Acute Effects of Food Products Containing Pulse Flours or Fractions, on Glycemic Response, Insulin, Appetite, and Food Intake in Healthy Young Adults

by

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ABSTRACT

Background: The glycemic benefits of consuming whole pulses alone are retained when consumed in a mixed meal, pureed, and ground into flours; however, whether pulse flours and ingredients retain the benefits of whole pulses when incorporated into extruded products is unknown.

Objective: Assess the effects of replacing corn in extruded snacks and oat in extruded cereals with pulse ingredients on postprandial glycaemia, insulin, appetite, physical comfort, energy/fatigue, and food intake.

Design: In two randomized, repeated-measures crossover studies, adults consumed six extruded snacks (n = 26) or cereals (n = 26). 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), oat plus starch plus protein (starch+protein). Participants completed validated visual analog scale (VAS) questionnaires to measure subjective appetite, physical comfort, and energy/fatigue. Blood samples were taken throughout each session and food intake was measured at a pizza meal at 120 min. Blood and VAS measures were assessed pre-pizza (0-120 min) and post-pizza (140-200 min) meal.

Results: Pinto bean and chickpea snacks led to lower (p < 0.05) pre-pizza meal blood glucose (BG) incremental area under the curve (iAUC), compared with control, whole yellow pea and green lentil snacks. Consumption of the pinto bean snack also led to lower (p<0.05) pre-pizza meal BG iAUC compared with corn control, whole yellow pea, and split yellow pea snacks.
The protein, protein+fibre, and the fibre+starch+protein cereals led to lower (p<0.05) pre-pizza meal BG iAUC compared to the starch and control. The starch+protein cereal led to a lower (p<0.05) iAUC BG response compared to starch. For pre-meal overall mean insulin, fibre+protein led to a lower insulin response compared to control (p<0.05), starch+protein (p<0.05), and protein (p=0.001) cereals. Fibre+starch+protein also led to lower insulin compared to protein cereal (p<0.05). Fibre+protein resulted in lower (p<0.05) insulin iAUC compared to control and protein cereal. There were no differences in appetite, food intake, energy/fatigue, or physical comfort in response to the extruded snacks or cereals.

Conclusion: The benefits of replacing corn or oat with pulse ingredients in extruded snacks or cereals on BG and insulin are dependent on pulse and fraction type utilized.
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LIST OF ABBREVIATIONS

ANOVA- analysis of variance
BG- blood glucose
BP- blood pressure
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
HOMA-IR- homeostatic model of assessment, insulin resistance
iAUC- incremental area under the curve
Kcal- kilocalories
PYY- peptide YY
RCT- randomized controlled trial
VAS- visual analog scale
y- year
CHAPTER 1 - INTRODUCTION

As a result of the obesity epidemic in North America, individuals are increasingly predisposed to chronic diseases such as type 2 diabetes and metabolic syndrome. Foods and ingredients that spontaneously increase satiety and reduce energy intake could be potential countermeasures. The marketplace is flooded with processed foods that are convenient, offer little nutrition, are high in calories, and do not satiate. More than half (55%) of calories consumed by Canadians are ultra-processed foods; these foods lead the consumer to eat more and do not aid in “tiding them over” until their next meal. The lack of readily available, nutritious, and satiable snacks and cereals offers few options to consumers striving to make nutritious choices to support their health. Therefore, an interest exists in developing commercially available products such as extruded snacks and cereals containing pulse ingredients so that consumers can attain the health benefits of pulses from products they buy at the grocery store.

Pulses (chickpeas, beans, lentils, and yellow peas) and pulse ingredients (fractions and flours) have many positive nutritional attributes. Regular consumption of pulses (1/2 cup/day) is related to higher quality diets, including higher intakes of fibre, protein, folate, zinc, iron, and magnesium and lower intakes of saturated and total fat compared to diets low in pulses. Pulses contain high amounts of protein which makes them somewhat unique in comparison to other plant foods. In fact, the protein content of pulses ranges from 17-30% of dry weight which is typically twice the amount found in cereals. Studies have shown that protein increases satiety to a greater extent than carbohydrate or fat and increases BG control. Pulses also contain vitamins, minerals and dietary fibre that support human health. Pulses
contain high amounts of complex carbohydrates including both soluble and insoluble fibre\textsuperscript{9}, as well as resistant and slowly digestible starch and oligosaccharides\textsuperscript{9,10}. A variety of health benefits are associated with intake of dietary fibre including increased satiety and reduced energy intake\textsuperscript{11–13}, improved glycemia 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\textsuperscript{14,15}. The high amounts of fibre, as well as resistant and slowly digestible starch in pulses contribute to their low glycemic index (GI)\textsuperscript{16–18}. The glycemic index is based upon the incremental area under a two-hour BG response curve (AUC) following a 12-hour fast and ingestion of a food\textsuperscript{19,20}. For GI studies, the test food has a certain quantity of available carbohydrate and is compared relative to a control food containing the same amount of available carbohydrate. A lower GI suggests slower rates of digestion and absorption of the carbohydrate in the test food, and usually equates to a lower insulin demand.

The effects of whole pulses have been examined alone and in mixed meals. Mixed meal studies have shown favorable effects on BG\textsuperscript{21,22,23,24}, food intake (FI) and appetite\textsuperscript{22}. Despite these glycemic control and satiety benefits, very few Canadians eat whole pulses\textsuperscript{25}. There is thus the potential that pulse ingredients may be used to develop nutritious and healthy snacks. Pulse flours and fractions are dietary ingredients that possess the potential to provide health benefits and may be used as food based strategies for the control of body weight and BG\textsuperscript{11}. Although limited, pulses have also been examined as flours in tomato sauce\textsuperscript{25} and bread\textsuperscript{26,27} and shown that the favourable BG effects of whole pulses can be retained. However, further research is needed to determine whether pulse flours and fractions do retain the health
benefits of whole pulses when incorporated into different food products. The effects of extruded products containing pulse ingredients have not yet been assessed.

Our objective is to determine whether pulse ingredients in extruded products retain the acute benefits of whole pulses. Our research is anticipated to aid in the development of extruded pulse foods. The development of pulse containing products will support Canadian pulse growers. Pulses are largely an exported commodity; thus a tremendous opportunity presents for this Canadian grown crop to be harnessed within its own economy. Our results may encourage industry to move forward with developing readily available products that contain pulse components as value added ingredients.

The following literature review examines current knowledge on pulses and their health benefits, and how those health benefits may vary depending on the type of processing pulses undergo. Human in vivo studies are the focus of the review. Potential mechanisms explaining the beneficial effects of pulses will also be discussed.
CHAPTER 2 - LITERATURE REVIEW

2.1 WHOLE PULSES - ACUTE POSTPRANDIAL GLYCEMIC and SATIETY EFFECTS

The effects of whole pulses on short term glycemic control have been assessed in over 80 published human studies including GI studies and postprandial glycemic response studies. A variety of whole pulses have been studied in these GI and postprandial glycemic studies. The following paragraphs discuss key studies and their findings.

Jenkins et al (1980) examined the low BG response to pulses by investigating the BG response to 35 carbohydrate rich foods. The carbohydrate rich foods included varieties of potatoes, grains, breads, pasta, cereals, and biscuits. Groups of 5-10 healthy volunteers participated. Each participant completed an oral glucose tolerance test to ensure the groups of participants were similar in glycemic responsiveness. Participants fasted overnight prior to the sessions. Meals were consumed in 10-15 min and were accompanied by tea. There were eight dried and boiled legumes tested containing 50 g carbohydrate. The legume treatments included butter beans, haricot beans, kidney beans, soya beans, black eye peas, chickpeas, marrowfat peas, and lentils. Cereals that were tested were consumed with milk. Legumes, millet, buckwheat, spaghetti, and rice that were tested were served with tomato. BG samples were obtained by fingerpick at baseline, 15, 30, 45, 60, 90, and 120 min following the consumption of the treatment. The results showed that the mean BG 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 peak in BG were significantly lower for all dried legumes than the other treatments. Strengths of this study are that participants were normal weight and overweight, and completed glucose tolerance tests to ensure they were
normoglycemic. The volume of each test meal was kept constant, and a variety of high carbohydrate foods were compared to pulses. Some weaknesses of the study are that the total number of participants, their sex, and the length of time between sessions was not stated. Without the sex of participants being disclosed it is unknown whether results can be generalized to both sexes. Another limitation is that, although matched for carbohydrate, the meals were not matched for calories. This study establishes that beans are low glycemic compared to other common carbohydrate foods.

Potter et al (1981) examined the effect of varying dietary fibre content of foods on plasma insulin and BG levels. Eight healthy sedentary men aged 22-45 y participated in the study. The treatments were a glucose formula (control), brown rice, pinto beans, and all bran cereal. Protein and fat were equalized between treatments by adding corn oil and casein. Beans and rice were cooked by steaming, all meals contained 75 g total carbohydrate. The meals were cooked to acceptable palatability and were then blended and water added to equalize volume. Blood was drawn at baseline, 30, 60, 120, and 180 min following consumption of the treatment. The bran and pinto bean treatment resulted in lower BG than the control at 30, 60, and 180 min. For insulin, the response to bran was lower at 30 and 60 min compared to the control. Consumption of the pinto treatment had lower insulin at 30 min compared to control. Bran and pinto bean both resulted in lower insulin compared to rice at 30 min. The authors concluded that dietary fibre was most likely responsible for the differences in insulin and glucose levels. Fibre was analyzed for each treatment, and the higher the BG and insulin the lower the non-digestible fibre (lignin, hemicellulose, and cellulose). Pinto beans and all bran were very similar in non-digestible fibre content which corresponds to their similar BG and insulin responses.
Strengths of this study are that the treatments were consumed one per week in a randomized order, and that protein, fat, and carbohydrates were equalized. A limitation of this study is that it only included men; including women would make the results more generalizable. This study demonstrates that the fibre content of pinto beans is comparable to that of bran and possesses acute insulin and BG lowering effects.

Jenkins et al (1982) also examined the effects of processing on lentils in eight healthy participants (2 men, 6 women) aged 21-37 y. To ensure participants were normoglycemic they completed two 50 g glucose tolerance tests. Treatments were served as breakfast after an overnight fast. The treatments were white bread (control), boiled 20 min lentils, boiled 20 min blended lentils, 1 hour boiled lentils, and 1 hour boiled 12 hour oven dried lentils. Meals were served with tomato and 500 mL of added water, tea or coffee, and were consumed within 10-15 min. Blood samples were obtained by finger prick and ran on an auto analyzer. Samples were drawn at baseline, 15, 30, 45, 60, 90, and 120 min following consumption of treatment. The BG response to consuming the bread treatment and 12 hours dried lentil treatment were very similar. The BG response to the bread and 12-hour dried lentil treatment were higher than the 20 minute boiled lentil treatment at 15, 30, and 45 min following treatment consumption. There was no difference in BG response between 20 min boiled lentils, compared to the 1 hour boiled, or 20 min blended lentils.

The authors performed in vitro digestion studies on the treatments and found that the carbohydrates were more rapidly digested from the lentils that were dried for 12 hours compared to the lentils boiled for 20 min which explains the higher BG response. It was concluded that blending does not appear to increase the BG response to lentils but prolonged
exposure to heat does $^{30}$. Strengths of this study are that participants completed glucose
tolerance tests, that men and women were both included, and that all treatments contained 50 g of carbohydrates. A weakness is that the proportion of men and women was not balanced. This study establishes that 12 hours of dry heat cooking raises the BG response to lentils.

Traianedes et al (1986) examined whether commercial canning increases the digestibility of beans. Six healthy normal weight participants (4 men, 2 women) aged 25-40 y completed the study. The treatments were randomized and consumed after a 12 hour fast. The three treatments were a glucose control, home cooked baked haricot beans, and Heinz vegetarian baked haricot beans. The meals each contained 50 g carbohydrate $^{31}$. The home cooked beans were prepared by soaking overnight, boiling for 1 hour, and then were added to a sauce of molasses, mustard, and tomato and baked for 2 hours. The canned beans were pressured cooked for 2 hours at 121°C and 15 psi. The treatments were consumed within 5-15 min $^{31}$. Blood sampling was conducted at baseline, 15, 30, 45, 60, 90, 120, 150, and 180 min following consumption of the treatment. The glucose treatment resulted in the highest BG response following consumption, and home cooked beans had the lowest. The canned beans resulted in BG peaking at 30 min and was significantly higher than home cooked beans that peaked at 45 min. Home cooked beans had a flatter, slower declining BG profile than the other treatments. The other two treatments resulted in a BG response that fell below fasting 2 hours after consuming the treatment. The home cooked beans BG AUC was significantly lower than canned beans. The insulin response was very similar to BG. The insulin AUC for home cooked beans was significantly lower than canned beans. The insulin response for home cooked beans peaked at 45/60 min and the canned/glucose at 30/45 min $^{31}$. The authors simulated the
different aspects of the canning process and tested the digestibility in vitro. The way that pulses are cooked can impact their digestibility, when this occurs the BG response may also be affected. They concluded that the digestion of starch from legumes is directly related to the duration of time that they are pressure cooked, and temperature due to pressure. Strengths of this study are that both men and women were included though not in balanced proportions, and that the meals all contained 50 g of carbohydrates. Another strength is that the authors simulated the canning process which allowed them to investigate the effects of varying cooking conditions. This allowed them to provide additional support for the result they found 31. A weakness is the small number of participants and that sex was not accounted for in the statistics. A greater number of participants could have allowed for increased significance to be found and would make the findings more robust. This study provides evidence that pressure and dry heat combined increases the digestibility of pulses and increases the BG response healthy men and women.

Wong et al (2009) conducted 3 studies: study I focussed on processing (n=14), study II focused on recipe (n=14) and study III focused on pulse type (n=15). The studies were randomized crossover studies conducted in healthy young men aged 18-35 y with a BMI of 20-25 kg/m². The treatments were consumed after a 12 hour fast. Measurements of BG and appetite using a VAS questionnaire were taken at baseline, 15, 30, 45, 60, 90 and 120 min following the consumption of the treatment. An ad libitum pizza meal was served at 120 min to measure FI. The treatments in study I (processing) were canned navy beans manufactured in Canada, canned navy beans manufactured in the UK, homemade navy beans made following a recipe, and a glucose drink as control. All of the bean types resulted in a significantly lower BG
response compared to control with the exception of 90 and 120 min. There was no difference between bean treatments. There were no significant differences in appetite across treatments. The Canadian navy beans and the UK navy beans led to lower FI at the pizza meal compared to control (p<0.05) \(^3^2\). The treatments in study II (recipe) were canned navy beans in tomato sauce, canned navy beans maple style, canned navy beans pork and molasses, homemade navy beans pork and molasses, and white bread as control. Homemade navy beans led to the lowest BG response at 15, 30 and 45 min, whereas the canned pork molasses navy beans and canned maple style navy beans led to a higher BG 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 BG iAUC compared to control. No effects on appetite or FI were observed across treatments \(^3^2\). The treatments in study III (pulse type) were chickpeas, lentils, navy beans, yellow peas, white bread as control, and water as control. BG iAUC was significantly reduced compared to white bread control for all beans except navy. The lentil and chickpea treatments resulted in the lowest BG response from 0-60 min compared to the other pulses. At 90 and 120 min there were no differences between any treatments or control. All treatments including control decreased appetite AUC compared to water, and among caloric treatments there were no differences. Navy bean, lentil, yellow pea, and bread control led to lower FI compared to the water control \(^3^2\). Study I demonstrates that canned beans can retain a low GI. Study II demonstrates that the recipe of canned beans can affect the BG response. At two time points the canned pork and molasses, and canned maple beans led to higher responses than the white bread control. In contrast, the homemade pork and molasses beans resulted in a significant reduction in BG, suggesting the recipes of beans may impact glycemic response. The
homemade beans contained 3 g more protein and 3 g less sugars than the canned beans. Study III demonstrated the chickpea and lentil were the most effective pulses among those tested at reducing BG acutely. Strengths of the study are that available carbohydrate and serving volume were kept constant, and that the effects of processing, recipe, and pulse type were explored. Limitations are that women were not included. The preceding papers have demonstrated that compared to common carbohydrate foods beans are low glycemic and possesses acute insulin and BG lowering effects. It was found that processing can significantly affect the BG effects observed. Lentils that were dried for 12 hours compared to boiled for 20 min resulted in carbohydrate leading to an increased BG response due to being more rapidly digested. Blending does not appear to increase BG but prolonged exposure to heat does. The digestion of starch was also affected by the duration of pressure cooking, and temperature. Blood glucose response was also dependent on pulse type and commercial recipe.

2.2 PULSES IN MIXED MEALS - ACUTE POSTPRANDIAL GLYCEMIC and SATIETY EFFECTS

Pulses in mixed meals have been demonstrated to retain the glycemic benefits of whole pulses consumed alone. The degree of glycemic reduction appears to be dependent on the pulse type and composition. Previous studies have indicated that meals containing yellow peas, lentils and navy beans are more satiating, however, not all pulses have shown this effect.

