Acute effects of pulse ingredients in food products on blood glucose, insulin, and food intake response following an endurance exercise session in healthy adults

by

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Abstract

Background: Whole pulses have been shown to provide favourable post-prandial glycemic responses and increase satiety. It is unknown whether pulse ingredients retain the health benefits of whole pulses when incorporated in different food matrices and if they provide any benefits before or after exercise.

Objective: Study objectives were to examine the effects of extruded pulse snack and cereal products on blood glucose (blood glucose), insulin and appetite responses before (0-60 min) and after (120-140 min) endurance exercise and food intake 2 hours (240 min) following exercise compared to a non-pulse product.

Design: Two acute trials were conducted following a repeated-measures crossover design. In trial 1, adults randomly consumed extruded six snacks (n=25) and in trial 2, adults consumed extruded cereals (n=27). Extruded snacks were made with corn flour (control), whole yellow pea flour, split yellow pea flour, green lentil flour, chickpea flour, and pinto bean flour. Extruded cereals were made with oat flour (control), oat plus pea starch (starch), oat plus protein (protein), and oat plus starch plus protein (starch+protein).

Results: Trial 1: Green lentil led to a lower blood glucose response compared with split yellow pea at 150 min, whereas at 165 min and 180 min, green lentil snacks led to lower blood glucose compared to the control. There were no differences between treatments for pre-or post-exercise blood glucose netAUC. There was time ($p<0.0001$), treatment ($p=0.02$), and treatment-by-V02 ($p<0.0002$) effects over the entire session for insulin, but no time-by-treatment interaction ($p=0.10$). There were no differences between treatments for pre-exercise insulin netAUC. Post-exercise appetite netAUC showed treatment effects for both split yellow and chickpea ($p<0.05$); split yellow and chickpea showed lower appetite scores compared to whole yellow pea and pinto

bean. There was no difference in food intake. Trial 2: Pre-exercise blood glucose netAUC was lower following the starch + protein + fibre and protein + fibre compared to control ($p<0.05$). However, post-exercise blood glucose netAUC showed no differences among treatments ($p=0.33$). Appetite pre-exercise netAUC showed differences in treatments ($p=0.028$); protein + starch and protein showed lower appetite compared to control ($p<005$). There was no difference in post-exercise netAUC appetite ($p=0.21$). There were no differences in food intake or insulin. Conclusion: These studies show the potential for pulse ingredients to impact the glycemic and appetite responses prior to and following exercise, however it is dependent on pulse type.

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Dedication

I dedicate this thesis to my family, my husband Ben and children Amara, Ava and Amelia. Over the course of this degree, Ben and I have gotten engaged, married, had 3 amazing children, moved 3 times and bought our first home. This road has come with a lot of surprises and challenges and without your love and support I couldn't have done it. Thank you for pushing me to be better and never letting me give up. I also dedicate my work to my parents and siblings, thank you for allowing me the time to do this and helping me with the kids. I'm so lucky to have such an amazing family.

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List of Abbreviations

ANOVA- analysis of variance

blood glucose - blood glucose

BMI- body mass index

CCK- cholecystokinin

CVD- cardiovascular disease

Db- dry basis

FBG - fasting blood glucose

FBI-fasting blood insulin

FI- food intake

G-grams

GI- glycemic index

GLP-1-glucagon-like-peptide-1

H- hours

HbA1C- glycated hemoglobin type A1C

HOMA-IR- homeostatic model assessment for insulin

IAUC- incremental area under the curve

netAUC- net area under the curve

Kcal- kilocalories

PYY- peptide YY

RCT- randomized controlled trial

VAS- visual analog scale

Y- year

Chapter 1: Introduction

The World Health Organization defines overweight and obesity as "abnormal or excessive fat accumulation that presents risk to health" (WHO, 2021). Obesity predisposes individuals to chronic diseases such as metabolic syndrome, type-2 diabetes mellitus and cardiovascular disease. There has been a drastic increase in the proportion of overweight and obese Canadians over the last few decades. Based on self-reported data, 63.9% of Canadian adults were overweight or obese in 2018 (Statistics Canada, 2018). Diabetes rates have also increased over the years, with 7.8% of Canadians living with diabetes (Statistics Canada, 2017). This increase in obesity and diabetes is believed to be influenced by the availability of ready-to consume, highly processed, pre-packaged foods, accounting for over half of Canadian's diets along with a sedentary lifestyle (Poti, Braga and Qin 2017; Nardocci, Polsky and Moubara 2019). These foods often are low in protein and dietary fiber with high amounts of sugar and sodium (Statistics Canada, 2018).

Increasing consumption of food ingredients that can increase satiety and reduce energy intake, along with increased physical activity, may prevent weight gain and promote weight loss. Replacing foods that consumers would normally reach for with healthier alternatives is needed in the marketplace, as consumers aren't ready to give up convenience foods altogether. Pulse flours and fractions are dietary ingredients that possess the potential to provide such benefits and currently there are more and more foods being developed to contain pulse ingredients. In 2020, 7% of all snack foods contained pulses (Pulse Canada 2021).

Dietary pulses (chickpeas, beans, lentils and yellow peas) and pulse ingredients (fractions and flours) have many positive nutritional properties. Regular consumption of pulses (½ cup/day) is associated with higher quality diets, including higher intakes of fibre, protein, folate,

zinc, iron, and magnesium and lower intakes of saturated fat and total fat, compared to diets that are low in pulses (Tosh and Yada 2010). Pulses are nutrient dense with high fibre, protein and low-fat content making them an excellent choice to incorporate into food products (Tosh and Yada 2010; Hoover et al. 2010).

In addition to having beneficial health effects, pulses are of interest as value-added ingredients for another reason. Canada is the largest producer of lentils; in 2017 it produced the majority of the world's supply at 3.73 million metric tons. Additionally, in 2011 Canada had the largest amount of dry pea production in tonnes globally (Statistics Canada: Pulses in Canada). However, Canadians only consume a small fraction of the pulses they produce, as the Canadian Community Health Survey reported only 13% of Canadians were consuming pulses each day (Health Canada 2009; Anderson et al. 2014). Pulse consumption has increased over time; however, it is still low compared to other countries. Consumption may be impacted by a lack of knowledge about the health benefits, limited visual appeal and the preparation that pulses require (Health Canada 2009). If pulse ingredients are incorporated into foods commonly consumed and readily available, such as in a snack or cereal form, these foods may increase satiety and improve glycemic control compared to foods without pulse ingredients. This may also support Canadian farmers, food processors, ingredient suppliers and food manufacturers.

Chapter 2: Literature Review

2.1 Introduction

The following is a review of the current evidence regarding the relationship between pulses, glycemic response, and satiety. It begins with an overview of the nutritional content of pulses. This review covers research related to the effects of whole pulses, pulses within mixed meals, pulse flakes, pulse flours, pulse fractions and lastly, reviews published on glycemic and satiety control.

2.2 Overview of pulses nutritional content

Pulses contain high amounts of complex carbohydrates including both soluble and insoluble fibre (Tosh and Yada 2010), as well as resistant and slowly digestible starch and oligosaccharides (Tosh and Yada 2010; Hoover et al. 2010). A variety of health benefits are associated with the intake of dietary fibre, including improved glycemic response and insulin sensitivity in non-diabetic and diabetic individuals from increased intake of soluble fibre, as well as enhanced weight loss due to fibre supplementation in obese individuals (Nugent 2005; Anderson et al. 2010; Bodinham, Frost and Robertson 2010).

Similar to dietary fibre, resistant and slowly digestible starches have been shown to improve glycemic and insulinemic responses, increase satiety and reduce energy intake. Resistant starch is not digested and therefore it is not absorbed as glucose in the small intestine. It can be fermented by microflora in the colon which produces various short-chain fatty acids. This allows for a delayed glycemic response in comparison to digested starch. The high amounts of fibre, resistant starch and slowly digestible starch in pulses contributes to its low glycemic index (GI). The glycemic index is defined as incremental area under a two-hour blood glucose

area under the curve (AUC) following a 12- hour fast and ingestion of a food (Jenkins et al.1976; Jenkins et al. 1978; Marinangeli et al. 2009). If a food has a GI number higher than 70, it is classified as a high GI food; if a food has a GI number lower than 55, it is classified as a low GI food. Medium GI foods have GIs in the range of 55-70 (Foster-Powell, Holt and Brand-Miller 2002). Pulse GI values range from 29 to 48, compared to 42-72 for grains (McCrory et al. 2010).

Depending on the GI of a food product, the blood glucose response will differ. Consumption of low GI foods results in slower absorption from the gastrointestinal tract in comparison to high GI foods, which are rapidly absorbed (Boye, Zare and Pletch 2009). This slower absorption results in a lower release of glucose into the blood stream. Substantial scientific evidence supports the notion that low GI foods can influence many physiological processes, which are relevant to health, including blood glucose control, appetite regulation, energy balance and aerobic performance (Augustin et al. 2015; Anderson 2014; Maringangeli et al.2009)

Pulses also contain high amounts of protein which makes them somewhat unique in comparison to other plant foods. The protein content of pulses ranges from 17-30% of dry weight which is typically twice the amount found in cereals and similar to meat (Boye, Zare and Pletch 2010). Studies have shown that protein increases satiety to a greater extent than carbohydrate or fat and increases blood glucose control (Anderson and Moore 2002; Hutchison et al. 2015; Veldhorst et al. 2017). Based on the nutritional composition of pulses they are a very compatible food ingredient for glycemic control, satiety, and endurance.

2.3 Blood glucose and satiety regulation

Glucose is the body's primary energy source. (O'Keefe JH, Gheewala N and O'Keefe JO, 2007) Blood glucose a tightly regulated process to ensure proper functioning of the brain. Too little glucose hypoglycemia will set in, and too much hyperglycemia will happen. Post-prandial blood glucose can be affected by multiple factors, such as secretion of hormones, quantity and composition of nutrients, glucose absorption rate and gastric emptying rate. Post-prandial glucose levels can be major determinants for several diseases (O'Keefe JH, Gheewala N and O'Keefe JO, 2007).

Insulin is the primary regulator of glucose homeostasis. Insulin is produced in the liver by beta cells and is released when blood glucose levels rise. It manages glucose levels by signalling the insulin-sensitive peripheral tissues to increase their uptake, acts on the liver to promote glycogenesis and inhibits glucagon secretion. Insulin binds to insulin receptors on skeletal muscle which responsible for the majority of glucose uptake.

Satiety measures the feeling of fullness following or between a meal and leads to the cessation of eating. Many signal pathways will let the central nervous system know an individual is full. The primary satiety hormone regulators are cholecystokinin (CCK), glucagon-like peptide (GLP-1), peptide tyrosine tyrosine (PYY) and gastric inhibitory polypeptide (GIP). (D'Alesssio 2008) CCK is an important hormone produced in the endocrine cells of the small intestine. It is released following ingestion of a food, partially after fat and protein are ingested. CCK's effects are synergized by the action of leptin (Barrachina et al 1997). GLP-1 is produced from L-cells in the ileum and colon. GLP-1 helps stimulate insulin secretion and inhibits hyperglycemia as well as inhibits gastric emptying which suppress food intake (D'alessio 2009). Carbohydrates have the greatest impact on GLP-1 secretion. Similar to CCK, GLP-1 is dependant on leptin to induce

satiety. PYY is also produced by the L-cells. Like CCK and GLP-1 it is also dependant on leptin (Moran et al 2005). GIP is released in the duodenum following a meal.

Ghrelin plays an important role in the regulation of food intake and appetite. It is secreted before a meal to begin gastric motility and increase gastric acid. Secretion then declines within one hour after the start of a meal. Amylin is secreted with insulin to inhibit glucagon secretions. It also works to delay gastric emptying after ingestion a food or meal to decrease blood glucose levels. It also plays a role in telling the brain one is full after a meal. Leptin is produced by white adipose cells in proportion to overall fat mass. Leptin maintains homeostasis in response to high fat levels through decreased food intake, decreased metabolic rate, increase activity level, increase temperature and inhibition of insulin production and release.

2.4 Effects of physical activity on glucose levels

The Canadian Physical Activity Guideline recommends 150 minutes of moderate to vigorous aerobic physical activities per week (Public Health Agency of Canada, 2019). Physical activity can reduce blood glucose by increasing insulin and non-insulin dependent glucose uptake into skeletal muscle (Banjpeyi et al 2009; Brestoff et al 2009; Christ-Roberts et al 2003). One way that glucose is taken up following exercise is by the increase of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) ratio which stimulates AMPK. This stimulation of AMPK leads to downstream translocation of GLUT 4 which upregulated glucose uptake into cells.

With regular exercise there can be increases in a concentration of GLUT 4 in the muscle cells and an increase in mitochondria in the cell. (Henriksen 2002; Rockl, Witczak and Goodyear

2008). Blood glucose uptake remains higher post-exercise with the contracted mediated pathway continuing for hours after.

2.5 High versus low glycemic index foods before physical activity on blood glucose and insulin

A specific macronutrient profile is desired for a pre-exercise meal to improve endurance and performance during exercise or sporting events. Dietitians of Canada recommend moderate protein, a high carbohydrate content, and low fat before participating in exercise. Timing of meals prior to exercise is based on the amount and type of food. One to two hours prior to exercise is optimal if consuming a snack or beverage with 2-3 hours for a small meal and 3-4 hours for large meal. Pulses nutrition content and low glycemic index supports the potential to be an optimal pre-exercise snack or meal.

The field of sport nutrition is vast and constantly evolving. Low versus high glycemic index meals before exercise as a tool to improve performance has been of interest in recent years. The majority of studies are conducted using a small sample size of young, healthy athletes but vary on outcomes measured and the sport/exercise protocol performed. This makes it hard to compare studies and evidence has been inconclusive. There have been some studies that investigated the effects of high versus low glycemic consumption before exercise on glycemic control, these are reviewed below.

Little et al. (2010) served a low glycemic index treatment of lentils or a high glycemic index meal of potatoes and egg whites as a pre-exercise meal in 13 male athletes with a mean age of 22.8 years and $V0_2$ of 55.4 ml. Treatments were served within a mixed meal, isocaloric and a fasting control was used. Participants had 20 mins to consume the meals and received a baseline finger prick blood sample to determine blood glucose before starting. Blood was also measured

at 15, 30, 60 and 120 mins after the test meal. Blood samples were taken during exercise at the end of the first 15 min, at 45 and at 90 mins. The exercise treatment was two 45 min periods with a 15 min break in-between. Serum fatty acids and insulin were found to be lower following the fasted state. No differences between treatments were found for blood glucose during exercise. Lentils resulted in improved total sprint distances as well as the high glycemic index pre-exercise meal, compared to fasted control conditions. In a subset of the participants (n=5) muscle glycogen was greater in the lentil meal and high GI conditions than in the control. Rating of perceived exertion was found to be lower following lentils. The finding in this study support that both low GI and high GI meals consumed before exercise can improve performance in high intensity exercise.

Moore and colleagues (2013) investigated the effects of high and low glycemic index (GI) carbohydrate pre-exercise meals (2.5 g CHO/kg body mass) on cycle performance in 10 untrained females. Participates came in fasted and consumed either a low or high glycemic index meal (HGI 423.4 ± 54.6 kcals and LGI 422 ± 50.8 kcals) which were prepared in equal weights and similar macronutrient composition. The HGI meal was a white bagel with honey and a glucose energy drink. Bran flakes cereal with skimmed milk was the LGI meal. Participates were given 15 mins to consume the meal. Blood was measured at rest, postprandial, during and after exercise. Participants cycled at 60% VO2 max to exhaustion. It was reported at 15mins the high glycemic index meal had a significant increase in glucose. The low glycemic index meal did not see this increase. Additionally, during exercise there was a large decline in blood glucose for the high glycemic meal. This study recommended individuals to consume a low glycemic index prior to endurance exercise.

In untrained individuals, a study by Stevenson et al (2009) looked at low versus high glycemic index breakfast consumed before a 60 min (walking) exercise session in 8 sedentary women. The low GI breakfast had an index off 44 and HGI breakfast of 78. Treatments were matched for calories and carbohydrates. Participants ate a standardized evening meal the day before sessions. They came in after an overnight fast and consumed the treatment breakfast. They completed a 60-min exercise session 3 hours after consuming their breakfast. Exercise intensity was set to 50% of their $V0_2$ peak. A standard lunch was then served 15 min after finishing exercise. The participants were then followed for another 2 hour post prandial period. Plasma glucose and insulin responses were higher following the HGI breakfast compared with the LGI breakfast. Participants reported that they felt significantly fuller after lunch when they had the LGI breakfast. The authors also investigated satiety hormones GLP-1, PYY and ghrelin but no differences were found. This supports the position that pulses are a low glycemic food and can be an optimal pre-exercise snack with satiety benefits.

A meta analysis conducted in 2017 by Burdon and colleagues analyzed studies looking at the effects of glycemic index of a pre-exercise meal on exercise performance. All trials included trained cyclists or active participates. Fourteen trials were included that didn't not provide carbohydrates during exercise. Out of these 14, 7 found no difference in glucose between a low and high glycemic index meal. Of the 7 that had glucose differences, all but 1 found lower glucose for the higher glycemic meal. Insulin was measured in 13 trials with 11 finding no differences. Two trials reported higher insulin in the first 20 mins of exercise after the high index meal. It is noted that these 2 trials had a short time interval between meal and exercise (30 and 45 mins) and 13 out of 14 exercise protocols were longer than 65 mins.

There is a research gap whether low glycemic foods consumed prior to an endurance 60 min exercise session can decrease blood glucose and insulin during and after exercise as well as increase satiety following exercise. Based on pre-exercise food recommendations and previous literature, extruded pulse products have the potential to be an optimal pre-exercise food as they have higher protein than other grains, provide complex carbohydrates, and a low glycemic index. Consuming a snack or cereal prior to exercise would be a convenient way for individuals to increase their pulse consumption while proving health and possible exercise benefits. Whole pulses and pulse ingredients have been shown to have a beneficial effect on glycemic control, which is summarized in the sections to follow.

2.6 Effect of whole pulses on glycemia and satiety

The effect of whole pulses on acute glycemic control has been widely investigated and assessed in numerous published human studies including GI studies, as well as, postprandial glycemic response studies.

Jenkins et al. (1980) conducted a study with 5 groups of 10 healthy volunteers to investigate the effects of different foods on blood glucose. The authors investigated the blood glucose response to 35 carbohydrate rich foods. The carbohydrate rich foods included varieties of potatoes, grains, breads, pasta, cereals, and biscuits. Each treatment was consumed by a minimum of 6 participants and a maximum of 10. Each participant completed an oral glucose tolerance test to ensure the groups of participants were similar in glycemic responsiveness. Participants were asked to fast overnight and instructed to keep prior day meals and exercise constant. Fifty grams of carbohydrate portions of 8 different dried and boiled legumes were tested. The legume treatments were butter beans, haricot beans, kidney beans, soya beans, black

eye peas, chickpeas, marrowfat peas, and lentils. Participants were instructed to consume the meal within 15 minutes. All meals were served with tea. Cereals were consumed with milk; legumes, millet, buckwheat, spaghetti, and rice were served with tomato. Blood glucose samples were obtained by fingerpick at baseline, 15, 30, 45, 60, 90, and 120 min following the consumption of the treatment. Results of the study showed mean blood glucose for each legume treatment was significantly lower than the mean curves of all other foods tested for at least two time points each. The AUC and mean spike in blood glucose were significantly lower for all dried legumes than the other treatments. These findings indicate that beans are low glycemic compared to other common carbohydrate foods.

Potter et al. (1981) found similar results in their study. The authors studied 8 healthy sedentary men aged 22-45 years to determine the effect of varying dietary fibre content on measures of plasma insulin and blood glucose. The treatments included a glucose control, brown rice, pinto beans pinto beans, and all bran cereal which were consumed once a week for 4 weeks. Protein and fat were matched by adding corn and casein, and all treatments had 74g of carbohydrates. Rice and beans were cooked by steaming. Blood was drawn at baseline, 30, 60, 120, and 190 min following consumption of treatments. Results showed that blood glucose concentrations for bran and pinto bean were significantly lower than control at 30, 60 and 180 min. Bran also led to significantly lower plasma insulin at the 30 min timepoint. Pinto bean elicited a significantly lower glucose concentration at 30 min. Bran and beans also had a lower blood glucose response compared to rice at 30 min. The researchers concluded that dietary fibre was most likely responsible for the reduction in blood glucose and plasma insulin concentrations. Fibre was analyzed in each treatment and results showed that the higher glucose and plasma insulin, the lower the non-digestible fibre, lignin, hemicellulose, and cellulose. The pinto beans

and all-bran cereal contained similar non-digestible fibre amounts, leading the authors to speculate that this was why these treatments resulted in similar blood glucose and insulin responses.

Another study conducted by Jenkins et al. (1982) examined lentils in 8 healthy participants (6 women and 2 men). Treatments were served as breakfast after an overnight fast. The treatments were white bread (control), lentils boiled for 20 mins, blended lentils boiled for 20 mins, lentils boiled for 1 hour, and dried lentils that were boiled for 1 hour then put in the oven for 12 hours to dry and ground into a powder. Meals were served with tomato and 500 mL of added water, tea or coffee, and were consumed within 10-15 min. Blood samples were collected by finger prick at baseline, 15, 30, 45, 60, 90, and 120 min. The blood glucose response to bread and 12 hours dried lentils were both significantly higher than the boiled 20-minute lentils at 15, 30, and 45 min. No significant differences in blood glucose responses between 20 min boiled lentils compared to the 1 hour boiled or 20 min blended lentils were found. This study confirmed the previous notion that after eating lentils, there will be a decrease in blood glucose response compared to white bread. In vitro digestion studies on the treatments were conducted and the results provided evidence that the 12-hour dried lentil carbohydrates were digested rapidly compared to the 20-minute lentils, thus potentially explaining the higher blood glucose response. Thus, blending lentils had no significant effect on blood glucose , however prolonged exposure to heat did. This trial established that 12 hours of dry heat cooking raises the blood glucose response to lentils, indicating that the type of processing is an important factor to consider.

