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The dose effect of lentil flour incorporated in a snack on blood glucose, satiety, and food intake in young male adults

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Abstract:

Functional foods are seeing increased potential for use in improving the health outcomes of Canadians, specifically in a population experiencing an aging demographic with increased rates of overweight, obesity, and chronic diseases. Pulses have been recognized for their added health benefits, when consumed as both a whole pulse, or as a processed ingredient added to foods. Many novel methods of processing pulses have been developed to increase their access as an added functional ingredient in food product development. While there are some differences in the functional properties of pulse ingredients based on the processing methods, there is little translation of the method of processing used to produce pulse ingredients to the consumer. The current investigation sought to quantify the dose needed to elicit a positive effect on post-prandial glycaemic control and short term food intake in male subjects/participants following consumption of a solid snack food product formulated with whole lentil flour. The secondary objective was to measure the subjective feelings of hunger in participants. There were four treatments used in this study: an energy-free water control, a snack bar formulated with 24g wheat flour, a bar replacing 12g of wheat flour with lentil flour, and a snack bar with 24g of lentil flour. The wheat and lentil flours used for this study were controlled for particle size, with 79µm and 94µm grind sizes, respectively. A sample of healthy men (n=12), aged 19-30 were recruited, and consumed one of the four treatments after completing an overnight fast followed by consumption of a standardized breakfast. Blood samples were collected at time 0,10, 20, 30, 60, 90, and 120 min, and analysed for glucose. Short term intake was measured via an *ad libitum* pizza meal consumed at the end of the 2 hour study period. There was an effect of 12g lentil flour on suppressing subjective feelings of appetite by ~10% compared to the wheat-based control. While not significant, this effect appeared to significantly impact short term food intake ($p<0.05$).

The bar formulated with 12g lentil flour resulted in a non-significant reduction of subsequent food intake by 122kcal compared to the wheat-based control, and 230 kcal compared to the water control. There was no effect of treatment for both caloric treatments and water free control on mean 2 hour blood glucose or tAUC, iAUC or niAUC values. While there were no significant effects seen on blood glucose over the 2-hour study period, both treatments containing lentil flour appeared to delay the post-prandial glycaemic response, resulting in a smaller increase in blood glucose concentration compared to the wheat control. The addition of as little as 12g of pulse flour may play a role in satiety, food intake, and delayed post-prandial glycaemia.

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Introduction

Non-communicable Diseases and their Risk Factors in Canada

Non-communicable diseases (NCD), or chronic diseases, are defined by the World Health Organization as diseases that progress slowly and are of long duration (1, 2). In Canada, the incidence and prevalence of NCD has been steadily increasing for many years, with NCD now reported to be the leading cause of death amongst Canadians (2). Examples of some of the most commonly NCD diagnosed in Canada include Diabetes Mellitus (DM), cardiovascular disease (CVD), chronic respiratory diseases (CRD), and cancer, and there are many risk factors associated with the development of chronic diseases (2). This represents a significant and growing risk to the health and wellbeing of Canadians, as well as a substantial burden on the healthcare system. From 2000-2011, the most prevalent NCD in Canada were cancer, hypertension, diabetes, and chronic obstructive pulmonary disease, all of which showed an upward trend over the same time period (3-5).

As an example of the impact of the NCD on Canadians, about 10% of Canadian adults are living with diagnosed DM (Type 1 and Type 2), with a dramatic increase in the age standardized prevalence of the disease over the past decade from 4.8% of the population in 2000-2001, to 7.6% in 2011-2012, with an annual increase of over 4% (5). The direct and indirect costs of diabetes was estimated at $11.7 billion in 2010, and this is expected to rise to an annual cost of $16 billion by 2020 (6). Within the Canadian population, the healthcare costs of the proportion of the population living with DM is three to four times higher than populations without the disease (7). While direct costs associated with DM are around 20% of the total cost, consisting mainly of costs associated with hospitalization and primary care physicians, almost 80% of the total cost of diabetes is accounted for by mortality and disability alone, due to the

impact of complications associated with a diagnosis of DM (6). This includes adverse health outcomes from both the macrovascular and microvascular outcomes of a diagnosis of DM, such as CVD, non-traumatic limb amputations, kidney disease, and blindness (7).

Further complicating the burden of NCD in Canada, there is also an increase in the prevalence of the diagnosis of multiple morbidities, or people living with two or more NCD, with 3.6% of Canadian adults falling into this category (5). As Canada continues to experience an aging demographic, with more Canadians now over the age of 65 than under the age of 15 as of 2015, when looking specifically at Canadians aged 65 years and older, the occurrence of more multi-morbidities jumps to nearly 12% of the population living with two or more of the four most common NCD, namely CVD, cancer, CRD, and DM (5, 8). While there are demographic differences across Canada, as well as different trends in growth and aging within sub-populations living in Canada, in general, the overall population is continuing to experience an increasingly older population, and therefore the incidence of multi-morbidities is expected to continue to increase moving forward (5, 8).

Risk Factors Associated with the Development of Chronic Diseases in Canada

There are many factors associated with the increasing trend of the prevalence of NCD in Canada. While the above numbers seem to paint a bleak picture in the health outcomes of Canadians, it is important to also consider the risk factors associated with the risk of developing NCD amongst Canadians. Broadly, risk factors may be loosely categorized into two main categories: non-modifiable risk factors, including traits like sex, genetic predisposition, and age; and modifiable risk factors, including social conditions and lifestyle choices (9, 10). While the overlap of the effects of these two categories is substantial, and is an area of increasing interest in understanding the health outcomes of populations world-wide who are experiencing increasing

trends in NCD, it is important to also examine the potential individual impacts of each category on chronic disease risk.

Modifiable Lifestyle factors

There are many lifestyle factors associated with an increased risk of diagnosis with a chronic disease. In 1986, the underlying conditions required to support an environment that is conducive with a healthy outcome were underscored with the creation of the Ottawa Charter for Health Promotion (11). Health promotion is defined as the process of enabling individuals to increase control over and improve their own health, and therefore seeks to address barriers to this process (11). Recognizing the complexity of situations involved in this, the Social Determinants of Health (SDoH) list factors associated with health outcomes, and in Canada, include categories such as: income and social status; social support networks; education level and literacy; working conditions and employment status; social and physical environments; health services; personal health practices and coping skills; and culture (12, 13).

While the SDoH seek to identify and address broad disparities amongst Canadians that relate to health outcomes, there are also several individual modifiable lifestyle factors that should be discussed in relation to NCD. Five of the most common lifestyle factors associated with an increased risk of diagnosis with an NCD include obesity, alcohol consumption, smoking, diet, and physical activity (10, 14). While these factors are related to the SDoH, as can be seen from a broader analysis of the relative stage of development in a country, at an individualized level, they are of interest for modifying lifestyles to reduce their influence on health outcomes (10).

The role of these modifiable lifestyle factors is significant, and adherence to multiple healthy factors is associated with lower adverse health risks. In a recent meta-analysis, it was found that individuals who have healthy behaviours in at least four of the five lifestyle factors

had an overall reduction in risk of mortality by 66% over those with no healthy lifestyle factors (14). Further to this, when controlling for markers of elevated risk of CVD, adherence to multiple healthy lifestyle factors had an increasing protective effect on health risk over those who participated in only 1-2 healthy behaviours (15).

It is important to consider that there is a complex relationship between modifiable and non-modifiable risk factors associated with NCD, and that reducing the incidence of NCD in Canada will take a multi-pronged approach targeting many areas of modifiable risk factors. As the incidence of NCD continues to rise in Canada, it is important to understand the underlying factors that may be associated with these observable trends. Further to this, there is a need to develop new strategies in health promotion to combat the rising levels of NCD and the associated modifiable lifestyle factors

Metabolic Syndrome

Metabolic syndrome is a term used to describe a group of risk factors associated with an increased risk of NCD, and includes a large waist circumference (WC), high triacylglyceride levels (TAG), low high density lipoprotein (HDL) levels, high blood pressure, and high fasting blood glucose levels (16, 17). Specifically, a large WC is defined as >102cm and >88cm in men and woman respectively; elevated TAG is defined as >1.7mmol/L; reduced HDL cholesterol as <1.0mmol/L and <1.3mmol/L in men and women respectively; a blood pressure of >130mm Hg systolic and/or >85mm Hg diastolic; and an elevated fasting blood glucose level of ≥5.6mmol/L (17). An individual is considered to have metabolic syndrome when they have three or more of the above five risk factors, or are taking medication to treat TAG, HDL cholesterol, or blood pressure (16). Metabolic syndrome is associated with a higher risk of developing NCD (17).

Overweight and Obesity in Canada

Concurrent with the increased incidence of NCD and their associated lifestyle factors, the Canadian population is also experiencing an increase in the proportion of the population that is classified as overweight or obese. Overweight is classified by the World Health Organization (WHO) as a Body Mass Index (BMI) of 25.0 kg/m^2 or greater, while obesity is classified by the WHO as a BMI 30.0 kg/m^2 or greater (18). Canada has been experiencing an increase in the prevalence of overweight individuals, with 40% of men, and 28% of women classified as overweight in 2014 (19). Further to this, over 20% of Canadians were classified as obese in 2014, a slight increase from 2006 survey results (19). These numbers are based on self-reported data, and are therefore subject to some self-reporting bias, including an overestimation of weight reported by men, and underestimation of weight reported by woman, however still offer an insightful look into the burden of the issue in Canada (19). While a recent survey has suggested that the percentage of the population who self-report as overweight in Canada appears to have stagnated, the subsequent continued increase in the rates of obesity indicate that the Canadian population is continuing to experience an overall weight gain, which is in stark contrast to the efforts being taken to reduce overweight and obesity in Canada (19).

