, -0.88]	Low
PPGR in T2DM	6	136	Not serious	Serious [a]	Serious [b]	Not serious	none	-2.89 [-4.61, -1.18]	Low
Fasting glucose healthy	16	1037	Not serious	Not serious	Very serious [c]	Serious [d]	none	-0.06 [-0.12, 0.00]	Very low
Fasting glucose DM	11	808	Not serious	Serious [a]	Very serious [c]	Not serious	none	-0.51 [-0.79, -0.24]	Very low
Hba1c DM	7	524	Not serious	Serious [a]	Very serious [c]	Very serious [e]	None	-0.20 [-0.36, -0.05]	Very low
HOMA	5	431	Not serious	Serious [a]	Very serious [c]	Not serious	none	-0.47 [-0.80, -0.14]	Very low

a. Due to high unexplained heterogeneity
b. Due to differences in control
c. Due to substantial differences in interventions and comparisons
d. The 95% CI included benefits as well as no effect
e. The 95% CI included benefits and harms

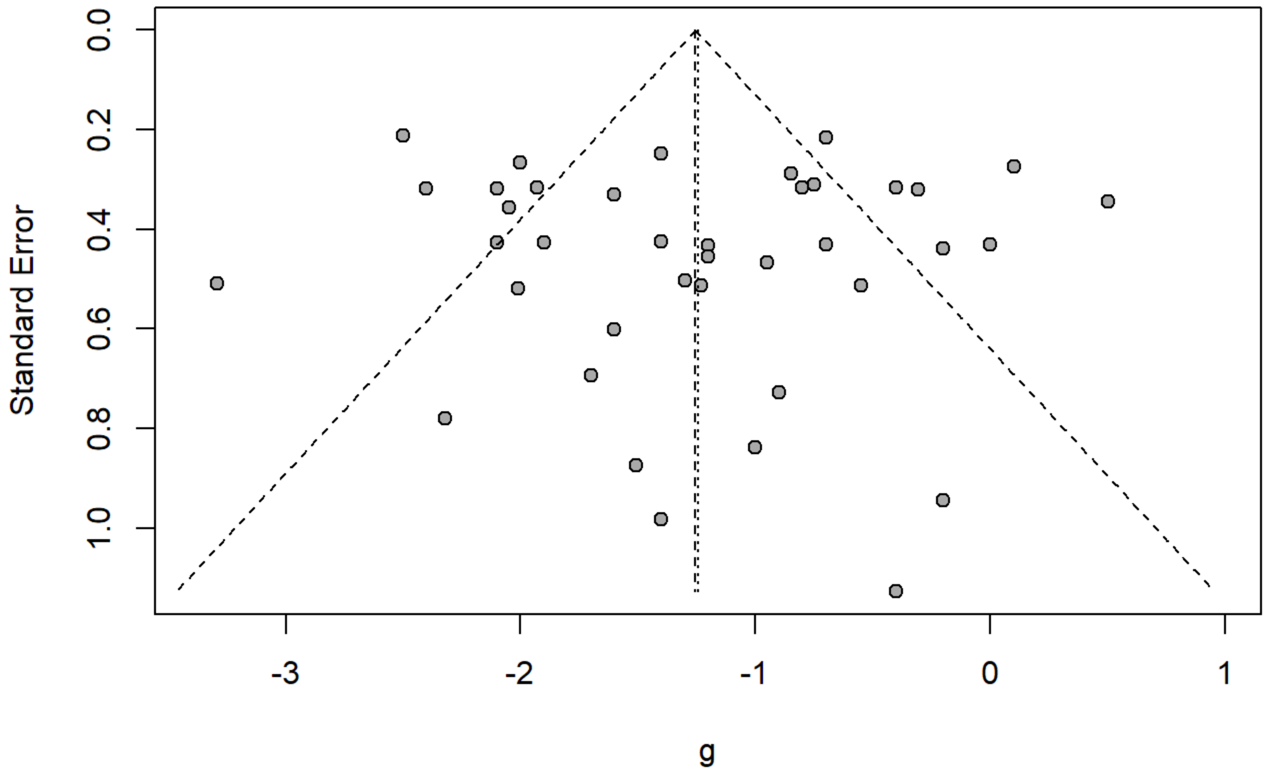


Figure A 1. Funnel plot of acute studies included in meta-analysis investigating acute glucose response after pulse consumption in adults without T2DM