Traianedes et al. (1986) studied 6 normal weight participants (4 men, 2 women) aged 25-40 years to determine whether commercial canning increases the digestibility of beans. The

treatments included a glucose control, home cooked baked haricot beans, and Heinz vegetarian baked haricot beans. All treatments contained 50g of carbohydrates. Preparation of the home cooked beans included soaking overnight, boiling 1 hour, addition to a sauce of molasses, mustard, and tomato and baking for 2 hours. The canned beans were pressure cooked for 2 hours at 121C and 15psi. All treatments were consumed after a 12 hour fast and served randomly. The treatments were consumed within 5-15min. Blood sampling occurred at baseline, 15, 30, 45, 60, 90, 120, 150 and 180 min. Results showed the control (glucose) elicited the highest blood glucose response, with the home cooked beans eliciting the lowest glucose response. At 30 min, the blood glucose for those consuming the canned beans peaked and was significantly higher than the blood glucose levels in those consuming the home cooked beans, with the latter peaking at 45min. The glucose AUC for the home cooked beans treatment was significantly lower than that for the canned beans treatment. Insulin AUC for the home cooked beans treatment was significantly lower than that for the canned beans and peaking at 45/60 mins. The study tried to simulate canning and to test digestibility *in vitro*. The authors concluded that the digestion of starch from legumes was directly related to the duration of time that they are pressure cooked and to the processing temperature due to pressure processing.

Wong et al. (2009) conducted a series of randomized crossover studies to investigate the effects of pulse processing, recipe and pulse variety on food intake and satiety. All treatments in the study had 50g of available carbohydrates and were consumed after a 12 hour fast. Blood glucose and VAS motivation to eat rating were taken at baseline, 15, 30, 45, 60, 90- and 120-min following consumption of the treatments. VAS measuring comfort was completed at 60 mins and 120 mins. A hot pizza meal was served at 120 mins to measure food intake.

Experiment 1 consisted of 14 healthy normal weight men aged 18-35 years. The treatments were 1) canned navy beans manufactured in Canada or United Kingdom (UK) compared with 2) homemade navy beans 3) and 300ml of glucose drink. All bean treatments resulted in a significantly lower blood glucose compared to control with the exception at times 90 and 120 min. No differences were found between bean treatments for appetite ratings. Canadian navy beans and the UK navy beans led to lower food intake compared to the control during the pizza meal appetite rating procedure. Experiment 2 included 14 men as test subjects with treatments consisting of: 1) canned navy beans in tomato sauce, 2) canned navy beans maple style, 3) canned navy beans with pork and molasses, 4) homemade navy beans with pork and molasses, 5) and white bread. Homemade navy beans led to the lowest blood glucose response at 15, 30 and 45 min, whereas the canned pork molasses navy beans and canned maple style navy beans led to a higher blood glucose response compared to control at 45- and 60-min. Homemade pork and molasses navy beans and canned navy beans in tomato sauce led to a reduced blood glucose iAUC compared to the control. No effects on appetite or food intake were found within treatments. Experiment 3 consisted of 15 men with treatments composed of 4 isocaloric (300 kcal) treatments 1) chickpeas, 2) lentils, 3) navy beans, 4) yellow peas, 5) white bread (control) 6.) and water (control). All treatments were canned and from commercially available sources. Blood glucose iAUC was significantly reduced compared to white bread control for all beans except navy. Lentil and chickpea led to the lowest blood glucose response from 0-60 min compared to the other pulses. All treatments including the control decreased appetite AUC compared to water. No differences were found among the isocaloric treatments. Navy bean, lentil, yellow pea, and the bread control led to lower food intakes compared to the water control. These studies concluded that canned beans have a low GI, that the recipe of

canned beans can affect the blood glucose response and that chickpea and lentils are the most effective pulses at reducing blood glucose response.

2.7 Effect of pulses consumed within mixed meals on glycemia and satiety

Pulses consumed in a mixed meal have been investigated in a few studies. The glycemic benefit of pulses has been shown to be retained under this study design, however it is dependent on pulse type.

Dilawari et al. (1981) performed a randomized study investigating the blood glucose responses of rice, red kidney beans, wheat flour, chickpeas, and dextrose (control) on 6 healthy men. All treatments had 50g of carbohydrates and were consumed with 50g of tomato, 10g lime, and tea without milk or sugar. The control was served the same meal but with dextrose in the tea. Subjects had 6-12 min to consume the test meals. There was at least 2 days between sessions. Blood was taken at baseline, 15, 40, 45, 60, 90, and 120 min. There was a significantly lower blood glucose concentration at 15, 30, 45 and 60 min for the chickpea treatment compared to the control. At 15 and 30 min, the kidney bean treatment also led to a significantly lower blood glucose concentration in comparison to the control. During the first 60 min, chickpea and kidney beans significantly reduced blood glucose AUC compared to control. A significantly lower blood glucose AUC was also seen in the wheat but not rice compared to the control. The investigators found that the reduction in blood glucose showed a direct relationship with the dietary fibre content of each food.

Mollard and colleagues (2011) investigated the first and second meal effects of pulses on food intake, blood glucose , and appetite. The study was randomized with 25 healthy normal weight men with 3 treatments: chickpea, lentil and yellow pea. Each was treatment was isocaloric and served with macaroni and homemade tomato sauce with a macaroni and cheese

control. There was about 100g of available carbohydrates in each treatment. Participants fasted overnight before the study sessions and, upon arrival, completed visual analog scale (VAS) questionnaires assessing their appetite. blood glucose was measures by fingerpick at: baseline, 20, 40, 60, 80, 110, 140, 200, 260, 280, 300, 320 and 340 min following consumption of the respective treatment. Participants had 20 min to consume the treatment at the start of the session and 20 min to consume an *ad libitum* pizza meal at 260 min. The study showed that the consumption of yellow pea and lentil significantly lowered food intake and pre-pizza meal appetite compared with the control, while the chickpea did not. No differences in blood glucose AUC were seen pre-pizza meal. At 20 mins all pulse treatments showed a lower blood glucose response compared with control and, at 140 min, the lentil treatment exhibited lower blood glucose compared to control. Post-pizza, at 280 min, the lentil and chickpea treatments both resulted in a lower blood glucose compared to the yellow pea treatment. The chickpea treatment showed the lowest overall blood glucose response compared to the control.

Another study conducted by Mollard (2012) examined the acute effects on blood glucose and satiety following a pulse meal and at a second meal later. The study had 24 healthy males aged 20-30 years of normal weight. The treatments were chickpeas, lentils, navy beans, and yellow peas served with pasta and homemade tomato sauce. Treatments were cooked on low heat for 20mins following a standardized recipe. Participants were given 20 min to consume the treatment. Blood samples were measured by glucometer at baseline, 20, 40, 60, 110, 140, 200 and 260min. Appetite was measured by VAS at these timepoints as well. At 260 min, an *ad libitum* pizza meal was served, and participants were instructed to eat until comfortably full. Blood glucose and appetite were measured following the pizza meal at 280, 300, 320, and 340 min. The treatment meal that included lentils was found to be the most satiating (lower food

intake) in comparison to the chickpea and control. Participants also ate less of the navy bean meal compared to the chickpea meal. Differences were not due to palatability, as all treatments had similar ratings. All treatments resulted in significantly lower blood glucose compared with control over 260 min, and navy beans resulted in significantly lower blood glucose as compared to chickpeas. There was no difference for blood glucose after the pizza meal and no effect on food intake at the second meal or appetite. However, the lentil treatment showed lower cumulative food intake compared to the control. This study provides evidence that pulses within a mixed meal retain their low glycemic properties, and that pulse type contributes to satiety and blood glucose responses.

Thompson (2012) performed a study to examine the effects of 3 pulse varieties on blood glucose in a randomised crossover trial that included 17 obese adults (BMI 31.8 ± 1 kg/m^2) diagnosed with type-2 diabetes aged 30-70y, using both men and women. Diabetes was managed by either diet and exercise or with metformin. Treatments were white long grain rice (control), pinto beans (50g)/rice (34.7g), black beans (50g)/rice (34.7g), red kidney beans(50g)/ rice (34.7g). All treatments were matched for 50g available carbohydrates; however, protein and fibre were much lower in the control. Before each session, participants consumed the same frozen dinner to control for the second meal effect. Participants fasted for 12 hours and then consumed the treatment and received blood sampling. Blood sampling occurred at baseline, 30, 60, 90, 120, 150 and 180 min following the respective treatment. Overall blood glucose was lower following the consumption of the black bean and pinto bean treatments compared to the red kidney bean treatment. The authors suggested that fibre differences may have been the reason behind the observed treatment differences. This study concluded black bean and pinto bean have glucose lowering properties for individuals with type 2 diabetes.

Glycemic response from pulses in a mixed meal appear to be dependent on the type of pulses and composition. Chickpea, kidney bean, black bean, pinto bean, and lentil all led to lower blood glucose within acute studies (Dilawari et al 1981; Mollard et al 2011; Mollard et al 2012; Thompson et al 2012). However, only yellow pea and lentil lowered appetite before an *ad libitum* meal.

2.8 The effects of pulse flakes on glycemia and satiety

The majority of research on pulses has been conducted on whole pulses. Research on other forms of pulses on glycemic response has been scarce but promising.

Pulse flakes and their role in glycemic response and satiety has only been investigated in one study to date. Pulse flakes are processed in a variety of ways and put through a roller to be thin and flat. Bourdon et al (2001) measured satiety within a bean meal in 8 normal and overweight men in a randomized crossover trial. Participants fasted for 12 hours before their study sessions and had 15 mins to consume the treatment. Sessions were 1-3 weeks apart. Treatments included a high and a low fibre meal. The high fibre meal contained white bean flakes with 11.8g of fibre and low fibre meals contained instant rice and non-fat milk powder. Blood was drawn at baseline, 30, 45, 60, 120, 180, 240, 300, and 360 min. plasma blood glucose, insulin and CCK were analyzed for each timepoint. Post prandial blood glucose peaked at 30 min for both treatments and there were no significant differences in blood glucose or insulin. However, the high fibre meal resulted in a significantly higher CCK response than the low fibre meal. The bean meal produced almost twice the CCK, measured by AUC as the control meal (25±4pmol/L•h; 14±2pmol/L•h). CCK has been suggested to influence satiety by stimulating pancreatic enzymes and inhibiting gastric emptying. More research is needed on hormone effects

on satiety, as well using different pulse-type flakes and amounts incorporated into products to see if their glycemic response lowering properties are retained.

2.9 Effects of pulse flours on glycemia and satiety

Pulse flours are an easy way to incorporate pulses into a cooked product. However, there is very limited research in this area of pulse flours. The following studies will discuss their glycemic and satiety benefits.

Hall et al (2005) conducted a study on the effects of lupin bread on blood glucose response in 11 healthy participants (9 males, 2 females) aged 25-45 years with a BMI of 20.9-28.6 kg/m^2. Lupin is considered a pulse crop as it fixes its own nitrogen and mostly grown in Australia. White bread was used as a control against a white bread with 7.7.g of added Australian lupin flour (ASLF). Treatments contained 50g of available carbohydrate and had less than a 100-calorie difference. Treatments were consumed with margarine, apricot jam and decaffeinated tea and were consumed within 10 min. The control bread was consumed twice leading to a total of three sessions. Participants fasted for 12 hours prior to coming to the morning session. A questionnaire similar to VAS questionnaires were used to measure satiety and sensory perception. Blood glucose response was significantly lower for ASLF bread compared to control and insulin was significantly higher for the ASLF bread compared to control. There were no differences in satiety or food intake. Potential limitations for this study include the fact that it may have been underpowered to detect differences and men and women were not balanced. Strengths of the study included using both men and women as well as separating sessions by at least 7 days. The blood glucose lowering effects of lupins were retained when milled into a powder and combined into a bread.

Another study examining glucose and insulin response to pulse flours was a study conducted by Johnston et al. (2005). Chickpea flour and extruded chickpea flour incorporated into breads were compared to white bread (control). Eleven healthy participants (9 men, 2 women) aged 25-45 completed the study. Both chickpea flours replaced 24.3g of wheat flour in the bread and were all served as part of a breakfast. This amount was chosen to keep the bread palatable. The breakfast provided 50g of available carbohydrates. A scale similar to a VAS was used to measure satiety at baseline, 10, 25, 40, 55, 85, 115, and 175 min postprandial. Blood was collected through an IV at baseline, 15, 30, 45, 60, 90, and 120 min postprandial. At 175 min post-prandial participants were provided buffet lunch. Food was weighed prior to participants eating and participants were also instructed to record what they ate for the rest of the day. The study results showed that, for all treatments, blood glucose peaked at 30 min and returned to and fell beneath baseline between 60-90 min. The blood glucose response to regular chickpea bread was lower than that of white bread at 90 minutes. The blood glucose for extruded chickpea bread was significantly lower than the control at 120 min. A trend towards lower blood glucose AUC for regular chickpea bread versus white bread was also observed. Insulin peaked at 30 min for the extruded flour and control bread and at 45 min for the chickpea bread. The insulin response was significantly higher for chickpea bread at 60 min than the control and the extruded flour bread. Insulin AUC was also significantly higher for chickpea bread than the control bread. There were no significant differences in satiety or sensory properties between the three treatments. The authors suggest that possibly the incorporation of chickpea flour was not enough or that the particle size was too small to observe the glycemic and insulinemic benefits seen by whole chickpeas. Further studies with varying particle size and greater amounts of chickpea flour in food products are needed.

Marinangeli et al (2009) examined postprandial blood glucose responses to whole yellow pea flour and pea fibre incorporated into pasta, biscotti and banana bread. It was conducted with 22 healthy men and women aged 22-67 BMI ranging 21-42 kg/m2. Banana bread and biscotti were made with 100% whole yellow pea flour, whereas the spaghetti was made with 30% whole yellow pea flour: 70% wheat flour. Each food was consumed twice, totaling 8 treatments. The control was boiled whole yellow peas and white bread. Participants consumed the treatment after a 12 hour fast. Blood measurements were conducted at baseline, 30, 60, 120. And 150 mins. Whole yellow pea flour banana bread and biscotti reduced the blood glucose response compared to white bread. In addition, whole yellow pea flour biscotti produced a lower blood glucose compared to the whole-wheat flour biscotti. For the whole yellow pea flour pasta, the blood glucose response was comparable to white bread. Carbohydrates were matched for the treatments and both men and women were included. However, hormones were not controlled for in pre-menopausal women in this study. This study also supports that pea flour can lower blood glucose and retains its benefits when used as a flour.

Anderson et al (2014) examined the effects of commercially prepared pulse powders on blood glucose responses before and after the consumption of a meal in healthy young men. There were 3 experiments conducted, each using a control of whole-wheat flour. In experiment 1 (n=17) the treatments were whole navy bean, pureed navy bean, and navy bean powder. In experiment 2 (n=17), whole lentil, pureed lentil, and lentil powder were used. In experiment 3, (n-12) participants consumed whole chickpea, pureed chickpea, and chickpea powder. All treatments were matched for carbohydrate content. Participants were allowed 15 min to consume the treatment and blood was drawn at baseline, 15, 30, 45, 60, 90, and 120 min. A fixed-energy pizza meal at 120 min was provided and blood was drawn at 140, 155, 170, 185, and 200 min. In

all 3 experiments, blood glucose was significantly affected by time and time by treatment interaction. In experiment 1 (beans), the bean powder did not differ from the control at 15 min, whereas all bean treatments resulted in lower blood glucose values at 30 minutes compared to the control. At 45 minutes, the navy bean powder resulted in a lower glucose than pureed, and at 60 min whole wheat had lower glucose than whole navy beans. The difference in pre-pizza meal blood glucose netAUC was significant for the navy bean powder compared to the control and was intermediate for the other two treatments. In experiment 2 (lentils), all treatments resulted in a significantly lower mean blood glucose compared to the control. Also, blood glucose netAUC was lower following the whole lentils and navy bean powder compared to the control. In experiment 3, blood glucose was significantly lower for powdered, pureed, and whole chickpeas compared to the control. A limitation to this study was only men were used. This study concluded that pureeing and commercial processing does not reduce the glycemic benefits of consuming pulses because there were overall no significant differences seen between the treatments due to processing.

Greffeuille et al. (2015) investigated faba bean flour incorporated into a pasta on glycemic and insulin response. Fifteen healthy participants (8 males and 7 females) completed the study. Four preloads of 50g glucose in 250 ml of water were given as a reference for glycemic and insulin responses. Three types of pasta were used as treatments, including durum wheat pasta dried at low temperatures (control), 35% faba bean flour pasta dried at low temperature and 35% faba bean flour pasta dried at high temperature. Glucose was measured by finger prick and VAS questionnaires at baseline, 5, 10, 15, 30, 45, 60, 90, 120 and 180 min. No significant differences were reported between the 3 pasta treatments however all 3 pastas produced significantly lower insulin levels than the glucose solution until 120mins. Palatability

was significantly lower following the faba bean flour pasta dried at high temperatures. There was no difference between the other pastas. Appetite scores were significantly lower following the faba bean flour dried at high temperatures compared to the other two pastas. This is thought to be due to texture differences. Our current study does not include faba beans and should be further investigated in an extruded product and prior to exercise to see if glycemic benefits are retained.

Yoshimoto et al. (2020) recently studied yellow peas in noodles on glycemic and insulin responses in 8 participants. The treatments included dehulled yellow pea flour noodles (YP), unshelled yellow pea (YP-U) flour noodles, with a pressurized white rice control. Blood was taken at 0, 15, 40, 45, 60, 90 and 120 min after consumption. Change in blood glucose was significantly lower for YP compared to YP-U. Blood glucose at 45, 60 and 90 mins was significantly lower follow both pea treatments compared to white rice. Blood glucose IAUC was significantly lower after both noodles compared to white rice as well with no differences between IAUC after consumption of YP or YP-U. For insulin, at 30 min YP-U levels were significantly higher than YP. At 60 mins insulin levels were significantly lower for YP compared to white rice YP-U. IAUC was lower YP compared to YP-U and WR. No differences were found between the two pea noodle types which is hypothesized to be weakening of structural strength of the coat when processed. No sensory differences were found between the two pea types. Yellow pea decreased both glucose and insulin concluding that it makes an optimal food ingredient for glycemic control.

These studies support that glycemic benefits are still retained when pulses are processed. Results are promising but further research is needed into pulse fractions and flours on glycemic response and satiety.

2.10 Effects of pulse fractions on glycemia and satiety

Investigating pulse fractions will support which component(s) within the pulse is responsible for the acute glycemic benefits and if these benefits are retained when fractionated. There is limited research in this area.

Smith and colleagues (2012) investigated the effects of yellow pea protein and fibre on post-prandial glycemia, and appetite in 19 normal weight men. Two randomized, single blind, repeated measure experiments were conducted and the same treatments; tomato soup, tomato soup with 10 g yellow pea fibre, tomato soup with 20 g yellow pea fibre, tomato soup with 10 g yellow pea protein, and tomato soup with 20 g yellow pea protein. Five treatments were consumed once per week, with sessions being a minimum of 7 days apart. Treatments had 9.5-10.5g of available carbohydrates. VAS questionnaires and blood samples were collected at baseline, 15, 30, 50, 65, 80 95, 110, 140 and 170 min for experiment. VAS and blood samples for experiment 2 were collected at baseline, 15, 30, 45, 60, 90, 120, 140, 70, 185 and 200 min. An *ad libitum* pizza meal was served in experiment one at 30 min and at 120 min for experiment 2. In the first experiment, blood glucose was significantly lower overall for both protein treatments compared to control. A protein dose of 20g resulted in lower blood glucose compared to the control and fibre 10g treatments. Following the pizza meal, both protein treatments resulted in significantly lower blood glucose. In experiment 2, there were significant differences due to time but not for treatments. Investigators postulated that lack of significance in the findings may have been due to the fact that fibre was being taken from the hull only and not from the entire pea, as pea hull contained 45.8% more insoluble fibre and 21% more soluble fibre compared to dehulled peas. A limitation for this study was only men were included. Results from

this study support yellow pea's ability to decrease blood glucose, increase satiety and reduce food intake, however the responses are dose dependent.

Another study supporting yellow peas for glycemic response was published by Mollard et al (2014). In a randomized single blind crossover study in 15 healthy normal weight young men, the authors looked at the consumption of pea fractions on food intake, appetite and blood glucose. Treatments included yellow peas, pea hull fibre and pea protein, pea protein, fibre alone and tomato sauce and noodles (control). Pea fibre and protein were consumed with noodles and tomato sauce and yellow peas were consumed with tomato sauce. Participants fasted overnight before coming in to consume their treatment once a week. Blood measurements and VAS questionnaires were taken at baseline, 15, 30, 45, 60, 75, and 135 min before an *ad libitum* pizza meal. Blood glucose at 30 min was significantly lower with protein plus fibre and yellow pea treatments. No differences were found for appetite or food intake. The authors agreed with Smith et al. 2012, suggesting higher doses of hull fibre and a longer period of time (4-12h) may be needed to see acute benefits on blood glucose, appetite and food intake. This study supports incorporating pulses fractions into food products for post prandial blood glucose benefits.

Chan et al. (2019) investigated glycemic response following addition of faba bean flours and fractions into pasta in 54 healthy adult males. Treatments were isocaloric and included a pasta made from 100% durum wheat semolina (DWS) or substituted with 25% faba bean split flour (FBF), high starch fractions (FBPC), protein concentrate (FBPC), or protein isolate (FBPI). Pasta was served with 125g tomato sauce. Three separate experiments were conducted using a single blind, within subject, randomized, repeated measures control design. Exp. 2A served an ad libitum pizza meal at 120 mins to measure food intake. Exp 2B a fixed quantity of pizza was

provided to determine food intake. Blood glucose measurements were taken at 0, 15, 30, 45, 60, 90, 120, 140, 155, 170, 185 and 200 min. Treatment FBPC and FBPI decreased blood glucose and appetite along with an increased protein content but had no effect on plasma insulin or GLP-1. Findings from this study supports previous literature on pulse flours ability to decrease glycemic response and increase satiety.

2.11 Review on effects of pulses on glycemic control and satiety

To date, there has been two systematic reviews and meta-analyses conducted on the effects of pulse consumption on glycemic response and satiety.

Sievenpiper et al (2009) conducted a systematic review and meta-analysis with 41 random control trials investigating the effects of pulses on glycemic control. They grouped the trials into 3 groups; pulses alone, pulses incorporated into low-GI diets, and pulses incorporated into high-fibre diets. The authors looked at the medium and long-term effects of pulses in the diet compared with a non-pulse diet. The group with whole pulses showed that fasting blood glucose was significantly decreased, but a homeostatic model assessment for insulin (HOMA-IR) and glycosylated blood proteins (GP) were not. The low GI group GP was significantly decreased but HOMA-IR, fasting blood insulin, and fasting blood glucose were not. The high-fibre group showed a significantly decreased fasting blood glucose as well, but HOMA-IR were not. The report also showed over the long term, pulses in the low-GI and high-fibre diet can significantly reduce fasting blood glucose and HbA1C in people with diabetes to levels comparable with oral anti-hyperglycemic therapies.