Overweight and obesity are related to many adverse health outcomes, and represent a growing burden on the healthcare system in Canada. Some of the health consequences directly associated with excess body weight include type 2 DM, dyslipidemia, hypertension, CVD, some cancers, and gallbladder disease (20, 21). With over 60% of the Canadian population reported to be in a weight category that places them at an increased risk for developing health problems, this is an important area of focus for health promotion and disease risk reduction (20).

As rates of overweight and obesity continue to remain high, the estimated direct cost of overweight and obesity in Canada was $6.0 billion in 2006, with $3.9 billion attributed to obesity, and $2.2 billion attributed to overweight (22). As overweight and obesity are risk factors associated with a higher risk of developing NCD, it is important to also consider the added indirect burden that is associated with excess body weight. For example, overweight is the strongest risk factor associated with the development of Type 2 DM, and the complications also associated with the disease (7). It is important to consider not just the direct costs associated with overweight and obesity, but also how high levels may impact the further development of NCD, and the increased costs and healthcare implications associated with those.

While there are some differences between the risk associated with overweight versus obesity, it is recognized that excess body weight in general is associated with a higher risk for adverse health outcomes (20). Based on data collected using direct measurements of height and weight, a 2004 survey found that less than 10% of normal weight Canadian adults had high blood pressure, compared to 15% of those who were overweight, with a further increase to over 20% amongst obese individuals (20). This is a similar trend seen in other NCD, such as heart disease, with a similar trend of less than 2.8% occurrence among normal weight men, 6% among overweight men, and 8% among obese men (20). These numbers directly relate to the odds-ratio of developing an adverse health condition associated with overweight and obesity when compared to normal weight individuals. For example, as addressed previously, excess weight is the strongest predictor for developing Type 2 DM, with an odds ratio of 3.8 and higher for obese individuals, compared to 1.6 for overweight (20). This increases the importance of the trends seen in Canada of increasing obesity, , and therefore highlighting the importance of addressing

this rising healthcare concern, given the higher association of adverse health effects seen with obesity in particular (20).

While BMI is an important method of categorizing weight and its associated risk factors, a growing area of concentration in understanding the health risks associated with excess weight as body fat is in considering its distribution around the body.

Recognizing that there are a number of complex factors involved in predicting the risk of becoming overweight or obese, which involve broad environmental and social factors, many individual and epidemiological approaches to addressing high rates of overweight and obesity have been underway in Canada for some time (23). While much efforts at addressing adult overweight and obesity are aimed at individuals, such as through health counselling and medical procedures, there are several community and national level initiatives underway, recognizing the broad challenge that the overweight and obesity rates are presenting (23). These efforts may be aimed several factors, including: improving physical activity in communities, such as the Canadian Obesity Network's (CON) national pedometer challenge (24); improving access to healthy and affordable foods, such as the Toronto Food Policy Council (25); and improving access and use of active forms of transportation, such as Walk and Roll (26). Other initiatives include changes to food packaging to encourage healthier food choices, such as the Front of Packaging (FOP) labelling initiative (27). Despite these, and other initiatives, rates of overweight and obesity have remained high in Canada, with some groups, such as overweight in men, continuing to increase (19).

Glucose Metabolism, Post-prandial Glycaemia, and Implications for Health

Glucose is an important nutrient for normal and healthy functioning of the human body. During normal metabolism, glucose is derived from one, or a combination of three different

sources. This includes the break down and absorption of starch and glucose containing foods that occurs in the small intestine, the break down of glucose stored as glycogen in the body through glycogenolysis, and the use of non-carbohydrate metabolites, inclusively protein, to generate glucose through gluconeogenesis (28). The concentration of glucose found circulating in the blood is tightly regulated, controlled via several different mechanisms and hormones (28). Normally, the uptake and regulation of glucose happens in two states: the post-prandial period after consuming a glucose-containing meal, and a fasting state which occurs between meals (28). During a fasted state, the regulation of blood glucose concentration occurs mainly through the controlled release of glucose from either body stores of glycogen, or, increasingly after longer periods of fasting, through the generation of glucose through gluconeogenesis (28). The post-prandial response to glucose depends on a number of factors, including how quickly glucose is isolated from an ingested meal, and absorbed into the blood stream, which is determined in large part by the matrix of the food in the small intestine, as well as the type of carbohydrates contained in the meal (28).

There are many conditions and diseases that may affect how the concentration of glucose is regulated during fed and fasting states, many of which were eluded to above, including metabolic syndrome, diabetes, and overweight and obesity (28). While these conditions may affect glucose regulation and metabolism, there is also recent evidence to suggest strong links between the type of food, and therefore the type of post-prandial glycaemic response that it induces, and an increased risk factor for developing these same conditions, pointing to a strong association with post-prandial glycaemic response and risk factors associated with NCD.

Abnormal post-prandial blood glucose levels specifically are increasingly recognized for their direct and indirect associations with adverse health outcomes. There is an increasing body

of evidence suggesting that high post-prandial glycaemic response is related to increased risk of weight gain, as well as increased risk of NCD. For example, an offspring study based on the Framingham report found that, for every increase of 2.1mmol/L over the 2-hour post prandial period from baseline, this resulted in an increase in vascular disease risk by 12%-46% (29). It is important to note that a significant proportion of the surveyed individuals showed this risk even though they exhibited normal fasting blood glucose levels (29-31). This, combined with the increased understanding of the role of post-prandial blood glucose in risk factors associated with NCD, points to the importance of regulating post-prandial blood glucose response.

The relationship between food and post-prandial glycaemic response is complicated. There have been several methods proposed to predict post-prandial glycaemic response, both acutely and in long term regulation. For example, the glycaemic index is a classification tool that can be used to assess the blood glucose raising potential of a controlled amount of carbohydrates when compared to a control food within individuals and is therefore a unitless value relative to a control (32). Given the unitless nature of the glycemic index, it is not able to be imposed on food intake, while food intake amounts have been directly correlated with post-prandial blood glucose (33-35). This has led to the development of several other methods of classifying the potential to raise post-prandial blood glucose. These include glycemic glucose equivalents, which is a method of measuring the amount of glucose in a food that would impact post-prandial glycaemic response, controlled for by the amount of food consumed, as well as the glycemic load, which is a theoretical cumulative response over time to the glycemic index of foods (36). Despite the varied methods of measuring the potential of a food to raise post-prandial glycaemic response, there does seem to be agreement that foods that undergo higher

amounts of processing tend to increase post-prandial glycaemic response over foods with minimal amounts of processing (37, 38)

While these methods may be effective at evaluating foods for their potential to impact post-prandial glycaemia, there are also a number of other factors that have been associated with improved post-prandial glycaemic control, such as exercise, weight loss, limiting alcohol intake, or using food ingredients such as cinnamon or vinegar to impact glycaemic control through other methods (37). Therefore, it is important to consider not just an individual component of a food source, but also how the food is consumed, what ingredients are consumed with it, and the amount consumed, when considering post-prandial glycaemia.

Food Consumption Patterns in Canada

With the understanding of the role of foods, and specifically the role of processed foods, with their potential to impact post-prandial glycaemia, it is important to consider what the intake trends of foods are amongst Canadians. While a caloric surplus has been highly associated with the weight gain and subsequent increase in NCD in the United States of America, there is little data to support such a conclusion in Canada. Survey data for populations in Canada specifically is limited, and survey methods have changed considerably between subsequent iterations of the Canadian Community Health Survey, resulting in poor ability to compare data between survey iterations. Despite this, caloric intakes, when adjusted for measurement methods, show little relative differences amongst Canadians spanning a 30 year difference, and are not as drastic as would be expected to explain the subsequent increase in BMI amongst Canadians (39). This suggests that caloric intake alone is not responsible for the subsequent changes in post-prandial glycaemia, incidence of NCD, and obesity, seen in Canada, despite the implications suggested in data from the USA (39).

While this comparison has many limitations inherent in how it was conducted, it does point to the importance of not just considering amount of calories, but also the food sources of those calories, when examining the changes in health status over the past several years in Canada. Using a novel classification of categorizing foods based on their level of processing, known as the NOVA classifications foods can be generally classified into one of three categories (40). Category 1 includes unprocessed or minimally processed foods, category 2 includes processed foods and processed culinary ingredients, while category 3 includes ultra-processed foods, which are often ready to eat with minimal to no preparation required, and contain ingredients from both categories 1 and 2 (40). Although this is a novel method of classification, it has had widespread uptake, including within Canada.

Using survey data collected between 1938 to 2011 from Canadian consumers food purchasing habits, when food purchased was categorized into the three categories outlined above, there were clear changes in food purchasing patterns (41). Specifically, the proportion of ultra-processed foods purchased rose with each subsequent survey, from just over 37% in 1938 to almost 55% in 2011, and, consequently, the proportion of unprocessed foods, saw a decline over the same period (41). This change in purchasing trends translated to a caloric share of ultra-processed foods accounting for well under a third of calories consumed in 1938, to over 60% of calories consumed in 2011 (41). Further to this, when comparing the nutritional quality of unprocessed foods to ultra-processed foods in Canada, diets high in processed foods were found to be nutritionally inferior to their counterparts in both macro and micronutrient composition (42).

The relative poor nutritional quality of ultra-processed foods, and the growing body of evidence linking foods high in readily digestible carbohydrates to adverse health outcomes, has

led to many recent and proposed changes to how food is marketed, and how nutrition information is displayed. For example, Health Canada is looking to impose mandatory front of package labelling for all packaged processed foods, which would readily identify the potential impact of a food on health outcomes in an easy to consume and recognizable format among consumers (27). Further to this, Health Canada has introduced changes to how nutrition labels are displayed on food packages, including more realistic serving sizes, as well as adding in a daily value for sugars, combined with simple messaging on interpreting daily value percents (43).