Dilawari et al (1981) examined the BG responses of rice, red kidney beans, wheat flour, chickpeas, and dextrose (control) in six healthy young men. All treatments contained 50 g of
carbohydrate, were randomized, and there were at least two days between sessions. Six healthy men aged 25-47 y participated. Blood draws were taken at baseline, 15, 40, 45, 60, 90, and 120 min following consumption of treatment. The meals were consumed with 50 g tomato, 10 g lime, and tea without milk or sugar. The control was served the same meal except the tea contained the dextrose. Participants consumed the meal within 6-12 min. There was a significantly lower BG response at 15, 30, 45, and 60 min to chickpea and at 15 and 30 min to kidney bean compared to the control. Peak BG in response to the chickpeas and red kidney beans was delayed for 15 min compared to rice, wheat flour, and control. Over the first 60 min, chickpeas and kidney beans significantly reduced AUC compared to control. A significantly lower AUC was also seen in individuals consuming the wheat but not rice compared to the control. The authors speculated that the insoluble fibre may been responsible for the BG decrease. A strength is that all treatments contained 50 g of carbohydrates. Weaknesses are that there were that the day and time of sessions were not kept constant which could increase within subject variability, and that the study contained only six men and no women. This study demonstrates that chickpeas and red kidney beans lower BG AUC for the first 60 min postprandial compared to rice and wheat flour, and that peak BG is delayed by 15 min.

Mollard et al (2011) examined first and second meal effects of pulses on FI, BG, and appetite in a randomized study with 25 healthy normal weight men. Treatments were chickpea, lentil, and yellow pea served with macaroni and homemade tomato sauce. The pulses were canned and prepared on the stove with a sauce. The control treatment was macaroni and cheese. The treatments were isocaloric and closely matched for available carbohydrates at about 100 g. Participants fasted overnight before each session. VAS questionnaires assessing
appetite and BG measurements by finger prick were completed at: baseline, 20, 40, 60, 80, 110, 140, 200, 260, 280, 300, 320, and 340 min following consumption of treatment. Participants had 20 min to consume each treatment meal at the start of the session, and 20 min to consume an ad libitum pizza lunch at 260 min. It was found that consumption of yellow pea and lentil treatments significantly lowered FI and pre-pizza meal appetite compared to the control, and that chickpea did not. No differences in BG AUC were observed pre-pizza meal. However, at 20 min all pulse treatments exhibited a lower BG response compared to control, and at 140 min lentil was lower compared to control. Post-pizza, chickpea and lentil treatments significantly lowered BG AUC compared to the yellow pea treatment. At 280 min lentil and chickpea resulted in a lower response compared to control. In overall BG AUC, chickpea exhibited a lower response compared to control.

Strengths of the study are that the meals were isocaloric. Limitations of the study are that only healthy young men were included making the results less generalizable. Differences were also noted in weight, volume, and energy density that could have affected the pre-pizza appetite ratings and FI. This study demonstrates that there are differences in appetite, FI, and BG depending on the type of pulse. The researchers suspected that the differences are due to variations in pulse composition. Further research is needed to understand different effects of different pulses.

Mollard et al (2012) investigated the acute effects of BG and satiety following a pulse meal and at a second meal four hours later. The study followed a repeated-measures, within subject, balanced design, and included 24 healthy normal weight males aged 20-30 y. The five treatments were received once a week over five weeks. The treatments were chickpeas, lentils,
navy beans, and yellow peas served with pasta and homemade tomato sauce. The control was the pasta and sauce alone. The meal was served in three portions made fresh every six min. Participants were given 20 min to consume the ad libitum meal. At baseline and at 20, 40, 60, 110, 140, 200, and 260 min following consumption of the treatment appetite was measured. At 260 min, an ad libitum pizza meal was served to measure FI. BG and appetite were measured following the pizza meal at 280, 300, 320, and 340 min. At the treatment meal, participants consumed less of the lentil meal indicating that it was more satiating compared to the chickpea meal and the control. Participants also ate less of the navy bean meal compared to the chickpea meal. These effects were not due to how much the participants enjoyed the treatments; the treatments had similar palatability ratings. All treatments resulted in a significantly lower BG compared to the control over 260 min, navy beans also resulted significantly lower compared to chickpeas. Following the pizza meal, BG was not affected by treatment. In response to all pulse treatments, BG before the pizza meal was significantly lower compared to control. There was no effect of treatment on FI at the pizza meal (second meal) or on appetite before or after the pizza meal. However, the lentil treatment did result in a lower cumulative FI compared to control. This study demonstrates that certain pulses may lead to satiation sooner and decrease FI at the meal they are served with. A limitation of this study is that women were excluded.

Complementing the previous study, Thompson et al (2012) examined the effects of three pulse varieties on BG in a randomized crossover study in 17 obese (aged 35-70 y, BMI 31.8 ± 1 kg/m²) adults diagnosed with type 2 diabetes (nine men, eight women). The treatments were long grain rice (control), pinto beans, black beans, and red kidney beans. The bean treatments were served with rice and were matched for 50 g of available carbohydrate.
Prior to each session participants consumed the same frozen dinner to control for the second meal effect. Participants fasted 12 hours and then consumed the treatment in 5-10 min. BG sampling was done at baseline, 30 60, 90, 120, 150, and 180 min following consumption of the treatment. The BG response was significantly lower compared to the control for all bean treatments at 90, 120, and 150 min and for pinto beans at 180 min. The black and pinto bean treatments led to a lower overall BG response compared to the red kidney beans though black beans had lower total fibre. The authors expected the bean treatments to result in a more uniform BG response because they were matched for CHO content, however, the fibre differences between the beans may have been responsible for the effects observed. The study concluded that bean and rice meals elicit an attenuated BG response compared to rice served alone, and that the difference in BG between bean types warrants further investigation.

Strengths of the study were that men and women were included in balanced proportions and that all treatments contained 50 g of carbohydrates. Another strength was that participants consumed the same frozen meal for supper the night before each session. A weakness was that some participants were using diet and exercise to control their diabetes, whereas others were taking medication, which may have increased variability in the results. This study demonstrates that beans have BG lowering effects not only individuals with normal BG control, but also for individuals who have type 2 diabetes.

In summary, the degree of glycemic reduction from consuming pulses in mixed meals appears to be dependent on the pulse type and composition. Chickpeas and kidney beans have resulted in reduced BG AUC compared to control while black and pinto bean have shown to reduce overall BG response compared to red kidney beans. Chickpea and lentil treatments
were shown to significantly lower post-pizza meal BG compared to the control, while the yellow peas did not. However, yellow pea and lentil treatments lowered FI and pre-pizza meal appetite compared to the control while chickpea did not 22. The lentil meal was indicated to be more satiating as less of the treatment was consumed compared to the chickpea and the control meal, also FI was lower for navy bean meal compared to the chickpea meal 24. All pulse treatments significantly lowered BG compared to the control over 260 min, navy beans also resulted in significantly lowered BG compared to chickpeas. Though some treatments resulted in a decreased FI of the treatment there was no effect of treatment on FI at the pizza given as a second meal 24.

2.3 PULSE FLAKES - ACUTE POSTPRANDIAL GLYCEMIC and SATIETY EFFECTS

Pulses in mixed meals have been demonstrated to retain the glycemic benefits of whole pulses consumed alone 21–24. To determine if pulses in the form of flakes could retain the same benefits, Bourdon et al (2001) conducted a randomized crossover design study with eight men aged 21-45 y of normal and overweight BMI. Participants fasted 12 hours before their session and had 15 min to consume the treatment. The two meals were high fibre and low fibre, with the high fibre meal containing 11.8 g of dietary fibre. The meals were matched for protein, carbohydrate and fat, the difference was the high fibre diet contained bean flakes and the low fibre diet contained instant rice and non-fat milk powder 33. Sessions were 1-3 weeks apart and six hours long. BG, insulin and CCK were assessed at baseline and 30, 45, 60, 120, 180, 240, 300, and 360 min postprandial. At 30 min postprandial BG peaked for both treatments. No significant differences were seen in post-prandial BG or insulin, nor were there differences in
AUC. The high fibre meal did, however, result in a significantly higher CCK response than the low fibre meal, 100% greater. CCK is associated with satiety. The authors speculated that the increase in the CCK may have been because of the trypsin inhibitor found in most legumes. It might also be due to the effects of the high fibre content delaying gastric emptying which would contribute to the higher CCK response.

In summary, this research highlights a satiety associated hormone response to a high fibre treatment compared to a low fibre treatment. The increased CCK response to the bean flake treatment could help explain the satiating effects of pulses and should be explored further.

2.4 PULSE FLOURS – ACUTE POSTPRANDIAL GLYCEMIC and SATIETY EFFECTS

Limited pulse flour research exists, however, research has demonstrated that varying benefits of whole pulses are retained compared to control. The following paragraphs discuss pulse flour findings.

Hall et al (2005) examined the effects of lupin bread on glycemic response in 11 healthy participants (nine male, two female) aged 25-45 y. The two treatments were white bread as a control or white bread with a 7.7 g substitution of Australian sweet lupin flour (ASLF). The control bread was consumed twice so there were three sessions in total. Sessions were at least seven days apart, and carried out in the morning after a 10-12 hour fast. Satiety and sensory perception were measured on a scale similar to VAS questionnaires. The treatment breakfasts were 3-4 slices of toasted bread with margarine, apricot jam, and decaffeinated tea. The meal was eaten within 10 min. There was less than 100 calorie difference between treatments and both treatments contained 50 g of available carbohydrate. The BG peak was seen at 30 min
for both treatments and the BG response of the ASLF bread was significantly lower than the control. The peak insulin response was seen at 30 min for both treatments and the insulin response was significantly higher for the ASLF bread than the control. Peak satiety was reached at 10 min following consumption of breakfast for the control and at 25 min for ASLF. The perception of satiety between the treatments was not statistically different. Post-meal FI and palatability of breakfasts also did not differ significantly. The authors expected satiety to be greater with ASLF as it has been in other studies and suggested that the study may have been underpowered to detect differences 26. Strengths are that men and women were both included, and that sessions were done at least seven days apart. Weaknesses of this study are that the number of men and women were not balanced and that there were insufficient proportions of each sex to measure potential sex differences. This study demonstrates that the acute beneficial BG effects of lupins are still observed when milled to a powder and made into bread. Having whole lupins as a third treatment would have strengthened the research, as it could have shown just how much of the beneficial effects of whole lupins were retained compared to the lupin bread.

Another aspect of processing was examined by Johnson et al (2005) by incorporating chickpea flour, and extruded chickpea flour into bread and comparing it to a white bread as control. Eleven healthy participants (nine male, two female) aged 25-45 y completed the study. The study was a single blind randomized crossover design with three treatment meals, with the control session conducted twice for a total of four sessions. The treatments were chickpea flour bread, extruded chickpea flour bread, and control white bread. The treatment meals were eaten as breakfast after an overnight fast, and sessions were at least seven days apart.
Participants were also given a list of foods they were allowed to eat the night prior to control for second meal effects\textsuperscript{27}. A scale similar to 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 a buffet was provided and participants consumed lunch. Food was weighed/measured prior to participants eating. Participants were also instructed to record what they ate for the rest of the day\textsuperscript{27}. The treatments all had 50 g of available carbohydrate and were very similar in energy, protein, and fat. The control bread contained 3 g total fibre, chickpea flour bread contained 5 g total fibre, and extruded chickpea flour bread contained 6 g total fibre. The chickpea bread replaced 24.3% of the white bread flour in the chickpea bread treatments, this amount was chosen because it kept the bread palatable. Breads were served with equal amounts of water, margarine, jam, and milk. Tea was also served, the quantity was adjusted to make the weight of the breakfasts equal\textsuperscript{27}. Blood glucose was analyzed on an auto analyzer and insulin on an RIA kit. For all treatments BG peaked at 30 min and returned to and fell beneath baseline between 60-90 min. There were no differences in BG iAUC for any treatments. Consuming chickpea bread resulted in a significantly lower BG compared to the control at 60 min, and at 120 min the extruded chickpea bread resulted in lower BG compared to control (p<0.05). At 60 min both treatment breads had lower insulin compared to the control (p<0.05). The insulin iAUC was also significantly higher for chickpea bread than the control bread. There were no significant differences in satiety, sensory, or FI between the three treatments. The authors suggested that perhaps the incorporation of chickpea flour was too low or the particle size too small to observe the glycemic and insulin benefits seen by whole chickpeas. They believed that further studies with varying particle size...
and greater incorporation of chickpea flour are needed. It was surprising to see a hyperinsulinemia effect from the chickpea flour, which also warrants further investigation.

Strengths of the study are that efforts were made to reduce the second meal effect from the previous day and that sessions were conducted at least one week apart. Weaknesses are that the number of men and women were not balanced and that some measurements were underpowered. It was surprising that the participants eating the non-extruded chickpea flour had a high insulin response and only elicited a significantly lower BG at a single time point compared to the white bread. Further investigation is required.

Marinangeli et al (2009) explored the glycemic response of whole yellow pea flours in novel food products in healthy males (n=7) and females (n=13) aged 22-67 y with BMIs between 21-42 kg/m^2. Participants consumed eight treatments in total. Treatments were banana bread, biscotti, and pasta. Each food product was given twice, consisting of whole yellow pea flour (WYPF) once and whole wheat flour (WWF) once. Controls were boiled whole yellow peas and white bread. Participants consumed the treatment after a 12 h fast and had BG measured at baseline, 30, 60, 120, and 150 min following consumption of the treatment by glucometer. WYPF biscotti and WYPF banana bread resulted in a significantly lower (61.9% and 55.1%) iAUC BG response compared to white bread (p<0.001). The WYPF biscotti resulted in a 29.2% BG reduction compared to the WWF biscotti. However, WYPF pasta resulted in significantly higher BG iAUC than boiled yellow peas. The WYPF pasta did not result in a lower BG response compared to WWF. This difference in BG response suggests that the manner in which the treatments are cooked affects the glycemic effect exhibited. It was not indicated whether WYPF possesses the same beneficial effects as whole yellow peas, but the cooking
method utilized appeared to be responsible for the beneficial effects of WYPF. Strengths are that both sexes were included and that available carbohydrate was kept constant across treatments. Limitations are that variations in hormones were not controlled for in pre-menopausal women. This research supports the use of whole pea flour in novel foods products intended to be low glycemic.

In another study examining pulse flours, Anderson et al (2014) found that the beneficial effects of whole beans are not diminished when ground to a powder in healthy young men. Three experiments were conducted, each with a control of whole-wheat flour. In experiment I (n=17) the treatments were whole navy bean, pureed navy bean, and navy bean powder. In experiment II (n=12) whole lentil, pureed lentil, and lentil powder were provided. In experiment III (n=12) whole chickpea, pureed chickpea, and chickpea powder were provided. There were 38.8 g of available carbohydrate in each treatment and water was added so treatments were of equal weight. Participants were allowed 15 min to consume the treatment with blood drawn at baseline, 15, 30, 45, 60, 90, and 120 min following consumption of the treatment. Subsequently participants consumed a fixed-size pizza meal, and blood was drawn at 140, 155, 170, 185, and 200 min. All three experiments had BG significantly affected by time and by a time by treatment interaction. In experiment I, prior to the pizza meal there was a significant time by treatment interaction. At 15 min the bean powder treatment did not differ from the control in BG response. At 30 min the three bean treatments resulted in a significantly lower BG compared to control. At 45 min the navy bean powder resulted in lower BG compared to the pureed treatment. At 60 min whole wheat resulted in lower BG compared to the whole navy bean treatment. The difference in pre pizza meal BG net AUC was significant for the navy bean
powder compared to the control, and was intermediate for the other two treatments. In experiment II there was a significant time by treatment interaction for BG, mean BG was also significantly lower compared to the control for all lentil treatments at all time points preceding the pizza meal. Compared to the control, the BG net AUC was lower for whole and powdered lentils. In experiment III, time points preceding the pizza meal had a time by treatment interaction and BG was significantly lower for powdered, pureed, and whole chickpeas compared to the control. The authors concluded that pureeing and commercial processing does not negate the acute glucose regulation benefits of consuming pulses as there were overall no significant differences seen between the treatments due to processing. Strengths of the study are that the amounts of available carbohydrates were kept constant between studies, limitations were that women were excluded.

The four published studies discussed above are encouraging in that it is evident that the BG lowering effects of consuming whole pulses are retained when pulses are processed to flours. It is unclear whether additional benefits such as appetite reduction and satiety are retained as some of these measurements may have been underpowered statistically. More studies with a greater number of participants are needed to determine the magnitude of beneficial effects retained from whole pulses, as well as studies exploring pulse flour incorporation into other food matrices.

2.5 PULSE FRACTIONS - ACUTE POSTPRANDIAL GLYCEMIC and SATIETY EFFECTS

Pulse fractions are the next step in understanding what compositional aspect of pulses are responsible for the acute postprandial glycemic and satiety effects, and determining
whether these benefits are retained when whole pulses are fractionated. Limited pulse fraction studies exist thus far, however, studies that have been conducted are summarized in the following paragraphs.