Li et al (2014) performed a systematic review and meta-analysis on pulses and their effect on satiety and second meal food intake. Nine acute, randomized, isocaloric trials that

served whole pulses as treatments were included in the review. The treatments were whole pulses and ground pulses used in breads or a spread. The dose of pulses ranged from 7.6 - 311g and the study session durations lasted from 120-220 min. It was concluded that the pulse meals compared to a control increased satiety by 31% and did not significantly affect second meal intake.

A recent review evaluated the effects of pulse intake on glycemic control in normoglycemic adults in acute randomized control clinical trials (Hafi et al. 2020). The review included 30 acute trials with varying processing methods. Whole, flour and pureed pulses were included. The authors concluded pulse intake did improve glycemic control, with lentils having the greatest effect. Other pulse types still showed to significantly lower post prandial blood glucose. They ran a meta-analysis on the effects of processing and found pulse flours were 50% less effective in reducing blood glucose compared to other forms of pulses. These trials had 25-35% pulse flour of the final product which could have led to a lower effect whereas whole pulses are mostly consumed alone (Hafi et al. 2021).

2.12 Literature Review Tables

Table 1. Studies on the effect of pulse before exercise and satiety.

Study	Participants	Treatments	Results
Little et al (2010)	13 healthy male athletes	Lentils (low GI high protein), potato and eggs (high GI high protein), fasted control	Serum fatty acids and insulin be lower following the fasted state. No differences between treatments were found for blood glucose during exercise. Lentils resulted in improved total sprint distances as well as the high glycemic index pre-exercise meal, compared to fasted control conditions.
Stevenson et al (2009)	8 untrained healthy women, sedentary	LGI or HGI breakfast 3 hrs before walking 60 mins. -Treatments matched for CHO, fat, and protein but not fibre. Provided standardized lunch 15 mins after exercise completed	Plasma glucose and insulin higher following HGI than LGI breakfast. Fat oxidation lower following both treatments but remained higher for LGI Felt fuller after lunch following LGI breakfast
Moore et al (2013)	10 untrained females	HGI white bagel with honey and glucose energy drink and LGI food of bran flakes with skimmed milk HGI 423.4 ± 54.6 kcals and LGI 422 ± 50.8 kcals	At 15mins high glycemic index meal had a significant increase in glucose. The low glycemic index meal did not see this increase. During exercise there

		Treatments has similar macronutrient profile	was a large decline in blood glucose for the high glycemic meal.
Burdon et al (2017)	Physically active, healthy, >16years old,	LGI or HGI meal 30-240 min prior to exercise, included some form of endurance exercise (>60mins)	19 trials included. 14 included glycemic outcomes. Out of these 14, 7 found no difference in glucose between a low and high glycemic index meal. Of the 7 that had glucose differences, all but 1 found lower glucose for the higher glycemic meal. Insulin was measured in 13 trials with 11 finding no differences. Two trials reported higher insulin in the first 20 mins of exercise after the high index meal

Table 2. Studies on the effect on consumption of whole pulses on glycemic and satiety.

Study	Participants	Treatment	Results
Jenkins et al (1980).	5 groups of 10 healthy volunteers	50g carbohydrate in 8 dried and boiled legumes (butter beans, haricot beans, kidney beans, soya beans, blackeye pea, chickpeas, marrowfat peas, lentils), and 35 other high carbohydrate foods.	Mean blood glucose for each dried legume was significantly lower than the mean curve of all other foods in at least two time points. The AUC and mean spike in blood glucose were significantly lower for dried legumes than other treatments.
Potter et al (1981).	8 healthy sedentary men 22-45yrs	Glucose formula (control 102g) Brown rice (97g) Pinto beans (118g) All bran (106g) Meals were blended with water, so they were equal volume. Match for fat, protein and carbohydrates.	Glucose: Bran and pinto bean were significantly lower than control at 30,60, ad 180min. Insulin: Bran significantly as lower at 30,60, and 120 min compared with control. Beans significantly lower at 30 mins. Bran and beans also lower compared with rice at 30mins. Insulin response peak of control nearly twice that of pinto and bran.
Jenkins et al (1982).	8 total; 6 healthy men and 2 females.	1. White bread control 2. Boiled 20 min lentils 3. Boiled 20 min blended lentils 4. One hour boiled lentils 5. One hour boiled, 12-hour	There were no significant differences in blood glucose between 20 min lentils compared to blended lentils or one hour boiled lentils.

		oven dried lentils Meals were served with tomato, 500 ml of water, as well as tea or coffee.	12-hour dried lentils had a significantly higher blood glucose response than 20 min lentils at some time points.
Traianedes et al (1986).	6 normal weight participants	1. D-glucose 2. Home cooked baked haricot beans 3. Heinz vegetarian baked haricot beans The home cooked beans were served with a sauce made of molasses, mustard and tomato.	Homecooked beans blood glucose AUC was significantly lower than canned beans.
Wong et al (2009).	Exp. I n=14 men Exp. II n=14 men Exp. III n= 15 men	I: Canned navy beans made in Canada, canned beans made in UK, homemade navy beans, glucose drink II: Canned navy beans in tomato sauce, canned navy beans pork and molasses, homemade navy beans pork and molasses, white bread (control). III: chickpeas, lentils, navy beans, yellow peas, white bread (control), water (control).	I: All beans reduced blood glucose with exception of at 90 and 120min compared to control. Lower food intake at pizza meal from navy beans made in Canada and UK compared to control. II: Homemade pork and molasses navy beans and canned navy beans in tomato sauce led to a reduced blood glucose netAUC compared to control. III: blood glucose netAUC was significantly reduced compared to white bread control for all beans except navy.

			Navy bean, lentil, yellow pea, and bread control led to lower food intake compared to water control.

Table 3. Studies on the effects of consumption of pulses in mixed meals on glycemic and satiety effects.

Study	Participants	Treatment	Results
Dillawari et al (1981)	6 healthy men 25-47y	1. Dextrose dissolved in tea 2. Rice 3. Red kidney beans 4. Wheat flour 5. Bengal gram (chickpea) Meals were consumed with 50 g tomato, 10 g lime, and tea without milk or sugar.	Chickpea and kidney bean resulted in significantly lower postprandial blood glucose compared to rice and wheat.
Mollard et al (2011)	25 men aged 20-30y BMI 20-24.9kg/m2	1. Chickpea 2. Lentil 3. Yellow pea 4. Macaroni & cheese (control) Pulse treatments were canned and served with macaroni pasta and served with homemade tomato sauce. All pulse treatments contained 40 g available carbohydrate. Overall available carbohydrate was matched between	Yellow pea and lentil treatments significantly lowered food intake and pizza meal appetite compared to control, chickpea did not. No differences in pre-meal pizza AUC. Chickpea and lentil significantly lowered post-pizza meal blood glucose AUC, yellow pea did not.
Thompson et al (2012)	17 obese (BMI 31.8 +/-1 kg/m2) adults (9 men and 8 women) diagnosed with type 2 diabetes aged 35-70 y.	1. White long grain rice 2. Pinto beans (50 g) /rice (34.7 g)	Blood glucose was significantly lower at 90, 120, and 150 min for all bean treatments compared to the control.

	Diabetes was managed by either diet and exercise or with metformin.	3. Black beans (50 g) /rice (34.7 g) 4. Red kidney beans (50 g) /rice (34.7 g) Matched for available carbohydrates. Protein and fibre were much lower in the control.	
Mollard (2012)	24 healthy males Normal weight ages 20-30y	Chickpeas 2. Lentils 3. Navy beans 4. Yellow peas 5. Control (pasta and tomato sauce) Treatments were served with pasta and homemade tomato sauce. 44% of energy from the meals was from pulses. The meals were isocaloric.	Blood glucose effect of treatment was significantly lower compared to control for all treatments to 260 min. AUC was significantly lower compared to the control for all treatments.

Table 4. Studies on the effects of consumption of pulses flakes on glycemic and satiety effects.

Study	Participants	Treatment	Results
Bourdon et al (2001)	8 men ages 21-45. BMI 22.6-29.4 kg/m2.	1. High fibre diet with bean flakes 2. Low fibre diet with instant rice and non-fat milk powder. Protein, fat, and carbohydrates were matched. Meals were identical except the high fibre diet contained beans and the low fibre diet contained instant rice and non-fat milk powder.	High fibre meal had a significantly higher response of CCK than the low fibre meal. No significant difference in postprandial blood glucose or insulin. Blood glucose and insulin concentrations remained above baseline longer than the low fibre meal.

Table 5. Studies on the effect of consumption of pulses flours on glycemic and satiety.

Study	Participants	Treatment	Results
Hall et al. (2005)	11 participants (9 men,2 women) ages 25-45, BMI of 20.9-28.6 kg/m2.	1. White bread 2. White bread with 7.7 g of Australian sweet lupin flour (ASLF) added. Consumed with margarine, apricot jam and decaffeinated tea.	Blood glucose response of the ASLF bread was significantly lower than the control. Insulin response was significantly higher for the ASLF bread than control.
Johnson et al (2005)	11 healthy participants (9 men and 2 women) ages 25-45y	1. White bread 2. Chickpea flour 3. Extruded chickpea flour (	Blood glucose of the chickpea bread was significantly lower than the control at 90 min. Extruded chickpea bread blood glucose was lower than control at 120min.
Anderson et al (2014)	Exp 1: 17 healthy young men; Exp 2: 12 healthy young men; Exp 3: 12 healthy young men	Ex. I/II/ III 1. Whole navy bean/ lentil/chickpea 2. Pureed navy bean/ lentil/chickpea 3. Powdered navy bean/ lentil/ chickpea	Lentil and chickpea treatments mean blood glucose was lower over 120min compared to control. Overall no significant differences seen between the treatments due to processing.
Greffeuille et al. (2015)	8 healthy men and 7 females	50g glucose in 250 ml as preloads, treatments were durum wheat pasta dried at low temperatures, 35% faba bean flour dried at low temperature and 35% faba bean flour pasta dried at high temperature	Palatability lower for faba bean flour dried at high temperature Appetite scores lower following faba bean dried at high temperatures No glucose differences

Yoshimoto et al. (2020)	8 healthy participants	Dehulled yellow pea flour noodles (YP), unshelled yellow pea(YP-U), pressurized white rice control	Change in BG lower for YP compared to YP-U. BG at 45, 60, 90 lower following both pea treatments compared to control. At 30 mins insulin higher for YP-U than YP. At 60 mins insulin levels were lower for YP compared to white rice YP-U. Insulin IAUC was lower YP compared to YP-U and WR.

Table 6. Summary of the effect on consumption of pulses fractions on glycemic and satiety effects

Study	Participants	Treatment	Results
Marinangeli et al (2011)	23 participants who were overweight (BMI 25-40 kg/m2), hypercholesterolemic (7 men, 16 women).	Banana and apple muffins 1. Whole pea flour (26.4 g) 2. Fractionated pea four, hulls only (6 g plus 20.4 g of white wheat flour) 3. Whole wheat flour control (26.4) Muffins were matched for energy and carbohydrate.	Fasting insulin was significantly reduced compared to the control by whole pea flour treatment. Insulin resistance estimates were educed by 25% in both treatment groups compared to the control. Women were found to have a 4.7% decrease in android:gynoid fat ratios for both treatments compared to control. There was no change for men. No effect on TAG, TC, HDL-C, LDL-L or glucose.
Smith et al (2012)	Experiment I (pizza meal 30 min) N= 19 healthy men. Experiment II (pizza meal at 120 min) N= 20 healthy men.	Experiment I and II had the same 5 treatments: 1. Tomato soup 2. Tomato soup, 10 g yellow pea fibre 3. Tomato soup, 20 g yellow pea fibre 4. Tomato soup, 10 g yellow pea protein 5. Tomato soup, 20 g yellow pea protein	Ex I: blood glucose significantly lower for protein 20g treatment compared to control and fibre 10g. Protein 20g food intake was lower than all treatments. Ex II: No blood glucose or food intake differences found
Mollard et al (2014)	15 healthy men	1. Yellow peas 2. Pea hull fibre and pea protein 3. Pea protein	Pea protein + fibre treatment lowered glycemic response compared to control.

		4. Tomato sauce and noodles (control) 5. Fibre alone	
Chan et al (2019)	54 healthy adult males	100% durum wheat semolina (DWS) pasta or substituted with 25% faba bean split flour (FBF), high starch fractions (FBPC), protein concentrate (FBPC), or protein isolate (FBPI). Pasta was served with 125g tomato sauce.	Treatment FBPC and FBPI decreased blood glucose and appetite. No inulin differences found.

Table 7. Summary of reviews on pulses glycemic and satiety effects.

Study	Participants	Treatment	Results
Sievenpiper et al (2009)	41 randomized controlled human studies that looked at the effects of pulses (alone, as part of a high-fibre intervention, or low GI study) in the diet.	Pulse treatment dosages ranged from 15.5-465 g/day. Treatments were given as whole pulses, flakes, and flours.	Pulses were concluded to improve primary markers of glycemic control in the long term given alone, or as part of a high-fibre or low GI diet.
Li et al (2014)	9 acute studies. Participants were aged 18-53 y, normal weight, overweight, and obese.	Pulse treatment dosages ranged from 17-292.5 g/day. Treatments were given as whole pulses, bread, or in a spread. Controls were given as white bread, potato puree, or mac and cheese.	Pulses were found to increase satiety compared to a control by 31%, and were found to not affect second meal intake.
Hafi et al (2020)	65 random control trials. Participants were healthy and had T2D	Whole pulses, pureed, flakes, flours and fractions were. Treatment sizes varied.	Acute pulse intake significantly attenuated fasting glucose in healthy adults and adults with T2D

Chapter 3: Rationale, Objectives, Hypothesis, And Design

These two studies are part of the P.E.R.F.E.C.T project, which was a large project with 6 acute studies funded by Saskatchewan Pulse Growers to investigate pulse flours and fractions in extruded and baked products. The project had two parts; part 1 was looking at the acute effects of pulse ingredients in food products on appetite, blood glucose in adults. It was comprised of three acute studies; the first study focused on extruded snacks made with pulse flours, the second study focused on extruded pulse cereals made with pea fractions (starch, protein and fibre) and the last study focused on pulse bagels made with pulse flours and fractions. Part 2 observed the acute effects of pulse ingredients in food products on the blood glucose , insulin, lactate and response following an endurance session in adults as well as on aerobic endurance and substrate oxidation during exercise. As in part 1, the first study focused on extruded pulse snacks made from pulse flours, the second study focused on extruded pulse cereals made with pea fractions and the third study focused on pulse bagels made with pulse flours and fractions.

From our lab, Johnston et al. (2021) conducted research from Part 1; study 1 and 2. It was concluded that in study 1, pinto bean and chickpea snacks led to lower pre-meal blood glucose . Consumption of the pinto snack also led to lower pre-meal iAUC insulin compared with corn (control). In study 2, the protein, protein + fibre, and the fibre + starch + protein cereals led to lower pre-pizza meal blood glucose iAUC compared to the starch and control treatments. The starch + protein cereal led to a lower iAUC blood glucose response compared to starch. For pre-meal overall mean insulin, fibre + protein led to a lower insulin response compared to control, starch + protein, and protein cereals. Fibre + starch + protein also led to lower insulin compared to protein cereal. Fibre + protein resulted in lower insulin iAUC compared to control and protein cereal. No food intake or appetite differences were found in

either study 1 or 2.

3.1 Rationale

Whole pulses have been shown to provide favourable post-prandial glycemic response and increase satiety. Post-prandial glycemic control is a crucial aspect in the prevention of chronic diseases such as cardiovascular disease and the progression of diabetes (Berry et al. 2020). It is unknown whether pulse ingredients retain the health benefits of whole pulses when incorporated in different food matrices and if they provide any benefits before or after exercise. This research will assist in addressing this gap in the literature, as well as investigating the novel area, specifically in determining whether pulse ingredients consumed before an exercise session will lead to lower blood glucose, insulin, appetite and food intake.

Johnston et al. 2021 demonstrated pulses' glycemic benefits are retained when incorporated into an extruded snack product, however the amount is dependent on pulse type. They found pinto bean to significantly lower blood glucose incremental area under the curve compared with control, whole yellow pea, and green lentil. It was also found that there is a synergistic effect when combining fractions of yellow pea on glycemic and insulin response. The studies presented here are a follow up to Johnston et al.'s work and will provide support to their findings and future health claims.

Previous literature has been focused on high versus low glycemic index foods and the glycemic response during and after exercise as well as exercise performance/endurance. The majority of the studies to date focused on athletes and performance. Studies looked at the effects of a liquid carbohydrate drink or bars on glycemic response before and after exercise. These studies do not include pre-exercise meals and exclude individuals from the general population (Hargreaves, Hawley and Jeukendrup 2004; Jentjens, Gutch, Jeukendrup 2003). Research has

established the higher the glycemic index the higher the glycemic response (Esfahani et al. 2009). There is no research to date on consumption of pulses or pulse ingredients before exercise and the response following exercise. Pulses are a very low glycemic index food, with values ranging from 29 to 48, compared to 42-72 for grains (McCrory et al. 2010). The addition of pulse ingredients to a product would create a lower carbohydrate product with higher fibre and protein content, making it an excellent food prior to exercise.

There is interest in increasing the amount of food products in the marketplace containing pulse ingredients. Snack foods allow for on the go convenience without any preparation and dried cereals are a common breakfast food consumed in North America. Market trends show there has been a significant increase in growth in sales for both products. There is a demand for higher protein and healthier alternatives when it comes to these products (Industry research reports Canada, 2021). Extruded products retain their nutrients when processed and are shelf stable. Thus, investigating different types of pulse ingredients and their impact on post-prandial glycemia and satiety will aid food companies when developing healthy extruded products while supporting Canadian farmers.

3.2 Research Hypothesis

It is hypothesized that the consumption of extruded food products containing pulse flours and fractions will lead to lower blood glucose, insulin, appetite and food intake, following a 60 min aerobic exercise session compared to the same exercise session following the ingestion of a non-pulse food. It is hypothesized in the second study that when pulse fractions are consumed in combination, rather than alone, they will provide greater reductions in the glycemic response.

3.3 Research Objectives

The objectives of study I and II were to assess the acute effects of five extruded snacks made with pulse flour, and 5 different pulse fraction extruded cereals on: 1) response of blood glucose, insulin and appetite to an aerobic exercise session, and 2) food intake two hours following the exercise session.

Chapter 4 – Trial 1: Acute Effects of Extruded Snacks Containing Pea Fractions on Blood Glucose, Insulin, Appetite and Food Intake Following an Aerobic Exercise Session - A Randomized Crossover Trial

4.1 Abstract

Studies indicate that whole pulse consumption is beneficial for post-prandial glycemic and satiety control and may impact the glycemic response following exercise. However, it is unknown whether extruded snack products containing pulse flours have these effects. The objective of this trial was to examine of the effects of extruded pulse snacks on blood glucose , insulin and appetite responses before and after an endurance exercise compared to a non-pulse snack. Food intake was measured 2 hours following exercise. Following a repeated-measures crossover trial, adult females (n=12) and male (n=13) randomly consumed extruded snacks made with: 1) whole grain yellow pea flour, 2) split yellow pea flour, 3) green lentil flour, 4) chickpea flour, 5) pinto bean flour, and 6) corn flour (control). Exercise consisted of walking or running for 50 mins at a pre-determined speed based on $V0_2$ max. Pulse extruded snacks contained 40% pulse flour and 60% corn flour, whereas the control was 100% corn flour. blood glucose and appetite were measured pre-exercise (0-60 min) and post-exercise (120-240 min) and food intake was measured at 240 min in all participants. Insulin was measured in a subset (n=15). There was a time ($p<0.0001$), treatment ($p<0.0001$) and treatment-by-V02 ($p<0.001$) effect over the entire session on change from baseline blood glucose , but no time-by-treatment interaction ($p=0.84$). Green lentil led to lower blood glucose response compared with split yellow pea at 150 min, whereas at 165 min and 180 min, green lentil snacks led to lower blood glucose compared to the control. There were no differences between treatments for pre-or post-exercise blood glucose netAUC. There was a time ($p<0.0001$), treatment ($p=0.02$), and treatment-by-V02 ($p<0.0002$) effect over the entire session for insulin, but no time-by-treatment interaction ($p=0.10$). There were no differences between treatments for pre-

exercise insulin netAUC. Post-exercise appetite netAUC showed treatment effects for both split yellow and chickpea ($p<0.05$); split yellow and chickpea showed lower appetite scores compare to whole yellow pea and pinto bean. There was no difference in food intake. This data shows the potential for pulse flours to impact the glycemic and appetite responses following exercise, however further investigation is required. Results are dependant on pulse type, as green lentil had the most significant reduction in blood glucose.

Key words: pulses, bean, pea, lentil, chickpea, glycemia, postprandial, blood glucose , insulin, appetite, exercise

4.2 Introduction

The World Health Organization defines overweight and obesity as "abnormal or excessive fat accumulation that presents risk to health" (WHO, 2021). Obesity predisposes individuals to chronic diseases such as metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease. There has been a drastic increase in the proportion of Canadians who are overweight and obese over the last few decades. Based on self-reported data, 63.91% of Canadian adults were overweight or obese in 2018 (Statistics Canada, 2018). This increase is believed to be influenced by the availability of ready-to-consume, highly processed, pre-packaged foods, accounting for as much as 62% of Canadians' diets, as well as a decrease in physical activity (Poti, Braga and Qin 2017; Nardocci, Polsky and Moubara 2019). These foods often provide little nutrition and are low in protein and dietary fiber, with high amounts of sugar and sodium (Statistics Canada, 2018). Additionally, a large proportion of Canadians are sedentary most of their day and do not meet the Canadian physical activity guidelines (>150 minutes per week of moderate to vigorous activity in >10 min bouts) (Public Health Agency of Canada, 2019).

Over the past decade pulses and pulse ingredients have gained popularity in the food industry. Increasing consumption of food ingredients in processed foods that improve glycemia and satiety in response to exercise, may improve glycemic control and support weight management in individuals. Replacing foods that consumers would normally reach for with healthier alternatives is needed in the marketplace, as consumers are not ready to give up convenience foods altogether. Pulse flours and fractions are dietary ingredients that possess the potential to provide such benefits.

Dietary pulses including chickpeas, beans, lentils, and yellow peas, as well as pulse ingredient fractions and flours, have many strong nutritional properties. Regular consumption of pulses (½ cup/day) is associated with higher quality diets, including higher intakes of fibre, protein, folate, zinc, iron, and magnesium and lower intakes of saturated fat and total fat compared to diets that are low in pulses (Johnson, Thomas and Hall 2005). Pulses are nutrient dense with a low glycemic index, contain high fibre, protein and low-fat content, thus making them an excellent choice to incorporate into food products (Hoover, Hughes and Chung 2010).