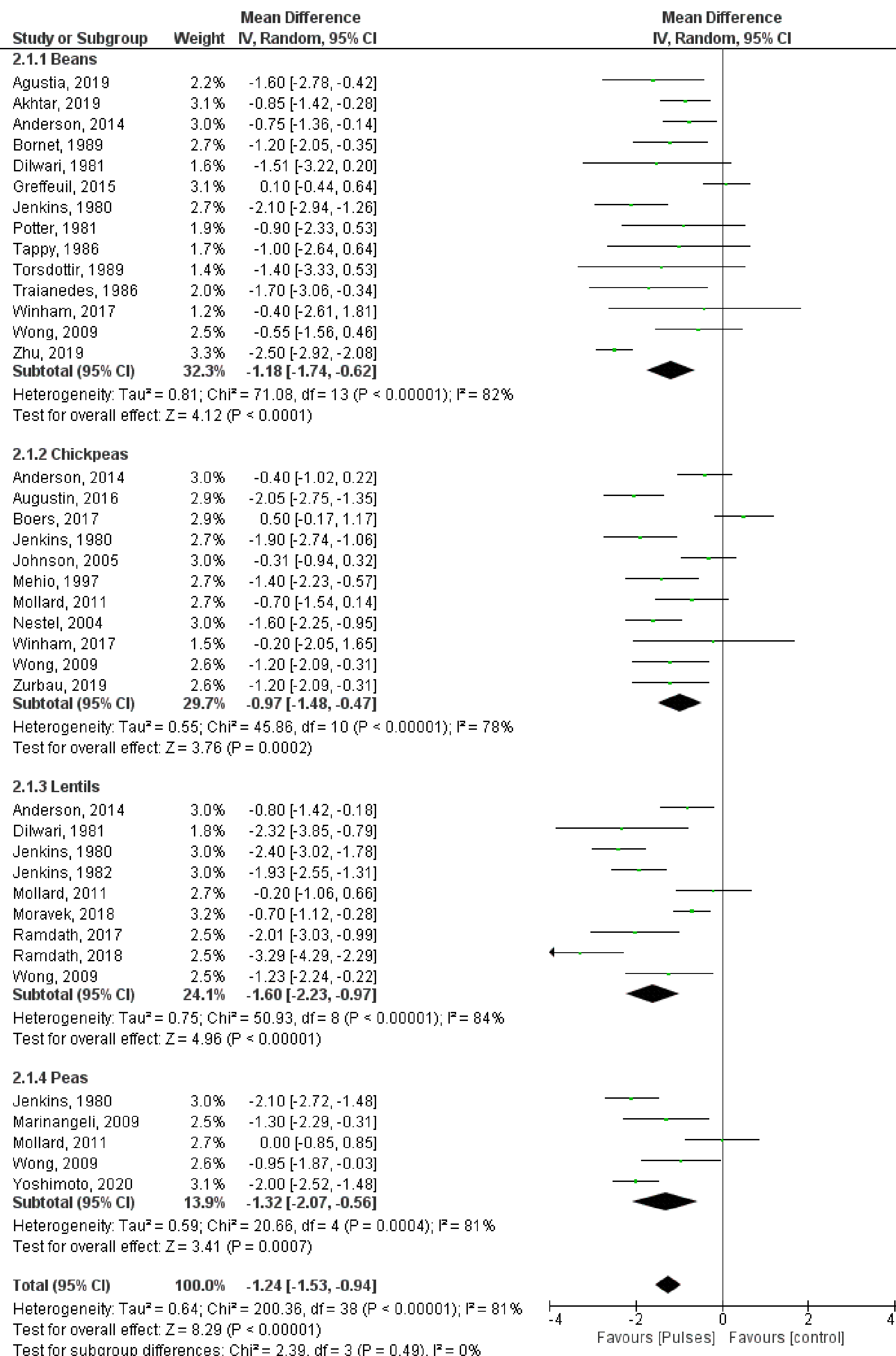


Figure A 2. Subgroup analysis by pulse type on acute studies investigating acute glucose response after pulse consumption in adults without T2DM

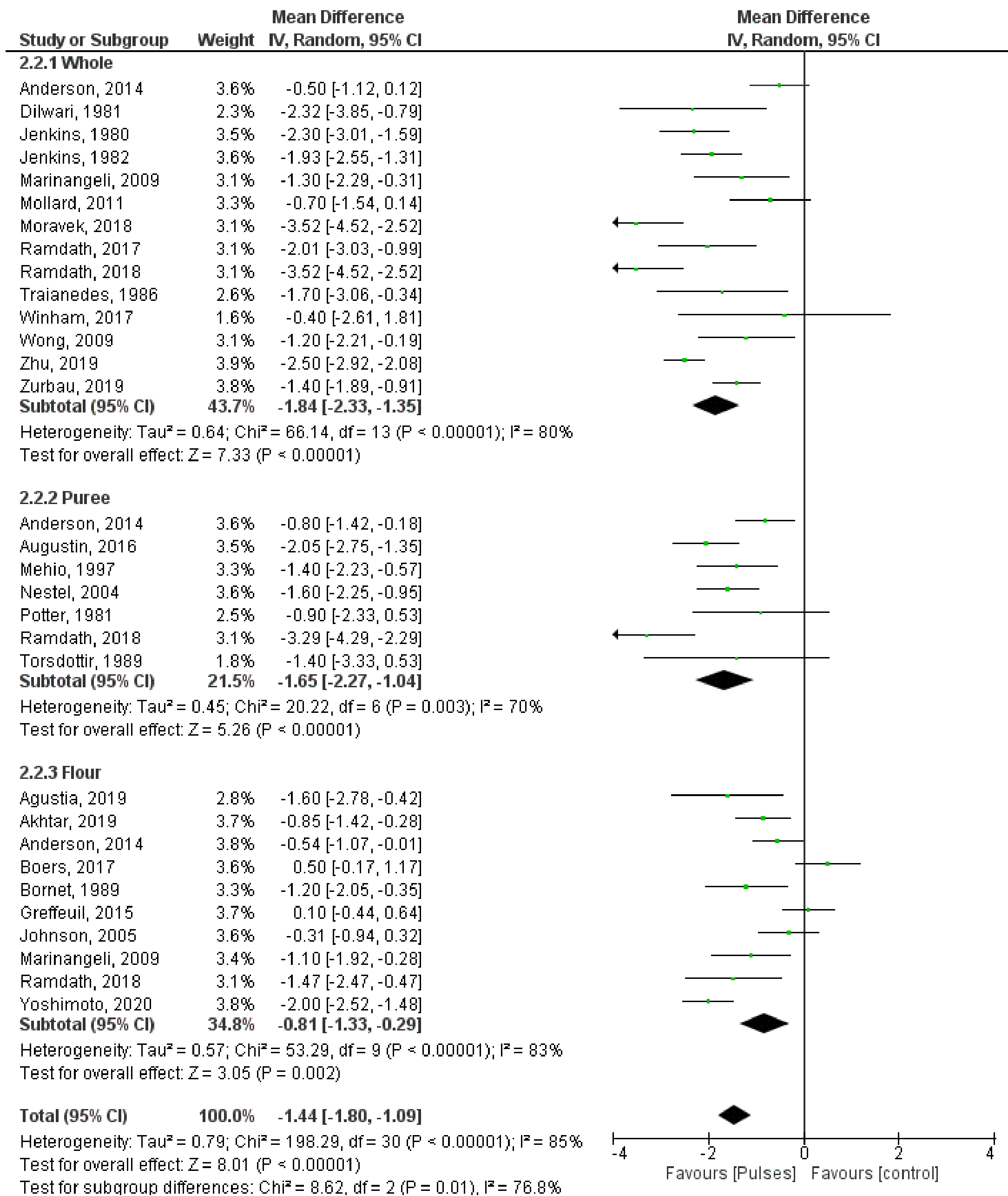


Figure A 3. Subgroup analysis by physical form of pulse on acute studies investigating acute glucose response after pulse consumption in adults without T2DM