Despite these changes, and efforts to improve messaging on packaging, there is little data to date in Canada as to their potential impact, with no indication that the use of ultra-processed foods has slowed (41). This may indicate a need to not only raise consumer awareness of the nutritional composition of foods, but also increase the amount of readily available foods that have added health benefits, such as functional foods.

Functional Foods and their Potential Use in Health Promotion

Functional foods are foods are defined as foods with added functional properties that have demonstrated health benefits, and are produced and consumed for health reasons beyond the basic caloric and nutrient content of typical foods (44, 45). Within Canada, functional foods are regulated as conventional foods or foods similar in appearance to conventional foods consumed as part of a normal diet (46). Further to this, functional foods within Canada should demonstrate an added physiological benefit, or a reduction in chronic disease risk beyond basic nutrition (46).

Functional foods can fall under several categories: Conventional foods, or unmodified whole foods; Modified foods that are either fortified, enriched, or enhanced; Medical foods, or

foods formulated for the specific dietary management of a disease or condition; Foods for special dietary use, often including infant formulas or allergen-free foods (44). Functional food development and production in Canada is growing at a faster pace than other food production sectors, and Canada is a large supplier to domestic and international markets of both finished functional food products, as well as functional food ingredients (47). Interest in functional foods in Canada is increasing, and consumer surveys have shown greater than 95% of Canadian consumers have purchased functional foods (47). Consumed as part of a normal diet, functional foods may play a role in reducing risk of developing diet related chronic diseases (48).

Functional foods are emerging as a novel approach to attenuating and reducing risk of developing diet related diseases (49). Diet and lifestyle related chronic diseases place a huge economic and social burden on health care and social systems, and functional foods are uniquely positioned to both address these burdens as well as increase economic output for Canadian food manufacturing economy. While specific demonstrated health properties of functional foods may vary due to the type of added benefit, functional foods have the potential to address many different risk factors for the development of diet and lifestyle related diseases(49). Examples of specific risk factors associated with chronic illnesses are overweight and obesity, and poor post-prandial glycemic control (31, 50).

The potential benefits of the use and promotion of functional foods to reduce the occurrence of risk factors associated with NCD cannot be overstated. While several pharmacological agents have been developed to help promote weight loss with high levels of success, they are often associated with many adverse affects, such as gastrointestinal, psychiatric, neurologic, and cardiovascular complications (51). Functional foods do not experience these adverse affects, while still being grounded in evidence on potential benefits, making them an

ideal tool for promoting healthy weight loss. While the modest potential for affecting health outcomes through the use of functional food may be comparatively small, even small reductions in body weight, for example, as low as 5%, have been shown to improve glycaemic control (52). Combined with this, their wide market penetration, low cost, and increasing availability through the production of new functional foods make them an ideal candidate in addressing many adverse health concerns.

There are currently several functional claims for food products approved for use in Canada. One of the proposed health claims under review for functional foods and their properties in Canada to address concerns related to adverse health outcomes is in modulating post-prandial glycaemic response (53). In the draft guidance document, there are several proposed ways to incorporate new ingredients into food products aimed at reducing post-prandial glycaemia. One of these methods is to replace a traditional ingredient with the proposed functional ingredient, while maintaining all other ingredients and processing methods for preparing the food the same (53).

In order to substantiate the function claim related to post-prandial glycaemic response,, few criteria has been laid out as guiding principles for designing studies. The study population should consist of healthy individuals representative of the general population or individuals with impaired glucose tolerance not treated with any medications that may affect glucose metabolism (50). When testing a product for its effect on post-prandial glycaemia, it is recommended to control and standardize the pre-treatment meal and time lapse between pre-treatment meal and treatment to avoid any possible effects from previously consumed meals (50). Blood glucose should be analyzed over a sufficient amount of time, and with enough points to properly measure the effect of the functional product on post-prandial glycaemia (50). In order to be considered

for this potential claim, a reduction of post-prandial glycaemic control of 20% or greater must be achieved within a 2-hour window after consuming the proposed functional product, as measured by incremental Area Under the Curve (iAUC) (50). In addition to this, the insulin response to the test food should be proportional to the glycaemic response (50).

Front of Package Labelling

In addition to functional food claims, Canada is also in the process of adopting a system of front of package labelling, to help inform consumers of foods that may contain high amounts of nutrients such as sodium, sugars, and saturated fats that are more closely associated with adverse health outcomes (27). In particular, these nutrients have been recognized as increasing the risk of obesity, heart disease, and high blood pressure (27).

While the process of introducing these labels into the marketplace is ongoing, there is some preliminary information that has been released on the amount of sodium, sugars, and saturated fats a food product may contain before it is subject to receiving these labels. The use of the labels is based off of two thresholds, where a prepackaged food containing more than 15% of the daily value per serving of one of the three nutrients, or a prepackaged meal containing more than 30% of the daily value per serving of one of the three nutrients, will be subject to using the labels that will indicate that this food product should be avoided (27). Specifically, this translates to a content of 345mg of sodium, 3g saturated fat, and 15g total sugars for prepackaged foods, and 690mg sodium, 6g saturated fat, and 30g total sugars for prepackaged meals, each based on 1 serving size and reference amount. To ensure that food products with small serving sizes are included in the initiative, foods with a serving size of less than 50g or 50mL, will have their total DV based off of a reference amount of 50g or 50mL (27).

In addition to this, there will be additional labelling requirements placed on products marketed to children specifically. The reference amounts for cut offs for the front of package labelling for high amounts of sodium, saturated fats, and total sugars are 225mg, 1.5g, and 7.5g respectively (27).

Pulses

While Canada produces many different types of functional ingredients and finished products for both domestic and international markets, legumes, often referred to in Canada as pulses, are a crop of interest to the functional food market for the many types of added benefits they may possess. Pulses have a narrower definition than legumes, referring to grain legumes, which include chick peas, dry beans, dry peas, and lentils (54). Pulse production from Canadian farmers has seen massive growth in recent years, increasing in production 12 fold from 1991 to 2009, and representing over $2 billion in exports (54). While much of Canada's pulse production is exported to international markets, the availability of pulses, as well as their demonstrated health benefits, has given rise to interest of using pulses in functional food product development.

Pulse Consumption in Canada

Pulse consumption within Canada remains relatively low, despite the widespread availability and cost effectiveness of consuming pulses, along with the many demonstrated health benefits. Amongst Canadian consumers, only 20% of Canadians report consuming at least one type of pulse on a weekly basis, while this number was matched by Canadians who reported no pulse consumption over a 6-month survey period (55). Based on dietary survey data, just above 13% of Canadians reported consuming pulses within a given day, with cultural and regional differences noted (56). Among the 80% of Canadians who reported consuming some

form of pulses over the 6 month survey period, the consumption patterns of type of pulses consumed varied, with two-thirds of consumers consuming beans, half consuming chickpeas and peas, and around 4 in 10 consuming lentils (55).

There are many barriers to pulse consumption that have been identified in Canadian consumers. These barriers include socio-demographic factors, where low pulse consumption is linked to lower age, but also include factors around taste, knowledge around preparation and cooking pulses, and infrequent inclusion in meal plans (55). While improving knowledge of the health benefits of pulses and their role in disease risk reduction and disease management, as well as improving knowledge around preparation techniques for pulses, stand to improve pulse consumption in Canada, the role of novel function food production that incorporates pulses into the food matrix may be uniquely situated to improve pulse use and consumption by the greatest amount (47, 55). Consumption of processed foods and ultra-processed foods in Canada has risen drastically in recent years, where the share of processed foods for the Canadian consumer went from under 30% of foods to over 60% between 1938 to 2011 (38). Following this trend, increased consumption of novel processed foods with added functional properties stand to make the greatest impact on Canadian pulse consumption. While increasing frequency of home prepared meals is shown to improve both perception and actual overall quality of diet, many barriers exist to improving consumption of home prepared meals, and, while this doesn't excuse the need for education to reduce actual and perceived barriers of home prepared meals, especially those incorporating pulses, meeting consumer needs by improving selection and functional qualities of processed foods through the addition of pulse based ingredients is warranted (57).

Nutrient Composition of Pulses

Pulses have an optimal distribution of macronutrients, containing 17-30% protein by dry weight, ~45% starch content, and are low in fat (58, 59). The health benefits of pulses are attributed to their unique distribution of macronutrients, as well as the presence of bioactive components (60). In general, a diet rich in low glycemic foods that has high fiber and low energy density, made up of 40-50% carbohydrates, <30% fat, and increased protein portions is beneficial for long term weight reduction and management, and reducing risk factors associated with metabolic syndrome (61). Pulses also contain many bioactive components, including oligosaccharides, inositol phosphates, and protease inhibitors, among others, which have been suggested to have protective roles against hypocholesterolemia, cancer, and atypical glycemic control (60). Medium and long chain saccharide components, including soluble and insoluble fibers, have also been demonstrated to have prebiotic effects, which may improve the microbial biodiversity in the human microbiome, increasing production of short chain fatty acids in the large intestine, and improving transit time, all of which play important roles in reducing risk of diseases, including as colorectal cancer (59, 60).

Protein from pulses includes a well-balanced amino acid profile, although they are low in sulfur-containing amino acids, suggestion that pulses should be consumed in conjunction with complementary cereal grains to ensure consumption of complete proteins (62). In addition to their nutritional properties, pulse proteins are also responsible for important functional properties, including water binding, fat binding, foaming, gelation, thickening, and flavour binding (62).

In addition to the approximately 45% starch component of pulses, approximately 12-28% of pulses by dry weight are made up of fiber (63). Fibres from pulse sources consist of the non-

starch carbohydrate fractions, such as cellulose, lignin, and hemicellulose. In lentils specifically, the starch content is 42-49%, with the fiber fraction being 12-14% (63). The fiber contained in pulses have specific physiochemical and functional properties for consideration in functional food product development, including sensory considerations, such as colour and flavour, as well as functional properties, including fat absorption, water hydration and retention capacity, and swelling capacity (63).