In acute studies, pulses have shown mixed results for glycemic control, satiety and food intake. Studies investigating the effects of whole pulses have found that when consumed alone or in a mixed meal, they can improve the glycemic, appetite and food intake responses compared to a non-pulse control, however the results can vary between pulse types (Wong et al 2009, Mollard et al 2012, Mollard et al 2011). Research investigating the effects of pulse ingredients, including flours, powders and fractions is more limited with varying results. Studies using pulse flours or fractions (Anderson et al. 2014, Johnston et al. 2005; Mollard et al 2014; Marinangeli et al. 2009; Smith et al. 2012) found promising results in this area.

A recent study investigated the impact of pulse flours incorporated into extruded snacks on glycemic response, insulin, appetite, and food intake (Johnston et al. 2021). They found that pinto bean and chickpea snacks retained their acute glycemic benefits better than the other pulses tested. This trial builds on the Johnston 2021 trial investigating the same extruded snacks but includes exercise. The objectives of the present trial were to test the acute effects of extruded snacks containing different pulses on blood glucose, insulin and appetite response following an aerobic exercise session, and food intake two hours following the exercise session.

4.3 Trial Design

4.3.1 Participants

Participants were recruited from posters placed around Winnipeg, University of Manitoba campus and advertisements in Winnipeg newspapers (Appendix 1). Individuals contacted the research team to learn more information about the studies and if interested, were scheduled for an in-person session at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) to determine whether they were eligible. During the visit, participants reviewed and signed a consent form, asked any questions they had regarding the study and completed the screening questionnaire (Appendix 3) and measured body weight, height, blood glucose, and blood pressure following standard procedures. Body mass index was calculated from weight and height measurements. blood glucose was measured by finger prick blood sample by a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and assessed using a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). Inclusion criteria included: normoglycemic (<5.6 mmol/L), normotensive (systolic blood pressure <140 mm Hg and diastolic blood pressure below < 90 mm Hg), males and pre-menopausal females aged 18–50y, and a BMI of 18.5–29.9 kg/m^2. To be eligible, participants needed to be physically inactive not meet the

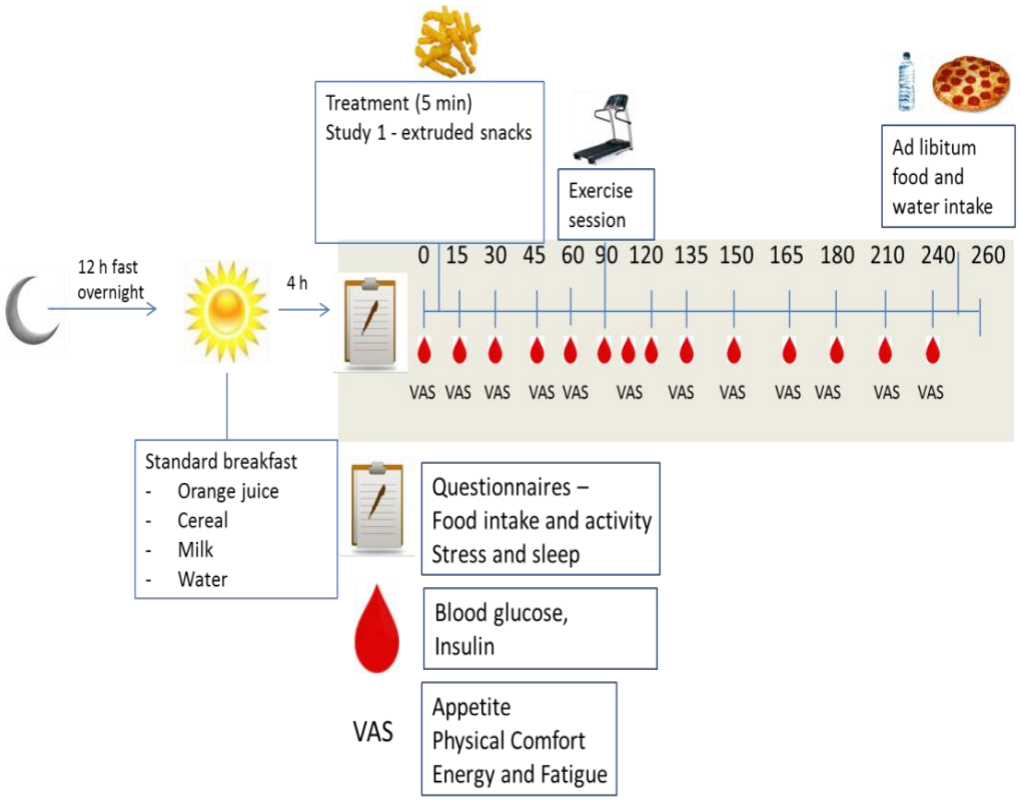
threshold criterion as per the Canadian Physical Activity Guidelines (≥150 minutes of moderate to vigorous physical activity accumulated in 10-minute bouts). Participants were excluded if they regularly skip breakfast, were restrained eaters (Appendix 4), smoked, on medications that may influence trial outcomes, or have experienced any gastrointestinal related health conditions/surgeries over the previous year. Participants were also excluded if they were unable to walk for an hour continuously. Restrained eaters were identified during screening through the use of an eating habits questionnaire (Mollard et al. 2012). Participants with a score >11 were excluded. Participants were asked if they have given a blood donation in the previous 4 weeks. If so, scheduling of the sessions accounted for this and ensured that there was a four-week period between the blood donation and the first session. Research staff also ensured that participants were willing to eat the food products and pizza meal (Appendix 5).

Baseline Physical Activity:

Once considered eligible, participants were scheduled for a treadmill test to determine maximal aerobic capacity ($V0_2$ max). This test was conducted by a Certified Exercise Physiologist to determine their $V0_2$ max using an adapted protocol designed to deal with the limitations of the treadmill used. Participants walked or jogged, depending on their ability, for 12 to 15 minutes where the speed and incline increased as the test progressed. The test was conducted with a metabolic cart (VMax Medical Graphics Corporation, St Paul, MN) that required the participants to wear a mouthpiece that collects O_2 and CO_2. This test allowed for participants' baseline aerobic VO_2 max, respiratory quotient and substrate utilization to be assessed. The treadmill test determined the level of intensity each participant must work at during the trial's exercise sessions. All exercise activities were conducted at the RCFFN at the University of Manitoba. Following the VO_2 max test, one to two weeks before starting the sessions, participants were given a physical

activity measurement tools (Actigraph activity monitor) to wear for one week to measure baseline physical activity. This evaluation provided confirmation that the participant did not exceed the physical activity exclusion criteria.

Figure 1. Trial protocol[1]



[1] Data collection schedule for each study visit. Red blood drops=time points (minutes) where blood samples were collected.

4.3.2 Treatments

The six different treatments consisted of a 35 g serving and 40% pulse flour. This amount was based upon Health Canada's reference amount and serving size for extruded snacks. Corn flour was supplied by Agricor (Marion, Indiana, USA) and the pulse flours used were provided by

Best Cooking Pulses (Portage la Prairie, Manitoba, Canada). Nutritional content data were determined by in vitro digestibility testing. In vitro digestibility testing was performed on 12 extruded snacks, the 6 flours selected for this trial were based on the in vitro results. Johnston et al. (2021) used the same 6 treatments for their trial. Table 1 displays the nutritional content of the snacks. Nutritional content data provided by Dr. Nancy Ames, Agriculture & Agri-Food Canada. Available carbohydrate was analyzed using Megazyme assay procedure K-ACHDE. Protein was measured using standard combustion methodology, using a Flash2000 protein analyzer. Total fat content was measured using modified method from AOAC (922.06). Fibre components are currently being analysed using AOAC method 2011.25. Total starch and starch damage were measured AACC method 76-31 and AACC method 76-31. For the extrusion process of the snacks, the maximum barrel temperature was 115°C with a screw speed 397rpm.

Table 8. Nutritional content of extruded snacks.

Treatments	Energy (kcal)	Available Carbohydrate % db	Protein % db	Resistant starch. % db	Insoluble fibre % db	Soluble fibre % db	Starch damage % db	Fat % db
Corn (control)	209.7	94.7	5.15	0.2	2.3	0.6	73.3	1.4
Whole Yellow Pea	198.8	78.0	12.1	0.2	6.7	1.7	62	2.0
Split Yellow Pea	204.9	83.6	11.5	0.3	3.9	1.4	65.7	2.1
Green Lentil	198.2	77.5	13.2	0.3	6.1	1.77	61.1	1.9
Chickpea	202	77.2	12.7	0.2	5.5	2.0	61.8	3.4
Pinto Bean	199.8	77.3	12.6	0.3	6.5	3.2	57.0	2.3

4.3.3 DESIGN

The trial followed a randomized crossover, double blind, balanced, repeated measures design. Participants consumed one treatment per week in randomized order. Randomized sequences of treatments were created using the rand() function in Excel, and a 6 by 6 Latin-square was created by co-author DM. These six sequences were printed on strips of paper and then put into opaque envelopes each containing six strips. One envelope for men, one envelope for women. Randomization was blocked by sex, with study coordinators drawing a sequence strip from the envelope for male or female participants, until the envelope was empty and another envelope containing six sequences was started. Treatments were provided to the research team in opaque individual serving size packages, numbered 1 to 6. Treatments were similar looking extruded puffed snacks, varying slightly in colour from light beige to greenish. Both the research staff and participants were blinded to the treatments. Sessions were scheduled with at least 5 days between. Women were seen during their follicular phase of their menstrual cycle, as observed insulin resistance has been showed to occur during the luteal phase (Escalante and Salazar 1999).

Subjective appetite was measured by a motivation-to-eat visual analog scale (VAS). This is a validated questionnaire (Flint et al. 2000) used in previous acute studies investigating the effect of pulse consumption on appetite (Mollard et al. 2012; Mollard et al. 2011; Wong et al. 2009). The questionnaire consists of 100 mm lines affixed with opposing descriptions at either end for each question. Participants mark an "X" on the line to depict their feelings at each time point. Scores are determined by measuring the distance (mm) from the left starting point to the intersection of the "X."

4.3.4 Session Protocol

Participants were asked to fast overnight for 10 hours and consumed a standardized breakfast 4 hour prior to the start of the session. The interval between breakfast and session was kept constant. Participants were allowed water one hour before the session. The day before the session, participants were asked to consume the same type and quantity of foods the night before each session and participate in their regular routine.

Upon arrival at the sessions, participants completed questionnaires assessing recent food intake and physical activity (Appendix 6). They also were asked additional questions concerning their previous night's sleep and if they were experiencing any stress (Appendix 6). If they reported significant deviations from their usual sleep pattern, they were rescheduled. Following the questionnaires, a blood sample was taken by finger prick by a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and blood glucose assessed by a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). Any reported significant deviations from the participants' usual blood glucose pattern or their level was > 5.6 mmol/L, they were rescheduled.

Participants then completed a motivation-to-eat visual analog scale (VAS) to measure subjective appetite (Appendix 8). Additional VAS questionnaires were administered to measure palatability (taste, texture) (Appendix 11), physical comfort (Appendix 9) and energy/fatigue (Appendix 10). Prior to treatment consumption, participants completed VAS questionnaires.

Following initial questionnaires, VAS, and finger prick blood glucose measurement, an indwelling intravenous catheter was inserted in the hand or arm by a registered nurse for blood sampling. Following baseline measures, participants consumed the pulse product (within 8-10 minutes kept constant for each participant), followed by completion of a VAS questionnaire

assessing palatability. Blood sampling took place at 15, 30, 45, 60, 90, immediately after exercise, 120, 135, 150, 185, 210 and 240 minutes. Appetite, energy and fatigue, and physical comfort VAS questionnaires were completed following each blood sample.

At 60 minutes, participants completed a treadmill endurance session which consisted of either running or walking. The exercise session lasted a maximum of 50 minutes and intensity was set at moderate intensity (established during VO_2 max test). Participants were set at 40% of their $V0_2$max for 35 mins then increased 70% for the remaining session. During the exercise session, exercise intensity (oxygen consumption) and substrate oxidation (respiratory quotient) was measured three times: 0-5 minutes, 15-20 minutes and at 35 minutes, at which the mask stayed on until exercise session was complete. Heart rate was recorded every 15 seconds to monitor exercise intensity and gave a measure of fatigue (cardiovascular drift). blood glucose was measured at 60 minutes (before exercise), during the exercise session at 90 minutes, and immediately after and at 120 minutes.

For measurement of food intake, participants were served an *ad libitum* meal at 240 minutes. Fresh hot pizza (McCain® Deep 'n Delicious®) was served every 5-7 minutes and participants were asked to eat until they were "comfortably full". Energy intake from the pizza meal was calculated from the weight consumed and the compositional information provided by the manufacturer. Once they finished eating pizza, participants rated its palatability to ensure that the pulse products did not influence the taste/enjoyment of the pizza.

4.3.5 Ethics

This trial followed the Declaration of Helsinki guidelines and all procedures involving human participants were approved by the University of Manitoba Research Ethics Board.

Informed written consent was received from each participant prior to their participation. Clinicaltrails.gov registration number was NCT02402517.

4.3.6 Sample Analysis

Blood samples were collected through intravenous catheters inserted by a registered nurse and allowed to sit at room temperature for 15-30 minutes. The samples were centrifuged at 1300g for 10 minutes and pipetted into micro-tubes. Samples rested in a fridge to cool before storing in -80˚C freezer. Serum glucose was measured by colorimetric slides on an Ortho Clinical Diagnostics (Raritan, New Jersey) Vitros 350 Auto-Analyzer. Quality controls were run each day prior to testing samples to ensure accuracy and precision of the measurements. Twenty percent of samples were performed in duplicate for additional precision confidence and if coefficient of variation was >10.5, then the sample analysis was repeated. Serum insulin was measured by radioimmunoassay in duplicate using MilliporeSigma (Etobicoke, Ontario) Human Insulin Specific RIA Kits (125 I-Insulin). Quality controls were run with each kit to ensure accuracy and precision of the measurements. All samples were performed in duplicate. If the result was >199.99 uU/mL the sample was diluted and remeasured. If the coefficient of variation between duplicate samples was >10.5, then the sample analysis was repeated.

4.3.7 Statistics

A sample size of 26 was determined based on previous literature on acute trials assessing the effects of foods and food ingredients on food intake following a similar acute design (Mollard et al. 2011; Mollard et al. 2012). A power analysis for a within-participant design from previous studies (Hamedani et al. 2009; Wong et al. 2009) indicated that a sample size of 26

would be sufficient to detect a treatment effect on food intake of 150 kcal, with a power of 0.80 and an alpha of <0.05.

Individual average treatment palatability, subjective appetite, physical comfort, and energy/fatigue were calculated from the average of the questions on their corresponding VAS questionnaire. Postprandial glucose and insulin responses were measured by netAUC and analyzed using the trapezoid method and included areas over and under baseline values. When there were two or less timepoint values missing (other than baseline, peak and last timepoint) AUC was calculated using Prism GraphPad (GraphPad Software, La Jolla California USA). Cumulative, pre-, and post-exercise netAUC for blood glucose, insulin, and subjective appetite were calculated for 0-240 minutes, 0-60 minutes and 120-240 minutes, respectively. Repeated measures ANOVA was used to determine the effects of treatments, time and the time-by-treatment interaction on blood glucose, insulin and subjective appetite, physical comfort, and energy/fatigue scores over the time of the trial followed by repeated measures ANOVA to determine the effects of treatment at specific time points. The effect of treatments on food intake at the meal and on blood glucose, insulin, appetite, physical comfort, and energy/fatigue AUC were determined by repeated measures ANOVA. Sex, session and $V0_2$ level were included as covariates in all analyses. Tukey-Kramer post-hoc tests were used to describe mean differences across treatments. Statistical analyses were performed using SAS (SAS Inc., Chicago, IL, USA).

4.4 Results

4.4.1 Participant Characteristics

Thirty-one participates were recruited for our trial with twenty-five completing. Twelve women with a mean age of 23.6. ± 4.2 y, mean BMI 23.0 ± 2.8 kg/m^2 and mean $V0_2$ max 39.1 ± 5.5

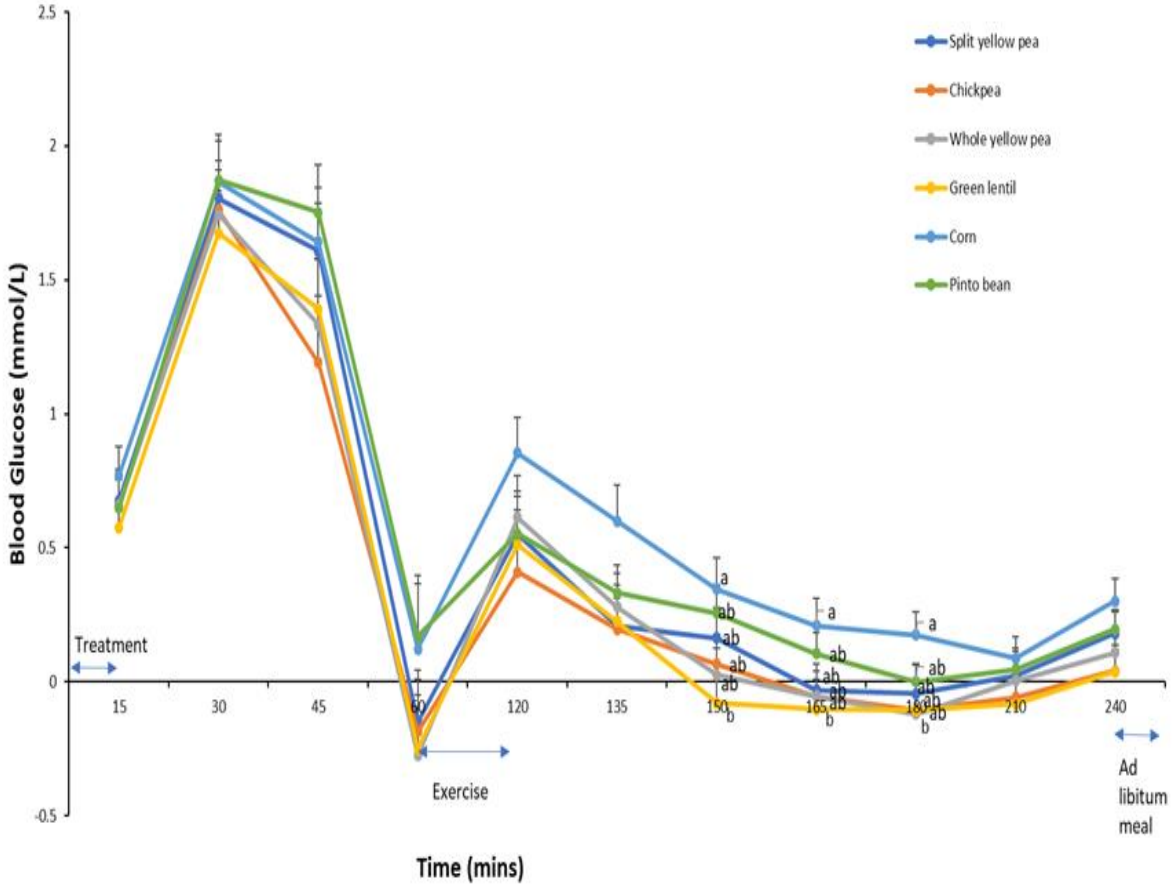
ml/kg/min. Thirteen men with a mean age of 24.5 ± 5.5 y, mean BMI of 25.0 ± 2.78 kg/m^2 and mean $V0_2$ max of 51.3 ± 9.0 ml/kg/min. Reasons for withdrawal from the trial included difficultly in drawing blood (n= 3), time commitment (n=1) or (n=2) could not complete due to surgery for pre-existing condition and injuries due to a car accident. Participants mean blood glucose calculated using the baseline value from the first session for each participant was 5.0 ± 0.9 mmol/L.

4.4.2 Blood Glucose

Figure 1 shows change from baseline blood glucose response over the entire session. There was significant time ($p<0.0001$), treatment ($p<0.0001$), and treatment-by-V02 ($p<0.001$) effects over the entire session on change from baseline blood glucose, but no time-by-treatment interaction. ($p=0.84$).

Change from baseline was used because there were differences between treatments at baseline. The pre-exercise period, using change from baseline, showed only a time ($p<0.0001$), but no treatment ($p=0.14$) or time-by-treatment effect. There was a significant time ($p<0.000$) and treatment ($p<0.0001$) effect for post-exercise. There was no significant time-by-treatment effect ($p=0.65$). The effects on blood glucose at specific time points were dependent upon pulse type. At 150, 165 and 180 minutes, blood glucose was lower ($p<0.05$) after consumption of green lentil compared to split yellow pea, whereas at 165 minutes and 180 minutes, green lentil consumption led to lower blood glucose compared to control snacks. There were no differences in treatments for pre-exercise netAUC ($p=0.12$), and post exercise netAUC ($p=0.42$). Green lentil snacks led to lower ($p<0.05$) cumulative blood glucose netAUC, compared with the control. (Table 9).

Figure 2. Change from baseline blood glucose response to extruded pulse snacks and exercise over 240 minutes[1]



[1]Note: All values are means ± SE (n=25). Values in the same column with different lowercase letters are significantly different from each other, $p<0.05$.