Smith et al (2012) studied the effects of yellow pea protein and fibre on post-prandial glycemia, short term FI, and subjective appetite in healthy men aged 20-30 y with a BMI between 20-24.9 kg/m². These authors conducted two experiments (n=19, n=20), both were randomized, single blind, and repeated measures design. Five treatments were received once a week, each separated by a week. Treatments were tomato soup used as a control, and tomato soup with 10 g or 20 g of fibre or protein. In the treatments there were 9.5-10.5 g of available carbohydrate. Visual analog scale questionnaires and blood samples were taken from 0-170 min for experiment one and from 0-200 min in experiment two. In both experiments participants consumed the treatment following the baseline measurements. In experiment I participants received an ad libitum pizza meal at 30 min, and in experiment II at 120 min. In experiment I pre-meal BG was significantly affected by time and treatment. BG was significantly lower over the whole treatment period for both protein treatments compared to the control. BG was overall significantly lower with protein 20 g treatment compared to control and fibre 10 g treatment. Both protein treatments had a significantly lower BG immediately following the pizza meal compared to the control and fibre 10 g treatment. Food intake in response to protein 20 g was significantly lower compared to all other treatments. The glycemic responses to yellow pea protein pre-meal were independent of dose, post-pizza meal they were dose dependent. In experiment II, pre- and post-meal BG was significantly affected by time but not by treatment. There was no significant difference in water or FI for any treatment. The authors
attribute the null results in experiment II to be due to pea protein’s rapid digestion. The authors suggested that the null results from the fibre treatments, though surprising, may have been because the fibre was taken from the hull only and not from the entire pea. Pea hull contains 45.8% more insoluble fibre and 21% more soluble fibre than dehulled peas that contain fibre derived from the cotyledon of the pulse. There may be different benefits derived from consuming hull fibre vs consuming cotyledon and hull fibre in combination. Strengths of this study are that treatments were isovolumetric. A limitation of that work is that women were excluded and that there was no whole pea treatment. The whole pea treatment would have provided a reference for the potential synergistic effects of the combined pea components. It is possible that had the serving sizes of the treatments been greater there would have been a significant difference in subjective appetite in both studies and in BG for experiment II. This study demonstrates that 20 g of pea protein induces high satiety and reduces FI at a meal 30 min following consumption.

Mollard et al (2014) conducted a study investigating the effects of pea fractions on FI, appetite and BG in a randomized single blind crossover study in healthy men aged 18-35 with a BMI of 20-24.9 kg/m². Five treatments included yellow peas, pea hull fibre and pea protein, pea hull fibre, pea protein, and the control. Treatments were served with tomato sauce and noodles, while the control was sauce and noodles alone. Treatments were received once a week following an overnight fast, and were consumed within 10 min. BG and appetite were measured at baseline, 15, 30, 45, 60, 75, and 135 min before an ad libitum pizza meal, and at 155, 170, 185, 200, and 215 min following pizza. Appetite was measured using a VAS questionnaire. For BG, the combined pea protein and fibre treatments lowered the response
compared to the control similar to yellow peas, this was, however, not seen by the fibre or protein treatment alone \(^\text{36}\). There were no differences in water or FI after the pizza meal, appetite ratings or palatability across treatments. No significant differences were observed in appetite. Strengths of the study were that the treatments were isovolumetric and isocaloric, a limitation was that women were excluded. The authors suggested that the dose of protein and fibre alone may have been too low to observe a difference in BG, however, the fibre treatment was observed to have resulted in the highest BG responses. This study establishes that fractions of pea fibre and protein combined retain the effects of whole peas, but suggests that fractions alone do not. Further investigation is required.

Results of the pulse fraction studies suggest that pea protein and fibre combined is more effective at reducing BG than protein or fibre alone. Smith et al (2012) found that 20 g of pea protein induces high satiety and reduces FI at a meal served 30 min following consumption of the treatment in a soup. It was found that protein at a dose of 10 g as well as 20 g reduced BG following the consumption of a second meal at 30 min. However, when the second meal was served at 120 min there were no differences \(^\text{35}\). Mollard et al (2014) served a pea protein treatment of similar protein content (18 g) with tomato sauce and noodles and found that the protein treatment alone did not reduce BG significantly, but that the combined protein + fibre treatment lowered BG compared to control. A second meal was supplied at 135 min and did not result in significant differences in BG post-meal \(^\text{36}\). These results suggest that effects of pea protein alone are transient and that pea protein and fibre combined may have synergistic effects that allow BG lowering effects to last longer than protein alone.
2.6 PULSE REVIEWS - ACUTE POSTPRANDIAL GLYCEMIC and SATIETY EFFECTS

The following pulse reviews focus on glycemic control and satiety and FI at a second meal. The review focusing on glycemic control considered medium and long term effects of pulses while the meta-analysis on the satiety effects of whole pulses focused on satiety and second meal FI.

Sievenpiper et al (2009) performed a systematic review and meta-analysis with 41 RCTs investigating the effects of pulses on glycemic control. Studies were pooled into three groups; pulses alone, pulses incorporated into low-GI diets, and pulses incorporated into high-fibre diets. The studies included examined medium and long-term effects of pulses supplemented to the diet compared to a non-pulse control. The whole pulse group contained 11 studies with 253 participants who were normoglycemic, hypercholesterolemic, or type 2 diabetics. The low GI group contained 19 studies with 762 participants who were normoglycemic or type 2 diabetics. The high fibre group contained 641 participants who were normoglycemic, or type 1 or 2 diabetics. The majority of studies were crossover in design. The group with whole pulses showed that FBG was significantly decreased, but that HOMA-IR and glycosylated blood proteins were not. The low-GI group glycosylated blood proteins were significantly decreased but HOMA-IR, FBI, and FBG were not. In the high-fibre group, FBG was significantly decreased and HOMA-IR and FBI was not. Strengths of the review are the vast number of studies included and that the studies were pooled into three categories to better examine the effects of pulses consumed in different ways. Limitations of the review are that acute postprandial glycaemia studies were not included. The review provides support that pulses may improve long and medium term glycemic control modestly.
Li et al (2014) completed a systemic review and meta-analysis examining the effect of acute pulse feeding studies on satiety and second meal FI\textsuperscript{38}. Nine acute, randomized, isocaloric studies with measurements of satiety and second meal FI were included. Only studies that contained whole pulses were included. The quality of the studies was examined using a Heyland methodological quality score and bias risk was assessed with the Cochrane risk of bias tool. Eight out of nine studies were considered high-quality, and high risk of bias was low.

Participants were generally healthy, normal weight, overweight, and obese individuals. There were slightly more men than women and 126 participants in total\textsuperscript{38}. The treatments were whole pulses, ground pulses used in breads, or a spread. The control was usually white bread, other controls were potato puree, placebo spread, or mac and cheese. The dose of pulses ranged from 7.6-311 g, and the session duration from 120-220 min. It was found that the pulse meals compared to a control increased satiety by 31% and did not significantly affect second meal intake\textsuperscript{38}. Strengths of the review are that the studies were conducted in three areas; Australia, Europe, and North America. Other strengths are the variety of ways pulses were served; as bread, as spread, as well as whole. Limitations are the small number of participants that the studies had and that the studies excluded children, youth, and older adults that were overweight or obese.

In summary, whole pulses when consumed alone or within a mixed meal have beneficial effects on post-prandial glycemic and satiety control, however, these effects depend upon pulse variety and processing. When pulses are incorporated into novel food products or meals as whole flours and fractions, they appear to retain the acute health benefits seen from whole pulses. The benefits retained vary by study which may be due to differences in study design.
### 2.7 LITERATURE REVIEW TABLE

Whole pulse treatment studies – Acute postprandial glycemic and satiety effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenkins et al (1980).</td>
<td>Groups of 5-10 healthy volunteers of 110% ideal body weight.</td>
<td>50 g carbohydrate portions of eight dried and boiled legumes (butter beans, haricot beans, kidney beans, soya beans, blackeye peas, chick peas, marrowfat peas, lentils), and 35 other high carbohydrate foods.</td>
<td>- Mean BG for each dried legume was significantly lower than the mean curves of all other foods at least two time points. The AUC and mean spike in BG was significantly lower for dried legumes than the other treatments.</td>
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<tr>
<td></td>
<td></td>
<td>2. Brown rice (97 g)</td>
<td>- Bran and pinto bean was significantly lower than the control at 30, 60, and 180.</td>
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<td></td>
<td></td>
<td>3. Pinto beans (118 g)</td>
<td>Insulin:</td>
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<tr>
<td></td>
<td></td>
<td>4. All bran (106 g)</td>
<td>- Bran was significantly lower at 30, 60, and 120 compared to the control, and beans was significantly lower at 30. Bran and beans were also significantly</td>
</tr>
</tbody>
</table>
The insulin response peak of the control was nearly twice that of pinto beans and bran.

- There were no significant differences in BG between 20 min lentils compared to blended lentils or one hour boiled lentils.
- 12 hour dried lentils had a significantly higher BG response than 20 min lentils at some time points.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Meals</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2. Boiled 20 min lentils</td>
<td>- The insulin response peak of the control was nearly twice that of pinto beans and bran.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Boiled 20 min blended lentils</td>
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<td>4. One hour boiled lentils</td>
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<td></td>
<td>5. One hour boiled, 12-hour oven dried lentils</td>
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<tr>
<td></td>
<td></td>
<td>Meals were served with tomato, 500 ml of water, as well as tea or coffee.</td>
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<tr>
<td>Traianedes et al (1986)</td>
<td>Six normal weight participants (four men, two women) aged 25-40 y.</td>
<td>1. D-glucose</td>
<td>- The home cooked beans BG AUC was significantly lower than canned beans.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Home cooked baked haricot beans</td>
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<td></td>
<td></td>
<td>3. Heinz vegetarian baked haricot beans</td>
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<tr>
<td></td>
<td></td>
<td>The home cooked beans were served with a sauce made of molasses, mustard and tomato.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>intervention</td>
<td>Results</td>
</tr>
<tr>
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</tr>
<tr>
<td>I</td>
<td>Healthy normal men aged 18-35 y.</td>
<td>Canned navy beans manufactured in Canada, canned navy beans manufactured in the UK, homemade navy beans made following a recipe, glucose drink (control).</td>
<td>All beans resulted in a reduced BG with the exception of at 90 and 120 min compared to control. Lower FI at the pizza meal resulted from the Canadian navy beans and UK navy beans compared to control.</td>
</tr>
<tr>
<td>II</td>
<td>Study I: n=14 Study II: n=14 Study III: n=15</td>
<td>Canned navy beans in tomato sauce, canned navy beans maple style, canned navy beans pork and molasses, homemade navy beans pork and molasses, white bread (control).</td>
<td>II: Homemade pork and molasses navy beans and canned navy beans in tomato sauce led to a reduced BG net AUC compared to control. III: BG net AUC was significantly reduced compared to white bread control for all beans except navy. Navy bean, lentil, yellow pea, and bread control led to lower FI compared to water control.</td>
</tr>
<tr>
<td>III</td>
<td>Study I: n=14 Study II: n=14 Study III: n=15</td>
<td>chickpeas, lentils, navy beans, yellow peas, white bread (control), water (control).</td>
<td></td>
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</table>
### Pulses in mixed meals - Acute postprandial glycemic and satiety effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Meals</th>
<th>Postprandial BG differences</th>
</tr>
</thead>
</table>
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 BG compared to rice and wheat. |
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 FI 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 BG AUC, yellow pea did not. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
</table>
| Thompson et al (2012)         | 17 obese (BMI 31.8 +/- 1 kg/m²) adults (nine men and eight women) diagnosed with type 2 diabetes aged 35-70 y. Diabetes was managed by either diet and exercise or with metformin. | 1. White long grain rice  
2. Pinto beans (50 g) /rice (34.7 g)  
3. Black beans (50 g) /rice (34.7 g)  
4. Red kidney beans (50 g) /rice (34.7 g) | -BG was significantly lower at 90, 120, and 150 min for all bean treatments compared to the control. |
2. Lentils  
3. Navy beans  
4. Yellow peas  
5. Control (pasta and tomato sauce) | -BG 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. |
meals was from pulses. The meals were isocaloric.

Pulse flake treatment study - Acute postprandial glycemic and satiety effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
</table>
| Bourdon et al (2001). | Eight men aged 21-45 with a BMI between 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. | - The high fibre meal had a significantly higher response of CCK than the low fibre meal.  
- There was no significant difference in postprandial insulin or BG. However, BG and insulin concentrations remained above baseline longer than the low fibre meal. |
Pulse flour treatment studies - Acute postprandial glycemic and satiety effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hall et al (2005).</td>
<td>11 participants (nine men, two women) aged 25-45</td>
<td>1. White bread</td>
<td>- BG response of the ASLF bread was significantly lower than the control.</td>
</tr>
<tr>
<td></td>
<td>with a BMI of 20.9-28.6 kg/m².</td>
<td>2. White bread with 7.7 g of Australian sweet lupin flour (ASLF) added</td>
<td>- Insulin response was significantly higher for the ASLF bread than the control.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consumed with margarine, apricot jam and decaffeinated tea.</td>
<td></td>
</tr>
<tr>
<td>Johnson et al (2005).</td>
<td>11 healthy participants (nine men, two women) aged</td>
<td>1. White bread</td>
<td>- BG of the chickpea bread was significantly lower than the control at 90 min, and the</td>
</tr>
<tr>
<td></td>
<td>25-45 y.</td>
<td>2. Chickpea flour</td>
<td>extruded chickpea bread BG was significantly lower than the control at 120 min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Extruded chickpea flour</td>
<td></td>
</tr>
<tr>
<td>Anderson et al (2014).</td>
<td>Healthy young men Ex. I (n=17) Ex. II (n=12)</td>
<td>Ex. I/II/ III</td>
<td>- Overall no significant differences seen between the treatments due to processing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Whole navy bean/ lentil/ chickpea</td>
<td>- For lentil and chickpea treatments mean BG was lower</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Pureed navy bean/ lentil/ chickpea</td>
<td></td>
</tr>
</tbody>
</table>
Ex. III (n=12)    3. Powdered navy bean/ lentil/ chickpea  over 120 min compared to control, for navy bean only peak BG was lowered compared to control.

**Pulse fraction treatment studies - Acute postprandial glycemic and satiety effects**

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
</table>
| Marinange li et al (2011). | 23 participants who were overweight (BMI 25-40 kg/m²) and hypercholesterolemic (7 men, 16 women). | Banana and apple muffins  
1. Whole pea flour (26.4 g)  
2. Fractionated pea flour, 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. The wheat flour treatment had less insoluble fibre. The whole pea flour treatment had about 3 grams more -Fasting insulin was significantly reduced compared to the control by the whole pea flour treatment. -Insulin resistance estimates were reduced 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. |
There were no effects on TAG, TC, HDL-C, LDL-C, or glucose between any of the treatments.  

<table>
<thead>
<tr>
<th>Study</th>
<th>Experiment Description</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment II (pizza meal at 120 min) N= 20 healthy men.</td>
<td>Experiment I and II had the same five 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</td>
<td>Ex I: BG was overall significantly lower with protein 20 g treatment compared to control and fibre 10 g treatment. Protein 20 g FI was significantly lower than all other treatments. Ex II: No BG differences. No significant difference in water or FI for any treatment.</td>
</tr>
</tbody>
</table>
## Pulse reviews – Acute postprandial glycemic and satiety effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sievenpiper et al (2009).</td>
<td>41 randomized controlled human studies that investigated effects of pulses (separately, as part of a high-fibre intervention, or low GI study) in the diet.</td>
<td>Pulse treatment dosages ranged from 15.5-465 g/day. Treatments were given as whole pulses, flakes, and flours.</td>
<td>-Pulses were found to improve primary markers of glycemic control in the long term given alone, or as part of a high-fibre or low GI diet.</td>
</tr>
<tr>
<td>Li et al (2014).</td>
<td>Nine acute studies (n=6-20). Participants were aged 18-53 y and were normal weight, overweight, and obese.</td>
<td>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.</td>
<td>-Pulses were found to increase satiety compared to a control by 31%, and were found to not affect second meal intake.</td>
</tr>
</tbody>
</table>
CHAPTER 3 - RATIONALE, OBJECTIVES, HYPOTHESIS, AND DESIGN

3.1 RATIONALE

Pulse flours and fractions appear to retain varying health effects of whole pulses and could be excellent additions incorporated into common foods making pulse consumption more intuitive for consumers. A current gap in the literature are that only select pulses have been tested as flours in novel food products or meals, and none have been tested in extruded products. Benefits of extrusion include increasing protein digestibility and decreasing antinutritional factors. Identifying the most effective pulse flours and fractions in extruded products could encourage the food industry to formulate new products which could lead to increased pulse consumption. More pulse types should be tested as flours to determine if pulses as a group retain their effects, or whether the effects are pulse dependent as some may be more resistant to processing than others. Another gap is whether a specific fraction, or synergistic effects of combined fractions, is responsible for the glycemic and satiety effects seen. Limitations of some studies conducted thus far are 1) appetite and FI measurements may have been underpowered, 2) only men were included or too few women to investigate whether there were sex differences making the results less generalizable, 3) variations in hormones were not controlled for in premenopausal women, and 4) treatments were not matched for calories.