Starches in pulses are stored in granules, often referred to as C-type granules due to the distinct x-ray pattern that they produce (64). While pulse starch granules differ in overall composition based on the source, pulse starch granules generally have a higher percentage of amylose to amylopectin (64). The ratio of amylose to amylopectin in starch sources is often used in inferring potential rate of rise in postprandial blood glucose concentrations from different starch fractions (65). A high portion of amylopectin, characterized by the presence of both α-1-4 and α 1-6 bonds resulting in a highly branched structure, results in a more rapid availability of glucose for absorption, while a high portion of amylose, characterized by a linear structure due to presence of α1-4 bonds results in a slower availability of glucose for absorption, due to limited access of intestinal hydrolytic enzymes to cleave off individual glucose units (65). Pulse starches are unique for their properties, namely high gelation temperatures, resistance to shear thinning, fast retrogradation, high resistant starch content, and high elasticity of starch gels (64). However, research is lacking on the specific functional characteristics of starches across different pulse cultivars, as well as the effects of grinding, isolating, and processing pulse flours and pulse starches, and more research is needed to better understand the specific roles of cultivar and processing methods on the functional characteristics of pulse starches.

The functional characteristics of pulse starches can be classified into one of three categories related to the release of glucose during digestion, namely rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS); resistant starch can be further subcategorized into 4 main components (66). The classification of starches into these categories relates to expected rate of postprandial absorption of glucose from these starch fractions into the bloodstream (66). RDS is characterized by a rapid rise in postprandial blood glucose concentration almost immediately after ingestion, with higher concentrations seen within 20 minutes followed by a rapid decrease in concentration (66). Conversely, SDS results in a delayed rise in blood glucose levels, with a more gradual increase in concentration, followed by a slower depletion in concentration, with sustained levels of blood glucose measurable at greater than 240min post-prandial (65). RS starch fractions are not metabolised in the small intestine, instead passing into the colon where they are fermented and broken down into by-products by bacteria present in the large intestine (67). While more research should be conducted to fully investigate the relationship of RDS, SDS, and RS starch fractions in pulses, in general, pulses tend to have a higher ratio of SDS and RS starch fractions when compared to other common sources of starch used in food processing (67).

Potential Health Benefits of Pulses

Pulses, and their components, have been shown to have numerous health benefit potential. Pulses affect satiety, food intake, subjective appetite, have a prebiotic effect, promote healthy intestinal transit, improve glycaemic control as eluded to previously, and reduce risk of some cancers and chronic diseases (60, 68).

Regular pulse consumption has been shown to improve both macro and micro nutrient intakes among Canadians (56). Overall, pulse consumers tend to consume higher amounts of

carbohydrates and protein, and lower consumption of fat, than non-pulse consumers (56). Pulse consumers also reported higher intakes of fiber than non-pulse consumers, even amongst the lowest pulse consumers, both in raw intakes, as well as when standardized for calories (56). When looking at micronutrients, pulse consumers had higher intakes of phosphorous, folate, magnesium, iron, and zinc, compared with non-pulse consumers, and although significant differences were only observed in the highest quartile of pulse consumers (>137.2g/day), there was an observable upwards trend in intakes of these micronutrients as pulse consumption increased (56).

Following the improvements in dietary composition as a result of improved macro and micro nutrient intakes, there are many observed health benefits to pulse consumption. Pulses may play an important role in managing weight and reducing obesity. Regular intake of pulses has an inverse relationship with BMI, reducing risk of obesity and overweight in pulse consuming populations, and frequent pulse consumption over short term interventions improves risk factors associated with metabolic syndrome in overweight and obese adults, such as fasting blood glucose, total and LDL-cholesterol levels, TAG levels, and reducing blood pressure, while lowering daily energy intake (69-71).

One of the potential health benefits of pulses is in relation to their effects on subjective feelings of appetite and food intake, and how this relates to short term and long term food intake. As excess weight gain may be due in part to excessive caloric intake, promoting appetite suppression through reducing subjective feelings of hunger has been an area of interest for health promotion by reducing weight gain and improving weight maintenance (72, 73). The mechanism of action for how different components of foods affect food intake and subjective appetite is an area of active research, with observable differences even within one topic area,

such as fiber (73-75). Despite this, pulses have been shown to reduce subjective feelings of appetite in short term investigations (69, 70, 76). There are mixed results on the impact of subjective appetite on food consumption, with lower subjective satiety not associated with lower short term food intake when different meals containing pulses were fed to participants (76). This disconnect between subjective appetite and food intake has been noted in past investigations (77). Despite this discrepancy, longer duration studies, including an intervention where participants consumed pulses daily over an 8 week period, and another investigation where participants consumed pulses over a 6 week period, demonstrated that sustained pulse consumption can reduce risk factors associated with NCD (70, 78).

While the exact mechanisms involved in improving outcomes of risk factors associated with NCD from pulses is unknown, whether it is through improved post-prandial glycaemic response, healthy weight promotion, improved metabolic markers, effects on appetite, or a combination of these, there is broad based agreement that regular pulse consumption is associated with improved health outcomes (69).

Impacts of Processing on the Characteristics of Pulses

Processed pulse ingredients have been shown to retain some of the beneficial health benefits seen in whole-pulse consumption. A crossover design study in which participants were fed blended lentils or whole lentils both showed significant reduction in post-prandial glycaemic response compared to a pulse-free control, despite close similarities in the energy and carbohydrate portion of the treatments (79). Similarly, participants in a crossover design study fed pulse containing meals where the pulse ingredients were subjected to different processing methods, including whole pulses, pureed pulses, or pulses processed into a flour, resulted in no significant differences in post-prandial glycaemic responses between treatments (80, 81). Pulse

consumption as both whole and processed ingredients have been shown to improve both post-prandial glycaemic control and risk of obesity (70, 80, 82).

Although whole pulses have been shown to have many health benefits, processing of pulses into ingredients consumed as part or whole of a food product impacts their composition, functional, and physiochemical properties (60, 83, 84). While the impact of processing in general may be observed, and is often beneficial, such as through the inhibition of antinutritional properties, the degree of change to functional properties of pulses differs with the type and level of processing used (85). There are differences seen in the functional properties of pulses depending on the type of processing method used.

Milling methods used in preparing pulse flours has been shown to result in some differences in yield and functional properties depending on the type of milling method used, whether pulses are wet or dry milled (86). As can be seen in Figure 4, a recent investigation at Mount Saint Vincent University into different grind size of pulse flours when controlled for by milling method, demonstrated a significant impact on potential availability of starch for digestion, based on an in vitro method that mimics human digestion and has been used to predict glycaemic response (87). Although these results have only been published in abstract form, the significant differences seen in glucose concentrations over the timeline of the digested flours demonstrate the potentially significant difference on post-prandial glycaemic response due to processing methods specifically (88).

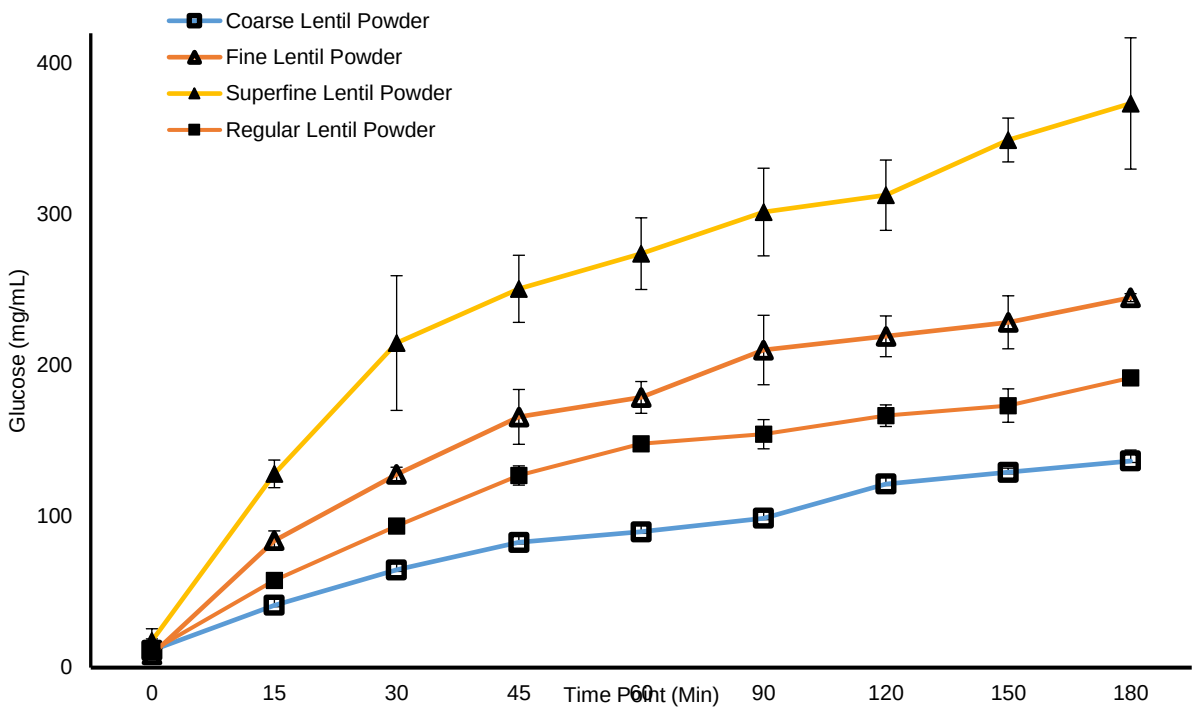


Figure 1 Glucose concentrations during the in-vitro digestion of different grind sizes of lentil flours over 180 minutes. One-way ANOVA with Tukey Kramer post-hoc test. Mean ±95% CI. Effect of Treatment: P<0.0001. Effect of Time: P<0.0001.