Table 9. Blood glucose area under the curve for different extruded pulse snacks and exercise session (mmol/min/L)[1]

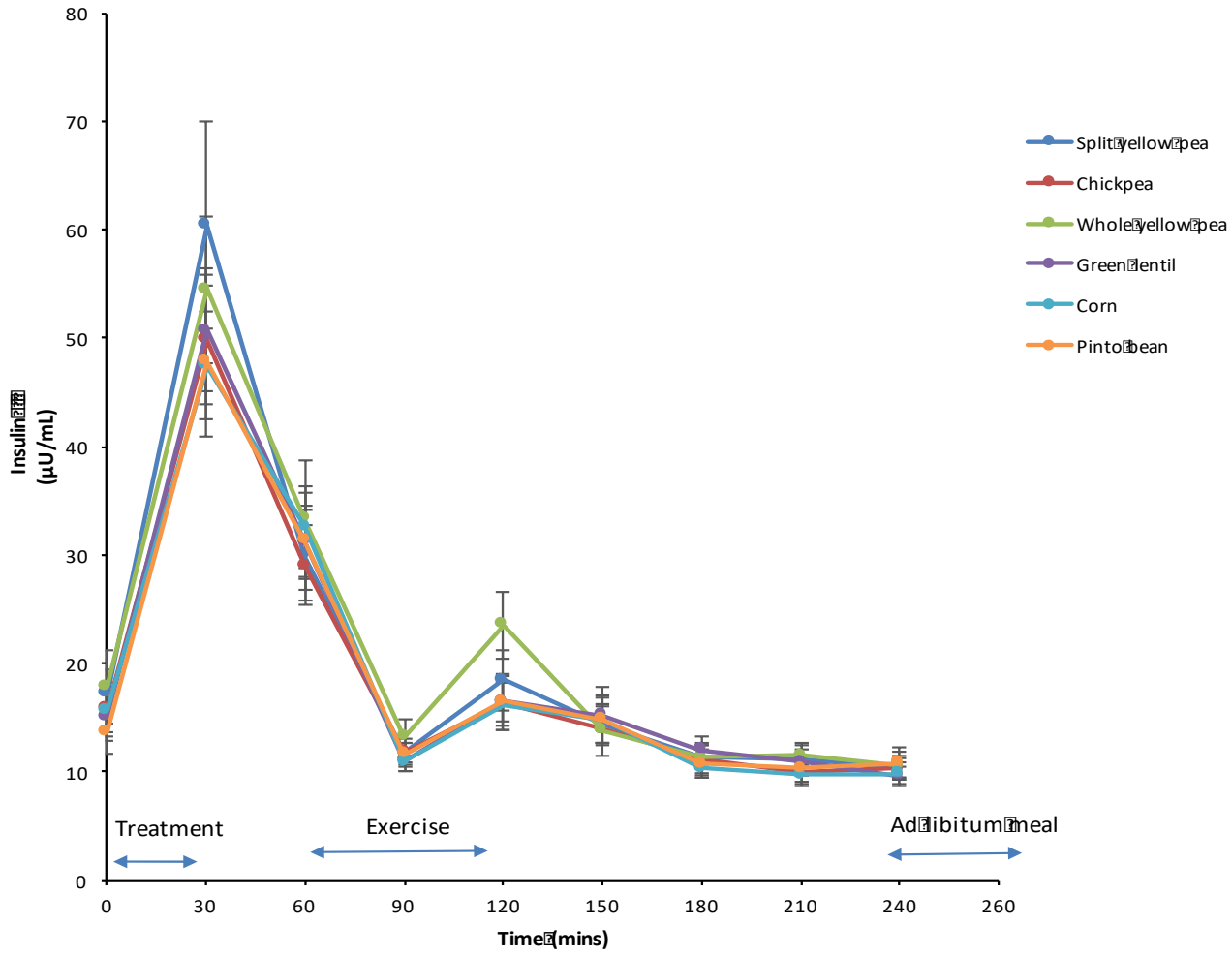
Treatments	Pre-exercise netAUC	Post- exercise netAUC	Cumulative netAUC
Corn	63.3±6.3	52.8±36.5	112.4±19.4[a]
Whole yellow pea	57.6±6.1	70.8±36.8	52.8±14.4[ab]
Split yellow pea	61.9±5.0	51.4±29.4	58.1±17.9[ab]
Green lentil	51.3±6.0	59.2±26.3	43.4±18.2[b]
Chickpea	56.4±6.8	42.7±29.5	51.1±17.1[ab]
Pinto bean	64.9±6.2	12.1±35.6	89.5±13.6[ab]

[1]Note: All values are means ± SE (n=25). Values in the same column with different lowercase letters are significantly different from each other, $p<0.05$. Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min.

4.4.3 Insulin

There were significant time ($p<0.0001$), treatment ($p=0.02$), and treatment-by-V02 ($p<0.0002$) effects over the entire session, however, no time-by-treatment interaction ($p=0.10$) was observed (Figure 2). For pre-exercise insulin (0-60 minutes), effects of time ($p<0.0001$), and treatment-by-V02 were observed ($p=0.0062$) but no treatment ($p=0.16$) or time-by-treatment ($p=0.28$) effects. During the post-exercise period, effects of time ($p<0.0001$), time-by-treatment ($p=0.01$) and treatment-by-V02 ($p=0.0034$) were observed. However, no treatment effects were seen ($p=0.09$). There were no differences in treatments pre-exercise netAUC ($p=0.46$), post exercise netAUC ($p=0.36$) or cumulative netAUC ($p=0.56$) (Table 10).

Figure 3. Insulin response to extruded pulse snacks and exercise over 240 minutes[1]



[1]Note: All values are means ± SE (n=15).

Table 10. Insulin area under the curve for different extruded pulse snacks and exercise session.

Treatments	Pre-exercise netAUC	Post- exercise netAUC	Cumulative netAUC
Corn	1738.7± 262.8	-3382.5± 557.4	865.7± 524.6
Whole yellow pea	1849.4± 287.7	-3135.6± 790.8	907.1± 569.2
Split yellow pea	1944.0± 331.7	-2706.7± 646.0	1071.8± 515.3
Green lentil	1784.1± 203.0	-3021.2±487.1	1175.1± 320.6
Chickpea	1744.9± 280.8	-2816.6± 598.3	1002.7± 450.2
Pinto bean	1826.0± 263.7	-2913.2± 735.0	1400.5± 286.0

[1]Note: All values are means ± SE (n=15). Values in the same column with different lowercase letters are significantly different from each other, $p<0.05$. Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min

4.4.4 Food and Water Intake

No effects of treatment on food intake (p=0.83) or water intake (p=0.77) were observed (Table 11).

Table 11. Food intake and water intake at second ad libitum pizza meal

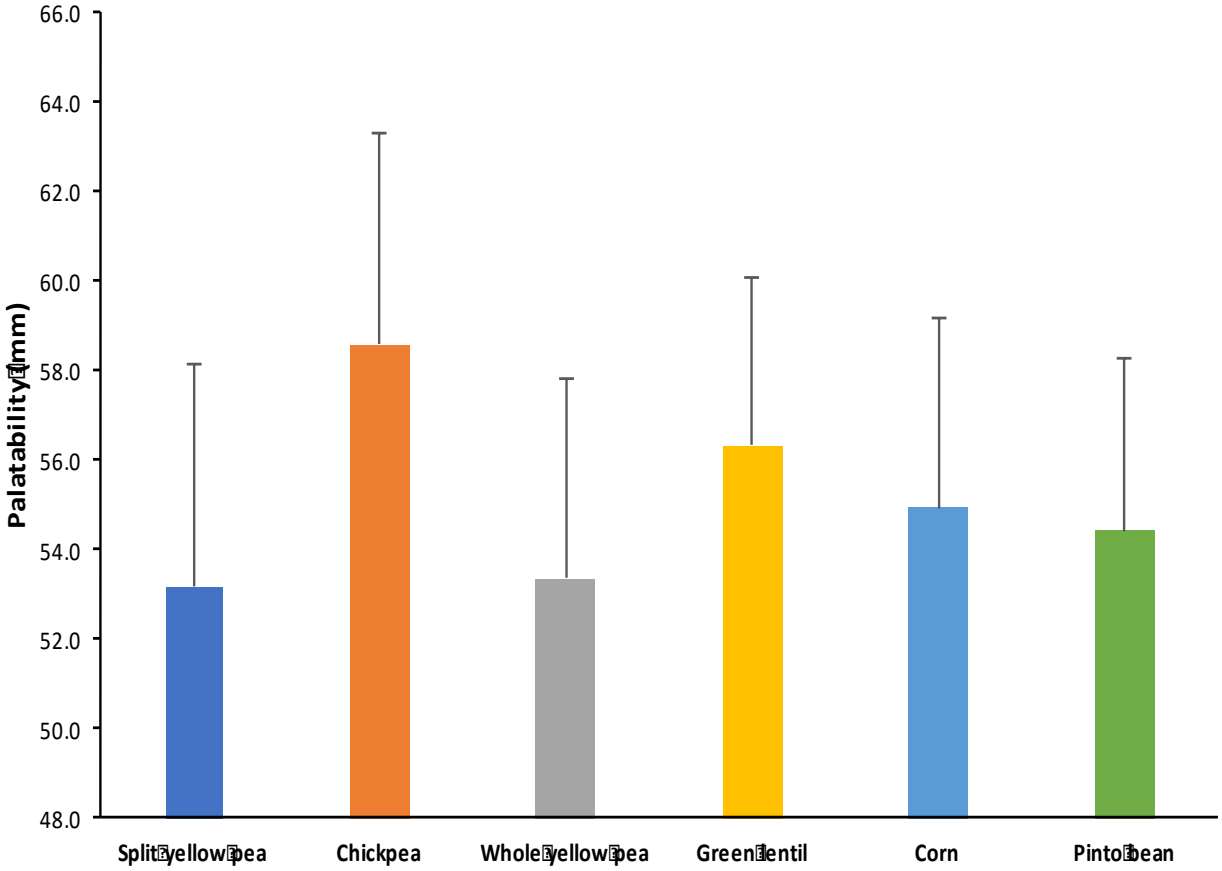
Treatments	Food intake (kcal)	Water intake (g)
Corn	784.5± 113.7	343.4±37.9
Split yellow pea	836.8 ± 106.0	376.7±47.9
Whole yellow pea	904.1± 101.0	377.0±51.8
Green lentil	842.7± 107.6	359.6±49.5
Chickpea	999.5 ± 87.9	405.6±45.8
Pinto bean	1048.5± 82.3	334.8±41.6

[1]Note: All values are means ± SE (n=25). Measured at an ad libitum pizza meal consumed at 240 min.

4.4.5 Palatability

There was no effect of treatment on palatability (p=0.24).

Figure 4. Extruded pulse snack treatment palatability ratings[1]

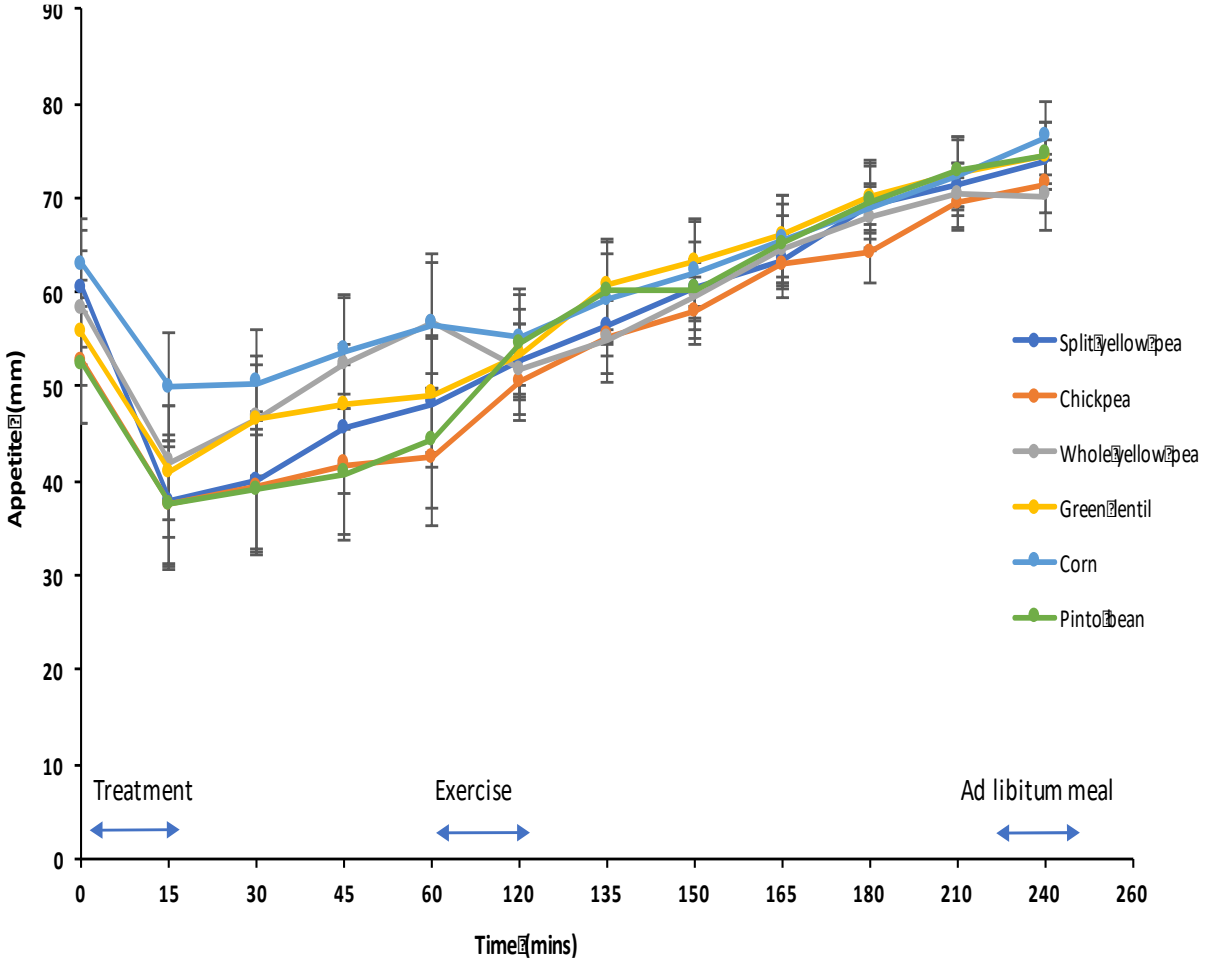


[1]Note: All values are means ± SE (n=25). VAS questionnaires were administered immediately after consuming treatment to measure palatability (taste, texture, how pleasant).

4.4.6 Appetite

There was an effect of time ($p<0.0001$) on appetite over the entire session, however, no effects of treatment ($p=0.99$) or time-by-treatment interactions ($p=0.50$) were observed. During the pre-exercise period, an effect on time ($p<0.0001$), but no treatment ($p=0.35$) or time-by-treatment interactions, were observed ($p=0.32$). During the post-exercise period an effect on time ($p<0.0001$), and treatment-by-sex ($p=0.03$) was observed, but no treatment ($p=0.59$) or time-by-treatment interaction. ($p=0.41$) **(**Table 5). Pre-exercise appetite netAUC ($p=0.03$) showed treatment differences but post hoc didn't find any differences. There were no differences in treatment post-exercise appetite netAUC ($p=0.24$) or for cumulative netAUC ($p=0.35$) (Table 12).

Figure 5. Subjective appetite response to extruded pulse snacks and exercise over 240 minutes[1]



[1]Note: All values are means ± SE (n=25).

Table 12. Appetite area under the curve response to extruded pulse snacks and exercise.

Treatments	Pre-exercise netAUC	Post- exercise netAUC	Cumulative netAUC
Corn	-553.9±161.1	2932.2 ± 603.7	376.1±717.9
Whole yellow pea	-906.5±226.0	3334.0±503.2	-385.2±904.3
Split yellow pea	-658.7±241.2	2168.1±437.0	-260.9±924.4
Green lentil	-521.9±197.7	2824.3±467.8	1110.8±605.1
Chickpea	-946.8±230.8	3848.4±597.1	-581.2±946.9
Pinto bean	-612.8±203.9	2312.5±402.6	528.1±794.3

[1]Note: All values are means ± SE (n=25). Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min

4.5 Discussion

This trial provides evidence that incorporating pulses into an extruded snack results in a lower glycemic response compared with corn control in healthy adults. No differences in blood glucose between treatments were found prior to the exercise session but occurred post-exercise. Green lentil had significantly lower blood glucose levels following exercise compared with control and split yellow pea.

All the snacks, aside from the corn control, possessed similar nutritional profiles. Chickpea had the lowest available carbohydrate (77.2%db), with second lowest being pinto bean (77.3%db), followed by green lentil (77.5%db). Corn (94.7%db) had the highest available carbohydrate out of all the snacks, followed by split yellow peas (83.6%db), both of which resulted in the highest blood glucose responses. Available carbohydrates could have played a role in glycemic response following exercise providing a rational for why split yellow pea and corn resulted in the highest response but does not fully explain why green lentil had the greatest reduction. Pulses are high in fibre and resistant starch. These factors are known to slow the rate of release of glucose in the body and affect appetite (Maringangeli et al. 2017). Green lentil had the second highest amount of resistant starch (31%db) and the third highest insoluble (6.11%db) and soluble fibre (1.77%db) amounts. Corn (73.3%) had the highest starch damage, with split yellow pea with the second highest (65.7%). These differences could play a role in explaining why they elicited a higher blood glucose response, as damaged starch is digested more quickly than non-damaged starch (Chung et al. 2008). Fibre amounts may have also played a role, but this feature does not completely explain the differences found. Amount of protein in the snacks ranged from 5.7- 6.6 g per serving. Protein has demonstrated having the greatest effect on

satiety, compared with carbohydrates and fat (Poppitt, McCormack and Buffenstein 1998). Out of the treatments, green lentil had the highest amount of protein compared to the other pulses. Chickpea and pinto bean followed. Corn had less than half the protein green lentil did. Split yellow pea had the second lowest amount. The amount of protein in the snacks is another possible reason why green lentil showed the greatest blood glucose lowering abilities within the treatments.

The glycemic benefits found were depended on pulse type. Specifically, the snack made with green lentils exhibited the greatest reduction in blood glucose compared to other pulses. The green lentil snack showed lower post-exercise blood glucose AUC at 150 minutes compared to split yellow pea, whereas at 165 and 180 minutes, green lentil consumption led to lower blood glucose compared to control. A similar trial done within our lab by Johnston et al. (2021), used the same treatments but with a second meal served at 60 mins and no exercise, found the pinto bean and chickpea snack to have lower pre-meal iAUC. No post- meal differences were found as well (Johnston et al.2021).

The glycemic benefits demonstrated by green lentil were not due to increased serum insulin. Johnston et al. (2021) found reduced insulin pre-meal iAUC (0-120 min) for pinto bean snack compared with corn control, whole yellow pea and split yellow pea. In this trial, the pre-meal period was from 0-60 min, and there were no treatment differences for insulin. However, there was a treatment-by-$V0_2$ interaction, which warrants further investigation.

A study by Johnson et al. (2005) tested regular chickpea flour bread, extruded chickpea flour bread and white bread (control) on blood glucose response and satiety. They replaced 24.3g of wheat flour in the breads and provided 50g available carbohydrates. blood glucose response to regular chickpea bread was lower than white bread at 60 min. At 120 min, blood

glucose from the extruded chickpea flour was lower than white bread. The authors suggested that the particle size could be why no glycemic benefits were observed and should be investigated further. They found insulin was higher for chickpea flour then control at 60 min, where our study did not see any differences in insulin. This study supports our results that pulses (specifically chickpea) glycemic benefits are retained when incorporated into a product or extruded.

It has recently been suggested that antinutrients and bioactive components play a role in glycemic control. These components include trypsin inhibitors, lectins, phytates, polyphenols (tannins) and raffinose oligosaccharides (Johnson, Thomas and Hall 2005). The efficacy of these compounds could degrade or enhance depending on processing method and pulse type. Phytic acid has been shown to reduce glycemic response and is higher in green lentils compared with chickpea (Singh et al. 2017). A study by Hernadnez-Salazar (2010) evaluated 3 pulse types for their indigestible fraction (IF), polyphenols content, antioxidant capacity and in vitro fermentability, including short chain fatty acid production. The study indicated lentils had higher polyphenol levels than chickpeas. Flavonoids are phenolic compounds that also may have glycemic benefits. Specifically, a-glucosidase and a-amylase, which play a role in digesting starch (Margier et al. 2018). The blood glucose lowering effects cannot be explained by one mechanism of action, but rather are multifactorial. The combination of macronutrients and anti-nutrient factors may play a role in why pulses possess glycemic benefits.

There were no differences in appetite or food intake in this study. In support of our findings, Johnston et al. (2021) also found no treatment effects for food intake or appetite following consumption of pulse snacks. In a study by Mollard et al. (2012), they found that whole green lentils led to lower food intake compared with whole chickpea mixed meals when

food intake was measured at 260 min. No appetite differences were found. Other studies also showed that whole pulses in a mixed meal can reduce food intake (Hernandez et al 2010, Mollard et al 2011). It is possible that there was insufficient pulse flour incorporated into the snacks in our current study to reflect the amount when consumed as whole pulses, or that processing impacts the effect of pulses on satiety. A study on pulse fractions, by Smith et al. (2012) found that yellow pea protein resulted in lower food intake at 30 mins compared to all other treatments. However, another study using yellow pea fractions found no impact on food intake (Mollard et al. 2014).

This study had several strengths including the use of a randomized double-blind crossover design, both male and females who were normal weight and overweight, and matching treatments for energy content. Treatments had no preparation before eating, making it realistic for consumers to incorporate into their diet in the same way this study did. Participants consumed the *ad libitum* pizza meal 4 hours after consuming the treatment and 2 hours following exercise. This long fasting period could have affected appetite results. I did not measure phytonutrients and particle size which was a limitation to this study.

The pulse flour did not adversely affect palatability of the snacks. A 50g serving size with 40% pulse flour was chosen based on previous literature. Increasing this amount too much may negatively impact palatability. However future research should investigate the impact on increasing amounts of pulse flours to find the more effective amount in extruded snacks. Additionally, hormone analysis including ghrelin, cholecystokinin (CCK), glucagon- like peptide 1 (GLP-1) could further explain the effects of the varying pulse flours on glycemic and satiety responses, as glycemia, satiety, gastric emptying is influenced by these hormones (Kristensen and Jensen 2011; D'souza et al. 2017; MacDonald et a. 2002).

In conclusion, this trial demonstrated significant changes in post-prandial glucose, providing evidence that pulses can be successfully incorporated into an extruded snack while not affecting palatability. Pulses' nutrient profile make them low glycemic and a promising functional ingredient in food products. Adding pulses to already consumed products will increase pulse consumption, as well as create a more nutritious and low glycemic food for consumers.

Bridge to Chapter 5

Whole pulses are widely known for their health benefits, including glycemic response. Literature has shown benefits are retained when pulses are incorporated into a flour or fraction when used in a food product, pureed or within a mixed meal. Chapter 4 demonstrated that the glycemic benefits are dependent on pulse type when consumed in an extruded food product after an exercise session. Green lentil had the greatest reduction in blood glucose compared with other pulses and control (corn). No insulin difference was found. Split yellow pea and chickpea led to lower appetite ratings however did not translate into differences.

It is unknown from the first study which fraction of the pulse is responsible for their glycemic benefit. The purpose of the following study was to assess this and determine what fraction elicited the beneficial effect, or if there is a synergetic effect when fractions are combined. Pea fractions were incorporated into an extruded cereal product to determine the response of blood glucose , insulin and appetite following an aerobic exercise session, and also in the two hours following exercise.

Chapter 5- Trial 2: Acute Effects of Extruded Cereals Containing Pea Fractions on Blood Glucose, Insulin, Appetite and Food Intake Following an Aerobic Exercise Session, A Randomized Crossover Trial.