The above mentioned gaps in the literature have been presently addressed by conducting two studies. Studies I (snack) and II (cereals) were conducted to assess the effects of different pulse flours and varying pea fraction combinations on postprandial BG, appetite, insulin, and FI two hours following treatment consumption. Study I addresses the first gap by
testing five pulse flours in an extruded snack to determine if some pulses perform more favourably than others. Study II addresses the second gap by testing yellow pea flour fractions in extruded cereals to determine if specific pea fractions performed more favourably than others. Our treatments were formulated as snacks and cereals because few Canadians consume pulses\textsuperscript{25}, adding pulses to foods that are already regularly consumed could add value to the diets of Canadians. It has been suggested that protein is a greater contributor to the health benefits of pulses\textsuperscript{35} and others suggest a combination of protein and fibre is optimal\textsuperscript{18,36}. Varying fraction proportions and combinations were tested in study II to investigate whether a fraction is responsible for acute health effects or whether the effects are synergistic of the combined fractions. Yellow pea fractions were selected for study II because of the preceding research supporting their use\textsuperscript{18,22,24,35,36} and industry interest.

The processing involved to create pulse flour snacks and pea fraction cereals could affect the treatment digestion and subsequent BG, appetite and FI response. The overall purpose of these studies were to assess whether a variety of pulse flours in extruded snacks retain the post-prandial glycemic, satiety, and FI effects of whole pulses, and whether pea fractions in extruded cereals retain post-prandial glycemic, satiety, and FI effects of whole peas.

3.2 OBJECTIVES

The objectives of study I and II were to assess the acute effects of five extruded pulse flour snacks, and 5 different pulse fraction extruded cereals on: 1) BG, appetite, and insulin before and after an ad libitum meal, and 2) FI at an ad libitum meal consumed two hours following consumption of the pulse products.
3.3 HYPOTHESIS

It is hypothesized that extruded food products containing pulse flours and fractions when compared to non-pulse products will result in a lower BG, insulin, appetite, and FI responses. It is hypothesized in the second study that these effects will be greater when the pulse fractions are consumed in combination, rather than as distinct isolated fractions, when provided as cereals.

3.3 EXPERIMENTAL DESIGN

Two acute studies were designed to address the objectives. The studies followed a randomized; double blinded, balanced, and repeated measures design. Study I investigated the acute effects of different pulse flours in extruded snacks on postprandial BG, appetite and insulin for two hours, and FI two hours following consumption of pulse products. Participants consumed 50 g of pulse snack or control once per week in randomized order. Study II investigated the acute effects of different pulse fractions in extruded cereals on postprandial BG, appetite and insulin for two hours, and FI two hours following consumption of pulse products. Participants consumed 35 g of pulse cereal or control once per week in randomized order.

Men and women were tested with a minimum of 5 days between sessions. Women were tested during the follicular phase of their menstrual cycle (the two weeks following menstruation), because of observed insulin resistance during the luteal phase \(^{40,41}\).

When conducting GI studies, the test food possesses an exact quantity of available carbohydrate (either 25 g or 50 g) and is compared relative to a control food (usually white
bread or glucose) containing the same amount of available carbohydrate. A lower GI suggests slower rates of digestion and absorption of the carbohydrate in the test food, and usually equates to a lower insulin demand. A practical limitation of the GI is that it does not take into account the amount of food actually consumed. In contrast, studies on postprandial glycemia do not necessarily provide 50 g of available carbohydrate in the test foods, can have different control foods besides glucose or white bread, and may run longer than two hours. This is the case for our study; sessions were conducted over a maximum of four hours; once started sessions were three hours and 20 min timed. Controls, provided as corn for the snack study and oat for cereal studies, reflect current products in the market place for which pulses have the potential to be value added ingredients. The treatment serving sizes were based upon guidance documents from Health Canada, study treatments were the same serving size as their control. Available carbohydrate contents for the pulse snacks range between 38.5-41.8 g (control 47.3 g), and for pulse cereals between 19.8-40.4 g (control 39.5 g) as was determined by Dr. Nancy Ames.

Blood samples were collected from an intravenous catheter by a registered nurse. The samples were centrifuged at 1300 g for 10 min after having sat for 15-30 min at room temperature. Serum was centrifuged and pipetted into micro-tubes continuously throughout the participants’ sessions. Samples were promptly put in a fridge to cool and were then stored in a -80°C freezer.

Serum glucose was measured by colorimetric slides on a Vitros 350 auto-analyzer. Quality controls were run each day prior to testing samples to ensure accuracy and precision of
the measurements. Duplicates were performed for 20% of the samples for additional accuracy and precision confidence.

Serum insulin was measured by radioimmunoassay in duplicate using Millipore 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 µU/mL the sample would be repeated and diluted. If the coefficient of variation was >10% the sample was repeated.
CHAPTER 4 - ACUTE EFFECTS OF EXTRUDED PULSE SNACKS ON GLYCEMIC RESPONSE, INSULIN, APPETITE, AND FOOD INTAKE IN HEALTHY YOUNG ADULTS

4.1 ABSTRACT

Background: Research indicates that the post-prandial glycemic benefits of consuming whole pulses alone are retained when consumed in a mixed meal, pureed, and ground into flours. However, it is unknown whether the glycemic benefits are retained when pulse flours are incorporated into extruded products and whether they can improve satiety control.

Objective: Assess the effects of replacing corn with pulse ingredients in extruded snacks on postprandial glycaemia, insulin, appetite, physical comfort, energy/fatigue, and food intake (FI).

Design: In a randomized, repeated-measures crossover study, adults (n = 26) consumed extruded snacks made with: 1) whole yellow pea flour, 2) split yellow pea flour, 3) green lentil flour, 4) chickpea flour, 5) pinto bean flour, and 6) corn flour (control). Food intake was measured at an ad libitum pizza meal consumed at 120 min. Blood glucose was measured by an auto analyzer and serum insulin was measured by RIA. Participants completed validated visual analog scale (VAS) questionnaires to measure subjective appetite, physical comfort, and energy/fatigue. Blood glucose, serum insulin and VAS were measured at pre-pizza (0-120 min) and post-pizza (140-200 min) meal.

Results: Pinto bean and chickpea snacks led to lower (p < 0.05) pre-pizza blood glucose (BG) incremental area under the curve (iAUC), compared with control, whole yellow pea and green lentil snacks. The effects on BG at specific time points were dependent upon pulse type. At 30 min, BG was lower (p < 0.05) after pinto bean compared to green lentil snacks, whereas at 45
and 60 min, pinto bean led to lower BG compared to whole yellow pea snacks. No differences were observed between treatments in post-pizza BG, FI or appetite.

Conclusion: These findings indicate that health benefits of replacing corn with pulse flours in extruded snacks on BG, and insulin are dependent on pulse type. This study supports the use of pinto bean and chickpea flours in extruded products designed to improve post-prandial glycemic control.

4.2 INTRODUCTION

Pulses are a low fat, protein rich plant food with high amounts of soluble and insoluble fibre \(^9\), as well as resistant and slowly digestible starch \(^9,10\). Pulses also contain vitamins, minerals and dietary fibre that support human health \(^5\), and contain 2-3 times the amount of protein that is found in cereals and other plant crops like wheat, corn, quinoa and rice \(^42\). Higher consumption of protein is associated with increased satiety \(^7\) and BG control \(^8\). Consuming a \(\frac{1}{2}\) cup of pulses per day improves diet quality by reducing saturated and total fat intake, and by increasing folate, zinc, magnesium, fibre, and protein \(^11\). Improved insulin sensitivity and glycemia are health benefits that are associated with consumption of dietary fibre, these effects have been seen in people with and without diabetes \(^15\). Reduced energy intake, increased satiety, and improved glycemic and insulin responses are health effects of consuming resistant and slowly digestible starches similar to dietary fibre \(^12,13,16\). Thus, the nutritional content of pulses makes them an excellent food to assist in the control of BG and satiety. Additionally, pulses are friendly to the environment. Pulses improve the health of the soil they’re grown in, are a protein source that is water efficient, and have a low carbon footprint.
Despite their nutritional benefits and environmental friendliness, very few Canadians eat whole pulses. About 10.7-13.1% of Canadians regularly consume pulses.

The current obesity epidemic in North America predisposes individuals to chronic diseases such as metabolic syndrome and type 2 diabetes. Potential countermeasures to the current obesity epidemic are to identify and recommend foods and ingredients that spontaneously increase satiety, reduce energy intake and improve glycemic control. Pulse flours possess the potential to provide health benefits and could be used as food based strategies in body weight and BG control. The effects of whole pulses have been extensively examined; however, the effects of pulse ingredients incorporated into extruded products have not been elucidated. Pulses consumed alone exhibit a low glycemic index when compared to white bread. Whole pulses consumed within meals have also been examined and have been shown to significantly decrease BG, FI, and appetite when compared to a meal without pulses. Although limited, research with pulse flours has shown that the favourable effects of whole pulses are retained. Both pulse flour and extruded pulse flour incorporated into bread resulted in a significantly lower BG response compared to a non-pulse bread. Another study comparing whole, pureed, and powdered pulses found there were no significant differences between treatments due to processing, all were able to decrease BG response compared to non-pulse control. Limitations of prior research include the inclusion of only young healthy males, which impacts the generalizability of the results. In addition, only select pulses have been tested as flours. Comparing different pulse flours will assist in determining whether beneficial effects are dependent upon pulse type. Many snack products currently available on the market are extruded, however, many of those products are energy-dense and
do not provide health benefits. Thus incorporating pulse flours into extruded products would make those products healthier and easier for consumers to consume pulses because they would be included into foods they already consume. The effects of extruded snack products containing pulse ingredients have not been assessed.

The objectives of this study were therefore to assess the effects of a variety of pulse flours when consumed in a snack on post-prandial BG, serum insulin, and appetite before (0-120 min) and after an ad libitum pizza meal (140-200 min). Food intake (FI) at the ad libitum pizza meal consumed at 120 min, and palatability of the treatments were also measured. It was hypothesized that extruded snacks containing pulse flours would reduce post-prandial BG and appetite 2 hours following consumption and reduce FI at a later meal.

4.3 STUDY DESIGN

4.3.1 Participants:

Healthy female and male participants were recruited from posters placed around Winnipeg and advertisements in Winnipeg newspapers (Appendix 1). Individuals contacted the research team for more information about the study and, if interested, were scheduled for an in-person screening session to determine study eligibility. At screening, prospective participants were given the consent form (Appendix 2) to review and ask questions. Following the signing of the consent, the research team completed the screening forms (Appendix 3, 4, and 5) and measured body weight, height, BG and BP following standard procedures. Body mass index was calculated from weight and height measurements. Blood glucose was measured by finger prick blood sample with a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and
assessed by a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). The inclusion criteria were normoglycemic (<5.6 mmol/L), normotensive (systolic BP <140 mm Hg and diastolic BP below < 90 mm Hg), males and pre-menopausal females aged 18–50 y, with a BMI of 18.5–29.9 kg/m². Participants were excluded if they regularly skipped breakfast, were identified as restrained eaters, smoked, were participating in high intensity organized athletic activities, or were training for an athletic event, on medications that may have influenced study outcomes, or had experienced any gastrointestinal related health conditions/surgeries over the past year. Restrained eaters were identified during screening through the use of an eating habits questionnaire. Participants with a score >11 were excluded. Participants were asked if they had given a blood donation in the previous four 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 ensured that participants were willing to eat the food products and the pizza meal.

4.3.2 Treatments:

The six treatments were given to each participant once in a 50 g serving. The serving size was based upon Health Canada reference amount and serving size for extruded snacks. Participants were given one cup of water to consume with the treatment. Corn flour was supplied by Agricor (Marion, Indiana, USA) and pulse flours were provided by Best Cooking Pulses (Portage la Prairie, Manitoba, Canada). Pulse flour treatments contained 40% pulse flour.
Table 1. Nutritional content of extruded snacks

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Energy (kcal)</th>
<th>Available Carbohydrate % db</th>
<th>Protein % db</th>
<th>Resistant starch % db</th>
<th>Insoluble dietary fibre % db</th>
<th>Soluble dietary fibre % db</th>
<th>Starch damage % db</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (control)</td>
<td>209.7</td>
<td>94.7</td>
<td>5.15</td>
<td>0.17</td>
<td>2.29</td>
<td>0.55</td>
<td>73.3</td>
</tr>
<tr>
<td>Whole Yellow Pea</td>
<td>198.8</td>
<td>78</td>
<td>12.1</td>
<td>0.23</td>
<td>6.68</td>
<td>1.74</td>
<td>62</td>
</tr>
<tr>
<td>Split Yellow Pea</td>
<td>204.9</td>
<td>83.6</td>
<td>11.5</td>
<td>0.35</td>
<td>3.91</td>
<td>1.38</td>
<td>65.7</td>
</tr>
<tr>
<td>Green Lentil</td>
<td>198.2</td>
<td>77.5</td>
<td>13.2</td>
<td>0.31</td>
<td>6.11</td>
<td>1.77</td>
<td>61.1</td>
</tr>
<tr>
<td>Chickpea</td>
<td>202</td>
<td>77.2</td>
<td>12.7</td>
<td>0.22</td>
<td>5.53</td>
<td>1.96</td>
<td>61.8</td>
</tr>
<tr>
<td>Pinto Bean</td>
<td>199.8</td>
<td>77.3</td>
<td>12.6</td>
<td>0.26</td>
<td>6.50</td>
<td>3.19</td>
<td>57</td>
</tr>
</tbody>
</table>

Nutritional content data determined by in vitro digestibility testing provided by Dr. Nancy Ames, Agriculture & Agri-Food Canada.

4.3.3 Study design:

The study followed a randomized; double blinded, balanced, and repeated measures design. Participants consumed one treatment per week in randomized order. Men and women were tested with a minimum of 5 days between sessions. Women were tested during the follicular phase of their menstrual cycle (the two weeks following menstruation), because of observed insulin resistance during the luteal phase.40,41

Blood samples were collected from an intravenous catheter by a registered nurse. The samples were centrifuged at 1300 g for 10 min after having been at room temperature for 15-
30 min. Serum was centrifuged and pipetted into micro-tubes continuously throughout the participants’ sessions. Samples were promptly put in a fridge to cool and were then stored in a -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. 20% of samples were performed in duplicate for additional precision confidence.

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.

4.3.4 Protocol:

Participants were asked to fast overnight for 10 hours, and consume a standardized breakfast 4 hours prior to the session. The breakfast contained Cheerios cereal (General Mills, Mississauga, Ontario), 250 mL of 2% milk, and 250 mL Tropicana orange juice (Tropicana Products Inc., Bradenton, Florida), along with a 500 mL bottle of water. The interval between breakfast and test sessions was kept constant. Participants picked-up or had the standardized breakfast delivered prior to their scheduled session. Participants were allowed water up to 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 maintain their regular routine.

Upon arrival at the sessions, subjects completed questionnaires assessing recent FI and physical activity (Appendix 6). They were also 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 pattern, they were asked to reschedule. Also, a blood sample was taken by finger prick by a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and BG was assessed by a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). If their BG 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). This is a validated questionnaire used in previous acute studies investigating the effect of pulse consumption on appetite. 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.” Additional VAS questionnaires were administered to measure palatability (taste, texture) (Appendices 9 and 10), physical comfort (Appendix 11) and energy/fatigue (Appendix 12). Prior to treatment consumption, subjects completed VAS questionnaires.

Following completion of the initial questionnaires, VAS, and finger prick BG measurement, an indwelling intravenous catheter was inserted in the arm by a registered nurse for blood sampling. Then a baseline blood sample was taken (0 min). Following baseline measures, participants consumed the pulse product along with a cup of water (within 5-10 min
kept constant for each participant), followed by completion of a VAS questionnaire assessing palatability. Blood sampling took place at 15, 30, 45, 60, 90 and 120 min. Appetite and physical comfort VAS questionnaires were completed following each blood sample. For measurement of FI, participants were served an ad libitum meal at 120 min\textsuperscript{22,24,32}. Fresh hot pizza (McCain\textsuperscript{®} Deep 'n Delicious\textsuperscript{®}) was served every 5-7 min and participants were asked to eat until they were “comfortably full”. Participants had a choice of pepperoni, deluxe, and cheese pizza. They were given the same pizza they selected at the initial session across all sessions. Energy intake from the pizza meal was calculated from the weight consumed and the compositional information provided by the manufacturer. Once they had finished eating pizza, participants rated its palatability to ensure that the pulse products did not influence the taste/enjoyment of the pizza. Following the pizza meal, BG, insulin, appetite, physical comfort and energy/fatigue were measured at 140, 155, 170, 185 and 200 min.

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 the commencement of their participation.