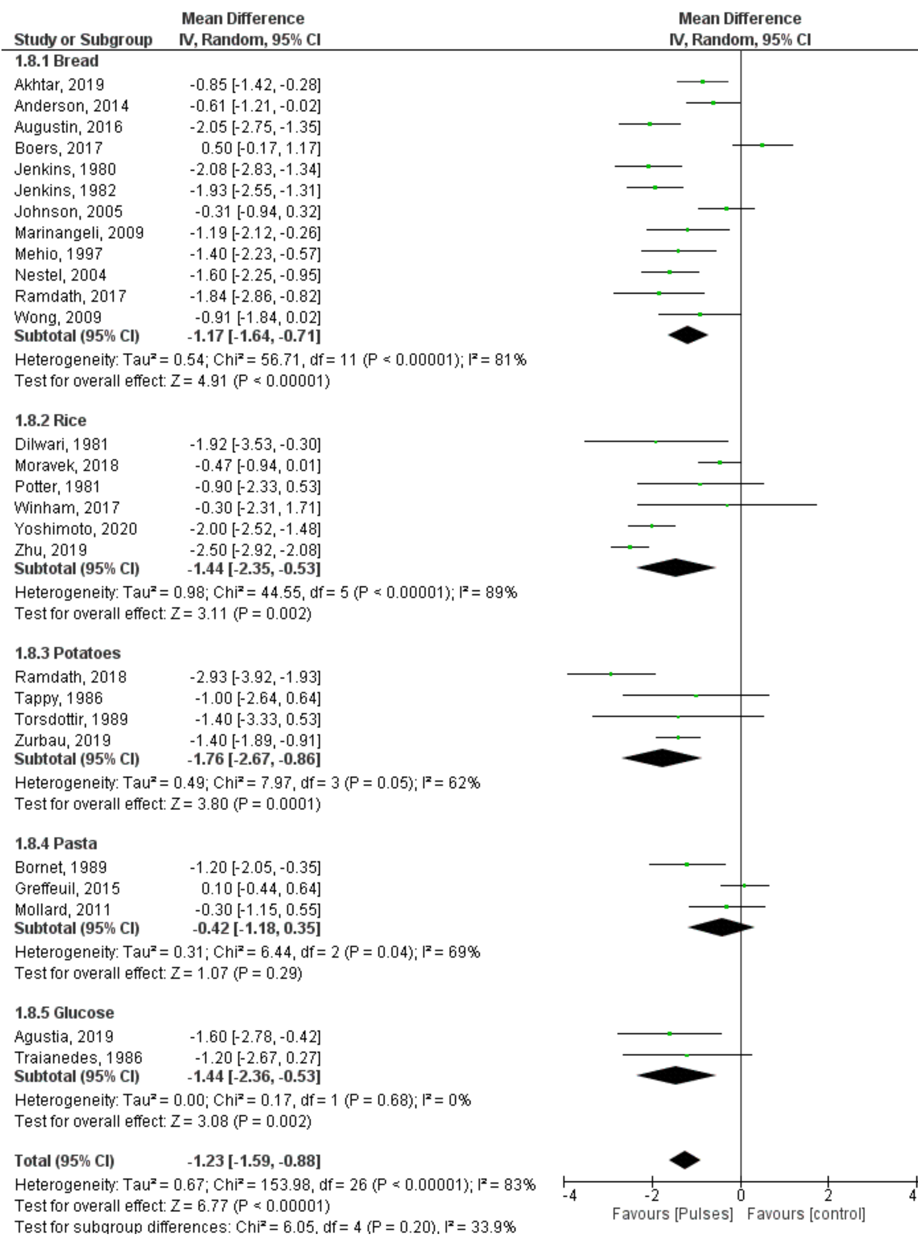


Figure A 4. Subgroup analysis by control group on acute studies investigating acute glucose response after pulse consumption in adults without T2DM

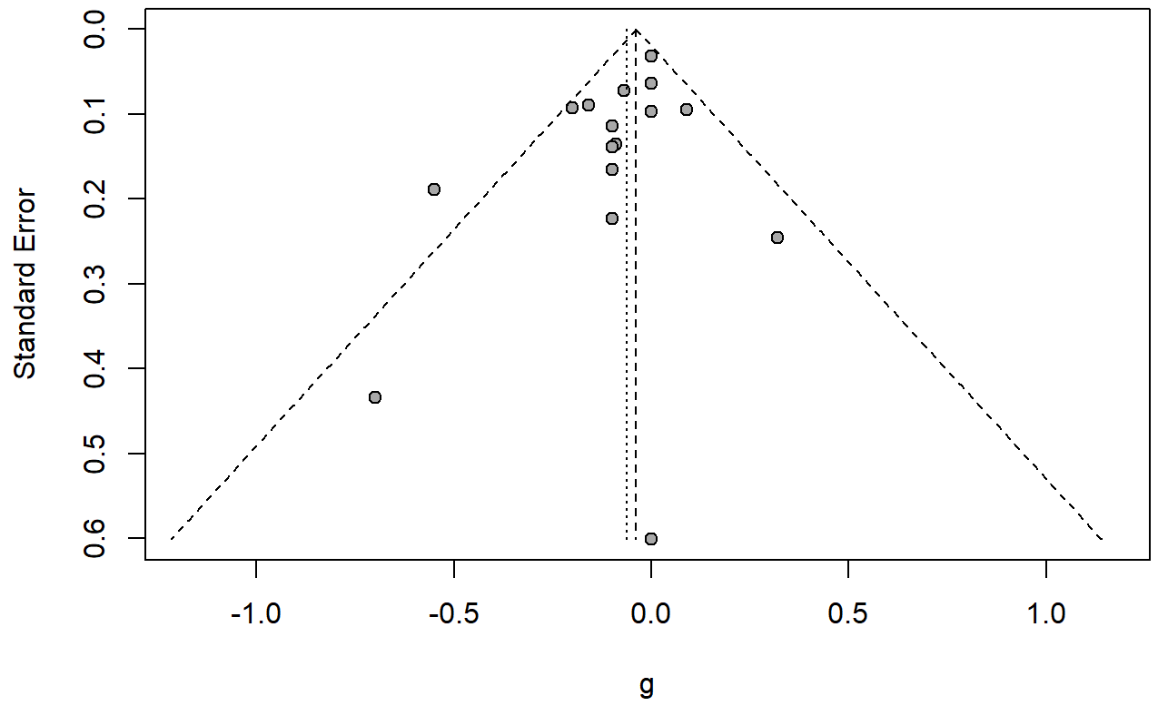


Figure A 5. Funnel plot of long-term trials included in meta-analysis investigating effect of pulse consumption in adults without T2DM

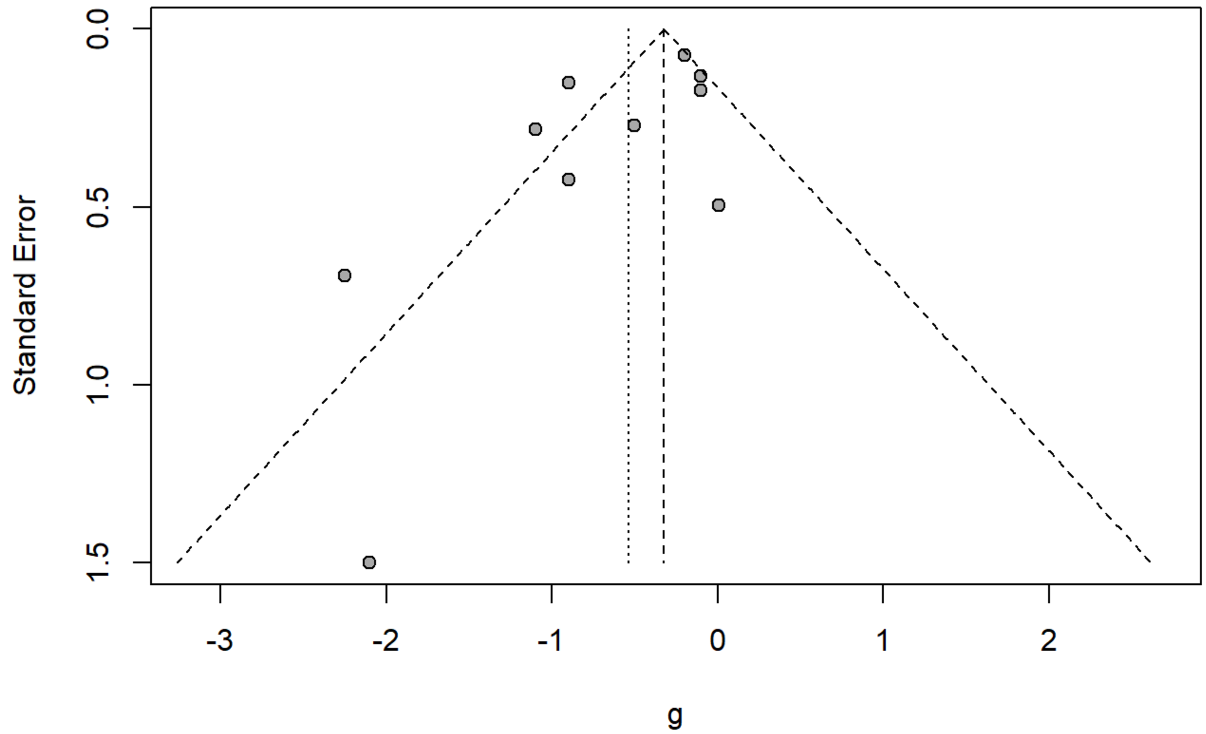


Figure A 6. Funnel plot of long-term trials included in meta-analysis investigating effect of pulse consumption in adults with T2DM