5.1 Abstract

Whether pulse fractions retain the glycemic and satiety effects of whole pulses when consumed in commercially available food products and if they provide any benefits following exercise is unknown. The objectives of this study were to examine the effects of extruded pulse cereals on blood glucose , insulin and appetite responses before (0-60 min) and after (120-140 min) endurance exercise and food intake 2 hours (240 min) following exercise compared to a non-pulse cereal. In a repeated-measures crossover trial, adults (n=27) randomly consumed extruded cereals (35g) made with 1) oat flour (control), 2) oat flour and pea starch (starch), 3) oat flour and pea protein (protein), 4) oat flour, pea starch and pea protein (starch+protein), 5) oat flour, pea fibre and pea protein (fibre+protein), and 6) pea fibre, pea starch and pea protein (fibre+starch+protein). Net area under the curve (netAUC) was calculated for blood glucose (blood glucose), insulin and appetite pre-exercise and post-exercise, whereas insulin was measured in a subset (n=15). Pre-exercise blood glucose netAUC was lower following the starch + protein + fibre and protein + fibre compared to control ($p<0.05$). However, post-exercise blood glucose netAUC showed no differences among treatments ($p=0.33$). Appetite pre-exercise netAUC showed differences in treatments ($p=0.028$); protein + starch and protein showed lower appetite compared to control ($p<005$). There was no difference in post-exercise netAUC appetite ($p=0.21$). There were no differences in food intake or insulin. Pulse fractions possess the ability to influence glycemic and appetite response pre-exercise, however further investigation after exercise is required.

5.2 Introduction

Beans, peas, lentils and chickpeas are known as “pulses”. They are the edible seeds of non-oilseed legumes and are nutrient dense with a high fibre, protein and low-fat content, making them an excellent choice to incorporate into food products (Hoover et. al 2019; Mitchell et al. 2009). Regular consumption of pulses (½ cup/day) is associated with higher quality diets, including higher intakes of fibre, protein, folate, zinc, iron, and magnesium and lower intakes of saturated fat and total fat compared to diets that are low in pulses (Mitchell et al. 2009). Canada is a top producer of yellow peas however human consumption and processing for food products is low (Anderson et. al 2014). Using pulses as value-added ingredients in easy to prepare or ready- to-eat foods is a strategy to increase pulse consumption and improve the nutritional profile of these foods, including increasing protein and fibre content, while also benefiting the pulse industry.

Obesity rates have been steadily rising throughout the world over the past few decades. Canada has followed this trend given the observed numbers of obese or overweight individuals. Based on self-reported data, 63.91% of Canadian adults were overweight or obese in 2018 (Government of Canada, 2019). Obesity has serious health implications as it increases the risk of many chronic diseases, as well as costs for our healthcare system. Many social and enviromental factors contrubute to obesity, including diet and physical activity. One dietary factor is the increase in availability of ready-to consume, highly processed, pre-packaged foods, accounting for over half of Canadians’ diets. (Poti, Braga and Qin 2017; Nardocci, Polsky and Moubara 2019). These foods provide little nutrition or satiety. Another factor is the decrease in physical activity levels and an increase in sedentary lifestyle (Statistics Canada, 2020). Many Canadians are sedentary most of their day, working indoors, and do not meet the Canadian physical activity

guidelines (>150 minutes per week of moderate to vigorous activity in >10 min bouts) (Tremblay et al. 2011).

Whole pulses have been widely investigated for their health benefits in various studies over the past decades. However, there are few studies to date on pulse fractions and their effect on glycemic and satiety responses. Smith et al. (2012) and Mollard et al. (2014) found when pea protein alone or protein and fibre were combined, they resulted in lowered blood glucose responses following consumption, but no differences in appetite or food intake. Chan et al. (2019) found when faba bean protein concentrate and protein isolate was added into a wheat pasta it led to lower blood glucose and appetite but no insulin or GLP-1 differences.

The effect on meal timing on exercise fatigue, glucose and insulin responses is a growing area of research, however, there has been minimal research conducted with pulses. The majority of the studies investigate the effects of liquid carbohydrate drinks or bars on glycemic response before and after exercise in athletes on performance. These studies do not include pre-exercise meals and individuals from the general populations (Hargreaves, Hawley and Jeukendrup 2004; Jentjens, Gutch, Jeukendrup 2003). Previous literature also has been focused on high verse low glycemic index foods and the glycemic response during and after exercise as well as exercise performance and endurance. Research has established the higher the glycemic index the higher the glycemic response (Esfahani et al. 2009). There is no research related to the consumption of pulses or pulse ingredients before exercise and the food response following exercise. Pulses are a very low glycemic index food, with values range from 29 to 48, compared to 42-72 for grains (McCrory et al. 2010). The addition of pulse ingredients to a food product would create a lower carbohydrate product with higher fibre and protein content, making it an excellent food prior to exercise.

A recent study by Johnston et al. (2021) investigated the impact of pulse fractions incorporated into extruded cereals on glycemic response, insulin, appetite and food intake. They found that the combination of protein + fibre and fibre + starch + protein treatments resulted in decreased blood glucose and insulin levels. This study builds on the Johnston 2021 study investigating the same extruded cereals but includes an exercise component. The study objectives were to test the acute effects of extruded cereals containing pea fractions on the response of blood glucose , insulin and appetite following an aerobic exercise session, and the food intake two hours following the exercise session.

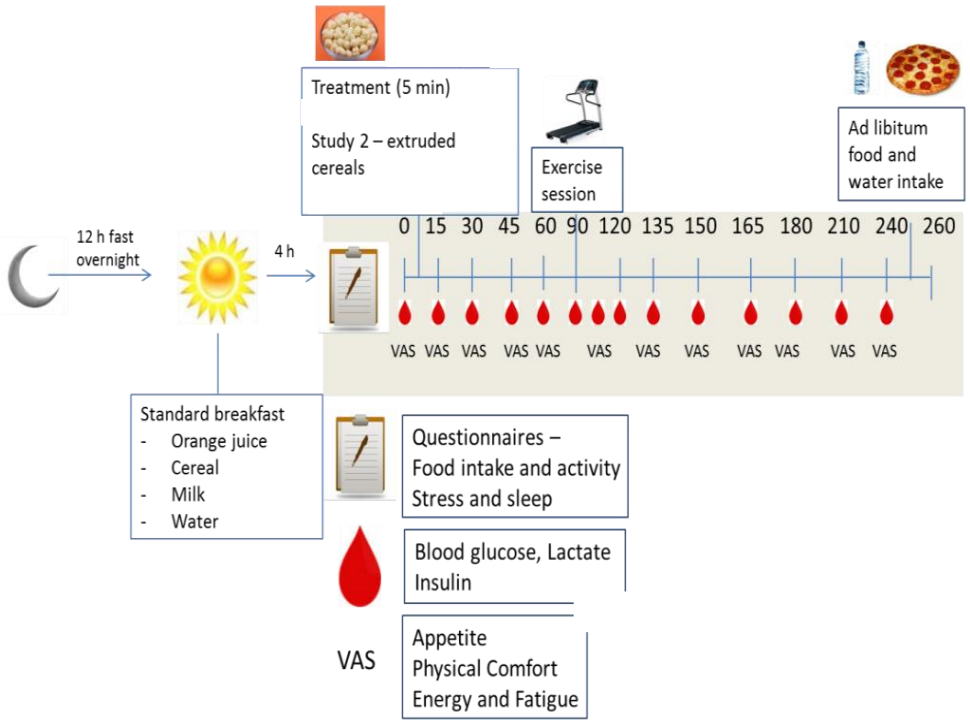
4.3 Trial Design

4.3.1 Participants

Participants were recruited from posters placed around Winnipeg, University of Manitoba campus and advertisements in Winnipeg newspapers (Appendix 1). Individuals contacted the research team to learn more information about the studies and if interested, were scheduled for an in-person session at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) to determine whether they were eligible. During the visit, participants reviewed and signed a consent form, asked any questions they had regarding the study and completed a screening questionnaire (Appendix 3). blood glucose was measured by finger prick blood sample by a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and assessed using a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). Inclusion criteria included: normoglycemic (<5.6 mmol/L), normotensive (systolic blood pressure <140 mm Hg and diastolic blood pressure below < 90 mm Hg), males and pre-menopausal females aged 18–50y, and a BMI of 18.5–29.9 kg/m^2. To be eligible, participants needed to be physically inactive and not meet the

threshold criterion as per the Canadian Physical Activity Guidelines (≥150 minutes of moderate to vigorous physical activity accumulated in 10-minute bouts). Participants were excluded if they regularly skipped breakfast, were restrained eaters (Appendix 4), smoked, on medications that may influence study outcomes, or had experienced any gastrointestinal related health conditions/surgeries over the previous year. Restrained eaters were identified during screening through the use of an eating habits questionnaire (Velangi, Fernaes and Wolever 2005). Participants with a score >11 in the eating habits questionnaire were excluded. Participants were also excluded if they were unable to walk for an hour continuously. Participants were asked if they had given a blood donation in the previous 4 weeks. If so, scheduling of the sessions accounted for this and ensured that there was a 4-week period between the blood donation and the first session. Research staff also ensured that participants were willing to eat the food products and pizza meal (Appendix 5).

Figure 6. Trial Protocol[1]



[1] Data collection schedule for each study visit. Red blood drops=time points (minutes) where blood samples were collected.

Baseline Physical Activity:

Once deemed eligible, participants were scheduled for a treadmill test to determine one's maximal amount of oxygen that can be used during exercise ($V0_2$ max). This test was conducted using an adapted protocol designed to deal with the limitations of the treadmill used. Participants walked or jogged, depending on their ability, for 12 to 15 minutes where the speed and incline increased as the test progressed. The test was conducted with a metabolic cart (VMax Medical Graphics Corporation, St Paul, MN) that required the participants to wear a mouthpiece that measures the volume and concentration of inspired and expired air. This test allowed for participants' baseline aerobic max capacity (VO_2 max), respiratory quotient and substrate utilization to be assessed. The treadmill test determined the level of intensity for each participant during the study's exercise sessions. All VO_2 max tests were conducted at the RCFFN at the University of Manitoba and supervised by a Certified Exercise Physiologist. Following the VO_2 max test, one to two weeks before starting the sessions, participants were given a physical activity measurement tools (Actigraph activity monitor) to wear for one week to measure baseline physical activity. This evaluation provided confirmation that the participant did not exceed the physical activity exclusion criteria.

4.3.2 Treatments

The six different treatments consisted of a 35g serving. This amount was based upon Health Canada's reference amount and serving size for extruded cereals. Oat flour was supplied by Aveena Foods (Regina, Saskatchewan, Canada), corn starch by ADM (Chicago, Illinois USA),

pea hull fibre by Best Cooking Pulses (Portage la Prairie, Manitoba, Canada), and pea starch and pea protein by Parrheim Foods (Saskatoon, Saskatchewan, Canada). Nutritional content data is provided in Table 13. *In vitro* digestibility testing was performed on 8 cereals, the 6 cereals selected for this study were based on the *in vitro* results. Nutritional content data provided by Dr. Nancy Ames, Agriculture & Agri-Food Canada. Available carbohydrate was analyzed using Megazyme assay procedure K-ACHDE. Protein was measured using standard combustion methodology, using a Flash2000 protein analyzer. Total fat content was measured using modified method from AOAC (922.06). Fibre components were analysed using AOAC method 2011.25. Total starch and starch damage were measured AACC method 76-31 and AACC method 76-31. For the extrusion process, the maximum barrel temperature was 120°C with a screw speed 397rpm.

Table 13. Nutritional Content of extruded cereals.

Treatment	Energy (kcal)	Available Carbohydrate (% db)	Protein (% db)	Resistant starch (% db)	Insoluble dietary fibre (% db)	Soluble dietary fibre (% db)	Starch damage (% db)	Fat (%)
80% oat (control)	150.2	79.0	9.6	0.2	6.2	3.6	63.3	5.77
39% oat, 50% pea starch	143.4	80.8	8.2	0.5	6.7	2.9	66.7	3.67
47% oat, 40% pea protein	135.3	53.5	24.3	0.2	10.0	4.0	43.4	5.31
32% oat, 18% pea hull, 40% pea protein	121.2	39.8	23.2	0.2	23.1	4.9	31.0	4.17
6% oat, 50% pea starch, 40% pea protein	130.2	57.9	22.6	0.6	8.9	3.1	47.4	2.86
16% pea hull, 45% pea starch, 36% pea protein	124.4	46.8	20.8	0.81	20.3	4.8	40.2	2.47

4.3.3 DESIGN

The study followed a randomized crossover, double blind, balanced, repeated measures design. Participants consumed one treatment per week in a randomized order. Randomized sequences of treatments were created using the rand() function in Excel, and a 6 by 6 Latin-square was created by co-author DM. These six sequences were printed on strips of paper and then put into opaque envelopes each containing six strips, with one envelope for men and one envelope for women. Randomization was blocked by sex, with study coordinators drawing a sequence strip from the envelope for male or female participants, until the envelope was empty and another envelope containing six sequences was started. Treatments were provided to the research team in opaque individual serving size packages, numbered 1 to 6. Treatments were similar looking, small torus shaped cereal, varying slightly in colour from light beige to greenish. Both the research staff and participants were blinded to the treatments. Sessions were scheduled at least 5 days apart. Women were seen during their follicular phase of their menstrual cycle, as observed insulin resistance has been showed to occur during the luteal phase (Escalant and Alpizar 1999).

Subjective appetite and palatability were measured by visual analog scales (VAS). The latter is validated questionnaire (Flint et al. 2000) used in previous acute studies investigating the effect of pulse consumption on appetite (Mollard et al. 2012; Mollard et al. 2011; Wong et al. 2009). The questionnaire consisted of 100 mm lines affixed with opposing descriptions at either end for each question. Participants marked an "X" on the line to depict their feelings at each time point. Scores were determined by measuring the distance (mm) from the left starting point to the intersection of the "X."

4.3.4 Session Protocol

Participants were asked to fast overnight for 10 hours and consume a standardized breakfast 4 hour prior to the start of the session. The interval between breakfast and the treatment session was kept constant. Participants were allowed to consume water until one hour before the session. The day before the session, participants were asked to consume the same type and quantity of foods the night before each session and participate in their regular routine.

Upon arrival at the sessions, subjects completed questionnaires assessing recent food intake and physical activity (Appendix 6). They also were asked additional questions concerning their previous night's sleep and if they were experiencing any stress (Appendix 7). If they reported significant deviations from their usual sleep pattern, they were rescheduled. Following the questionnaires, a blood sample was taken by finger prick by a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and blood glucose assessed by a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). If there were any reported significant deviations from the participants' usual blood glucose pattern or if their level was > 5.6 mmol/L, they were rescheduled.

Participants then completed a motivation-to-eat visual analog scale (VAS) to measure subjective appetite (Appendix 8). Additional VAS questionnaires were administered to measure palatability (taste, texture) (Appendix 9), physical comfort (Appendix 10) and energy/fatigue (Appendix 11). Prior to treatment consumption, participants completed VAS questionnaires.

Following initial questionnaires, VAS, and finger prick blood glucose measurement, an indwelling intravenous catheter was inserted in the hand or arm by a registered nurse for blood sampling. Following baseline measures, participants consumed the pulse product (within 8-10 minutes kept constant for each participant), followed by the completion of a VAS questionnaire

assessing palatability. Blood sampling took place at 15, 30, 45, 60, 90, immediately after exercise, 120, 135, 150, 185, 210 and 240 minutes. Appetite VAS questionnaires were completed following each blood sample.

At 60 minutes, participants completed a treadmill endurance session which consisted of either running or walking. The exercise session lasted a maximum of 50 minutes and intensity was set at moderate intensity (established during VO_2 max test). During the exercise session, exercise intensity (oxygen consumption) and substrate oxidation (respiratory quotient) were measured three times: 0-5 minutes, 15-20 minutes and at 35 minutes, at which the mask stayed on until exercise session was complete. Heart rate was recorded every 15 seconds to monitor exercise intensity and give a measure of fatigue (cardiovascular drift). blood glucose was measured at 60 minutes (before exercise), during the exercise session at 90 minutes, immediately after completing exercise and at 120 minutes.

For measurement of food intake, participants were served an ad libitum meal at 240 minutes. Fresh hot pizza (McCain® Deep 'n Delicious®) was served every 5-7 minutes and participants were asked to eat until they were "comfortably full". Energy intake from the pizza meal was calculated from the weight consumed and the compositional information provided by the manufacturer. Once they finished eating pizza, participants rated its palatability to ensure that the pulse products did not influence the taste/enjoyment of the pizza.

4.3.5 Ethics

This study followed the Declaration of Helsinki guidelines and all procedures involving human participants were approved by the University of Manitoba Research Ethics Board. Informed written consent was received from each participant prior to their participation. Clinicaltrial.gov registration number was NCT02370927.

4.3.5 Sample Analysis

Blood samples were collected through intravenous catheters inserted by a registered nurse and allowed to sit at room temperature for 15-30 minutes. The blood samples were centrifuged at 1300g for 10 minutes and serum fraction pipetted into micro-tubes continuously throughout the participants' session. Serum samples were allowed to rest in fridge to cool before storing in a -80C freezer.

Serum glucose was measured by colorimetric slides on an Ortho Clinical Diagnostics (Raritan, New Jersey) Vitros 350 Auto-Analyzer. Quality controls were run each day prior to testing samples to ensure accuracy and precision of the measurements. Twenty percent of samples were performed in duplicate for additional precision confidence and if coefficient of variation was >10.5, then the sample analysis was repeated.

Serum insulin was measured by radioimmunoassay in duplicate using MilliporeSigma (Etobicoke, Ontario) Human Insulin Specific RIA Kits (125 I-Insulin). Quality controls were run with each kit to ensure accuracy and precision of the measurements. All samples were performed in duplicate. If the result was >199.99 uGu/mL the sample would be repeated and diluted. If the coefficient of variation was >10.5, then the sample analysis was repeated.

4.3.6 Statistics

A sample size of 26 was determined based on previous literature on acute trials assessing the effects of foods and food ingredients on food intake following a similar acute design (Mollard et al. 2011; Mollard et al. 2012). A power analysis for a within-participant design from previous studies (Hamedani et al. 2009; Wong et al. 2009) indicated that a sample size of 26

would be sufficient to detect a treatment effect on food intake of 150 kcal, with a power of 0.80 and an alpha of <0.05.

Individual average treatment palatability, subjective appetite, physical comfort, and energy/fatigue were calculated from the average of the questions on their corresponding VAS questionnaire. Postprandial glucose and insulin responses were measured by netAUC and analyzed using the trapezoid method and included areas over and under baseline values

When there were timepoint values missing (other than baseline, peak and last timepoint) AUC was calculated using Prism GraphPad (GraphPad Software, La Jolla California USA). Cumulative, pre- and post-exercise netAUC for blood glucose , insulin, and subjective appetite were calculated for 0-240 minutes, 0-60 minutes and 120-240 minutes, respectively. Repeated measures ANOVA was used to determine the effects of treatments, time and the time-by-treatment interaction on blood glucose , insulin and subjective appetite, physical comfort, and energy/fatigue scores over the time of the study followed by repeated measures ANOVA to determine the effects of treatment at specific time points. The effect of treatments on food intake at the meal and on blood glucose , insulin, appetite, physical comfort, and energy/fatigue AUC were determined by repeated measures ANOVA. Sex, session and $V0_2$ level were included as covariates in all analyses. Tukey-Kramer post-hoc tests were used to describe mean differences across treatments. Statistical analyses were performed using SAS 9.3 (SAS Inc., Chicago, IL, USA).

4.4 Results

4.4.1 Participant Characteristics

Thirty participants were recruited for the study with 27 completing. Fifteen male participants completed the study with mean age of 21.7 ± 3.5 y, mean BMI of 24.5 ± 2.4 kg/m^2 and mean $V0_2$ max of 51.8 ± 6.5ml/kg/min. Twelve female participants completed the study with mean age of 22.6 ± 3.6 y, mean BMI of 22.6 ± 2.0 kg/m^2 and mean V02 max of 43.2 ± 7.1 ml/kg/min. Participants withdrew from the study because of the time commitment (n=1), moved away (n=1) and didn't like the product (n=1). Participants mean blood glucose calculated using the baseline value from the first session for each participant was 4.6 ± 0.7 mmol/L.

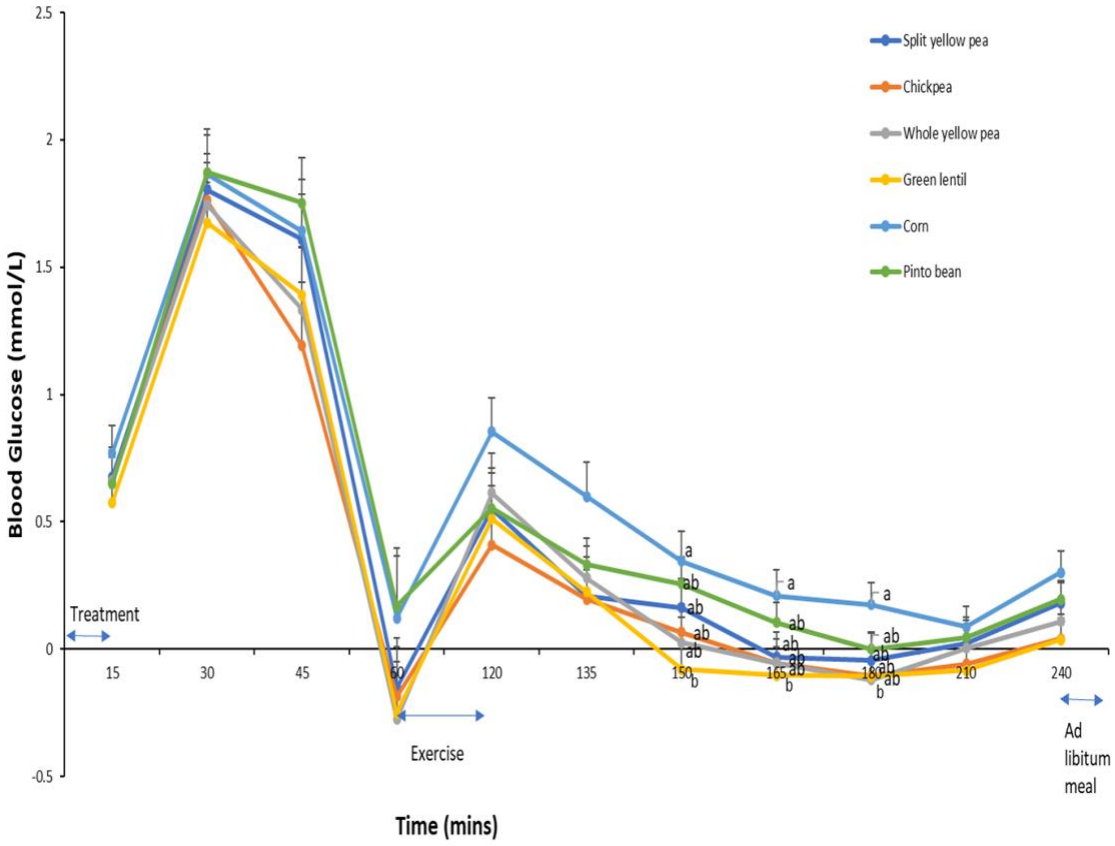
4.4.2 Blood Glucose

There was a significant time ($p<0.0001$), treatment ($p=0.002$), time-by-treatment ($p<0.0001$), treatment-by-session ($p<0.0001$), and treatment-by-$V0_2$ ($p=0.0047$) effect over the entire session (Figure 5). Thirty minutes after treatments were consumed blood glucose peaked. Pre-exercise time ($p <0.001$) treatment ($p=0.0098$) and time-by-treatment ($p<0.001$). Post-exercise there was a time ($p<0.0001$) and time-by-treatment ($p<0.0001$) effect but no treatment ($p=0.80$).