Thermal processing has also been shown to have an impact on the characteristic of pulse ingredients. Specifically, type of thermal processing, including roasting and boiling, as well as when thermal treatment is applied during the processing method, including before or after grinding pulses into a flour, has an impact on the functional properties of pulses (84). Significant changes were observed in the functional properties of the processed pulse flours in trypsin inhibitor activity, fat and water absorption, gelling, and emulsifying activities, which all have the potential to impact how pulse ingredients perform in formulated food products (84). Further to this, thermal processing may have a significant impact on the availability of carbohydrates for digestion in pulse flours. A recent in vitro investigation at Mount Saint Vincent University

showed a significant change in carbohydrate digestion of raw versus thermally processed pulse flours, as well as thermally processed pulse flours differing in particle size (88, 89). This is despite evidence that shows a beneficial effect on post-prandial glycaemic response with the consumption of pulses that are processed using different methods, independent of processing methods (79, 80, 89). This disparity shows a gap in knowledge, where there is an effect of processing of pulses on glycaemic properties, while there also appears to be a benefit of processed pulse consumption. While the study by Anderson and others (2014) shows that there is a benefit despite processing methods, it did not control for the particle size of the pulse ingredients in the final treatment (80). Specifically, the study did note a reduction in post-prandial glycaemic response after a second meal was served only after the whole pulse treatment, suggesting that the larger particle size from whole pulses versus pureed or ground pulses, may have additional benefits, despite each treatment controlling for available carbohydrates (80)

The use of processed pulse ingredients has been shown to differ from whole pulses in their impact on subjective feelings of fullness and short term food intake. Treatments containing blended pulses were shown to increase feelings of appetite over whole pulse consumption (79).

While it is important to consider the potential health benefits of using processed pulse ingredients in formulated products, it is equally important to consider the acceptability of the product to the consumer. A recent investigation showed that the addition of flaked lentil ingredients to a formulated lentil snack bar led to acceptable sensory properties among some of the formulations (90). This was based on a maximum inclusion of approximately 37% lentil ingredient inclusion by final product weight, for a maximum of approximately 17.4g of modified lentil ingredients included in the final recipe (90). 17.4g is well below the amount used to measure a demonstrable benefit in post-prandial glycaemic response in whole and processed

pulse ingredients of 25.0g, and it is therefore unclear if this product, though acceptable to a trained sensory panel, will add a measurable health benefit (80). Similarly, while a replacement of 24.3% of the tradition wheat flour with pulse based products in bread showed similar sensory acceptability when served as a breakfast meal, there was no significant differences in post-prandial glycaemic response, or satiety response (91).

Despite the mixed results on the effects of processed pulse ingredients on markers of health and sensory acceptability, the health benefits associated with pulse consumption has led to an increase in the amount of products being produced that incorporate pulse-based ingredients. Since 2016, there have been over 170 new snack based cereal and energy bar products developed in Canada and the US, with the majority (>80%) of these products developed for retail centres (92). Many of these products have been developed to meet changing consumer needs, such as reduced or free from gluten projects (92). In Canada, consumer preferences for ingredients with no added preservatives or additives and microwavable convenience products have been the two largest packaging claims on pulse-based products purchased in Canada, comprising over 20% of new products purchased over the past 10 years (92).

Meeting this demand, lentils, a type of pulse, are ideally situated. As the leading producer of lentils globally, with capacity for lentil production substantially increasing over the past several decades, Canada has an abundant supply of lentils to meet consumer needs (93, 94). Further to this, lentils have a lower seed hardness compared to peas, chickpeas, and beans, allowing them to be more readily milled as a whole seed, and specifically milled into finer fractions (95). This may increase their availability for use in different products. Lentils are also relatively low in antinutritional factors compared to other pulses, low in flatulence promoting constituents, and high in protein, and the ease of milling lentils compared to other pulses into a

finer flour size increases the protein fraction of the flour compared to other pulses (96, 97). High in protein, lentils complement well with cereal grains to produce a complete protein (96, 97). This makes them an ideal candidate for use as a processed ingredient in new food products.

Despite the increased demand, increased availability, and increased perception of health properties associated with lentil-based products, few investigations have been conducted into both the effective dose of lentils needed to obtain a detectible benefit, as well as the effect of processing on the potential benefits of processed pulse ingredients. While there is evidence suggesting that new products produced with lentil-based ingredients have acceptable sensory properties, there is a need to investigate both the effective dose needed of pulse ingredients when controlling for processing methods, as well as the threshold for sensory acceptability for addition of pulse-based lentil ingredients.

Rationale

There continues to be increases in the diagnosis of NCD, as well as associated risk factors, amongst Canadian consumers (2). Functional foods with demonstrated health benefits are positioned to address many of these rising healthcare concerns, especially considering the continued high intake of processed foods amongst Canadian consumers (48). Pulses, with high availability and economic affordability, along with substantial evidence showing health benefits associated with regular consumption, are uniquely situated for use as functional food ingredients. There is evidence that suggests that the consumption of processed pulse ingredients may maintain the benefits associated with whole pulse consumption (80, 81). However, there are observable effects on the functional properties of pulses due to processing, including the potential change in post-prandial glycaemic response and subjective appetite, despite past evidence (84, 88, 89). Further to this, the amount of processed pulse ingredients needed per

serving to observe a short-term benefit in post-prandial glycaemic response, subjective feelings of hunger, and short term food intake, has not yet been investigated, and specifically in amounts consistent with normal snack product consumption.

Hypothesis

The replacement of a portion of wheat flour with a processed lentil flour of similar particle size into a formulated solid snack bar matrix similar in recommended serving size with commercially available snack bars, will improve blood glucose control in human participants, while subsequently reducing short term food intake and subjective feelings of hunger. Further to this, the addition of pulse flours within a level that will positively impact blood glucose control will not negatively affect the acceptable sensory properties of formulated snack bars

Main Objectives

Our main objectives were threefold:

To determine the effect of two doses of raw lentil flour incorporated in a solid snack bar on:

1. Postprandial blood glucose
2. Subjective appetite
3. Short-term food intake

A snack bar recipe previously developed for commercial use was used in this investigation, with some modifications to the recipe. The hypothesis was considering a normal serving size as recommended by CFIA of 50g for a snack bar, and the expected dose response was tested by replacing the flour portion of the 50g snack bar (24g) with 50% and 100% replacement with lentil flour of the traditional wheat based flour. While a dose of 12g and 24g of lentil flour was not chosen based on past studies, it is within a reasonable amount that would

be expected to be consumed in a normal snack bar within serving size recommendations in Canada. Similarly, while there is no specific evidence to suggest a serving size of 12g to 24g of lentil flour may reduce subjective hunger or short term food intake, this falls within the recommended serving size.

As this investigation was exploratory in nature, a change in blood glucose, subjective appetite, and short term food intake, was investigated for any statistical differences between lentil flour containing treatments, and all other treatments. While it is recognized that statistical significance does not necessarily result in clinical significance, the exploratory nature of this investigation was set forth to determine the effect, if any, of replacing wheat flour with lentil flour in a serving size consistent with a snack food produce.

The secondary objective was to develop a commercially reproducible snack prototype using lentil flour, with acceptable sensory characteristics.

Methods

Study Protocol

The study was a randomized cross over design, consisting of 12 male adult participants. Treatments were given to participants using a balanced approach. The first participant had the first treatment as the low lentil flour treatment, followed by the high lentil flour treatment, followed by the wheat control, and lastly a water control. The second participant was given the high lentil treatment, followed by the wheat control, followed by the water control, and ending with the low lentil flour treatment. This method of balancing the beginning treatment for each participant was continued as participants were recruited into the study and adjusted as participants withdrew from the study.

Each treatment session began with an overnight fast beginning no later than 9pm the night before the session, after which participants consumed a standardize breakfast at home, before arriving to the lab for the treatment session. Participants were asked to bring in the empty containers from their breakfast to be handed to lab volunteers upon arriving to the lab, and were verbally asked the time that they consumed the treatment to ensure it was at least 2 hours prior. Upon arrival, participants completed a questionnaire assessing the previous 24 hour period on food intake and physical activity level, as seen in Appendix 1. The participants went through a screening process to ensure adherence to the protocol and to ensure informed consent was maintained throughout the study. Further to this, participants completed VAS questionnaires evaluating physical comfort and subjective appetite (appendix 1), before having an intravenous catheter inserted into their arm for blood collection, and an initial baseline sample was taken for time 0. A baseline blood glucose measurement was taken from a discard tube and checked with a handheld glucometer (Accu-check compact plus, model GT, Germany) to ensure baseline blood glucose was within an acceptable range, and those with baseline blood glucose under 4mmol/L or over 6mmol/L were asked to come back to repeat the session at a later time. Among all participants, two participants had to high blood glucose; no participants had more than one instance of high blood glucose at baseline among the four treatments.

Following this, participants consumed one of four treatments. Time 0 was marked for blood glucose as participants took the first bite of treatments, or sip of water. Participants were given 8 minutes total to consume the treatments. The caloric treatments were also served with 250mL of water. While participants were blinded to the treatment they were receiving, there was a noticeable difference in the visual appearance of the treatments, with the 100% wheat flour treatment much lighter and greater in volume compared to the 100% lentil flour treatment.

Similarly, the caloric free control, with only water, was obviously different than the treatments with bars.

Following consumption of the treatments, blood was collected at 10, 20, 30, 60, 90, and 120 min, with time point 0min set at the first bit of treatment or sip of water, as observed by a research assistant. At time points 15, 30, 45, 60, 75, 90, 105, and 120, VAS scales were completed by participants evaluating subjective comfort, subjective appetite, and gastrointestinal wellness (Appendix 1).