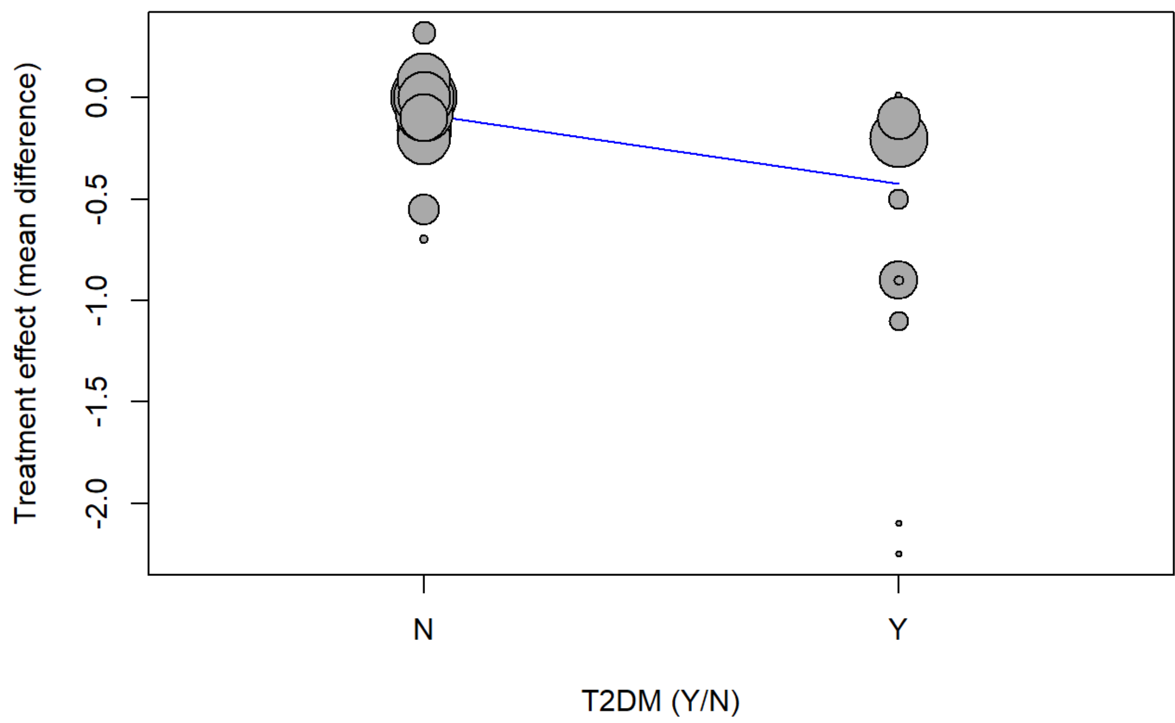
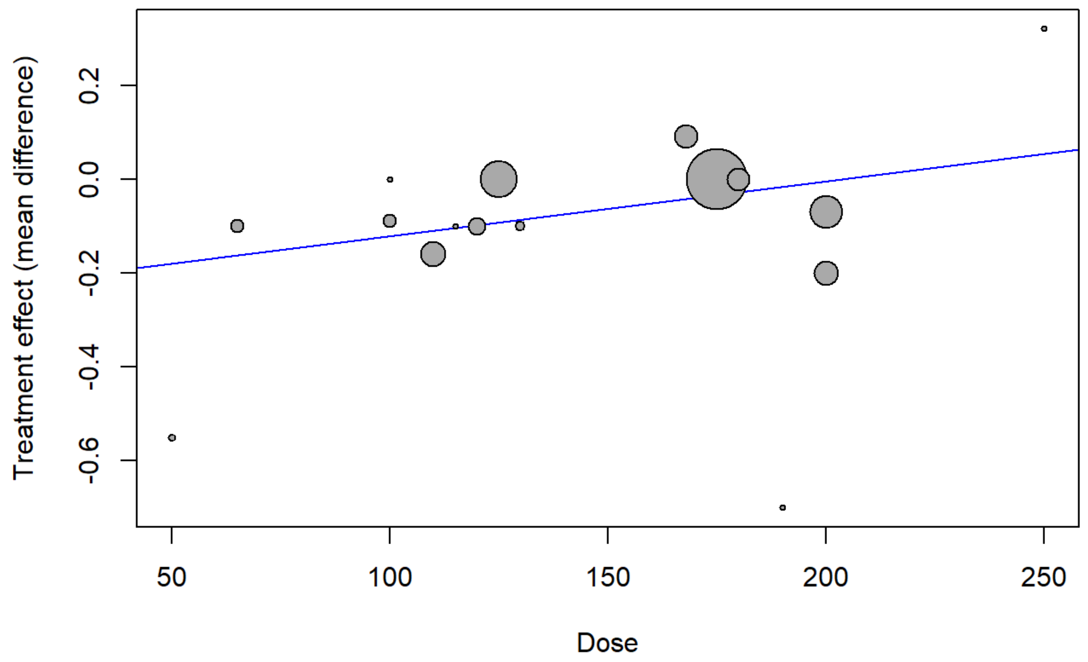


Figure A 7. Comparison of effect size in long-term trials between adults with and without T2DM