The effects on blood glucose at specific time points were dependent upon pulse type. At 30 min blood glucose was lower ($p<0.0001$) following protein + fibre + starch and protein + fibre compared to control and starch. However, control blood glucose was lower than protein + starch. At 45 min, blood glucose was lower ($p<0.0001$) following protein + fibre + starch, protein, and protein fibre compared to control. Control and starch blood glucose were lower than starch+ protein. Whereas at 60 min, blood glucose was lower ($p<0.0015$) following starch, protein, protein + starch, protein + fibre and protein fibre + starch compared to control. Control blood glucose was

lower than protein + starch though. There was a 120 min treatment effect (p=0.023) but post hoc analysis did not identify differences among treatments. Pre-exercise netAUC was lower following the protein+ fibre + starch and protein + fibre cereals compared to control (p<0.05). In contract, post-exercise blood glucose netAUC showed no differences among treatments (p=0.14). Cumulative AUC there were no differences in blood glucose netAUC (p=0.19). (Table 14).

Figure 7. Blood glucose response to extruded pulse snacks and exercise over 240 minutes[1]



[1]Note: All values are means ± SE (n=27). Values in the same column with different lowercase letters are significantly different from each other, p<0.05.

Table 14. Blood glucose net area under the curve for different extruded pulse cereals (mmol.min/L)

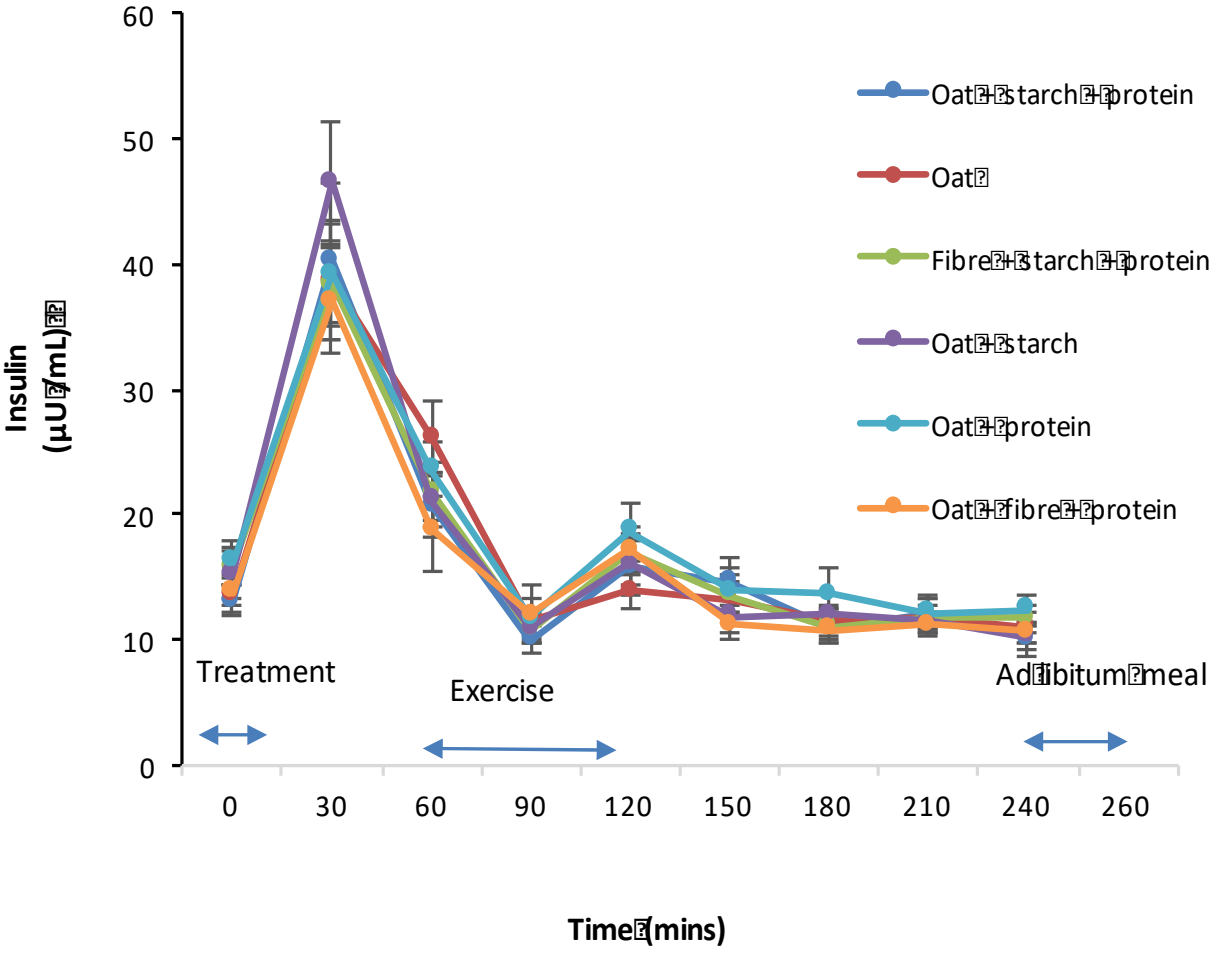
Treatments	Pre-exercise netAUC	Post-exercise netAUC	Cumulative netAUC
Oat	50.1±7.9	2.91±35.61	87.9± 18.9
Oat + starch	32.0±7.6	70.40±30.88	32.3±22.6
Oat + protein	25.3± 5.3	89.72±25	47.2±20.3
Oat + starch + protein	23.6±4.8	65.28±23.92	41.1±22.1
Oat + fibre + protein	23.7 + 5.9	52.17 + 28.95	44.1 + 17.3
Fibre + starch + protein	15.5±4.7	67.57± 28.75	32.2±19.6

Note: All values are means ± SE (n=27).Values in the same column with different lowercase letters are significantly different from each other, $p<0.05$..Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min.

4.4.3 Insulin

Insulin showed only a time ($p<0.001$) effect, but no treatment ($p=0.53$), time-by-treatment ($p=0.19$), sex ($p=0.06$) or treatment-by-$V0_2$ ($p=0.10$) interactions over the entire session (Figure 6). Pre-exercise values also showed only a time ($p<0.001$) effect, but no treatment ($p=0.08$), time-by-treatment ($p=0.08$) or treatment-by-V02 ($p=0.43$). Post-exercise values exhibited significant time-by-treatment ($p=0.0004$), sex ($p=0.0064$) and treatment–by-session ($p=0.0017$) interactions in insulin, but no treatment ($p=0.06$) or treatment-by-V02 ($p=0.29$) effects were observed. No differences were identified for insulin in pre-exercise netAUC ($p=0.30$), post-exercise netAUC ($p=0.66$), or cumulative netAUC ($p=0.78$) (Table 15).

Figure 8. Serum insulin response to extruded pulse cereals and exercise session over 240 minutes[1]



[1]Note: All values are means ± SE (n=27).

Table 15. Insulin area under the curve for different extruded pulse cereals and exercise session (µU·min/mL)

Treatments	Pre-exercise netAUC	Post-exercise netAUC	Cumulative netAUC
Oat	468.5± 68.1	-2120.3± 427.3	894.4± 328.2
Oat + starch	493.5± 55.9	-1455.5± 293.2	618.7± 298.4
Oat + protein	401.7± 60.1	-1607.3± 240.5	535.1± 235.7
Oat + starch + protein	462.6± 86.4	-1444.3± 290.4	914.6± 222.3
Oat + fibre + protein	385.4± 82.7	-1177.5± 588.8	599.0± 358.7
Fibre	381.4± 52.8	-1494.6±334.4	338.6± 271.4

Note: All values are means ± SE (n=27). Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min.

4.4.4 Food and Water Intake

There was no effect of treatment on food intake (p=0.86) or water intake (p=0.43). (Table 16)

Table 16. Food & water intake at second ad libitum pizza meal

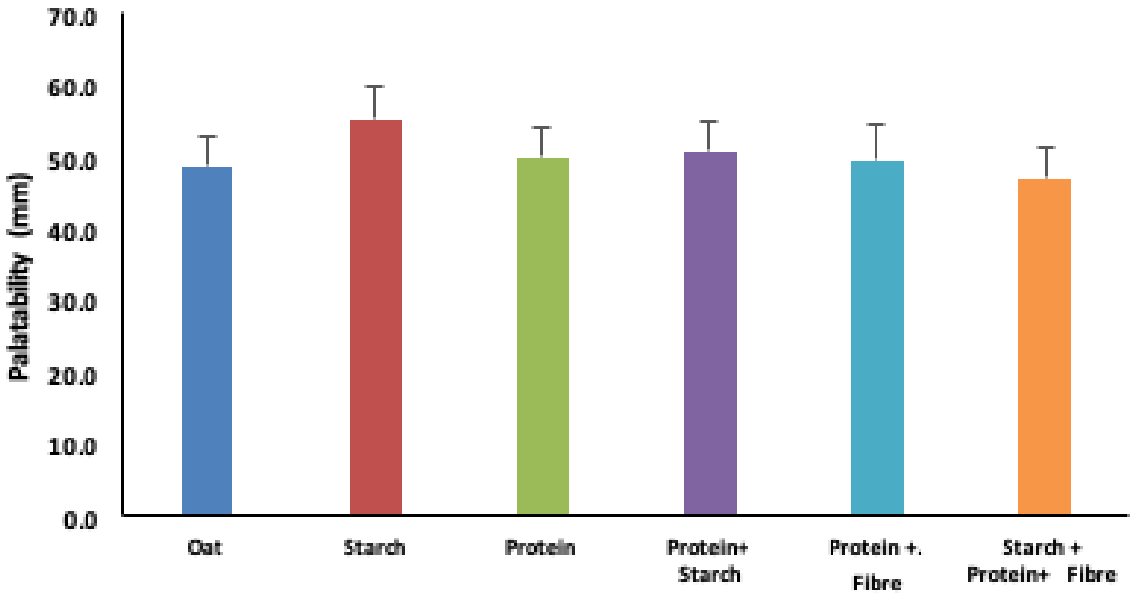
Treatments	Food intake (kcal)	Water intake (g)
Oat	1241.1± 101.4	504.1±47.1
Oat + starch	1259.2±83.1	539.1±39.9
Oat + protein	1190.1± 78.9	489.9±37.5
Oat + starch + protein	1213.7 ± 74.0	509.1±34.9
Oat + fibre + protein	1185.9 ± 86.2	491.3±40.9
Fibre + starch + protein	1219.5±83.1	501.1±38.3

Note: All values are means ± SE (n=27). Measured at an ad libitum pizza meal consumed at 240 min. Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min.

4.4.5 Palatability

There was no effect of treatment on palatability (p=0.43).

Figure 9. Extruded pulse cereal treatment palatability ratings[1]



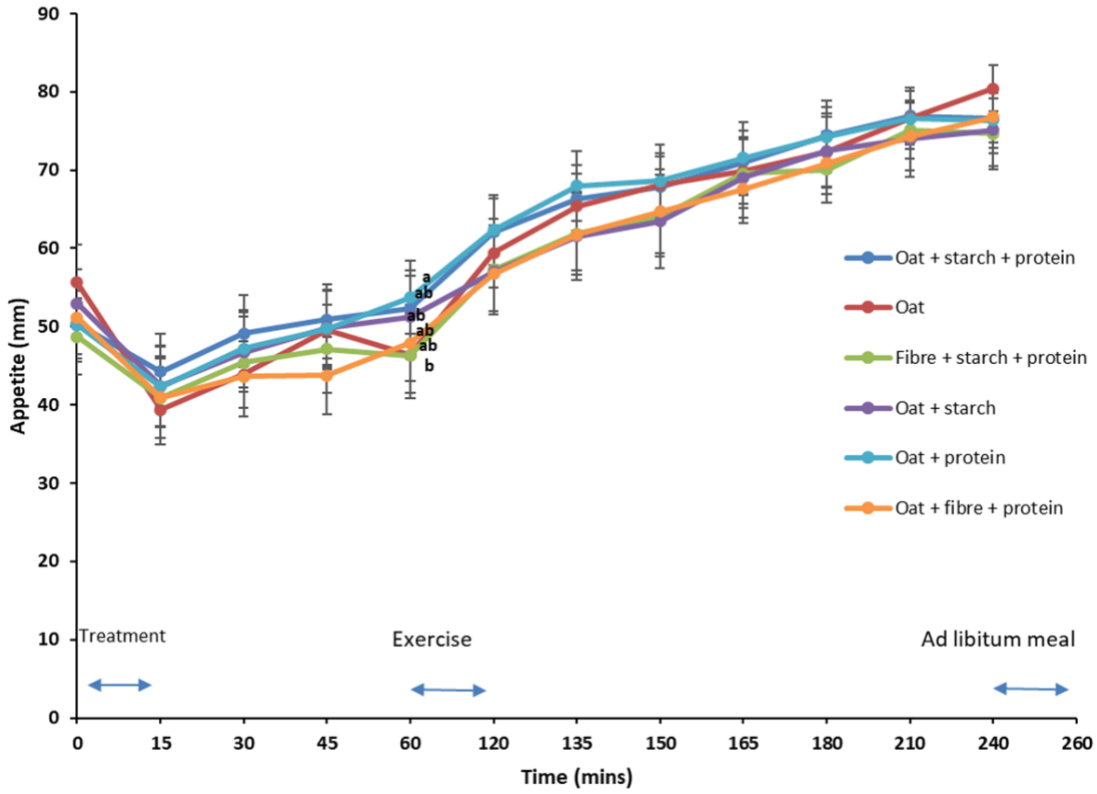
[1]Note: All values are means ± SE (n=27).

4.4.6 Appetite

There was an effect of time (p <0.0001), treatment (p=0.0271), session (p=0.0012), treatment-by-sex (p=0.0131), and treatment-by-session (p<0.0001) over the entire session on total appetite. There was a time (p<0.0001), time-by-treatment (p=0.03) for pre-exercise appetite. Whereas for post exercise appetite scores, there were time (p<0.0001), time-by-treatment (p=0.03) and treatment-by-session (p=0.03) interactions. At 60 min the treatment with protein led to lower appetite scores compared to control. At 180 min there was also a treatment effect (p=0.03) but

post hoc did not identify any differences among treatments. (Table 17). Pre- exercise netAUC (p=0.11), post-exercise netAUC (p=0.12) and total exercise netAUC (p=0.17) showed no differences between cereals.

Figure 10. Subject appetite response to extruded pulse cereals and exercise over 240 minutes[1]



[1]Note: All values are means ± SE (n=27). Values in the same column with different lowercase letters are significantly different from each other, $p<0.05$.

Table 17. Appetite area under the curve for different pulse cereals and exercise session (mm/min)[1]

Treatments	Post-exercise netAUC	Post-exercise netAUC	Cumulative netAUC
Oat	50.1±7.9	3447.6± 518.3	1061.3± 691.4
Oat + starch	32.0±7.6	2326.7± 305.1	1860.2± 497.0
Oat + protein	25.3± 5.3	2565.9±648.0	3131.9± 721.6
Oat + starch + protein	23.6±4.8	2535± 485.5	2714.6± 776.9
Oat + fibre + protein	23.7 + 5.9	2835.8± 573.7	1777.7± 646.1
Protein + fibre + starch	15.5±4.7	2917.9± 488.4	2123.4± 633.1

[1]Note: All values are means ± SE (n=27). Pre-exercise netAUC was calculated from 0-60 min. Post exercise was calculated from 120-240 min and cumulative was 0-240 min.

4.5 Discussion

This study investigated extruded cereals containing pea fractions in different combinations on the response of blood glucose , insulin and appetite following an aerobic exercise session and food intake two hours following the exercise session. The treatments protein + fibre and protein + fibre + starch resulted in the lowest blood glucose response, with control (oat) having the highest blood glucose response before exercise. No differences were found after exercise between treatments.

In response to all treatments, blood glucose peaked at 30 min and was lowest at 60 min compared to the entire session. During exercise, blood glucose climbed and peaked at 120 min before dropping again. The cereals containing protein resulted in the lowest blood glucose response whereas control as well as the starch cereal resulted in the highest. The cereals protein + fibre and protein + fibre + starch had lower amounts of available carbohydrates compared with control or starch. They also contained the most resistant and insoluble fibre compared to other treatments. Protein amounts were similar between all treatments with the exception of control and starch, having less than half the amount protein compared to the other treatments. A similar study from our lab, by Johnston et al. (2021) used the same treatments as our current study but followed a different protocol. Protocol differences included timing of the second meal; the second meal in Johnston et al. was served at 120 mins, whereas in the present study it was served it at 240 mins. Also, the Johnson study did not include exercise; our study had a 1-hour exercise session from 60-120 min in the study session. Johnson et al. found pre-meal protein + fibre and protein + fibre + starch and protein to have a lower blood glucose response compared with control, and starch post- meal blood glucose differences. The results from this current study are consistent with the Johnston *et al* findings (2021).

Smith et al (2012) assessed the effect of yellow pea protein, served in a soup, on glycemic response. They reported that doses of 10g and 20g of protein reduced pre-meal mean blood glucose with a second meal served at 30 min, but no differences found when served at 120 min. Only the 20g treatment of protein reduced blood glucose post meal. The treatments were not matched in calories, as the 20g protein treatment had 409 kcal more than the control. In our study kcals were closely matched (a maximum difference of 29 kcals between treatments). Chan et al. (2019) found when faba bean protein concentrates and protein isolate was added into a wheat pasta it led to lower blood glucose and appetite but no insulin or GLP-1 differences. High starch fractions or the addition of 25% fava bean split flour in the pasta did not reduce blood glucose. These findings support protein as a fraction having blood glucose lowering effects, as well as the benefits of protein being dose dependant. Another study investigating the effects of pea fractions on blood glucose control by Mollard et al (2014) found consistent results; yellow pea protein but not fibre alone reduced the blood glucose response. However, when consumed together protein and fibre produced a synergistic effect, lowering blood glucose more than the fractions alone. Our findings are consistent with this, as we found protein + fibre reduced blood glucose greater than protein or fibre alone. All the treatments containing protein resulted in a decrease in blood glucose , however when combined with fibre there is a greater impact. When fibre and protein are combined the post-prandial glycemic benefit may be due to protein stimulation of insulin, gastric emptying as well as fibres effect on carbohydrate and protein absorption (Mollard et. al 2014). Future studies should further investigate this synergistic relationship.

No differences were found for insulin pre or post exercise in this study. However, Johnston et al. (2021) found insulin differences in the pre-meal period (0-120 min), fibre +

protein had a lower insulin response compared to the oat control. The two treatments with pea fibre had the lowest insulin responses in Johnston's study. Study protocol differences in blood draw timing may have produced variable insulin results between the studies.

Appetite was lower following protein compared to control at 60 min. However, these differences in appetite did not translate into food intake differences hours later. There were no palatability differences found in this study or Johnston et al. (2021) indicating that the palatability of the treatments did not impact appetite or food intake. The lack of an effect on food intake could be dues to the timing of the meal. Johnston et al (2021) found no differences in appetite before or after a meal served at 120 min or food intake differences at 120 min. Smith et al (2012) found no food intake differences for 10g protein however 20g protein reduced food intake compared with control when a second meal was served at 30 min, but no differences when served at 120 min. The author suggests if the second meal was consumed sooner, differences may have been detectable, which may also explain why this trial did not see differences in food intake.

Our findings align with the previous notion that protein is more satiating compared to carbohydrate or fat (Holt et al. 1995; Chambers, McCrickerd and Yeomans 2015). The lack of an effect on food intake could also be due to the dose of pulse fractions. Other acute studies have reported varied results on pulses ability to suppress food intake. Johnston et al (2005) and Mollard et.al (2014) both failed to see any food intake or appetite changes when feeding pulse fractions. The Johnston study (2005) used chickpea flour in a white bread and Mollard used pea fibre (2014), protein or hull fibre consumed with noodles and pasta sauce. The dose of pulse fractions in previous studies may not have been enough to see an impact on food intake. Another potential explanation for the lack of an effect on food intake may be the fact that pulses were

provided as fractions and/or the pulse type. In response to consumption of whole pulses, Mollard et al (2011) found appetite and food intake was reduced after being served whole pulses in a mixed meal, however it was depended on pulse type. They found that participants served lentil and yellow peas 4 hours before an ad libitum meal had suppressed appetite, but chickpea did not. Yellow pea and lentil significantly lowered food intake and pre-pizza meal appetite compared with control. Future research could include investigate the effects of pea fractions on food intake within 30 – 60 min following exercise, the optimal amount of pulse fractions for appetite and food intake control and well as the type of pulse (i.e. yellow pea vs. chickpea vs. lentil).

Exercise length and intensity may play a role in why no food intake differences were found. Moderate intensity (50-70% Vo_2 max 30-90 min) as well as high intensity > 75% Vo_2 max both may suppress hunger and energy intake via changes in hormones, but studies have shown conflicting results (Mollard et al. 2011; Schubert et. 2012). CCK, GLP-1, Ghrelin and PYY are all hormones which can influence glucose and appetite regulation. A review done by Schubert et al. (2012) reported exercise does not change post-exercise food intake but results may vary in different exercise modes, intensity and different populations. More research into this area is necessary.