4.3.6 Statistics:

Average treatment palatability, as well as subjective appetite, physical comfort, and energy/fatigue were calculated from the average of the questions on their corresponding VAS
questionnaire. Statistical analysis was performed using SAS (SAS Inc., Chicago, IL, USA). Postprandial responses were measured by incremental AUC (iAUC = total AUC – fasting concentrations of respective parameter) analyzed using the trapezoid method. Cumulative, pre- and post-test meal iAUC for BG, insulin, and subjective appetite were calculated for 0–200 min, 0–120 min and 140–200 min, respectively. Repeated measures ANOVA were used to determine the effects of treatments, time and the time-by-treatment interaction on BG, 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 the specific time points. The effect of treatments on FI at the meal and on BG, insulin, appetite, physical comfort, and energy/fatigue AUC were determined by repeated measures ANOVA. Sex and session were included in all analyses. Tukey-Kramer post-hoc tests were used to describe mean differences among treatments.

4.4 RESULTS

4.4.1 Participant Characteristics: Thirty-nine (23 women, 16 men) participants were recruited, and 26 (12 women, 14 men) completed the study. Participants who withdrew from the study did so because of the time commitment (n=3), difficulty drawing blood (n=6), or discomfort of the IV (n=3). One participant withdrew because they were moving. Participants mean age was 24.7 ± 5.72 y and BMI was 23.5 ± 2.41 kg/m².

4.4.2 Blood Glucose: Figure 1 shows the BG response over the entire session. Time (p<0.0001), treatment (p<0.0001) and time-by-treatment (p<0.0001) effects were observed on total BG
over the session. For pre-meal BG (0-120 min), time (p<0.0001), treatment (p<0.0001) and time-by-treatment effects (p<0.0001) were observed, whereas only time (p<0.0001) and treatment (p<0.05) effects were noted on post-meal BG (120-200 min), but no time-by-treatment interaction (p=0.10). The effects on BG at specific time points were dependent upon pulse type. At 30 and 45 min, BG was lower (p<0.05) after consumption of pinto bean compared to whole yellow pea and green lentil snacks, whereas at 60 min, pinto bean consumption led to lower BG compared to whole yellow pea snacks. Pinto bean and chickpea snacks led to lower (p<0.05) pre-pizza BG iAUC, compared with control, whole yellow pea and green lentil snacks. There were no differences in post-meal BG iAUC (p=0.49).

Figure 1. Blood glucose response over 200 min.
Table 2. Pre- and post-pizza BG iAUC (mmol·min/L)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative</th>
<th>Pre-pizza</th>
<th>Post-pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>217±35</td>
<td>142.2±17.6a</td>
<td>53.8±10.7</td>
</tr>
<tr>
<td>Whole yellow pea</td>
<td>250.8±34.6</td>
<td>148.8±18.6a</td>
<td>69±9.87</td>
</tr>
<tr>
<td>Split yellow pea</td>
<td>230.1±38.6</td>
<td>128.1±19ab</td>
<td>63.9±10</td>
</tr>
<tr>
<td>Green lentil</td>
<td>237.7±46</td>
<td>140±22.6a</td>
<td>68.7±11.5</td>
</tr>
<tr>
<td>Chickpea</td>
<td>193±31.6</td>
<td>110±15.4b</td>
<td>70.2±9.59</td>
</tr>
<tr>
<td>Pinto bean</td>
<td>198.6±29.9</td>
<td>102±14.3b</td>
<td>70.2±9.59</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26). Values in the same column with different lowercase letters are significantly different from each other, p<0.05.

4.4.3 Insulin: Significant time (p<0.0001), but no significant treatment or time-by-treatment effects were seen over the entire session (Figure 2). In the pre-meal period effects of time (p<0.0001) and time-by-treatment (p=0.001) were observed but no effect of treatment (p=0.61). Post-meal, an effect of time (p<0.0001) but no effects of treatment (p=0.66) or time-by-treatment (p=0.66) were observed. However, consumption of the pinto bean snack led to lower pre-meal iAUC compared with corn control, whole yellow pea, and split yellow pea snacks (p<0.05). No effect of treatment was observed in post-meal iAUC (p=0.19). One participant was excluded from the insulin dataset as they exhibited an exceeding high insulin response following the pizza meal.
Figure 2. Blood insulin response over 200 min.

Table 3. Pre- and post-pizza insulin iAUC (µU·min/mL)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative (µU·min/mL)</th>
<th>Pre-pizza (µU·min/mL)</th>
<th>Post-pizza (µU·min/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>5898.7±691.7</td>
<td>2256.4±251.5a</td>
<td>2758.9±391.1</td>
</tr>
<tr>
<td>Whole yellow pea</td>
<td>5106.1±579.5</td>
<td>2299.1±258.6a</td>
<td>2573.3±310.2</td>
</tr>
<tr>
<td>Split yellow pea</td>
<td>5858±864.2</td>
<td>2269.3±346.1a</td>
<td>3059.2±600.8</td>
</tr>
<tr>
<td>Green lentil</td>
<td>4995.6±602.3</td>
<td>1995.8±175ab</td>
<td>2768.5±470.9</td>
</tr>
<tr>
<td>Chickpea</td>
<td>5254.7±611.2</td>
<td>1952.21±224ab</td>
<td>3162.4±521.1</td>
</tr>
<tr>
<td>Pinto bean</td>
<td>4917.6±684.3</td>
<td>1698.4±207.3b</td>
<td>2993.2±490</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26). Values in the same column with different lowercase letters are significantly different from each other, p<0.05.
4.4.4 Food & Water Intake: No effects of treatment on FI (p=0.77) or water intake (p=0.12) were observed (Figure 3).

Table 4. Food & water intake (second meal/ab libitum pizza meal*)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Food intake (kcal)</th>
<th>Water intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>795.8±50.5</td>
<td>469.4±53.4</td>
</tr>
<tr>
<td>Whole yellow pea</td>
<td>787.4±47.5</td>
<td>394.8±35.8</td>
</tr>
<tr>
<td>Split yellow pea</td>
<td>822.9±60.6</td>
<td>444.5±49.5</td>
</tr>
<tr>
<td>Green lentil</td>
<td>816.6±47.1</td>
<td>420.7±45</td>
</tr>
<tr>
<td>Chickpea</td>
<td>796.3±55.9</td>
<td>471.8±45</td>
</tr>
<tr>
<td>Pinto bean</td>
<td>819.7±57.3</td>
<td>392.2±38.6</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26).

*Measured at an ad libitum pizza meal consumed at 120 min.
4.4.5 Palatability: No differences in palatability of the treatments (p=0.15) or pizza (p=0.06) were observed (Figure 4).

Figure 4. Treatment palatability

4.4.6 Appetite: An effect of time (p<0.0001) on total appetite was observed, however, no effects of treatment (p=0.14) or time-by-treatment interaction (p=0.44) were observed (Figure 5). An effect of time on pre (p<0.0001) and post-meal (p<0.0001) but no effects of treatment or time-by-treatment were observed. No effects of treatment on pre-meal, post-meal, or total appetite iAUC were observed.
Figure 5. Subjective appetite over 200 min.

Table 5: Average appetite total AUC (mm/min)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative</th>
<th>Pre-pizza</th>
<th>Post-pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>8376.2 ± 340.9</td>
<td>5544.4 ± 263.8</td>
<td>2769.6 ± 101.9</td>
</tr>
<tr>
<td>Whole yellow pea</td>
<td>8818.0 ± 354.5</td>
<td>6000.8 ± 254.9</td>
<td>2856.4 ± 141.7</td>
</tr>
<tr>
<td>Split yellow pea</td>
<td>8806.5 ± 348.6</td>
<td>5906.4 ± 262.8</td>
<td>2872.0 ± 128.2</td>
</tr>
<tr>
<td>Green lentil</td>
<td>8589.2 ± 267.8</td>
<td>5858.8 ± 213.7</td>
<td>2705.6 ± 120.9</td>
</tr>
<tr>
<td>Chickpea</td>
<td>8600.5 ± 302.9</td>
<td>5853.3 ± 240.4</td>
<td>2707.6 ± 111.9</td>
</tr>
<tr>
<td>Pinto bean</td>
<td>8584.0 ± 333.6</td>
<td>5830.2 ± 263.5</td>
<td>2753.8 ± 98.5</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26).
4.4.7 Physical Comfort: An effect of time (p=0.0001) on total physical comfort, and no effects of treatment (p=0.65) or time-by-treatment interaction (p=0.76) were observed. On pre-meal physical comfort an effect of time (p<0.001) was observed but no effects of treatment (p=0.89) or time-by-treatment (p=0.43). On post-meal physical comfort an effect of time was observed (p<0.05) but no effects of treatment (p=0.49) or time-by-treatment (p=0.99). In pre, post, and total iAUC no effect of treatment on physical comfort was observed (Data not shown).

4.4.8 Energy/Fatigue: No effects of time (p=0.15), treatment (p=0.37), or time-by-treatment interaction (p=0.34) were observed on total, pre and post energy/fatigue. In total iAUC an effect of treatment (p<0.05) was observed. Green lentil consumption resulted in a higher (p=0.01) rating of energy compared to pinto bean. In pre-meal iAUC or post-meal iAUC no differences were observed (Data not shown).

4.5 DISCUSSION

Despite their nutritional profile very few Canadians eat whole pulses. Dry pulses require significant time to prepare and Canadians may be unfamiliar with how to incorporate them into their meals. The results from our study support the use of pulse flours in extruded snacks as value adding ingredients, however, the effects on post-prandial glycemia were dependent upon pulse type. The variety of snacks tested were equally palatable and the pulse snacks contained higher amounts of protein and fibre compared to the corn control. The corn snacks are similar to what is currently available on the market. The present data suggest that
adding pulses to already existing extruded corn products could help consumers consume more pulses from foods they are already eating.

The BG results suggest that pinto bean and chickpea snacks retain their acute post-prandial BG lowering effects better than the other pulses tested. Pinto bean and chickpea snack had a lower pre-pizza BG iAUC, and at 30 and 45 min pinto bean had lower BG compared to whole yellow pea and green lentil snacks, and at 60 was lower than whole yellow pea snacks. The snacks with the exception of the corn control had similar macronutrient profiles. When comparing the nutritional content of the snacks, chickpea has the lowest available carbohydrate (77.2 % db) followed by pinto bean (77.3% db) and green lentil (77.5% db). If variations in available carbohydrate were responsible for the BG response, whole yellow pea and green lentil should have elicited a similar BG response to the pinto and chickpea treatments. This is not what occurred; therefore, variation in available carbohydrate does not explain the pre iAUC BG results. Not only does green lentil have the third lowest percentage of available carbohydrates (77.5%), split yellow pea has the highest available carbohydrate (83% db) of the pulse treatments and yet it does not cause one of the highest BG peaks. Surprisingly the green lentil treatment caused the highest BG peak followed by whole yellow pea, corn and split yellow pea.

Another possible explanation for the pinto bean and chickpea BG lowering effects could be starch damage that occurred during the extrusion process. Damaged starch is digested more quickly than non-damaged starch ⁴⁵. Pinto bean snack did have the lowest starch damage (57% db) compared to the other pulse snacks (61.1-65.7% db). Starch damage, however, does not explain why chickpea performed more favorably than the other pulses as green lentil and whole
yellow pea have very similar rates of starch damage. The treatments also have very similar resistant starch (0.22-0.35% db) and insoluble dietary fibre (3.91-6.68% db). Pinto bean has the highest soluble fibre (3.19 vs 1.38-1.96% db), chickpea, however, does not share this and therefore this finding does not conclusively explain the acute BG lowering effects observed. It is possible that pinto and snacks may have lowered BG in different ways.

The serum insulin results demonstrate that the reduction in pre-meal BG iAUC was not a result of an increased serum insulin response. The glucose-insulin relationship is regulated in response to the BG level. Following the consumption of a meal BG rises. The rise in BG stimulates the beta-cells in the pancreas to release insulin, which results in glycolysis and glycogen synthesis. BG then gradually decreases. Not only did pinto bean reduce BG in pre-meal iAUC, it also led to decreased pre-meal iAUC for insulin compared to corn control, whole yellow pea, and split yellow pea.

Fibre and resistant starch have been demonstrated to play a role in postprandial BG, insulin, and appetite control. A randomized single blind crossover study of 20 young adult men consumed a breakfast and lunch containing 24 g of resistant starch (48 g total). The authors found lower energy intakes at the ad libitum meal compared to the placebo but no effect on subjective hunger ratings. Lower postprandial insulin levels compared to the placebo were observed across the entire study. While resistant starch has shown to lower insulin levels, it alone does not account for the reduction in insulin seen in pre iAUC by the pinto bean snack. The yellow pea, and green lentil snacks contained more resistant starch than the pinto bean and chickpea snacks and yet did not show the same effect. A study has shown that pinto along with black beans were most effective at reducing BG compared to kidney beans and a rice
control. Soluble fibre was discussed as part of the explanation as it has shown to slow digestion and subsequently BG. Differences in snack composition alone do not explain the differences observed.

Along with their strong nutritional profile, pulses also contain anti-nutrients that can be destroyed when cooked. This may be a potential explanation as to why pinto bean and chickpea were the most effective in BG lowering. Some anti-nutrients have desirable effects such as α-glucosidase and α-amylase inhibition. α-glucosidase and α-amylase are important glucosidases needed for humans to digest starch. It is crucial to carbohydrate digestion as all carbohydrates other than monosaccharides require being broken down by enzyme activity prior to absorption. Indeed, α-glucosidase and α-amylase inhibition impedes carbohydrate digestion leading to lower postprandial BG levels. These anti-nutrients could have been more resistant to extrusion cooking in the pinto bean and chickpea snacks.

The BG lowering effects of α-amylase inhibitors were demonstrated in an animal study with male Wistar rats. The rats received 50 mg/kg of body weight α-amylase inhibitor or placebo for 21 days. The study showed that both acute and chronic consumption of α-amylase inhibitor resulted in significantly lower BG levels. Human studies have also demonstrated the acute insulin lowering effects of α-glucosidase inhibitors. Healthy men and women aged 18-39 consumed a herbal α-glucosidase inhibitor with a meal, then had blood sampling performed over two hours. Postprandial insulin was significantly reduced.

The heating used during extrusion can decrease or inactivate compounds that contain anti-nutrition factors such as the enzyme inhibitor alpha-amylase inhibitor which affects digestibility. Testing has shown that when pinto beans are extruded the majority of anti-
nutrition inhibitors are inactivated with the exception of alpha-amylase inhibitor. When pinto beans were processed at a barrel temperature of 140°C they were found to have 55% of alpha-amylase inhibitor remaining. At a final barrel temperature of 180 °C there was no remaining alpha-amylase inhibitor. The maximum barrel temperature used during the extrusion of our snacks was 115°C, therefore, it is plausible that a significant amount of alpha-amylase inhibitor may have remained in some of the snacks. Pale brown and brown pulses have the greatest amount of alpha-amylase inhibitor, followed by beige, dark brown and red. Specific pulses were not listed as examples, however, pinto beans could be described as beige with flecks of red and brown containing all of the colors highest in the inhibitor. Chickpeas could also be described as beige. The greatest effectiveness of pinto beans followed by chickpeas could be due to greater concentrations of α-glucosidase and α-amylase inhibitors. The pulse snacks underwent the same processing, however, the anti-nutrients in pinto beans and chickpeas may have been more resistant to the extrusion process than the other treatments in the study. This is an aspect that has not been examined in our treatments thus far and could be explored as a future direction.

One beneficial aspect of extrusion is that it improves pulse protein digestibility by causing the proteins to unfold and denature. Extrusion also increases protein digestion by inhibiting enzyme inhibitors that hinder protein digestion. Protein has been shown to increase satiety to a greater extent than fat or carbohydrates. The protein content of the pulse snacks ranged from 5.7-6.6 g. This is substantially higher than the corn control which contains 2.5 g. Increasing the protein content of snacks is desirable because it could increase satiety and decrease FI, however, in this study the dose of protein may have been too low, or
the digestibility may have been affected by extrusion negating the potential appetite or FI effects. The ease of digestion of protein could help explain why there were no postprandial differences in BG, insulin and appetite, or in FI at the pizza meal. This is similar to what was seen in Smith et al (2012) with pea fractions. Smith et al (2012) performed two experiments with protein (10 g, 20 g) and fibre (10 g, 20 g) treatments. When the second meal was served at 30 min the pre-pizza BG reduction effects were independent of protein dose and both 10 g and 20 g led to a reduction compared to the control and fibre 10 g treatment. In the second experiment the second meal was served at 120 min. In this experiment no effects of BG pre and postprandial were observed. The authors concluded that the protein in pea flour may be similar to whey in the sense that it is rapidly digested. Our treatments contained less than 10 g of protein, as well as fibre, and we also followed the participants over 120 min. Our study observed differences in BG from 0-120 min. Protein and fibre in combination may be more effective at reducing BG than protein alone. Important to note is that our treatments were fairly matched for kcal and the described study was not. Future studies should assess FI 30-60 min following consumption as well as extruded snacks containing higher percentages of pulse flours. Despite there being no effect on FI or appetite, it is beneficial to incorporate pulses into snacks. The incorporation of pulses into the study snacks increased the protein and fibre content. If incorporating pulse fractions was adopted by industry, it would improve the nutritional profiles of readily available snacks for consumers. Unlike most snacks currently on the market, the snacks in this study were not intensely flavoured. Our palatability rating results suggest that the pulse containing snacks were equally as palatable as the corn control making them excellent value adding ingredients. Additionally, there were no differences in physical
comfort ratings between the treatments indicating that the pulse snacks were well tolerated and did not result in discomfort over 200 min. Pulses contain resistant starch that ferments in the large intestine and produces gas that may cause discomfort. Individuals who do not regularly consume foods containing resistant starch may experience discomfort, however, this was not seen in this study. Participants also experienced no differences in perceived energy/fatigue for any of the treatments, this is evidenced by no effect of treatment on energy/fatigue in total iAUC.