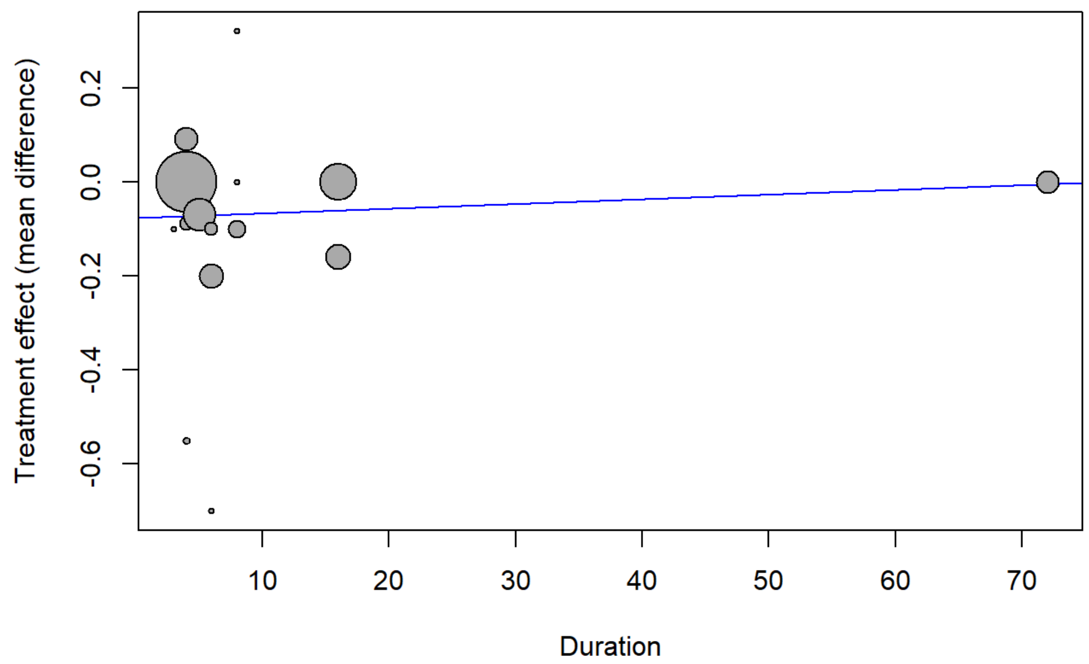
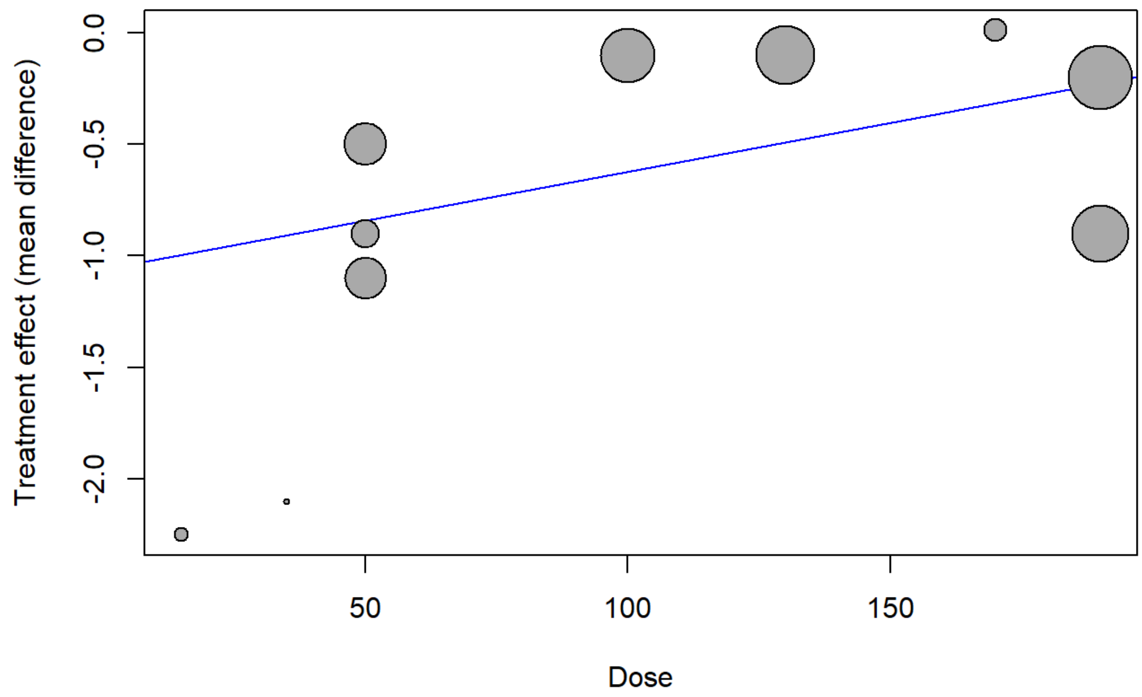


Figure A 8. Meta-regression of affect of dose and duration on modifying effect size in long-term trials on normoglycaemic adults



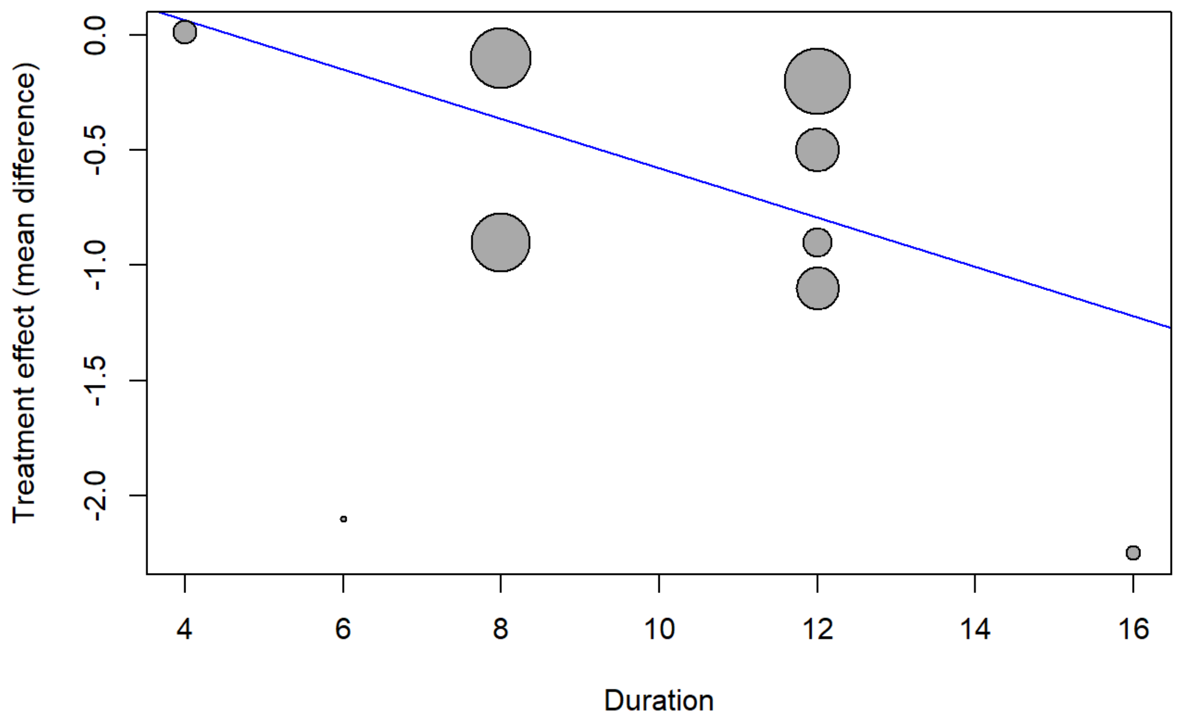


Figure A 9. Meta-regression of affect of dose and duration on modifying effect size in long-term trials on T2DM adults

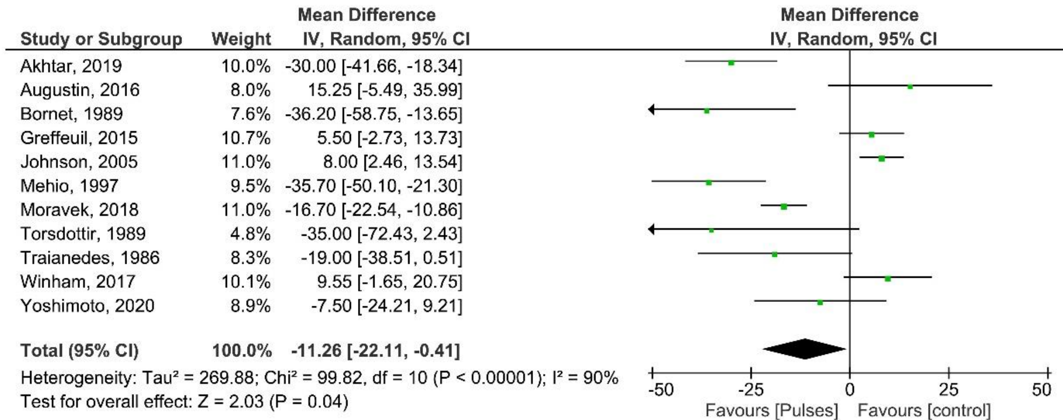


Figure A 10 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of acute trials investigating pulse intake on postprandial insulin in healthy population