Limitations in this study were the inclusion of only healthy young individuals. Further research is needed on the health effects in other populations, such as those who are obese, have diabetes or metabolic syndrome. Another limitation is that only yellow pea fractions were used in this study and other pulse types need to be examined to see if their benefits are retained when used as fractions in food ingredients while still considering palatability. Finally, hormones were not examined in this study and are a critical part of understanding glycemic regulation. Strengths

of this study include the inclusion of both males and females, use of a randomized double-blind crossover design, and inclusion of exercise.

Our results support the low glycemic and high satiety properties of pulses when used as fractions in extruded food products. Incorporation of pulse protein into extruded cereals results in a lower post-prandial glycemic response compared with an oat cereal, however no differences between treatments were seen following exercise. Future studies should investigate different serving sizes/doses of pulse fractions, timing of post exercise meals, exercise length and intensities. Combing the effects of nutrition and exercise together may help to reduce post-prandial glucose levels to manage one's long term risk of developing obesity, diabetes, or CVD. Incorporating yellow pea fractions, into convenient ready-to-eat foods that are shelf stable, may allow for increased pulse consumption and health benefits to Canadian consumers and increased marketing for pulse growers.

Chapter 6: Conclusions

The research presented investigated the acute effects of consumption of an extruded snack or cereal product containing pulse ingredients on blood glucose , insulin, appetite and food intake, following a 60 min aerobic exercise session, compared to the ingestion of a non-pulse food. Trial I demonstrated pulses reduce glycemic response more than corn following an exercise session but are dependent on type. Green lentil led to significantly lower blood glucose at 150 min compared with split yellow pea, and again at 165 and 180 min compared with corn (control). Split yellow pea and chickpea led to lower pre-exercise appetite. No insulin differences were found in this study. Trial 2 found blood glucose to be significantly lower following the protein + fibre + starch and protein + fibre cereals compared to control pre-exercise. Post-exercise blood glucose did not have any differences between treatments. The cereal with protein led to lower appetite scores at 60 min. No food intake or insulin differences were seen in this study as well. This may be to the treatment serving size or timing of second meal. Overall, this research agrees with previous literature and supports at acute consumption of pulses and pulses ingredients for the control of postprandial glycemia. The synergistic effect produced when pea fibre and protein are consumed together on blood glucose response should be investigated further. Varying types of exercise and intensities should be examined on post-prandial glycemia. Incorporating pulse ingredients into a ready-made product will allow for increased consumption as well as increased nutritional value of the products. Additionally, increasing demand for pulses will support Canadians pulse growers and processors.

Chapter 7: Future Directions

The results from this study shows pulses can be value added food-ingredients and provide glycemic benefits before and after an exercise session. Replacing the commonly used corn or oat flour in food products with pulses will create a more nutritious product. Food intake differences were not detected in our studies; however, this may be due to the amount of pulse ingredients. Therefore, determining the highest amount of pulse ingredients that can be incorporated into an extruded product without compromising palatability is needed.

There are multiple hormones that are involved in the regulation of glycemic and appetite control. Additionally, there is little research on how exercise affects appetite and satiety. Investigating these hormones to better understand pulse ingredients influence on glycemic and appetite control will support improved weight management strategies. Examples of these hormones include glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), leptin, ghrelin, C-peptide, peptide tyrosine tyrosine (PYY) and cholecystokinin (CCK). Also, there is little research on how exercise- induced responses in blood glucose or appetite regulating hormones differ between sexes or different ages.

There are several studies that compare low and high glycemic index foods effects on exercise performance and post-exercise glycemia. No research to date has been conducted on pulses or extruded products prior to an exercise session on glycemic and satiety control. Our research is novel and will add to the literature. The timing of the pizza meal, timing between treatment and exercise as well as exercise intensities needs to be investigated further. Food products using pulse ingredients will be more nutritious, with higher protein and fibre content compared to corn or oat, as well as more satiating than alternatives currently on the market.

Chapter 8: References

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Appendix 1

Poster, Version 1
Appendix 1
October 6th, 2014

UNIVERSITY OF MANITOBA

Your Health Begins With The Food You Eat!

The University of Manitoba is looking for participants for a study examining the effects of healthy food products on:

- **Blood Sugar**
- **Food Intake**
- **Appetite**
- **Exercise Endurance**

For more information please call (204) 480-1042

Visit us online at: www.rcffn.ca

Richardson Centre for Functional Foods and Nutraceuticals 196 Innovation Drive · University of Manitoba · Winnipeg, MB · R3T 6C5

Dr. Peter Jones and Dr. Danielle Bouchard, Principal Investigators

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Appendix 2

RESEARCH SUBJECT INFORMATION AND CONSENT FORM

Pulse EnRiched Food and Exercise Clinical Trials; PERFECT project

Part 2. Acute effects of pulse ingredients in food products on aerobic endurance and substrate oxidation during exercise, as well as, the blood glucose, insulin, lactate and food intake response following an endurance exercise session in adults.

Protocol number: B2014:114 (2)

Investigators:
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Winnipeg, Manitoba R3T 6C5
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318 Max Bell Centre, University of Manitoba
Winnipeg, Manitoba, R3T 2N2
Phone: 204-474-8627

Sponsors: Saskatchewan Pulse Growers, 116 Research Dr, Saskatoon, SK, S7N 3R3
Alberta Pulse Growers, 5007B - 49 Avenue, Leduc, AB, T9E 6M6

The following consent form is for three studies; however you are only agreeing to participate in one of the three studies. You are consenting to participate in (identified with a check mark)

☐ Study 1: Extruded snack products, 6 sessions

☐ Study 2: Extruded cereal products: 6 sessions

☐ Study 3: Bagels: 6 sessions

If you agree to participate in the above checked study, you are not obligated to engage in the entire study or the other two studies. An additional consent form will need to be signed before starting any other studies. Please take your time to review this Information and Consent Form and discuss any questions you may have with the study staff. You may discuss it with your regular doctor, friends and family before giving consent. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

Purpose of study
Many Canadians suffer from high blood sugar, high blood pressure, obesity, and high cholesterol. When combined these are related to a chronic condition called metabolic syndrome, which has the potential to be treated by changing what we eat and how much we exercise. This study will test the effect of different food products made from pulses (beans, yellow peas, chickpeas, lentils) on risk factors of metabolic syndrome.

Pulses (lentils, chickpeas, yellow peas and beans) are produced into flours and fractions (protein, fibre, starch). These flours and fractions are used as ingredients in foods. This project includes studies that will investigate the effects of three different food products containing pulse ingredients on appetite, blood sugar control, food intake and exercise in generally healthy adults. A total of 30 participants will participate in each acute study.

Screening procedures
To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, smoking habits, eating habits, exercise habits, your health, and if you are on any medications. Your height and weight will be measured. We will also measure your blood sugar using a finger prick blood sample.

Study procedures
If you can take part, you will be required to attend sessions at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) for about for a maximum of 5 hours. Men will be seen once a week over six weeks. Women will be seen during the follicular phase of their menstrual cycle (the 2 weeks following menstruation), 6 times over 12 weeks.

One week before starting the sessions, following an overnight 10 hour fast (no eating or drinking for 10 hours except water), you will be asked to come to the RCFFN to assess how many calories you burn at rest. This test is called resting metabolism and will take about 30 minutes. After receiving a continental breakfast, your maximal exercise capacity will be assessed with a treadmill test. During the treadmill test we will determine how much oxygen you consume while doing the test. The test should take between 10 and 15 minutes and the speed of the treadmill will be adjusted according to your fitness level. The treadmill session will be conducted by a Certified Exercise Physiologist who is trained to do exercise with people at risk. After this visit that will last a total of 60 minutes, you will be scheduled for your next session. Before leaving the facility, you will receive two tools to wear for the next seven days to measure your physical activity level and your next visit (session) will be scheduled.

Before each session, you will be asked to fast for 10 hours the night before (no eating or drinking for 10 hours except for water) and then eat a breakfast in the morning four hours before you meet with us. Please only eat the breakfast provided before meeting with us in the morning. You will be asked to stick to your normal routine, including exercise and to eat a regular meal the evening before each session. You can drink water up to one hour before meeting with us. You

will receive your breakfast a day or two before the session. You will be asked to arrive at the RCFFN between 10:45 am to 12:45 pm on the day of the session. When you arrive one finger prick blood sample will be taken to measure blood sugar.

At each session, you will be asked to eat a novel food product (snack, cereal, bagel), give blood samples and to complete questionnaires at the times outlined in the table below. The order of the food products you will receive will be assigned by chance, but you will receive them all by the end of the study. Blood draws will be used to measure blood sugar, lactate and hormones that control your blood sugar, including insulin. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite, energy level and physical comfort as well as how much you liked the treatment and pizza throughout the study session.

A total of 14 blood samples will be taken during each experimental session. To obtain blood samples, a trained phlebotomist will insert a catheter (a needle attached to a plastic tube) into a vein in your arm. The catheter will remain in your arm and be used to sample blood in small amounts during the session so you only have a catheter inserted one time per session. After the phlebotomist collects the first sample at baseline (0 minutes), you will consume one of the foods within five minutes. After you finish, we will collect blood samples at multiple time points over the next hour and then you will complete a treadmill endurance test. Each session will last up to 5 hours.

Following the exercise test, blood will be sampled and questionnaires will be completed for another two hours. After that a pizza meal will be served to measure food intake. You will be asked to consume pizza until you are comfortably full.

Time	**Activity**
7:00	Consumption of breakfast (provided 1-2 days before)
10:45	Arrive at the laboratory
10:50	Fill in food intake, physical activity, sleep, stress, and VAS questionnaires and take first blood sample
11:00-11:05	Eat the treatment (0 minutes)
11:15-12:00	Blood sampling and VAS questionnaires at 15, 30, 45, and 60 minutes
12:00-1:00	Exercise with one blood sample during exercise and one as soon as you are done exercising
1:00-3:00	Blood sampling and VAS questionnaires at 120, 135, 150, 165, 180, 210 and 240 minutes after baseline
3:00-3:20	Eat the ad libitum (eat until comfortably full) pizza meal

Risks and discomforts

As with any study, there may be some risks of taking part. You may feel dizzy following the overnight fast, but this is rare. If this happens, you will feel fine once you eat the breakfast provided to you.

Some discomfort might be experienced as a result of a sharp momentary pain caused when the venous catheter or syringe needle is put into your arm by the phlebotomist. The pain felt will be similar to skin puncture during vaccination or if a blood sample is taken by a needle at your doctor's office. There is very little risk of infection. Before the catheter or needle is inserted, the area is cleaned with antiseptic (alcohol) by the phlebotomist. There might be slight bruising under the skin, but this will be minimized by applying pressure after the catheter or needle is removed. The amount of blood taken during each visit is 70 ml (2.3 ounces), which is 420 ml (13.2 ounces) for the entire study. These amounts are lower than the amount collected in a single blood donation (450 ml or 15.2 ounces). However, we recommend that you do not donate blood during or within one month of the end of the study. You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the treatments. This is more likely if you are not used to eating pulses, however this is rare and there is no health risk linked with these effects. During the treadmill test or the exercise sessions, risks will be reduced because a Certified Exercise Physiologist will perform the test. Also, the test will be stopped when you reach your maximum capacity (until you cannot go any longer). In addition, during the exercise sessions, the intensity will be aligned with your fitness level. As when you go for a workout, we are asking you to wear running shoes during the test and the exercise sessions.
There is always a possibility that you will become ill following consumption of food, but that is very unlikely in this study. The pizza is freshly prepared at the time of your session. The pizzas are stored frozen and cooked accordingly to the manufacturer's instructions immediately before you are served.

Benefits
You may not directly benefit from participation in this research; however, the study should contribute to a better understanding of the effects of pulse products on blood sugar and appetite. You will also have the opportunity to try new novel food products and may benefit from these food products if they become available on store shelves. You will also receive supervised exercise sessions for 6 weeks and you may feel energized. We will also provide you with a summary of the findings of the study once it is done.

Costs
There will be no cost to participate in this study.

Remuneration for participation
You will receive $36 per session. If you do not complete all sessions, you will be paid for the number of sessions you completed

Alternatives
You are not obligated to participate.

Confidentiality
Personal records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba. The RCFFN staff involved with your care may

review/copy information that may reveal your identity. The Biomedical Research Ethics Board at the University of Manitoba may also review your research-related records for quality assurance purposes. If the results of the trial are published, your identity will remain confidential. Personal information such as your name, address, telephone number and/or any other identifying information will not leave the RCFFN.

Blood samples will be stored in a locked freezer at the RCFFN. Only the study coordinators and the principal investigator will have access to the samples. Your samples will not be stored for any longer than 5 years, nor shared with any other group, other than is indicated in the protocol, without your prior specific consent.

Stored samples will be labelled with your participant code and the date of collection. They will not be labelled with any of your identifying information. These samples will be retained for study outcome analyses, as well as the potential analyses of hormones related to the regulation of blood sugar and appetite.

Voluntary participation/withdrawal from the study
Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time.

Your participation in this study may be terminated without your consent by the study coordinators, ~~physician~~ or principal investigator. The study staff will withdraw you if he/she feels that participation is no longer in your best interest, or if you fail to follow the directions of the study staff.

If you decide to participate, you will agree to cooperate fully with the study visit schedule, and will follow the study staff's instructions.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

Should you wish to withdraw your participation from the study, you must inform the study coordinators so that your file can be officially closed. Your previously collected data will be used unless you request otherwise by sending that request to the study coordinator Dr. Rebecca Mollard an email at Rebecca.Mollard@umanitoba.ca or call her at 204-474-8270.

Medical care for injury related to study
In the event of an injury that occurs to you as a direct result of participating in this study, or undergoing study procedures you should notify the principal investigator or study coordinator or go to your nearest emergency room to receive necessary medical treatment. You are not waiving any of your legal rights by signing this consent form nor releasing the investigator or the sponsor from their legal and professional responsibilities. If any health abnormalities are identified in the clinical tests conducted during this experiment, the principal investigator or study coordinator will be contacted, who will inform you of the results.

Questions
You are free to ask any questions that you may have about your treatment and your rights as a research subject. If any questions come up during or after the study or if you have a research-related injury, contact the study doctor and the study staff.

Investigator:	Dr. Peter Jones	Tel No.	204-474-9787
Investigator:	Dr. Danielle Bouchard	Tel No.	204-474-8627
Coordinator:	Dr. Rebecca Mollard	Tel No.	204-474-8270

For questions about your rights as a research subject, you may contact:

The Biomedical Research Ethics Board, University of Manitoba at 204 789-3389

Do not sign this consent form unless you have a chance to ask questions and have received satisfactory answers to all of your questions.

This study is registered on a publicly available Registry Databank at Clinicaltrials.gov under identifiers NCT02402517, NCT02370927, and NCT02365363. ClinicalTrials.gov is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Consent
1. I have read and understood this Information and Consent Form, and I freely and voluntarily agree to take part in the clinical trial (research study) described above.

2. I understand that I will be given a copy of the signed and dated Information and Consent Form. I have received an explanation of the purpose and duration of the trial, and the potential risks and benefits that I might expect. I was given sufficient time and opportunity to ask questions and to reflect back my understanding of the study to study personnel. My questions were answered to my satisfaction.

3. I agree to cooperate fully with the study coordinator and the principal investigator and will tell them if I experience any side effects, symptoms or changes in my health.

4. I am free to withdraw from the study at any time, for any reason, and without prejudice to my future medical treatment.

5. I have been assured that my name, address and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations.

6. By signing and dating this document, I am aware that none of my legal rights are being waived.

Signature:_____ Date: _____

Printed name of above:_____

I confirm that I have explained the purpose, duration and methods of this clinical trial, as well as any potential risks and benefits, to the subject whose name and signature appears above. I confirm that I believe that the subject has understood and has knowingly given their consent to participate by his/her personally dated signature.

Signature:_____ Date: _____

Printed name of above:_____ Study role:_____

ALL SIGNATORIES MUST DATE THEIR OWN SIGNATURE

Appendix 3

Screening Questionnaire

Please type

NAME: _____

ADDRESS:
_____

_____

PHONE #: (_____)_____ E-MAIL: _____

ID assigned: _____

To be kept separately from part 2 and other study forms

Screening Questionnaire

(NOTE: After you are recruited for the study, you will be assigned an ID# which will be used on your forms and data throughout the study.)

AGE: _____ HEIGHT: _____ WEIGHT: _____ BMI: _____

Participation in Athletics/Exercise:

ACTIVITY HOW OFTEN? HOW LONG? (HOURS)

_____

_____

_____

_____

_____

_____

Do you usually eat breakfast? ☐ YES ☐ NO

If YES, what do you usually eat?

_____

Health Status:

Do you have diabetes? ☐ YES ☐ NO

Do you have any other major disease or condition? ☐ YES ☐ NO

If YES, please specify:

_____

Are you taking any medications? ☐ YES ☐ NO

If YES, please specify: _____

Do you have reactions to any foods? ☐ YES ☐ NO

If YES, please specify:

_____

Are you on a special diet? ☐ YES ☐ NO

If YES, please specify:

_____

Have you recently lost or gained weight? ☐ YES ☐ NO

If YES, please specify:

_____

Do you smoke? ☐ YES ☐ NO

How many alcoholic beverages do you consume per day? _____Per week?

_____

Appendix 4

Eating Habits Questionnaire

Choose the appropriate answer to best describe your personal situation.

1. How often are you dieting?

   Never ____ rarely _____ sometimes _____ often _____ always _____

2. What is the maximum amount of weight (in pounds) that you have ever lost within one month?

   1 - 4 _____ 5 - 9 _____ 10 - 14 _____ 15 - 19 _____ 20+ _____

3. What is your maximum weight gain within one week?

   0 – 1 ____ 1.1 - 2 _____ 2.1 – 3 _____ 3.1 - 5 _____ 5.1+ _____

4. In a typical week, how much does your weight fluctuate?

   0 – 1 _____ 1.1 – 2 _____ 2.1 - 3 _____ 3.1 - 5 _____ 5.1+ _____

5. Would a weight fluctuation of 5lbs affect the way you live your life?

   Not at all _____ slightly _____ moderately _____ very much _____

6. Do you eat sensibly in front of others and splurge alone?

   Never _____ rarely _____ often _____ always _____

7. Do you give too much time and thought to food?

   Never _____ rarely _____ often _____ always _____

8. Do you have feelings of guilt after overeating?

   Never _____ rarely _____ often _____ always _____

9. How conscious are you of what you are eating?

   Not at all _____ slightly _____ moderately _____ extremely _____

10. How many pounds over your desired weight were you at your maximum weight?

0-1 _____ 2 - 5 ____ 6 - 10 _____ 11 - 20 _____ 21+ ____

Appendix 5

Food Acceptability

Please indicate with a rating between 1 and 10 how much you enjoy the following foods **(1 = not at all, 10 = very much)** and how often you eat them **(never, daily, weekly, monthly).**

	Enjoyment?	**How often?**
Pasta	_____	_____
Rice	_____	_____
Potatoes (mashed, roasted)	_____	_____
French fries	_____	_____
Pizza	_____	_____
Bread, bagels, dinner rolls	_____	_____
Sandwiches, subs	_____	_____
Cereal	_____	_____
Cake, donuts, cookies	_____	_____
Protein/breakfast shakes	_____	_____
Chips/puffs/crackers	_____	_____

At the end of each session, you will be provided with pizza. In order to provide you with a meal that you will enjoy, we ask that you rank the following pizzas according to your **personal preferences (i.e. 1st, 2nd, 3rd choice)** in the space provided. If you **do NOT** like a particular type of pizza, then do not rank it but instead write **"I don't like"** in the space provided.

Pepperoni (cheese, pepperoni) _____
Deluxe (cheese, pepperoni, peppers, mushrooms) _____
Three-cheese (mozzarella, cheddar, parmesan) _____

Appendix 6

Recent Food Intake and Activity Questionnaire

At what time did you have dinner? _____

Please describe your dinner last night (list all food and drink and give an estimate of the portion size):

_____
_____
_____

The following three questions relate to your food intake, activity and stress over the last 24 hours. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

How would you describe your **food intake** over the past 24 hours?

Much LESS than usual _____ Much MORE than usual

How would you describe your **level of activity** over the last 24 hours?

Much LESS than usual _____ Much MORE than usual

How would you describe your **level of stress** over the last 24 hours?

Much LESS than usual _____ Much MORE than usual

To be completed by staff only.

Comments/Notes: _____

Appendix 7

Sleep Habits and Stress Factors Questionnaire

1. Did you have a normal night's sleep last night? Yes_____ No_____
2. How many hours of sleep did you have? _____

3. What time did you go to bed last night? _____
4. What time did you wake up this morning? _____
5. Recount your activities since waking:
Time Activity
_____

_____

_____
_____
6. Are you experiencing any feelings of illness or discomfort, other than those from hunger?

Today: Yes ____ No_____ Past 24 hours: Yes ____ No_____

If yes, please describe briefly:
_____
_____
7. Are you under any unusual stress? (Exams/reports/work deadlines, personal, etc.)
Today: Yes ____ No_____ Past 24 hours: Yes ____ No_____

If yes, please describe briefly:
_____
_____
8. Have you been involved in any physical activity within the past 24 hours that is unusual to your normal routine? Yes_____ No_____

If yes, please describe briefly:

_____

To be completed by staff only.

Comments/Notes: _____

Appendix 8

Visual Analogue Scales
Motivation to Eat

Time: 0 _____

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. **How strong is your desire to eat?**

VERY weak _____ VERY strong

2. **How hungry do you feel?**

NOT hungry at all _____ As hungry as I have ever felt

3. **How full do you feel?**

NOT full at all _____ VERY full

4. **How much food do you think you could eat?**

NOTHING at all _____ A LARGE amount

How thirsty do you feel?

NOT thirsty at all _____ As thirsty as I have ever felt

Appendix 9

Visual Analogue Scales
Physical Comfort

Time: 0 min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

Do you feel nauseous?

NOT at all _____ VERY much

Does your stomach hurt?

NOT at all _____ VERY much

How well do you feel?

NOT well at all _____ VERY well

Do you feel like you have gas?

NOT at all _____ VERY much

Do you feel like you have diarrhea?

NOT at all _____ VERY much

Appendix 10

Visual Analogue Scales

Energy and Fatigue

Time: 0 min

These questions relate to your energy level and fatigue at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. How energetic do you feel right now?

NOT at all _____ VERY energetic

2. How tired do you feel right now?

NOT at all _____ VERY tired

Appendix 11

Visual Analogue Scales
Palatability: Treatment

This question relates to the palatability of the beverage/food you just consumed. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present findings.

1. How pleasant have you found the beverage/food?

NOT at all pleasant _____ VERY pleasant

2. How tasty have you found the treatment?

NOT at all tasty _____ VERY tasty

3. How did you like the texture of the treatment?

NOT _____ VERY
at all much