### 4.6 CONCLUSION

This study supports the use of pinto bean and chickpea flours in the control of postprandial glycemia. Results demonstrate that pulse snacks were equally palatable as the corn control similar to what is readily available on the market and did not cause physical discomfort. These data also show that pulse flours would make an excellent value added ingredient for extruded snacks. Pulses are a dynamic food containing a wealth of nutrition. Incorporating pulse flours into food products would not only increase the nutritional content of the food products, but increase the amount of pulses being consumed. Future research should include individuals who are obese and individuals with type 2 diabetes. Future research studies need to investigate the effects of pulse flours incorporated into extruded snacks on food intake at meals served earlier (30-60 min later), as well as investigate the effects of snacks containing higher percentages of pulse flours to find the optimal dose without affecting the palatability of the products.
4.7 ACKNOWLEDGEMENTS

The authors acknowledge the volunteers that participated in the study.

The authors’ contributions are as follows:

Alie Johnston coordinated the clinical study, performed laboratory analyses, compiled the data, performed statistical analysis, and contributed to the writing of the manuscript. Dianna Omer assisted on the clinical study. Dr. Rebecca Mollard designed the study, supervised the clinical study, performed statistical analysis and contributed to the preparation of the manuscript. Dr. Nancy Ames contributed the in vitro digestibility data and contributed to the preparation of the manuscript. Dr. Julianne Curran contributed to the preparation of the manuscript. Dr. Danielle Bouchard and Dr. Peter Jones assisted in the study design and coordination as well as contributed to the preparation of the manuscript.

The authors declare no conflicts of interest.
The beneficial effects of consuming whole pulses on glycemic control are well established. The benefits of consuming pulses have been found to be retained when served in mixed meals, when pureed, as well as when consumed as flours in a sauce or in bread. Prior to this study it was yet to be seen whether the glycemic benefits are retained when pulse flours are incorporated into extruded products.

From the previous chapter, we now know that when pulses are extruded that some perform more favourably than others. Pinto bean and chickpea reduced BG, and pinto bean also reduced insulin. Thus, it can be concluded that incorporating pulse flours into extruded corn flour snack products has a beneficial effect on postprandial glycemic control; however, this effect is dependent upon pulse type. From the previous chapter it is not possible to assess if a specific fraction of pulse flour is responsible for the acute BG and insulin effects observed, or whether synergistic effects of combined fractions are responsible. This gap in the literature is addressed in the cereal study. Pea fractions were incorporated into extruded oat cereal products to determine whether pea fractions impact postprandial glycemia and satiety control or whether the combination of different fractions have synergistic effects.
CHAPTER 5

ACUTE EFFECTS OF EXTRUDED PEA FRACTIONS ON GLYCEMIC RESPONSE, INSULIN, APPETITE, AND FOOD INTAKE IN HEALTHY YOUNG ADULTS

5.1 ABSTRACT

Background: The benefits of consuming pulses on glycemic control are well established; however, research examining the effects of pulse fractions incorporated into extruded products is limited.

Objective: To assess the effects of replacing oat flour with pea fractions in extruded cereals on postprandial glycaemia, insulin, and appetite before and after an ad libitum meal consumed at 120 min.

Design: In a randomized, repeated-measures crossover study, adults consumed cereals made with: 1) oat flour, 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). Blood glucose (BG), insulin and appetite iAUC was calculated pre-meal (0-120 min) and post-meal (120-200 min).

Results: For pre-meal overall mean BG, there were time (p<0.0001), treatment (p<0.0001) and time-by-treatment (p<0.0001) effects observed. Pre-meal BG iAUC was lower following the protein (p<0.0001), starch+protein (p<0.0001), protein+fibre (p<0.0001), and the fibre+starch+protein (p<0.0001) cereals compared to the starch. BG iAUC was also lower following the protein (p<0.05), starch+protein (p<0.05), protein+fibre (p<0.005), and the fibre+starch+protein (p<0.0001) cereals compared to the control. For pre-meal overall mean
insulin levels, time (p<0.0001), treatment (p<0.0005), and time-by-treatment interaction (p=0.001) effects were observed. Fibre+protein led to a lower insulin response compared to control (p<0.05), starch+protein (p<0.05), and protein (p=0.001) cereals. Fibre+starch+protein also led to lower insulin compared to protein cereal (p<0.05). There were also treatment effects on pre-meal insulin iAUC (p<0.05); fibre+protein resulted in lower insulin compared to control (p<0.05) and protein (p<0.05) cereal.

Conclusion: The benefits of replacing oat with pulse fractions in extruded cereals are dependent on fraction type. The greatest effect was in response to the combination of protein+fibre and fibre+starch+protein resulting in both decreased BG and insulin levels.

5.2 INTRODUCTION

The current rates of obesity in North America place many individuals at risk of developing chronic diseases such as metabolic syndrome and type 2 diabetes. Potential countermeasures to obesity and resulting chronic risk factors include consumption of foods and ingredients that increase satiety, reduce energy intake, and improve post-prandial glycemic control. Increased consumption of pulses could be one effective countermeasure. Pulses contain 17-30% of dry weight protein which is typically twice the amount found in cereals. The benefits of consuming protein include increased satiety 7 and BG control 8. Pulses also contain soluble and insoluble fibre 9, as well as resistant and slowly digestible starch and oligosaccharides 9,10. A variety of health benefits are associated with intake of dietary fibre such as increased satiety and reduced energy intake 11-13, as well as improved glycemia and insulin sensitivity in individuals with and without diabetes 15. The high amounts of fibre, as well as
resistant and slowly digestible starch in pulses contribute to their low glycemic index (GI) \(^{16-18}\).

Despite the glycemic control and satiety benefits, very few Canadians eat whole pulses \(^{25}\).

Canadians may be unfamiliar cooking with pulses, therefore adding pulses to food products that are already a part of Canadians diets such as extruded cereals may help increase pulse consumption and improve the diets of consumers. Merits of extrusion cooking include: greatly increased product shelf life, raw ingredients are cooked very efficiently \(^{56}\), protein digestibility is increased \(^{54,55}\), and anti-nutrition factors and micro-organisms are inactivated \(^{56}\).

Pulse fractions are dietary ingredients that may be used as food based strategies for the control of body weight and BG \(^{15}\). Although limited, pulses have been examined as flours and fractions where their favourable effects have been shown to be retained \(^{18,25-27,35,36}\). Pulse flour as well as extruded pulse flour consumed in a bread, resulted in a lower BG response compared to a non-pulse bread \(^{26,27}\). Another study comparing whole, pureed, and powdered pulses found there were no differences between treatments due to processing and that all had beneficial effects on post-prandial glycemic control \(^{25}\).

A previous study examined the effects of yellow pea protein (10 g, 20 g) and fibre (10 g, 20 g) on glycemic response, appetite and FI in two acute studies. Treatments were served in a tomato soup. In the first experiment, an ad libitum pizza meal was served 30 min following consumption of the treatment. Both protein treatments caused a significant BG reduction from 0-30 min as well as following the pizza meal compared to the control and fibre 10 g treatment. The protein 20 g treatment lowered FI compared to all other treatments. From 0-30 min the BG reduction was independent of protein dose, but after the pizza meal it was dose dependent. The protein 20 g treatment was the most effective at reducing BG. In the second experiment
the pizza meal was served at 120 min and there were no effects on pre or post BG or FI. The authors attribute this to pea protein digesting rapidly similar to whey 35.

Another study examined the effects of pea fractions on FI, appetite and BG. Treatments were yellow peas (18 g protein, 10 g fibre), pea hull fibre (10 g) plus pea protein (18 g), pea hull fibre (10 g protein, 10 g fibre), pea protein (18 g protein, 2 g fibre), and a control (10 g protein, 2 g fibre) 36. Treatments were served with tomato sauce and noodles. Results showed no differences in appetite or FI. Combined protein and fibre reduced pre and total BG AUC compared to the control, but the protein and fibre treatments alone did not. It may be that the dose of protein and fibre alone were insufficient to cause an effect when served in a mixed meal. There were no differences in FI or appetite. These studies suggest that both protein and fibre contribute to the acute BG reducing effects of pea flour, but are not definitive.

Pulse fractions may be used to impact postprandial glycemia, however, these effects are dose dependent and depend upon the timing of outcomes measured. Additionally, yellow pea is an attractive ingredient as its fibre and protein fractions are commercially available 35, abundant, and are the least expensive pulse 4. The next step is to test pea fraction treatments in varying fibre, starch, and protein proportions to determine whether a specific fraction is responsible for the effects, or if the effects are synergistic when fractions are combined. According to the Canadian Community Health Survey, 20% of adult Canadians regularly consume breakfast cereal 57. Incorporating pea fractions into common breakfast products could increase the protein and fibre content in the diet of Canadians. The amino acid profile of oats complements that of yellow peas making oat and pea fraction cereals a desirable combination. The objectives of this study were to assess the effects of varying pea fraction combinations
including protein, starch, and hull fibre on acute post-prandial BG, serum insulin, and appetite, physical comfort, and energy/fatigue responses before and after a second meal. Food intake (FI) at the second meal, and palatability of the treatments were also measured. It was hypothesized that cereals containing both pea protein and fibre would cause the greatest reduction in BG, appetite 2 hours postprandial and reduce FI at a later meal.

5.3 STUDY DESIGN

5.3.1 Participants:
Healthy female and male participants were recruited from posters placed around Winnipeg, Manitoba, Canada and advertisements in Winnipeg newspapers (Appendix 1). Individuals contacted the research team for more information about the study and, if interested, were scheduled for an in-person screening session to determine study eligibility. At screening, prospective participants were given the consent form (Appendix 2) to review and ask questions. Following the signing of the consent the research team completed the screening forms (Appendix 3, 4, and 5) and measured body weight, height, BG and BP following standard procedures. Body mass index was calculated from weight and height measurements. Blood glucose was measured by finger prick blood sample with a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and assessed by a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). The inclusion criteria were normoglycemic (<5.6 mmol/L), normotensive (systolic BP <140 mm Hg and diastolic BP below < 90 mm Hg), males and pre-menopausal females aged 18–50 y, with a BMI of 18.5–29.9 kg/m². Participants were excluded if they regularly skipped breakfast, were restrained eaters, smoked, were participating
in high intensity organized athletic activities, or were training for an athletic event, on medications that may have influenced study outcomes, or had experienced any gastrointestinal related health conditions/surgeries over the past year. Restrained eaters were identified during screening through the use of an eating habits questionnaire. Participants with a score >11 were excluded. Participants were asked if they had given a blood donation in the previous four 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 ensured that participants were willing to eat the food products and the pizza meal.

5.3.2 Treatments:
The six treatments were given to each participant once in a 35 g serving. The serving size was based upon Health Canada reference amount and serving size for extruded cereals. Oat flour was provided 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), pea starch and pea protein by Parrheim Foods (Saskatoon, Saskatchewan, Canada).
### Table 6. Nutritional content of extruded cereals

<table>
<thead>
<tr>
<th>Extruded cereal treatments</th>
<th>Energy (kcal)</th>
<th>Available Carbohydrate (% db)</th>
<th>Protein (% db)</th>
<th>Resistant starch (% db)</th>
<th>Insoluble dietary fibre (% db)</th>
<th>Soluble dietary fibre (% db)</th>
<th>Starch damage (% db)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% oat (control)</td>
<td>150.2</td>
<td>79.0</td>
<td>9.58</td>
<td>0.24</td>
<td>6.17</td>
<td>3.64</td>
<td>63.3</td>
</tr>
<tr>
<td>39% oat, 50% pea starch</td>
<td>143.4</td>
<td>80.8</td>
<td>8.22</td>
<td>0.52</td>
<td>6.74</td>
<td>2.88</td>
<td>66.7</td>
</tr>
<tr>
<td>47% oat, 40% pea protein</td>
<td>135.3</td>
<td>53.5</td>
<td>24.3</td>
<td>0.23</td>
<td>9.96</td>
<td>3.97</td>
<td>43.4</td>
</tr>
<tr>
<td>32% oat, 18% pea hull, 40% pea protein</td>
<td>121.2</td>
<td>39.8</td>
<td>23.2</td>
<td>0.16</td>
<td>23.1</td>
<td>4.94</td>
<td>31</td>
</tr>
<tr>
<td>6% oat, 50% pea starch, 40% pea protein</td>
<td>130.2</td>
<td>57.9</td>
<td>22.6</td>
<td>0.62</td>
<td>8.85</td>
<td>3.10</td>
<td>47.4</td>
</tr>
<tr>
<td>16% pea hull, 45% pea starch, 36% pea protein</td>
<td>124.4</td>
<td>46.8</td>
<td>20.8</td>
<td>0.81</td>
<td>20.3</td>
<td>4.77</td>
<td>40.2</td>
</tr>
</tbody>
</table>

Nutritional content data determined by in vitro digestibility testing provided by Dr. Nancy Ames, Agriculture & Agri-Food Canada.
5.3.3 Study design:

The study followed a randomized; double blinded, balanced, and repeated measures design. Participants consumed one treatment per week in randomized order. Men and women were tested with a minimum of 5 days between sessions. Women were tested during the follicular phase of their menstrual cycle (the two weeks following menstruation), because of observed insulin resistance during the luteal phase \cite{40,41}.

Blood samples were collected from an intravenous catheter by a registered nurse. The samples were centrifuged at 1300 g for 10 min after having been at room temperature for 15-30 min. Serum was centrifuged and pipetted into micro-tubes continuously throughout the participants’ sessions. Samples were promptly placed in a fridge to cool and were then stored in a -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. 20% of samples were performed in duplicate for additional precision confidence.

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.
4.3.4 Protocol:

Participants were asked to fast overnight for 10 hours, and consume a standardized breakfast four hours prior to the session. The breakfast contained Cheerios cereal (General Mills, Mississauga, Ontario), 250 mL of 2% milk, and 250 mL Tropicana orange juice (Tropicana Products Inc., Bradenton, Florida), along with a 500 mL bottle of water. The interval between breakfast and test sessions was kept constant. Participants picked-up or we delivered the standardized breakfast prior to their scheduled session. Participants were allowed water up to 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 maintain their regular routine.

Upon arrival at the sessions, subjects completed questionnaires assessing recent FI and physical activity (Appendix 6). They were also 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 pattern, they were asked to reschedule. Also, a blood sample was taken by finger prick by a Monojector Lancet Device (Sherwood Medical, St. Louis, MO, USA) and BG was assessed by a glucose meter (Accu-Chek Compact Plus, Roche Diagnostics, Laval, Que., Canada). If their BG 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). This is a validated questionnaire used in previous acute studies investigating the effect of pulse consumption on appetite. 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.” Additional VAS questionnaires will be administered to measure palatability (taste, texture) (Appendices 9 and 10), physical comfort (Appendix 11) and energy/fatigue (Appendix 12)\(^{22,24,32}\). Prior to treatment consumption, subjects completed VAS questionnaires.

Following initial questionnaires, VAS, and finger prick BG measurement, an indwelling intravenous catheter was inserted in the arm by a registered nurse for blood sampling. Then a baseline blood sample was taken. Following baseline measures, participants consumed the pulse product along with a cup of water (within 5-10 min kept constant for each participant), followed by completion of a VAS questionnaire assessing palatability. Blood sampling took place at 15, 30, 45, 60, 90 and 120 min, 5 mL was collected at each time point. Appetite and physical comfort VAS questionnaires were completed following each blood sample. For measurement of FI, participants were served an ad libitum meal at 120 min\(^{22,24,32}\). Fresh hot pizza (McCain\(^{®}\) Deep 'n Delicious\(^{®}\)) was served every 5-7 min and participants were asked to eat until they were “comfortably full”. Participants had a choice of pepperoni, deluxe, and cheese pizza. They were given the same pizza they selected at the initial session across all sessions. Energy intake from the pizza meal was calculated from the weight consumed and the compositional information provided by the manufacturer. Once they had finished eating pizza, participants rated its palatability to ensure that the pulse products did not influence the taste/enjoyment of the pizza. Following the pizza meal, BG, insulin, appetite, physical comfort and energy/fatigue were measured at 140, 155, 170, 185 and 200 min.
4.3.5 Ethics:

This study followed the Declaration of Helsinki guidelines, 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 the commencement of their participation.