At 120 min post-treatment, participants were given a pizza meal to consume ad libitum, with 250mL of spring water refilled ad libitum, and instructed to eat until comfortably full. More water was given to participants in 250mL increments if requested. Food intake was measured using a weight difference, with the weight of pizza consumed used to determine caloric intake based on information provided on the nutrient facts table from the manufacturer (Appendix 3). Water intake was also measured using a weight difference of the cup plus water before serving to the participant, minus the final weight of cup plus remaining water.

Participants were rescheduled for each subsequent treatment after a washout period of at least 1 week. A 1-week washout period was selected based on established lab protocols, and similar published studies investigating the effects of lentils on short term food intake, subjective appetite, and blood glucose (70, 76, 80).

Treatment Formulation and Ingredient Selection

The treatments were prepared on site at Mount Saint Vincent University using fresh ingredients. The treatments were formulated as a snack bar with a fruit filling on the inside. All treatments were baked over a period of one to two days to eliminate any variability introduced by

different batches of ingredients, and vacuum packaged and stored at a temperature of -18°C, before being defrosted and warmed up just prior to serving during each treatment session.

According to Canadian Food Inspection Agency (CFIA) guidelines of reference serving sizes for nutrient facts tables, grain-based bars with filling or partial or full coating should have a serving size of 20-50g, with a reference serving size of 40g (98). Following these recommendations and ensuring consistency with other commercially available grain based bars, the treatments were formulated to a cooked serving size of 50g, based on an established method of preparing the bars with finished weights of 50g ± 2g. A preliminary analysis of the finished cooked weights was performed to ensure achieving a consistent cooked weight was possible, with low deviation amongst treatments. Following a series of weighing raw and cooked bars, cooking loss was determined to have an average of 5.8% ± 0.3, when accounting for all formulations (low pulse, high pulse, and pulse free). As shown in Table 1, when accounting for this expected weight loss during cooking, it was possible to achieve a finished treatment weight of 49.3 ± 0.5 after multiple repetitions. The formulated snack bars contained 45g ± 0.1g of uncooked dough and 8.1g ± 0.2g of uncooked filling for a final.

Treatment weights before and after cooking		
Repetition	**Pre-cooked weight:**	**Post cooked weight:**
1	52.1	49.6
2	52.4	49.6
3	52.4	49.8
4	51.7	49.3
5	52.2	49.4
6	52.0	49.3
7	52.1	49.5
8	52.2	49.6
9	51.6	48.9
10	51.6	48.5
11	51.4	48.4
Mean	52.0	49.3
SD	0.3	0.5
CV	0.7	0.9
Recipe used: 12g lentil flour with 12g wheat flour. Raw weights: 45.0g (±0.1) dough and 8.1g (±0.2) filling SD = Standard deviation; CV= coefficient of variation		

Table 1 1 Raw and cooked weights of treatments prepared using 45g of dough with 8.1g of filling

To achieve consistent weights of cooked treatments, each individual bar was made by weighing a raw dough ball of 45.0g ±0.1, and then, following flattening this dough to a consistent thickness of 70mm among all treatments, was placed on a scale, and 8.1g ±0.2 of filling was placed onto the dough. The filling was prepared separately, and in a single batch sufficient to use with all three dough recipes, to eliminate any differences in ingredient use if prepared in multiple smaller batches. These amounts and margins of error were selected after several trials, to balance accuracy with feasibility. The dough was then rolled around the filling, and placed onto a lightly greased cookie sheet, before baking for 15 min at 350˚F. This allowed an internal temperature of greater than 85˚C to be reached, which should be sufficient to deactivate anti-nutritional components found in lentils (99).

In total, three different recipes were prepared. These consist of a pulse free control using all-purpose wheat flour (enriched all-purpose wheat flour, Creative Baker, lot 17061-10142, Canada), a low pulse dose treatment, replacing 50% of the all-purpose wheat flour in the recipe with lentil flour (whole laird lentil flour product #517, Best Cooking Pulses, lot # 0216, Canada), and a high pulse dose treatment, replacing 100% of the all-purpose flour in the recipe with lentil flour. In total, each treatment contained 24g of flour, and therefore the pulse free treatment had 24g of all-purpose flour, the low pulse dose treatment had 12g of lentil flour and 12g of all-purpose flour, while the high pulse dose treatment contained 24g of lentil flour. While this recipe was previously developed for commercial use, it is unclear if a flour portion of 24g, or approximately 50% of the bar weight, is normal for commercially available snack bars, given the proprietary nature of commercial recipes. Similarly, while this investigation used a serving size in line with CFIA guidelines, there is a lot of variability in the actual serving sizes of commercially available bars. A decision was made to follow CFIA guidelines for serving size,

which yielded results more in line with what government and packaging labelling were, if not different than commercial availability and actual eating habits of Canadian consumers.

To eliminate any variations due to different crop yields, fields, or years, both wheat flour and lentil flour were sourced from the same batch. Similarly, all other ingredients for the dough, (inclusively 2% milk, eggs, granulated sugar, cinnamon, vanilla, baking soda, salt, and oats), and all other ingredients for the filling (inclusively dried apples, cinnamon, granulated sugar, water, and cornstarch), were sourced from one batch, and purchased once, to ensure consistency amongst the ingredients for all recipes. All three treatments were prepared within a four day period, and individual bars were packaged in vacuum sealed packaging, and immediately frozen until removal from the freezer the day of the treatment session.

A particle size analysis was conducted by the Canadian International Grain Institute (Malvern Scirocco 2000 Mastersizer UK) to compare the grind size of both lentil flours and wheat all-purpose flour. The commercially milled lentil flour had a volume weighted mean grind size of 94.2µm. This was similar to the volume weighted mean of the all purpose flour of 79.9µm. The grind size was measured and samples were selected with similar grind sizes to eliminate the potential effects of grind size on biological outcomes, as well as differences in composition, which were measured in previous investigations (88, 89).

Nutritional Contents of Treatments

In order to meet the draft guidelines for functional health claim aimed at post-prandial glycaemic control, the treatment formulations were designed to contain the same amount of flour ingredients, with equal amounts by weight (g) of wheat flour replaced with lentil flour. Table 2 shows the nutrient composition of the treatment formulations. As the nutritional composition of wheat flour varies from lentil flour, the nutritional composition of the bars varies slightly across

the three caloric treatments as the proportion of lentil flour to wheat flour changes. However, as this investigation was designed to determine any effects of a snack bar formulated with lentil flour, it was important to use an ingredient substitution approach. As outlined in section 3.2 of the draft guidelines from Health Canada for the functional claim on blood glucose, the reference product should be the same as the treatment product, with the exception of the partial or total replacement of one ingredient with the proposed functional ingredient (53). While similar investigations have controlled for available carbohydrates, balancing carbohydrates between treatments would require multiple compositional changes, and therefore, the health claim would apply only to the food products investigated, rather than the role of the ingredient being replaced (100, 101).

As mentioned, there are clear compositional differences between treatments. Although components, such as fiber and protein, have been demonstrated to play a role in affecting post-prandial glycaemic response, it is important to consider the purpose of this investigation, which is to look at lentil flour as a possible functional food ingredient, so these compositional differences are valid for this analysis as they would also be expected in food products formulated using lentil flour to replace a portion, or all, of conventional flour sources (100-102). Further to this, while there is an effect of constituents such as protein and fiber, it has been demonstrated that there is still a strong correlation between available carbohydrate of foods and glycaemic response, even in the presence of these components (103).

Nutrient composition per one serving 50g Bar including 8.1g of filling			
Nutrient (g)	**Bar with wheat flour**	**Bar with a low dose of lentil flour**	**Bar with a high dose lentil flour**
Protein	4.6	6.7 (1.3 from wheat flour, 3.4 from lentil flour)	8.7
Total Fat	1.1	1.0	0.8
Saturated Fat	0.1	0.1	0.1
Carbohydrate	30.0	28.9	27.7
Total Sugars	11.8	12.3	12.9
Avail. Carb	28.6	26.4	24.3
Energy (kcal)	142.6	142.7	142.8
Fiber	1.5	2.5	3.4
Sodium	135.0	136.2	137.5

Table 2 Nutrient Composition of Different Treatments per 1 Bar

As is currently set forth in the preliminary guidelines for front of package labelling, snack products with serving sizes under 50g, will be subject to the newly proposed thresholds for sodium, total sugar, and saturated fat being equivalent to a 50g serving (27). As seen in Table 1, the average post-baked weight of the bars is 49.26g, so the nutrient composition seen in Table 2 can be used as a reference for determining if the bar is subject to the current proposed front of package warning labels. As previously discussed, the cut offs for front of package labelling to come into effect for a prepackaged food, of which this bar would be considered, are 345mg, 3 g, and 15g for sodium, saturated fat, and total sugars respectively (27). When referencing the nutrient content in Table 2, the bar is below these cut off points, with 135 – 137mg of sodium, 0.08 – 0.13g of saturated fat, and 11.75 – 12.92g of total sugars. All three formulations of the snack bar would therefore be considered a healthy snack choice based on the new proposed guidelines under development by Health Canada (27).

During preliminary investigations, it was determined that there is some loss of raw material that occurs during mixing, transferring, forming, and baking the treatments. The nutrient breakdown in Table 2 considers this loss. A loss of 1.59% was experienced between the sum of starting weights of raw ingredients, and the sum of the weight of the dough measured, portioned, formed, and transferred to the baking sheet just prior to baking. Table 3 shows the initial and adjusted weights for each ingredient, and the adjusted weight was demonstrated to make 12.65 servings. This serving number for one recipe was used to calculate the above nutrient composition of each treatment seen in Table 2, and all nutritional information was gathered from Canadian Nutrient File.