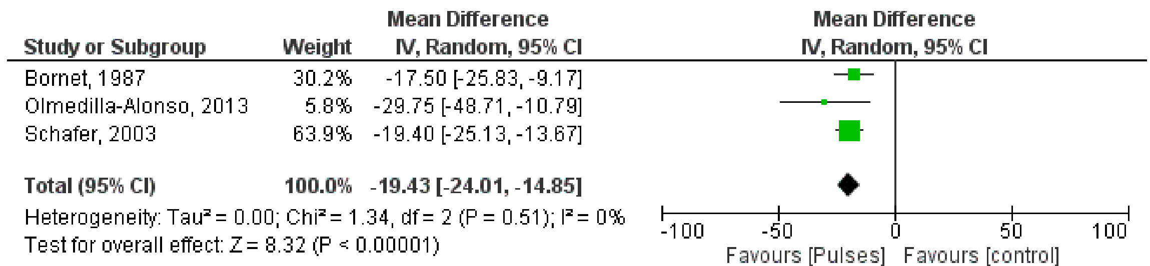


Figure A 11 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of acute trials investigating pulse intake on postprandial insulin in T2DM population

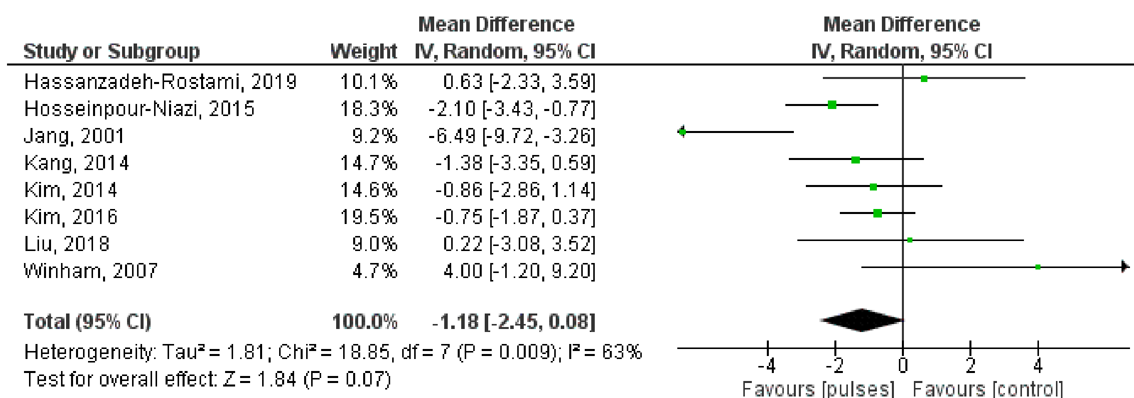


Figure A 12 Pooled effect using inverse-variance random effect model (mean difference and 95% CI) of long-term trials investigating pulse intake on postprandial insulin in T2DM population

Appendix B: Supplementary information of chapter 3

UNIVERSITY OF LEEDS

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MaPS and Engineering joint Faculty Research Ethics Committee (MEEC FREC)
University of Leeds

22 May 2019

Dear Maryam

Title of study	**Effect of acute consumption of legumes with different processing on post-prandial glycaemic profile**
Ethics reference	**MEEC 18-035**

I am pleased to inform you that the application listed above has been reviewed by the MaPS and Engineering joint Faculty Research Ethics Committee (MEEC FREC) and following receipt of your response to the Committee's initial comments, I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	*Version*	*Date*
Maryam_Hafiz_ethics_acute_RCT_April2019	3.0	16/05/2019
Maryam _Hafiz_CGM_instructions	3.0	16/05/2019
Maryam_HAfiz_Appendix for acute	3.0	16/05/209
Maryam_Hafiz_risk_assessment_form CB (1)	3.0	16/05/2019
Maryam_HafizIPAQ_short_v2.0_151116	3.0	16/05/2019

Please notify the committee if you intend to make any amendments to the information in your ethics application as submitted at date of this approval as all changes must receive ethical approval prior to implementation. The amendment form is available at http://ris.leeds.ac.uk/EthicsAmendment.

Please note: You are expected to keep a record of all your approved documentation and other documents relating to the study, including any risk assessments. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be audited. There is a checklist listing examples of documents to be kept which is available at http://ris.leeds.ac.uk/EthicsAudits.

We welcome feedback on your experience of the ethical review process and suggestions for improvement. Please email any comments to MEECResearchEthics@leeds.ac.uk.

Yours sincerely

Rachel E de Souza, Research Ethics & Governance Administrator, The Secretariat

On behalf of Dr Dawn Groves, Chair, MEEC FREC

Cc Student's Supervisor

Table B 0-1 Test meal randomisation sequence.

19 Participants	1st visit	2nd visit	3rd visit	4th visit
1	ChW	ChF	Con	ChPu
2	ChF	Con	ChW	ChPu
3	ChPu	ChF	ChW	Con
4	Con	ChW	ChPu	ChF
5	ChF	Con	ChW	ChPu
6	ChW	ChPu	ChF	Con
7	ChF	ChW	Con	ChPu
8	ChW	ChF	ChPu	Con
9	ChPu	ChW	Con	ChF
10	Con	ChF	ChW	ChPu
11	ChW	Con	ChPu	ChF
12	ChPu	ChF	Con	ChW
13	ChW	Con	ChF	ChPu
14	ChF	ChW	Con	ChPu
15	Con	ChW	ChPu	ChF
16	ChW	ChPu	ChF	Con
17	Con	ChF	ChPu	ChW
18	ChPu	ChW	ChF	Con
19	ChW	ChPu	Con	ChF

Con= mashed potatoes + 200 mL water

ChW = canned whole chickpeas + 200 mL water

ChPu = canned pureed chickpeas + 200 mL water

ChF = chickpea pasta + 200 mL water

Table B 0-2 Macronutrient composition of the standardised lunch.

Nutrition information [1]	Cheese sandwich	Salted crisps	Soft drink	Total
CHO, g	38.6	12.1	16	66.7
Fat, g	15.9	9.0	0	24.9
Protein, g	16.9	1.6	0	18.5
Salt, g	1.5	0.29	0	1.79
Energy, kJ	1548.1	573.2	263.6	2384.8

[1] The values are as per food labels.

Vascular Access Devices and Techniques:

1. PURPOSE AND APPLICABILITY

To establish a uniform procedure for the use of vascular access devices and techniques including venepuncture and cannulation to be used for research purposes within the School of Food Science and Nutrition. Venepuncture and cannulation are procedures that are routinely performed by trained phlebotomists to obtain single or serial venous blood samples.