4.3.6 Statistics:

Average treatment palatability, as well as subjective appetite, physical comfort, and energy/fatigue were calculated from the average of the questions on their corresponding VAS questionnaire. Postprandial responses were measured by incremental AUC (iAUC = total AUC – fasting concentrations of respective parameter) analyzed using the trapezoid method. Cumulative, pre- and post-test meal iAUC for BG, insulin, and subjective appetite were calculated for 0-200 min, 0–120 min and 140-200 min, respectively. Repeated measures ANOVA were used to determine the effects of treatments, time and the time-by-treatment interaction on BG, 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 the specific time points. The effect of treatments on FI at the meal and on BG, insulin, appetite, physical comfort, and energy/fatigue AUC were determined by repeated measures ANOVA. Sex and session were included in all analyses. Tukey-Kramer post-hoc tests were used to describe mean differences among treatments. Statistical analysis was performed using SAS (SAS Inc., Chicago, IL, USA).
5.4 RESULTS

5.4.1 Subject Characteristics: Thirty participants were recruited (12 women, 18 men) and 26 participants (11 women, 15 men) completed the study. Participants chose to withdraw because of dislike of the IV (n=2), for an unspecified reason (n=1), or because they ceased communication (n=1). Participants mean age was 23.8 ± 3.87 y and BMI was 22.6 ± 2.32 kg/m².

5.4.2 Blood Glucose: Time (p<0.0001) and time-by-treatment (p<0.001) effects on total BG were observed over the session, but no treatment effect (p=0.25). Figure 6 shows the BG response over the entire session. For pre-meal BG (0-120 min), effects of time (p<0.0001), treatment (p<0.0001) and time-by-treatment interaction were seen (p<0.0001). While the control and starch cereals resulted in the highest glycemic response, the cereals containing protein resulted in the lowest BG response. During the pre-meal period fibre + protein (p=0.0001, p=0.001), fibre + starch + protein (p<0.01), and protein cereal (p<0.001, p<0.01), resulted in a lower BG response compared to control, and starch. The starch + protein cereal also resulted in lower BG compared to control (p<0.05). At 30 min, protein + fibre exhibited a lower BG response compared to oat control (p<0.01), whereas the protein (p<0.05), protein + fibre (p<0.0001), and starch + protein + fibre (p=0.005) cereals exhibited a lower glycemic response compared to the starch cereal. At 45 min, the protein (p=0.0005), starch + protein (p=0.01), protein + fibre (p<0.0001) and the starch + protein + fibre (p<0.0001) cereals resulted in a lower BG response compared to the control cereal. Also at 45 min, the protein + fibre (p<0.001), starch + protein + fibre (p<0.001), and protein (p<0.05) cereals led to a lower BG response compared to the starch cereal. At 60 min, the protein, and fibre + starch + protein
cereal resulted in a lower BG response compared to the control (p<0.01, p=0.01) and starch cereals (p=0.01, p<0.05). The fibre + protein cereal also led to a lower response compared to control (p=0.01). There was a 60 min session-by-treatment effect (p<0.05) but post hoc did not identify differences among treatments on session or session-by-treatment. Pre-meal BG iAUC was lower following the protein, starch + protein, protein + fibre and the starch + protein + fibre cereals compared to the starch (p=0.0001) and control (p<0.05).

During the post-meal period, effects of time (p<0.0001) and time-by-treatment interaction (p<0.05) were seen, but no effect of treatment (p=0.71). Post-meal BG iAUC comparisons revealed no differences between treatments.

Figure 6. Blood glucose response over 200 min
Table 7. Pre- and post-pizza BG iAUC (mmol-min/L)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative</th>
<th>Pre-pizza</th>
<th>Post-pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>151.5±21.4ab</td>
<td>81.7±11a</td>
<td>53.6±8.75</td>
</tr>
<tr>
<td>Oat + starch</td>
<td>164.7±21b</td>
<td>98.6±9.88a</td>
<td>50.5±8.3</td>
</tr>
<tr>
<td>Oat + protein</td>
<td>119.4±18.9a</td>
<td>53.4±8.39b</td>
<td>56.3±8.85</td>
</tr>
<tr>
<td>Oat + starch + protein</td>
<td>127.4±17.3a</td>
<td>54.1±7.3b</td>
<td>63.3±8.86</td>
</tr>
<tr>
<td>Oat + fibre + protein</td>
<td>120.6±20.4ab</td>
<td>46.9±7.38b</td>
<td>62.9±9.94</td>
</tr>
<tr>
<td>Fibre + starch + protein</td>
<td>124.3±17.3a</td>
<td>44.6±6.71b</td>
<td>72.3±10.3</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26). Values in the same column with different lowercase letters are significantly different from each other, p<0.05.

5.4.3 Insulin: Time (p<0.0001) and time-by-treatment (p<0.05) effects on total insulin were observed over the entire session but no treatment (p=0.35) effect was seen. For pre-meal insulin (0-120 min), effects of time (p<0.0001), treatment (p<0.001) and time-by-treatment were observed (p=0.001). During the pre-meal period (0-120 min), the fibre + protein cereal resulted with the lowest insulin response, lower than the cereal control (p<0.05), starch + protein (p<0.05), and protein cereal (p=0.001). The fibre + starch + protein cereal was also lower (p<0.05) than the protein cereal. At 30 min, fibre + protein cereal led to a lower insulin response compared to starch + protein cereal (p<0.05). At 45 min, the fibre + protein cereal led to a lower response compared to starch + protein (p<0.001), control (p<0.05), and protein cereals (p=0.001). Fibre + starch + protein cereal also exhibited a lower insulin response than the protein cereal (p<0.05). At 120 min, starch + protein (p=0.001), fibre + starch + protein
(p<0.001), protein (p<0.05), and fibre + protein (p<0.05) cereals had a lower insulin response compared to the cereal control (Figure 7). Pre-meal iAUC was lower following fibre + protein compared to control (p=0.01) and protein cereal (p<0.05).

During the post-meal period, an effect of time was observed (p<0.0001), but no treatment (p=0.83) or time-by-treatment (p=0.51) interactions were observed. No differences were identified in post-meal iAUC.

Figure 7. Serum insulin response over 200 min
Table 8. Pre- and post-pizza insulin iAUC (µU·min/mL)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative</th>
<th>Pre-pizza</th>
<th>Post-pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>5563.5±452.4</td>
<td>1674.7±158.3a</td>
<td>3394.1±415.6</td>
</tr>
<tr>
<td>Oat + starch</td>
<td>5434.3±675.1</td>
<td>1455.8±178ab</td>
<td>3779.6±497</td>
</tr>
<tr>
<td>Oat + protein</td>
<td>4997.2±509.5</td>
<td>1595.5±199.4a</td>
<td>3397.5±380.9</td>
</tr>
<tr>
<td>Oat + starch + protein</td>
<td>5008.4±462.9</td>
<td>1545.8±204ab</td>
<td>3584±330.4</td>
</tr>
<tr>
<td>Oat + fibre + protein</td>
<td>4905±642.1</td>
<td>1125.5±154.8b</td>
<td>3827.9±512.2</td>
</tr>
<tr>
<td>Fibre + starch + protein</td>
<td>4866.4±503.3</td>
<td>1293.2±182.3ab</td>
<td>3679.9±365.2</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26). Values in the same column with different lowercase letters are significantly different from each other, p<0.05.
5.4.4 Food & water Intake: No effects of treatment on FI (p=0.053) (Table 8) or water intake was observed (p=0.058).

Table 9. Food & water intake at second meal ab libitum pizza meal*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Food intake (kcal)</th>
<th>Water intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>859.9±69.6</td>
<td>387.5±39.3</td>
</tr>
<tr>
<td>Oat + starch</td>
<td>794±64.4</td>
<td>464.7±45.8</td>
</tr>
<tr>
<td>Oat + protein</td>
<td>847.3±70.2</td>
<td>454.6±43.5</td>
</tr>
<tr>
<td>Oat + starch + protein</td>
<td>857.4±81.5</td>
<td>418.2±46.2</td>
</tr>
<tr>
<td>Oat + fibre + protein</td>
<td>854.7±62.6</td>
<td>433.4±49.1</td>
</tr>
<tr>
<td>Fibre + starch + protein</td>
<td>825.7±64.4</td>
<td>397.8±49.7</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26).

*Measured at an ab libitum pizza meal consumed at 120 min.
5.4.5 Palatability: No differences in palatability of the treatments (p=0.38) or pizza (p=0.16) were observed (Figure 9).

Figure 9. Treatment palatability

5.4.6 Appetite: An effect of time (p<0.0001) on total appetite was noted, however, no effects of treatment (p=0.84) or time-by-treatment interaction (p=0.39) (Figure 10). During the pre-meal period an effect on time (p<0.0001), but no treatment (p=0.39) or time-by-treatment interactions were observed (p=0.65). During the post-meal period an effect on time was observed (p<0.0001), but no treatment (p=0.86) or time-by-treatment interaction (p=0.85). For total iAUC, pre-meal iAUC, and post-meal iAUC no effects of treatment were observed.
Figure 10. Subjective appetite over 200 min

Table 10. Pre- and post-average appetite total AUC (mm/min)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative</th>
<th>Pre-pizza</th>
<th>Post-pizza</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>9032.6 ± 264.2</td>
<td>6235.0 ± 262.9</td>
<td>2731.9 ± 76.5</td>
</tr>
<tr>
<td>Oat + starch</td>
<td>8983.5 ± 307.0</td>
<td>6111.5 ± 258.7</td>
<td>2793.9 ± 90.9</td>
</tr>
<tr>
<td>Oat + protein</td>
<td>9025.8 ± 283.3</td>
<td>6021.4 ± 261.7</td>
<td>2783.5 ± 89.5</td>
</tr>
<tr>
<td>Oat + starch + protein</td>
<td>8862.2 ± 298.5</td>
<td>5945.0 ± 256.8</td>
<td>2834.3 ± 104.7</td>
</tr>
<tr>
<td>Oat + fibre + protein</td>
<td>8629.4 ± 343.7</td>
<td>5834.9 ± 293.2</td>
<td>2760.2 ± 123.1</td>
</tr>
<tr>
<td>Fibre + starch + protein</td>
<td>8912.9 ± 336.8</td>
<td>6102.3 ± 285.7</td>
<td>2756.0 ± 102.5</td>
</tr>
</tbody>
</table>

Note: All values are means ± SE (n=26).
5.4.7 Physical Comfort: An effect of time (p<0.01) on total physical comfort was observed but no effects of treatment (p=0.97) or time-by-treatment interaction (p=0.9). For pre-meal physical comfort, no effects of time (p=0.09), treatment (p=0.99), or time-by-treatment interaction were seen (p=0.65). For post-meal an effect of time (p<0.01) but no effects of treatment (p=0.08) and time-by-treatment were observed (p=0.41). No effects of treatment on pre-meal, post-meal, or total iAUC physical comfort were observed (Data not shown).

5.4.8 Energy/Fatigue: An effect of time (p<0.0001), but not treatment (p=0.21), or time-by-treatment interaction was seen (p=0.79) on total energy/fatigue. For pre-meal energy/fatigue an effect for sex (p=0.01) but no effects of time (p=0.14), treatment (p=0.32) or time-by-treatment interaction were observed (p=0.96). On pre-meal energy/fatigue no effect of treatment was observed for men (p=0.39), there was an effect for women (p<0.05) but post hoc analysis did not identify differences among treatments. For post-meal energy/fatigue an effect for time (p<0.05) but no treatment (p=0.08) or time-by-treatment interaction were observed (p=0.63). No effects of treatment on pre-meal, post-meal, or total iAUC energy/fatigue were observed (Data not shown).

5.5 DISCUSSION

Our results obtained through an acute randomized crossover study support the use of yellow pea protein and fibre in the regulation of acute postprandial glycemia. Although BG was lower in response to treatments containing protein compared to an oat control, there were no effects on subjective appetite or FI. It may be that 35 g is an insufficient serving to exert an
effect on satiety and FI two hours following the consumption of the cereal. A similar study demonstrated second meal FI effects when the second meal was served after 30 min of consuming a treatment containing 20 g of protein, but not after 120 min. Future studies should administer the second meal earlier to address this finding and determine whether there are beneficial pea fraction effects on satiety control.

The BG and FI effects of pea protein and fibre were investigated in two randomized crossover studies in healthy young men. The first study found that a dose of 10 g and 20 g of yellow pea protein served in a mixed meal reduced mean BG response up to 30 min following consumption compared to control, and fibre treatments of 10 g and 20 g in a mixed meal. A second meal was served at 30 min and only the protein 20 g treatment reduced mean BG post-meal. In a second study the same treatments were given except the second meal was served after 120 min of consuming the treatment. In this study there were no differences in pre or post-meal mean BG. These studies demonstrate that protein is the fraction responsible for the short term BG lowering effects of whole yellow peas, and shows that these effects are transient and dose dependent. In our study, the treatments were consumed in 35 g servings and contained 2.8-8.5 g of protein. The treatments that exhibited BG lowering effects contained 7.26-8.5 g of protein. Our results differ somewhat from those of the previous study as we observed an effect in pre BG (0-120 min), which maybe the results of the treatment formulation. Some of our extruded cereals contained both protein and fibre fractions with oat flour, whereas their treatments consisted of protein and fibre served as treatments separate from one another in tomato soup. It is also important to note that our treatments were fairly matched for kcal whereas in the other study they were not. Mollard et al (2014) examined
yellow pea fractions with the treatments fibre, protein, protein + fibre, and whole yellow pea compared to a control and served in a mixed meal. Protein + fibre and whole yellow pea led to a reduced cumulative iAUC BG compared with control and fibre. Cumulative iAUC BG was also reduced for protein compared to fibre. Results of this study suggest that protein is a greater contributor to BG reduction than fibre, but that protein and fibre have greater synergistic effects when consumed together. This mechanism of synergy is consistent with our findings. Our study found that all of the treatments containing pea protein resulted in a significantly decreased BG. Additionally the attenuated BG effects were greater for the treatments that contained pea protein and fibre, treatments that also reduced insulin. While the pea protein containing treatments led to lower BG during the pre iAUC period, this did not continue into the post iAUC period. These findings support the conclusion that protein is the fraction most responsible for the acute post-prandial BG lowering effects of yellow pea and that the effects do not last beyond a second meal served after 2 hours as demonstrated by Smith et al (2012).

The available carbohydrate content of the protein containing cereals ranged between 13.9-20.2 g. Despite the difference in available carbohydrate, the protein containing cereals performed very similarly; BG pre iAUC was significantly lower for all protein containing treatments compared to the control. Among the protein containing cereals there was a maximum difference of about 6 g in available carbohydrate. This did not seem to affect BG response as all protein treatments reduced BG and there were no BG differences among them. Starch + protein cereal contained 8 g less available carbohydrate compared to the starch cereal and resulted in decreased BG compared to the starch cereal. A difference of 6 g compared to 8
g is small, which suggests that available carbohydrate alone is not responsible for the glycemic reduction caused by the cereals containing protein.

Potential synergistic effects of pea protein and fibre were seen in both the insulin and BG results. Not all treatments that lowered BG also reduced insulin. Our results suggest that pea protein may be insulintropic when consumed alone. During the pre-meal period, the fibre + protein cereal resulted in the lowest insulin response, lower than the cereal control, starch + protein, and protein cereal. The fibre + protein cereal was also lower than protein cereal. The two cereals containing pea fibre had the lowest insulin response and contained the greatest amount of insoluble dietary fibre. Potter et al (1981) conducted a study in which the researchers concluded that dietary fibre was most likely responsible for the differences in BG and insulin reduction seen in pinto beans. It was found that the higher the insoluble dietary fibre, the lower the BG and insulin response. Our research supports this conclusion as the cereals containing pea fibre contained the highest insoluble dietary fibre and were the treatments with the lowest pre iAUC BG response. This effect is likely due to the ability of insoluble fibre to slow gastric emptying by means of its water retaining abilities, and the effects of soluble and insoluble fibre to cause slower and prolonged nutrient absorption through distension of the stomach. Our results indicate that consumption of pea protein combined with fibre is the most effective at reducing BG and insulin levels.

Intestinal satiety should also be considered as a modulator of BG response. Close interaction between nutrients and the intestinal wall causes the release of satiety signal hormones. Increasing the viscosity of digested food as it travels through the small intestine increases its residence time, and time that nutrients are being absorbed. Prolonged residence
of digested food in the small intestine also impacts gastric emptying through the release of satiety hormones. We did not measure intestinal satiety, however, future studies should do so when investigating the gut hormone response to pea protein and hull fibre. Such work will aid in determining the mechanism(s) responsible for their acute BG and insulin effects, as well as potential satiety effects.