Table 1 3 Raw weights of dough ingredients, adjusted for loss after preparation, for basic dough recipe used in all treatments

Ingredients	Starting Weight (g)	Weights Adjusted for Loss (g)	Per 1 bar (g)
Flour (wheat flour CNF# 6642; lentil flour CNF# 6183	284.0	279.5	22.1
Granulated sugar (CNF# 4318	100.0	98.4	7.8
Rolled oats (CNF# 5143)	24.0	23.6	1.9
Baking soda (CNF# 4005)	3.7	3.6	0.3
Salt, table (CNF# 214)	1.5	1.5	0.1
Cinnamon, ground (CNF# 178)	8.0	7.8	0.6
Vanilla (CNF# 217)	10.0	9.8	0.8
Milk, fluid 2% (CNF# 61)	90.0	88.6	7.0
Egg - large, raw (CNF# 126)	57.2	56.3	4.5

The total available carbohydrates per treatment were between 24g (high lentil flour treatment) to 28g (lentil free wheat control). While this amount is not standardized, and is less than some similar studies, it is in accordance with normal dietary patters for a snack bar, and is in line with serving size references in Canada (100, 101, 104).

The total protein content of the bars was 4.6g, 6.7g, and 8.7g for the wheat, low lentil flour, and high lentil flour treatments respectively. Of the total protein content, 2.0g comes from ingredients other than the flour fraction (mainly egg, milk, and rolled oats). For the wheat flour treatment, the remaining 2.7g of protein comes from the wheat flour. Similarly, for the high lentil flour treatment which didn't use wheat flour, 6.7g of protein is from the lentil flour. With the low lentil flour treatment, which used a mixture of wheat flour and lentil flour of 50% by weight, 1.3g of protein is due to the wheat flour, and 3.4g is due to the lentil flour.

To make a functional health claim, the beneficial effect of a functional ingredient has to be within reason of normal consumption patterns, including the amount consumed, acceptable sensory properties, and the time consumed (105). As this product is being developed to be consumed as a snack, participants were asked to consume a standardized breakfast consisting of one serving (26g) of honey nut cheerios with 250mL of 2% milk, and 250mL of Tropicana orange juice. Milk and juice were provided to participants in factory sealed, pre-portioned containers, as purchased in the store, to reduce variability between breakfasts. Cheerios were weighed out by a lab volunteer, and the weight of 26.0g was selected as this matches one serving size recommended on the packaging for cheerios.

Sensory Analysis

A sensory analysis was conducted on the three different formulated snack bars. In accordance with the guidelines set forth by Health Canada on making functional food claims,

products formulated as functional food products must demonstrate that they meet similar sensory acceptability compared to related, non functional food products of similar style (105). As recommended by Health Canada, sensory analysis should be conducted using hedonic or visual analogue scaled (VAS), measuring bars with the proposed functional ingredients compared to bars of similar makeup without the proposed ingredient (105). While there is no requirement that any of the food meets a minimum preference level, the proposed ingredient should not significantly change the sensory preference when added to a product compared to similar products without the ingredient (105).

The sensory analysis was conducted during the clinical trial, where all participants completed VAS forms measuring the pleasantness, taste, and texture of each treatment in the booths in which they consumed the treatments. Sensory booths were climate controlled, including control for aroma, temperature, lighting, and sound, so as to limit any potential influence on their subjective ratings, and reduce variability between treatments.

Measuring Subjective Feelings of Appetite

Subjective appetite was measured using VAS scales administered to participants at several time points. VAS scales were completed at 0, 15, 30, 45, 60. 75, 90, 105, and 120 min, and measured several objective outcomes, including desire to eat, hunger, feelings of fullness, feelings of thirst, and subjective food intake speculation. Further to this, subjective ratings of energy and tiredness were collected at each of these time points. Subjective feelings of physical comfort were measured at these time points using VAS scales. This included measuring subjective feelings of nausea, stomach discomfort, wellness, flatulence, and diarrhea. These forms were filled out within the blood collection area, as several time points overlapped with blood collection time points as well.

Appetite was measured using the following 4 questions (Appendix 1): 1) how strong is your desire to eat?, 2) how hungry do you feel?, 3) How full do you feel?, 4) How much food do you think you could eat?.

VAS measurements on subjective comfort and gastrointestinal wellness were collected using the following 8 questions (Appendix 1): 1) How thirsty do you feel?, 2) How energetic do you feel right now?, 3) How tired do you feel right now?, 4) Do you feel nauseous?, 5) Does your stomach hurt?, 6) How well do you feel?, 7) Do you feel like you have gas?, 8) Do you feel like you have diarrhea?.

Measuring Food Intake Following Treatment

At 120 min after consuming the treatment, participants were given bottled spring water and an *ad libitum* pizza meal and were instructed to eat until comfortably full. Food intake was determined via weight difference, measured through the weight of the pizza prior to serving, minus the weight of the pizza after serving. Caloric intake was measured using information provided from the manufacturer. According to previously published guidelines, objective average appetite was calculated via the following formula from the VAS scales: appetite score = [desire to eat + hunger + (100 – fullness) + prospective consumption]/4 (106).

While there may be some effect on blood glucose control at subsequent meals due to the consumption of the lentil based bars, this is not the primary outcome of this investigation. According to the draft guidelines on short term blood glucose control, an effect on post-prandial glycaemic response should be measured as an acute effect (53). Any potential affect beyond the 2 hour post-prandial period, and subsequent ad libitum pizza meal is beyond the scope of this project (53). The purpose of the pizza meal, in conjunction with the VAS scales, is to determine

if the addition of lentil flours to the bars have an effect on subjective feelings of appetite, and if so, if that effect translates to a difference in energy intake at the subsequent meal.

Participants

The participants were 12 healthy male adults between the ages of 18-35 y, with normal BMI (18.5-24.9) (107). This sample size is based on previous data indicating that 14 participants were required to detect 10% difference for peak blood glucose response at 30 min, with an attrition rate of 15% (alpha level of 0.05 and power level of 0.8). This sample size was sufficient to detect a 5-10% difference in appetite rating using VAS scales (108). Participants were recruited using convenience sampling by posters and advertisements placed within the Halifax area.

Interested participants were subjected to a pre-screening questionnaire to determine their eligibility for entry into the study. Inclusion criteria required that participants were healthy, non-smoking adults, aged 19-35, with a normal BMI between 18.5-24.9 (107). Exclusion criteria included food allergies, persons who are taking medications or have health conditions that may affect food intake or metabolic markers in their blood. In addition to this, participants who were deemed eligible, but had blood glucose concentrations outside of normal levels were asked to come back at a later time to complete the study the first time.

Participants were initially pre-screened using a telephone questionnaire, to ensure that no food allergies, recent abnormal weight changes, or behavioural or emotional issues existed, and then were invited into the lab to participate in an in-person screening session. All participants were weighed, and their height measured using a Tanita body composition analyser to determine BMI. A weight of 1.2kg was subtracted from measured weight to account for the additional weight from clothes (109).

Although the results of this study will be used to help promote increased access to evidence based, healthier foods, and all populations may stand to benefit from this, only participants who have a normal BMI were included in the study. This is due to the draft guidelines for the post-prandial glycaemic response functional food claim, stipulating that the test population must be healthy (53). While having a BMI greater than 24.9 is not an indication of poor health in isolation, overweight and obesity are strongly correlated with increased risk of pre-diabetes and diabetes (20, 21). Further to this, a large portion of the population may be prediabetic without a positive diagnosis, and BMI, along with other markers, is associated with the potential for undiagnosed conditions which may affect glucose metabolism (110). It was determined that screening participants based on BMI was a good indicator of overall health status in order to meet the draft guidelines, however, it is recognized that this is a limitation of the study, as it does limit the population that the results will be applicable to.

Blood Collection and Analysis

Blood was collected into serum SST tubes (BD vacutainer SST gold top tubes) by Registered Nurses, and aliquoted into cryoprotective tubes which were frozen at -18C until analysis. Established lab procedures outlining blood collection methods, handling, and storage, were followed.

Blood samples were analyzed for serum concentrations of glucose against a glucose standard solution of known concentration. Serum concentrations of glucose was used as a primary outcome of interest for this study, as blood glucose levels are directly related to carbohydrate consumption (111). The availability of the carbohydrate fraction of a food for digestion is directly related to blood glucose concentrations (111). All glucose concentrations

were analyzed on the same machine (YSI 2900) over a period of 2 days. Serum samples were compared against a glucose standard of 50mmol/L, with an acceptable calibration range of 47.5-52.5mmol/L. The equipment was recalibrated between participants (12 times).

Specifically, participants arrived at the lab for the treatment session day, and had a 20 gauge IV catheter inserted into a vein in their arm by a registered nurse. The nurse drew off 1-3mL of blood, and this was used to determine baseline blood glucose levels. As participants were instructed to consume the standardized breakfast at least 2 hours before consuming the test snack bar, their blood glucose had to read between 4.0mmol/L and 6.0mmol/L to continue with the treatment session, as this is considered a normal fasting blood glucose level based on previous established lab protocols. Participants who had high blood glucose readings at baseline were rechecked 15min after the catheter was initially inserted, provided this was before the start of the session, and if blood glucose levels remained high, they were rescheduled to repeat the session. Baseline blood glucose was measured using a hand held glucometer.

After consuming the treatment, and at each time point throughout the study session, the nurse flushed the IV catheter with saline solution, drew enough blood into a discard tube to remove any saline from the IV line, and then filled two 10mL SST tubes. A total of two 10mL tubes was collected at each time point (0, 10, 20, 30, 60, 90, and 120 min). These tubes were immediately inverted 5 times and allowed to clot at room temperature for 30 minutes, before centrifugation at 15˚C at 1300 g (RCF) for 10 minutes. This is in accordance with manufacturer directions, and established lab procedures.