2. PERSONNEL QUALIFICATIONS AND RESPONSIBILITIES

Venepuncture should be undertaken by personnel trained in phlebotomy technique and who are authorized to do so by the study Principle Investigator (PI). It is the responsibility of the PI to designate which staff shall conduct venepuncture and cannulation and subsequent blood sampling. If the PI has determined that training is necessary, all staff that perform activities within this SOP must have completed the training checklist and have their training verified and recorded in the training log.

- Principle Investigator:
  - Ensure that the researcher has necessary and up-to-date training by the local authorities for blood collection procedures.
- Researcher/Phlebotomist
  - Adhere to the procedures and follow the guidance outlined in this SOP.
  - Complete a venepuncture and cannulation training course. Newly qualified personnel must also be supervised for the first few samples taken and signed off as competent by an experienced researcher, before taking unsupervised samples. Documented records are to be kept on file within the School.
  - Ensure complete vaccination for Hepatitis B (and other blood borne virus' where necessary) through Occupational Health. Documents of the vaccination will be kept within the School and occupational Health.
  - Ensure that all blood collections are done in a manner that maximizes the safety of the participant, themselves, and any other staff or person(s) present during the procedure.
  - Report any accidents, incidents, or adverse events that occur during blood collection by following the appropriate School procedures. The PI

should always be informed of such events at the earliest possible opportunity.

- Ensure that participants are well informed of the study and its requirements, and, have signed the associated consent form.
- Ensure that participants must also have observed the blood transfusion services (BTS) guidelines for providing blood samples and be asked to withdraw if any of the outlined criteria applies (without need to disclose reasoning). This includes a maximum of 500ml of whole blood to be collected within a 16 week period.
- Ensure that all blood samples are labeled appropriately, and that records are kept including the participant's study code, date, storage, and analysis information (where appropriate).

3. EQUIPMENT AND RESOURCES

1. Equipment

- Access and use of an appropriate biochemistry space
- Centrifuge
- -80°C freezer
- Sharps bins
- Clinical waste disposal bags
- Vascular access devices
- Alcohol wipes
- Stop cocks
- Stylets
- Leur connector and barrel
- Tourniquet
- Heat packs
- Gauze
- Latex-free gloves
- Pre-filled 10ml saline syringes
- One-use plastic syringes
- Blood sampling tubes (vacutainers)
- Eppendorf tubes (labeled)
- Storage boxes for eppendorf tubes
- 1000ul and 100ul pipettes and pipette tips
- Blue roll

2. Other Resources

- Blood sample log (to be held by the PI)

4. HEALTH AND SAFETY

1. Precautions

Standard precautions must be observed at all times, including hand washing and decontamination. All items used must be sterile single use. All sharps used must be safety devices. Aseptic Non-Touch Technique (ANTT) should be used. Personal protective equipment must be used, including non-sterile latex-free gloves and aprons. Safety goggles or a face shield should be considered if the patient is known to have a blood borne virus and/or is likely to do anything (i.e. movement /exercise) that may increase risk of blood splatter. Skin must be cleansed using the appropriate aseptic technique and allowed to air dry.

2. Avoidance of contaminated phlebotomy equipment

Tourniquets are a potential source of methicillin-resistant Staphylococcus aureus (MRSA), with up to 25% of tourniquets contaminated through lack of hand hygiene on the part of the phlebotomist or re-use of contaminated tourniquets. To avoid contamination, any common-use items, should be visibly clean before use on a participant, and single-use items should not be reused.

3. Fainting

If a participant feels faint whilst a blood sample is being taken, the procedure should be stopped immediately and the participant reclined on the floor/chair with their legs raised. If the volunteer faints lie them down with their legs raised. Check they are breathing and for any injuries from fainting. Reassure them and once they are feeling better get them to sit up slowly and offer a glass of water for refreshment. If they do not regain consciousness within 1-2 minutes, but are breathing, put them in recovery position and call University security on 32222 and ask them to call an ambulance. If they are not breathing phone University security on 32222 and ask them to call an ambulance. Record details in the accident log and the adverse events (AE) form.

4. Needle stick injury

In case of a needle stick injury, bleed the wound, rinse under cold water and cover with a sterile dressing. Report the event to the Health and Safety Officer and immediately inform Occupational Health. Record the accident/incident in the accident book and adverse event (AE) form.

5. Blood spill

In case of a blood spill, wear protective gloves to mop up the spill immediately using blue roll tissue paper and a high level laboratory disinfectant e.g. Distel. Repeat the

process until all traces of blood have been removed. Discard the used blue roll tissue paper and gloves in the clinical waste bag.

5. PROCEDURE

1. Preparation of Blood Room

Ensure limited access except for relevant parties i.e. researchers and volunteer, with 'DO NOT ENTER' sign clearly displayed.

- Ensure that the environment is clean, tidy, well-lit, and has the following:
  - A working telephone for emergencies
  - Appropriate medical supplies
  - A reclining chair or bed for the participant
  - A hand-wash basin with soap, running water and paper towels
  - Sharps container(s) and appropriate hazardous waste containers for biological waste and softs
  - Alcohol hand-run / hand disinfectants
  - Instructions for proper aseptic technique are visible
  - Is set-up to run the research procedures appropriately

2. Preparation of Equipment

Collect all of the equipment as necessary for undertaking the procedure(s) and place within a safe and easy to reach self-contained tray or trolley, ensuring that all items are clearly visible. Ensure that any necessary laboratory forms are accessible.

3. General procedure

- Check all lighting, ventilation, privacy and positioning is adequate
- Check that the laboratory form matches the patient's identity
- Check whether the participant has allergies, difficulties with clotting, phobias, or has ever fainted during previous injections or blood draws
- Explain to the volunteer clearly and confidently the procedures involved and what can be expected
- Allow the volunteer to ask any questions and talk through any concerns
- Remind the participant of their right to withdraw at any time
- Check packaging of equipment to ensure equipment is not damaged and is in date in order to maintain asepsis
- Assemble necessary equipment and where applicable maintain asepsis
- Wash hands carefully with a bactericidal soap and perform appropriate aseptic technique. Ensure hands are dry before commencement to minimise the risk of infection

- Check hands for any broken skin and cover with a waterproof dressing to minimise the risk of blood contamination
- Wear well-fitting latex-free gloves, a disposal apron and goggles as/if necessary
- Ensure the participant's clothing is loose on the chosen arm and that the arm is supported to ensure blood flow is not restricted
- Palpate the participant's arm to select an appropriate insertion site. Where possible, choose the antecubital fossa on the non-dominant arm. If difficulties are encountered in palpating a vein, use a heat pack over the insertion site.
- Prepare the participant's insertion site and swab with an alcoholic wipe and let dry for ~2 minutes. Do not retouch or re-palpate the skin
- Apply the tourniquet to the upper arm on the chosen side
- The arm may be placed in a dependent position with the volunteer gently clenching and unclenching their fist to promote blood flow and improve prominence of the vein
- Inspect the device(s) carefully before use
- Anchor the vein by applying manual traction on the skin a few centimetres below the proposed insertion site. This will ensure smoother needle entry and immobilize the vein
- Perform venepuncture or cannulation technique as appropriate

4. Cannulation

- Insert the cannula needle, bevel edge upward at an angle of approximately 15-30 degrees to ensure pain free entry (note: a fragile vein usually requires a lower angle of insertion)
- Stop as blood is observed in the flashback chamber
- Lower the angle of insertion to correspond to the vein's depth and direction.
- Advance the cannula 5mm into the vein
- Stop and withdraw the needle up to 5mm from the cannula
- Secondary flashback should be seen along the length of the cannula
- Continue with the skin traction
- Slowly advance the cannula into the vein in short stages and at each stage withdraw the needle slightly – never fully remove the needle until the cannula is fully inserted.
- Once the cannula has been fully inserted into the vein remove the tourniquet

- Withdraw the needle and dispose immediately into the sharps bin keeping the luer lock cap
- Apply the luer lock cap to the cannula
- Secure the cannula in place with strips of micropore on each side of the cannula
- Once secure, place gauze under cannula, remove Luer cap and replace with either a stylet or three-way tap as quickly as possible
- Place sterile cannula dressing over the whole site

1. **Keeping the vein patent – Use of a stylet**

- Stylets are single use and can be placed directly into the catheter of the cannula. Ensure that stylets are discarded (into sharps) and replaced afresh after every blood sample.