This research demonstrates that the acute BG reducing effects of pea protein are independent of pea fibre. While protein treatments lowered BG without added fibre, the lowest BG responses were from the treatments containing both added pea protein and fibre. The lowest BG response was from the fibre + starch + protein cereal. This cereal had less protein than the protein and starch + protein cereals that also lowered BG significantly. Thus, these findings suggest that a synergistic effect of protein and fibre may be responsible for the lowest BG responses. A treatment containing only added pea hull fibre may have assisted in determining whether the greatest acute BG and insulin reductions were caused by synergistic effects or if hull fibre alone would cause the effects.

There were no differences in palatability, FI, appetite, physical comfort, or energy/fatigue across treatments. It may be that the 35 g serving was an insufficient dose to affect appetite and FI. Another explanation could be the timing of the second meal. A study investigating pea protein found FI differences when a second meal was served at 30 min, but not when a second meal was served at 120 min. The timing of the second meal may have been too long after the treatment meal to see FI effects in our study. Food intake should also be measured at 30 to 60 min following the treatment meal to determine whether there are FI effects earlier than 120 min.
A strength of the present study is that normal weight and overweight men and women participated. However, further research with other populations such as obese adults, and adults with hyperglycemia would be valuable in determining whether beneficial effects on post-prandial glycemia and insulin response would be greater in states of hyperglycemia. Treatments were processed under the same conditions, however, including whole peas as a treatment would have allowed for the extruded products to be compared to unprocessed peas. This would have been difficult because of the additional time commitment needed from participants. In addition, treatments were matched for serving size and reflected the Health Canada’s serving size for extruded cereal. However, the cereal serving size may have been too small to see an effect on satiety and FI at a second meal. This study had intentional differences in treatment composition to investigate the specific fraction of peas responsible for the acute health benefits observed in other studies.\(^1,2,24,35,36\)

5.6 CONCLUSION

In conclusion, the extrusion of pea fractions into a cereal product improves the postprandial glycemic response; thus the glycemic benefits of whole pulses are somewhat retained with pulse fractions. All treatments containing pea protein led to a reduced BG response. In particular, the two cereals containing pea protein and fibre were the most effective as they reduced both BG and insulin. The synergistic effects of pea protein and fibre require further investigation. The increased insoluble fibre content of these cereals is one explanation of the additional benefits, however, intestinal satiety as well as satiety hormone response should also be investigated for increased understanding of the underlying
mechanisms. This research supports the use of pea fractions fibre and protein to improve postprandial glycemic response and postprandial insulin response. It is anticipated that the commercial development of cereals containing pea fractions could be marketed for improving post-prandial glycemic control. Our research provides support for a glycemic control health claim for extruded pea fraction cereals. Pulse consumption could increase as pulses would be available in a ready to consume form that Canadians are familiar with, and many consume often.

5.7 ACKNOWLEDGEMENTS

The authors acknowledge the volunteers that participated in the study.

The authors’ contributions are as follows:

Alie Johnston coordinated the clinical study, performed laboratory analyses, compiled the data, performed statistical analysis, and contributed to the writing of the manuscript. Dianna Omer assisted on the clinical study. Dr. Rebecca Mollard contributed to the study design, supervised the clinical study, performed statistical analysis and contributed to the preparation of the manuscript. Dr. Nancy Ames contributed the in vitro digestibility data and contributed to the preparation of the manuscript. Dr. Julianne Curran contributed to the preparation of the manuscript. Dr. Danielle Bouchard and Dr. Peter Jones assisted in the study design and coordination as well as contributed to the preparation of the manuscript.

The authors declare no conflicts of interest.
6 CONCLUSION

This research is the first to examine the acute health effects of extruded pulse flours and fractions in snacks and cereals. Study I demonstrated that pinto bean and chickpea retain acute BG lowering effects and that these effects are not due to an increased insulin response. Study II demonstrated that pea protein causes acute BG lowering effects apart from fibre, but that the effects are greater when protein and fibre are consumed together. Our results suggest that pea protein may be insulintropic when consumed without pea fibre. When consumed together the protein and fibre cereal not only lower acute BG, but also insulin, indicating that the protein and fibre may have synergistic effects resulting in both glycemic and insulin benefits. Thus the post-prandial benefits of pulse ingredients are dependent upon pulse variety as well as fraction type.

The results of the present studies suggest that pulse flours, and pea fractions protein and fibre could be utilized in extruded products without affecting the palatability of the products. Our results provide support for future post-prandial glycemic health claims for pulse ingredients in extruded products, particularly for pinto bean and chickpea flour as well as pea protein and fibre. Incorporating pulse flours and fractions into commonly consumed and readily available snacks and cereals would improve the nutrition of those foods by increasing the amount of protein and fibre. It would also make it easier for consumers to consume pulses by having them in a readily available form. Pulse flours and fractions incorporated into extruded products have the potential to support Canadian pulse growers, increase the number of Canadians consuming pulses, and improve the health and nutrition of Canadians.
7 FUTURE DIRECTIONS

The results from both studies support the role of pulse flours and fractions in extruded products designed to improve post-prandial glycemic control. Our research demonstrates that pinto and chickpea extruded snacks reduce acute BG response and that pinto bean also reduces acute insulin response. Our research also adds to the current research on the health benefits of yellow pea fractions by demonstrating that extruded cereals containing pea protein reduce acute BG. It provides evidence that in combination pea protein and pea fibre further reduce the acute insulin response, and reduce acute BG to a greater extent than pea protein consumed alone.

Our inclusion criteria were designed to be generalizable and represent the average Canadian. The inclusion criteria for age was 18-50 y, however, there were few participants over 30 y which led to our population being younger than intended. Future studies could include an older demographic, participants with a BMI greater than 29.9 kg/m², and/or type-2 diabetes. Individuals with type-2 diabetes could benefit from readily available snacks and cereals that improve post-prandial glycemic control. Thus, further research is needed to determine if the beneficial effects of pinto and chickpea snacks, and pea fractions would be retained in other groups of the population.

A next step would be to incorporate a varying range of percentages of pulse flours and fractions in products to determine the minimum and optimal doses needed for acute health benefits and product palatability. While our research failed to observe FI or appetite effects, a higher incorporation rate of the flours/fractions could cause FI and appetite reduction. Additionally, future studies should measure FI earlier than in our study. It is possible that the
benefits on satiety may occur 30 or 60 min following consumption and that these benefits are no longer apparent at 120 min. Increasing the percentage of pulse flours and fractions in the extruded products could increase the health benefits seen. The percentage of pulse flours incorporated may have been too low in our studies to elicit some of the possible health benefits. However, higher doses of pulse ingredients may also increase the possibility of gastrointestinal discomfort for consumers who do not regularly consume pulses. Our treatments did not cause gastrointestinal discomfort but it remains a possibility when increasing the pulse ingredient percentage.

There is the opportunity for the pulse industry to pursue “function” or “structure-function” type health claims. Health claims related to the maintenance of normal postprandial glycemia levels, and satiety are examples of “function” or “structure-function” type claims. Broadly defined, function claims are claims about the specific beneficial effects that the consumption of a food or a constituent of a food (i.e. nutrient or other component) has on normal functions or biological activities of the body. Such claims relate to a positive contribution to health and to the maintenance of a physiological function. Our study protocol was designed in accordance to guidance documents from Health Canada. Our research provides scientific evidence in support of an application to substantiate BG control health claims for food products containing pulse ingredients.

From our research, it is still unclear why pinto beans and chickpeas were the only pulse flours to significantly reduce BG postprandial. The composition of the snacks were very similar. Pulse extrusion research from Martín-Cabrejas et al (1999) suggests that anti-nutrition factors could explain why pinto and chickpea performed best out of the treatments examined.
barrel temperature during the extrusion process can significantly impact the extrudate\textsuperscript{52}. At the temperature that our snacks were processed, it is possible that the alpha-amylase inhibitor that naturally occurs in pulses was more resistant to the extrusion process in some pulse types. This is a future direction that could be pursued.

Future research should also include hormone analysis. Interactions between nutrients and the intestinal wall causes the release of satiety signals hormones which affect gastric emptying. Analyzing the hormonal response to pulse flours and fractions could help further our understanding of the mechanisms behind the health effects of pulses\textsuperscript{60}, as well as glycemic regulation. This was a limitation of our research. We collected blood in both the pulse flour and pea fraction studies to perform hormonal analysis with the intention of analyzing these samples in the future.

This research adds to the growing body of evidence indicating which fraction of peas is responsible for acute post-prandial glycemic effects. Our research demonstrated that pea protein incorporated into an oat cereal causes acute BG reduction apart from effects of the pea fibre itself. However, when pea protein and fibre were incorporated into an oat cereal together there was a greater reduction in BG and a reduction in insulin that was not seen in treatments without pea fibre. This suggests that there are synergistic effects when pea protein and fibre are consumed together. This finding is supported by previous research\textsuperscript{18,36}.

Starch damage is a factor that could help explain differences. Starch damage was lower for the protein containing cereals compared to the non-protein containing cereals. The starch cereal contained 50% pea starch, the starch + protein cereal contained 50% pea starch, and the fibre + starch + protein contained 45% pea starch. This suggests that the BG lowering effects are
not negated by increased starch content as the starch + protein and fibre + starch + protein treatments reduced BG while the starch treatment did not. A limitation of our research is that we did not have an oat cereal with pea fibre alone. Future research could include a treatment containing only added pea fibre to determine if pea fibre decreases BG, or whether pea fibre furthers BG reduction through synergistic effects with pea protein.

Overall, this research indicates that pulse flours and pea fractions retain post-prandial glycemic effects of whole pulses when incorporated into extruded products. However, the mechanisms behind the acute BG and insulin effects of pulses and their fractions are not completely clear. Despite the fact that future research is needed to explore the mechanisms of action, our research demonstrates that it is beneficial to incorporate pulse ingredients into extruded products for glycemic control and improved nutritional content.
8 REFERENCES


45. Sandstedt RM, Mattern PJ. Damaged starch quantitative determination in flour.pdf.


Campos-Vega R, Loarca-Pina G, Oomah BD. Minor components of pulses and their


57. Barr SI, Difrancesco L, III VLF. Consumption of Breakfast and the Type of Breakfast Consumed Are Positively Associated with Nutrient Intakes and Adequacy of Canadian Adults 1, 2. *J Nutr.* 2013:86-92. doi:10.3945/jn.112.167098.other.


doi:10.1017/S0007114513000032.


http://www.inspection.gc.ca/food/labelling/food-labelling-for-industry/health-
Appendix 1.

Richardson Centre for Functional Foods and Nutraceuticals

Interested in being a nutrition study volunteer?

Are you between 18-45 years old?

If so, you may be eligible to participate in a study at the examining effects of pulse products on:
- Blood sugar
- Appetite and food intake
- Exercise

Contact us:
Richardson Centre for Functional Foods and Nutraceuticals
196 Innovation Drive, University of Manitoba
Phone: 204-480-1042
Email: pulsetrial@umanitoba.ca
Co-Investigators: Dr. Peter Jones and Dr. Danielle Bouchard
Appendix 2.

RESEARCH SUBJECT INFORMATION AND CONSENT FORM
Pulse EnRiched Food and Exercise Clinical Trials; PERFECT project

Part 1. Acute effects of pulse ingredients in food products on appetite, blood glucose and food intake in adults.

Protocol number: B2014:114 (1)

Investigators:
Peter J.H. Jones, PhD
Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba
196 Innovation Drive
Winnipeg, Manitoba R3T 6C5
Phone: 204-474-9787

Danielle R. Bouchard, PhD, CEP
318 Max Bell Centre, University of Manitoba
Winnipeg, Manitoba, R3T 2N2
Phone: 204-474-8627

Sponsors: Saskatchewan Pulse Growers, 116 Research Drive, 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 can be made into flours and fractions (protein, fibre, starch). These flours and fractions can be used as ingredients in foods that may be able to lower appetite and blood sugar. This project includes studies to investigate the effects of three different food products containing pulse ingredients on appetite, blood sugar control and food intake 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 meet with us at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) six times for a maximum of 4 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 10 weeks.

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 similar meal the night 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 or drink 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 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 12 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 or hand. The catheter will remain in your arm and be used to sample blood in small amounts during the session so you only need to 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 consuming the food product, we will collect blood samples at multiple time points. You will be served a pizza meal at 120 minutes, followed by additional blood sampling and questionnaires. You will be asked to consume pizza until you are comfortably full. Each session will last up to 4 hours.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00</td>
<td>Consumption of breakfast (provided 1-2 days before)</td>
</tr>
<tr>
<td>10:45</td>
<td>Arrive at the laboratory</td>
</tr>
<tr>
<td>10:50</td>
<td>Fill in food intake, physical activity, sleep, stress, and VAS questionnaires and take first blood sample</td>
</tr>
<tr>
<td>11:00-11:05</td>
<td>Eat the treatment (0 minutes)</td>
</tr>
<tr>
<td>11:15-1:00</td>
<td>Blood sampling and VAS questionnaires at 15, 30, 45, 60, 90 and 120 minutes</td>
</tr>
<tr>
<td>1:00-1:20</td>
<td>Eat the ad libitum (eat until you are comfortably full) pizza meal</td>
</tr>
<tr>
<td>1:20-2:20</td>
<td>Blood sampling and VAS questionnaires at 140, 155, 170, 185 and 200 minutes after baseline</td>
</tr>
</tbody>
</table>

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 72 ml (2.4 ounces), which is 432 ml (14.4 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.

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. 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 and metabolites related to the control 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, 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.

<table>
<thead>
<tr>
<th>Investigator:</th>
<th>Dr. Peter Jones</th>
<th>Tel No.</th>
<th>204-474-9787</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator:</td>
<td>Dr. Danielle Bouchard</td>
<td>Tel No.</td>
<td>204-474-8627</td>
</tr>
<tr>
<td>Coordinator:</td>
<td>Dr. Rebecca Mollard</td>
<td>Tel No.</td>
<td>204-474-8270</td>
</tr>
</tbody>
</table>

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 NCT02402504, NCT02366572, and NCT02385890. 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:

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>HOW OFTEN?</th>
<th>HOW LONG? (HOURS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

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.

0. How often are you dieting?
   Never ____ rarely _____ sometimes _____ often _____ always _____

1. 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+ _____

2. What is your maximum weight gain within one week?
   0 – 1 _____ 1.1 - 2 _____ 2.1 – 3 _____ 3.1 - 5 _____ 5.1+ _____

3. In a typical week, how much does your weight fluctuate?
   0 – 1 _____ 1.1 – 2 _____ 2.1 - 3 _____ 3.1 - 5 _____ 5.1+ _____

4. Would a weight fluctuation of 5lbs affect the way you live your life?
   Not at all _____ slightly _____ moderately _____ very much _____

5. Do you eat sensibly in front of others and splurge alone?
   Never _____ rarely _____ often _____ always _____

6. Do you give too much time and thought to food?
   Never _____ rarely _____ often _____ always _____

7. Do you have feelings of guilt after overeating?
   Never _____ rarely _____ often _____ always _____

8. How conscious are you of what you are eating?
   Not at all _____ slightly _____ moderately _____ extremely _____

9. How many pounds over your desired weight were you at your maximum weight?
   _____ 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**).

<table>
<thead>
<tr>
<th></th>
<th>Enjoyment?</th>
<th>How often?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pasta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Rice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Potatoes (mashed, roasted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. French fries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Pizza</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Bread, bagels, dinner rolls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Sandwiches, subs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Cereal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Cake, donuts, cookies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Protein/breakfast shakes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Chips/puffs/crackers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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: ____________

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 ____________________________ VERY weak
   strong

2. How hungry do you feel?

   NOT ______________________________ As hungry hungry
   at all ____________________________ as I have ever felt
   at all

3. How full do you feel?

   NOT ______________________________ VERY
   full ____________________________ full
   at all

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

   NOTHING ____________________________ A LARGE
   at all ____________________________ amount

5. How thirsty do you feel?

   NOT ______________________________ As thirsty
   thirsty ____________________________ as I have ever felt
   at all
Appendix 9
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 _______________________________ VERY pleasant
   at all pleasant

2. How tasty have you found the treatment?

   NOT _______________________________ VERY tasty
   at all tasty

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

   NOT _______________________________ VERY much
   at all much
Appendix 10
Visual Analogue Scales
Palatability: meal

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.

How pleasant have you found the beverage/food?

NOT at all ________________________________ VERY pleasant
pleasant
Appendix 11  
Visual Analogue Scales  
Physical Comfort

Time: ____________

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. Do you feel nauseous?
   NOT _____________________________ VERY
   at all much

2. Does your stomach hurt?
   NOT _____________________________ VERY
   at all much

3. How well do you feel?
   NOT _____________________________ VERY
   well well
   at all

4. Do you feel like you have gas?
   NOT _____________________________ VERY
   at all much

5. Do you feel like you have diarrhea?
   NOT _____________________________ VERY
   at all much
Appendix 12
Visual Analogue Scales
Energy and Fatigue

Time:  
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  ________________________________  VERY energetic
   at all

2. How tired do you feel right now?
   NOT  ________________________________  VERY tired
   at all