2. **Keeping the vein patent – Use of saline**

- If using saline, ensure that a three-way tap is secured to the Leur of the cannula.
- Flush the cannula with 5mls of sodium chloride 0.9% (flush with 10mls if you aren't taking the first sample immediately)

5. Taking blood samples - venepuncture

- The use of vacuum extraction tube systems as closed systems for blood collecting reduces the risk of direct exposure to blood and has made it easier to take multiple samples from a single venepuncture
- Double-ended winged butterfly needles have the end covered by a rubber cuff and are screwed into the sampling barrel. The barrel holds the sample collection tube in place and protects the phlebotomist from direct contact with blood.
- Following the General Procedures outlined in 5.3
- Insert the butterfly needle, bevel edge upward at an angle of approximately 15-30 degrees to ensure pain free entry (note: a fragile vein usually requires a lower angle of insertion)
- Stop as blood is observed in the flashback chamber
- Lower the angle of insertion to correspond to the vein's depth and direction.
- Advance the needle 5mm into the vein
- Secondary flashback should be seen along the length of the butterfly connector
- Continue with the skin traction and anchor the needle by placing two fingers either side of the butterfly tips downward to the skin

- Once the winged butterfly needle is in the vein, the tube is pressed on to the needle and the blood is drawn automatically into the sample tube by vacuum until the required amount is collected
- When sampling has finished, remove the vacutainer, followed by the tourniquet. Anchor the vein, remove the butterfly needle, and if possible use the safety-engineered device to cover the sharp end of the needle
- Discard the barrel and winged butterfly needle as a single entity where possible
- Following the Removing the Vascular Access Device procedures in 5.7

1. **Taking blood samples - cannulation**

- If using saline, flush the cannula with 5mls of sodium chloride 0.9% (do not repeat if you've just done this, only do this for new samples). Saline flush is not necessary if using a stylet
- Take 1ml of whole blood using a small syringe and discard as waste
- If using a syringe for blood sampling, choose an appropriate size syringe and insert this into the Leur. Draw back the syringe gently to ensure the vein does not collapse. If drawing blood becomes difficult, try gently moving the cannula or heating the site of insertion
- Take the required amount of blood. If using saline flush the cannula with 10mls of sodium chloride 0.9% immediately after sampling. Dispense the blood from the syringe into an appropriate blood collection tube
- If using a vacutainer, prepare a Leur connector and barrel, remove the stylet and discard (sharps) and insert this into the Leur. Insert the vacutainer into the barrel and fill until the necessary amount of blood has been collected
- Remove the vacutainer from the barrel and invert several times. Apply pressure to the vein whilst the Leur is removed and a fresh stylet is placed into the catheter of the cannula

6. **Removing the vascular access device**

- Remove the cannula/venepuncture needle as soon as it is no longer required
- Place firm pressure on the puncture site with gauze until the bleeding has stopped
- Cover the site with either a plaster or micropore
- Remove gloves and lab coat and wash hands thoroughly with appropriate anti-bacterial hand wash
- Record details of sampling in the blood-taking log book

7. **Preparing and storing blood samples**

- Ensure only the designated worker/s are present to minimise the risk of further contamination and infection (with the exception of the participant if they cannot be moved)
- Other staff should be notified when necessary that the room is in use
- Blood manipulation should take place in a designated environment ensuring that surfaces are clean.
- A clean lab coat should be worn
- Wash hands and put on clean gloves
- Take care to ensure that there is no possible transfer contamination. Only designated pens and materials to be retained in the designated work area. Anything removed from the designated area must be sterilized using appropriate methods

1. **Blood sample processing**

Blood processing should be carried out in-line with local procedures. The following serves as guidance:

- The centrifuging process must take place with only the designated worker/s in the room (with the exception of the participant if they cannot be moved)
- Centrifuging must only be performed in sealed buckets that are opened **only** in the safety cabinet to prevent contamination of other areas in case of spillage
- Place the blood tubes in the designated buckets and ensure that the arm is balanced sufficiently (do this by adding a tube with water)
- Spin the bloods according as per analysis requirements
- Should it be suspected that a tube has broken then leave the centrifuge for at least one hour before opening to reduce the risk of aerosol contamination and infection ANY BREAKAGE OR SPILLAGE IN THE CENTRIFUGE SHOULD BE DETAILED AND RECORDED IN THE ACCIDENT BOOK
- Remove the sealed buckets from the centrifuge and into the safety cabinet and then carefully remove the tubes from the buckets and finally remove the lid off the tube
- Pipette (using disposable pipettes) the required volume of plasma/serum from the tube and transfer to the appropriately labelled and identifiable (in terms of volunteer details i.e. ID) eppendorf tube
- Repeat this for the number of eppendorf tubes you need
- Seal carefully and transfer to an appropriate rack for storage
- Store in a secure designated area (preferably -80 freezer) ensuring that it is clearly labelled in terms of the study and researcher
- Avoid repeated thawing and freezing to avoid damaging samples

8. **Storage and Disposal of Clinical Waste**

- Dispose of needles in designated sharps bins
- Dispose of clinical waste in the designated yellow waste bags
- Dispose of solid waste in an appropriate suitable container for incineration
- The waste is then to be disposed of by approved methods in clinical waste disposal as identified by the University of Leeds guidelines. Arrangements must be made prior to removal from area and should not be left unattended elsewhere for disposal

9. Considerations

- All surfaces in the designated work area should be cleaned with 1% Trigene solution after each use
- All surfaces including the chair and floor should be thoroughly cleaned at the end of each week and this should be documented in the cleaning log
- The centrifuge should be cleaned (and disinfected) according to the manufacturer's instructions after each use and especially in the case of an accidental spill. Cleaning should also be recorded on a weekly basis or when necessary. Details of the method of cleaning used and researchers name should also be recorded
- To prevent the rotor from sticking, the drive shaft should be lubricated at least once a month and after cleaning
- Report all accidents to University Safety Advisory Services (SAS) and University Occupational Health (OHD). All incidents should be reported to the School's Safety Supervisor and recorded
- Lone working should be avoided where and if possible.
- Associated colleagues should be notified at the beginning and end of undertaking procedures to avoid disruption and possible contamination in the event of an accident