Each 10mL of blood yielded ~2mL serum, for a total of 4mL per time point of serum. This was aliquoted into six 2mL microtubes in equal amounts (0.70mL per tube), and immediately placed into storage at -18˚C.

All assays analyzing biomarkers of metabolism were conducted in duplicate, and an average of both scores was used in the data analysis.

Data Analysis

All data analysis was conducted on site at Mount Saint Vincent University using Statistical Analysis Software (SAS) studio, version 3.4 and GraphPad Prism.

All data was assessed for normality by considering the mean, kurtosis, skewness, and a histrogram output on the results for each area of analysis. A skewness and kurtosis of less than ±2 was used as a reference to assess normality, as well as general shape of the histograms (112). Where results partially met the categories outlined above, a judgement call at the researchers discretion was made based on the overall results to categorize a variable as normally distributed or non-normally distributed (112).

Specifically, for the analysis of serum concentrations of glucose, the average of concentrations for each time point run in duplicate, were used to find the incremental area under the curve (iAUC), net area under the curve (netAUC), and total area under the curve (totalAUC), for each participant. A one-way ANOVA with Tukey's post-hoc analysis was used to determine if there was a statistical difference between the iAUC, netAUC, and totalAUC between treatments at time 60, 90, and 120 min as per similar investigations, and for any significant treatment to treatment interactions (80). Further to this, 2-way ANOVA was conducted on the pooled mean glucose concentrations for each time point, to determine if there was a significant effect of both time and treatment.

VAS questionnaires were converted to a value between 0-100 by measuring to the point of the x along a 100mm line and analyzed for differences between treatments. The VAS scores

for each time point and treatment were pooled across all participants, and analyzed for normality, and a one-way ANOVA with Tukey's post-hoc analysis was used to determine if there were any significant treatment interactions.

The pizza weights were converted into calories using the weight consumed multiplied by the caloric information found on the Nutrition Facts Table on the product box found in Appendix 3, and the calories were then analyzed to see if there was difference between calories consumed based on treatment. A 1-way ANOVA with Tukey's post-hoc analysis was conducted to determine a significant difference between mean caloric intakes of pizza across all 4 treatment sessions.

Ethical Clearance

The protocol and procedures used in this study were reviewed and approved by the Mount Saint Vincent University Research Ethics Board.

Results

Study Participants

A total of 28 male participants were pre-screened, and recruited into the study, attending at least 1 session. Of these, 12 males completed all four treatment sessions during the study period. All data forms were filled out, with no missing values found for any of the results reported in this document. The average age of participants was 25 ± 4.5 years, and the average BMI was 22.4 ± 2.5 kg/m^2. At the start of each session, baseline VAS questionnaires assessed their self reported subjective food intake, activity level, and level of stress over the past 24 hours leading up to the treatment session, compared to a normal 24 hour period. There was no significant difference for self-reported food intake (P=0.158), activity level (P=0.194), or level of stress (P=0.565) between all four treatments in the 24 hours before each treatment session, among all participants (n=12).

Pizza Meal Preference by Treatment and Food Intake of Pizza Meal

Calories consumed in the pizza meal after each treatment were calculated by weight of pizza consumed. While pizza type (peperoni, 3 cheese, deluxe) served for each treatment session was based on preference that participants indicated during their initial screening session, of the twelve participants, two consumed more than one type of pizza over the four treatments, which was noted and adjusted for in the calorie calculations. VAS scales on palatability of pizza were completed by all participants after each of the four treatments, and there was no significant difference on preference of pizza between treatments (p=0.542), showing no impact on the type of treatment consumed on the preference for pizza. Figure 2 shows the total mean calories (±S.E.M.) of pizza consumed by treatment.

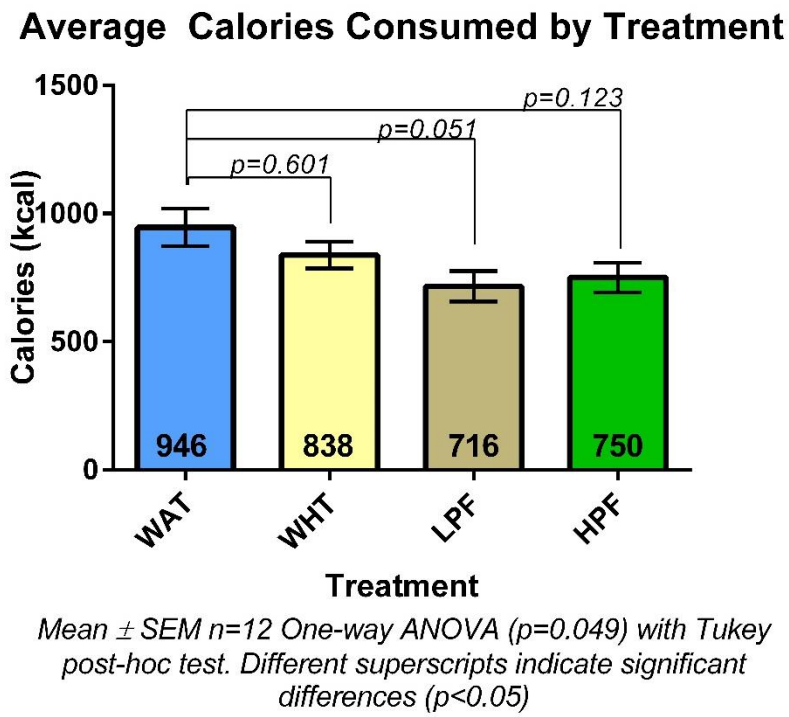


Figure 2 Average calories consumed from pizza meal by treatment type

Overall, there was a significant effect seen on total caloric intake between treatments (p=0.049). Tukey's post-hoc analysis indicated a very strong trend of reduced caloric intake between the water treatment and low pulse flour treatment (p=0.051), which, although not significant, was an important finding. The average calories consumed for the low lentil flour treatment was 230 kcal less than the average consumed for the water (caloric free control). While not as large of a difference, there were 196 kcal less consumed following the high lentil flour treatment, compared with the water free control. The calorie content of the snack bars is 142kcal per bar, therefore the consumption of a snack bar, either lentil flour containing or wheat flour bars, resulted in an overall reduction of calorie intake during the study session and pizza meal compared to the water free control. These two values, showing around 200kcal difference between the caloric free control and treatments containing pulse flour is approximately double the difference seen between the wheat flour and the two treatments containing lentil flour

(108kcal). While there was a difference between the calories consumed at the ad libitum pizza meal from the wheat bar to the two bars containing lentil flour, these were not significant. The reduced calories of 122kcal after consuming the bar with 12g of lentil flour, compared to the wheat flour only bar, was P=0.497. The reduced calories of 88kcal after consuming the bar with 24g of lentil flour, compared to the wheat flour only bar, was P=0.742. There was virtually no difference seen between the two lentil flour containing bars, with P=0.978.

Using the R-squared value from the one-way ANOVA of 0.1616 (r: 0.402; Cohen's d 0.88) as a measure of the effect size seen in this investigation on total calories consumed by treatment, a total of 13 participants would be required to see significant differences between treatments (alpha level of 0.05 and power of 0.8). The standard deviation for each treatment was 252kcal, 183kcal, 205kcal, and 201kcal for water, wheat, low pulse flour, and high pulse flour treatments respectively, suggesting a wide variability in the amount of calories consumed between participants.

Sensory Acceptability of Treatments

The palatability of the treatments for the bars formulated with wheat flour, low lentil flour, and high lentil flour, were assessed using the results from the VAS scales. The results were assumed to follow a normal distribution. There were no significant differences between the sensory evaluations of all three treatments on pleasantness (p=0.742), taste (p=0.682), or texture (p=0.440). The results are summarized in Figures 3-5.

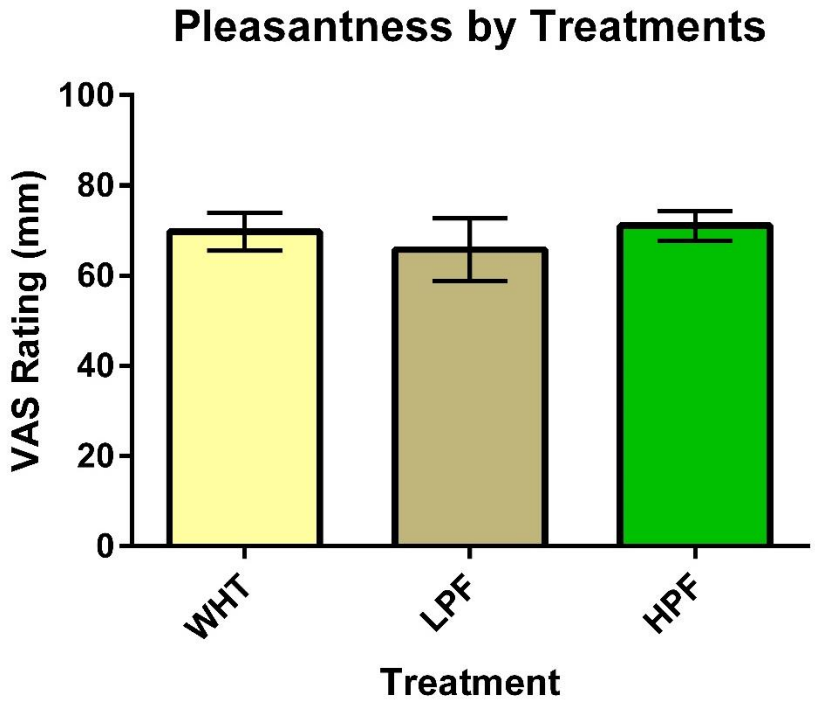


Figure 3 Subjective ratings of pleasantness of different caloric treatments

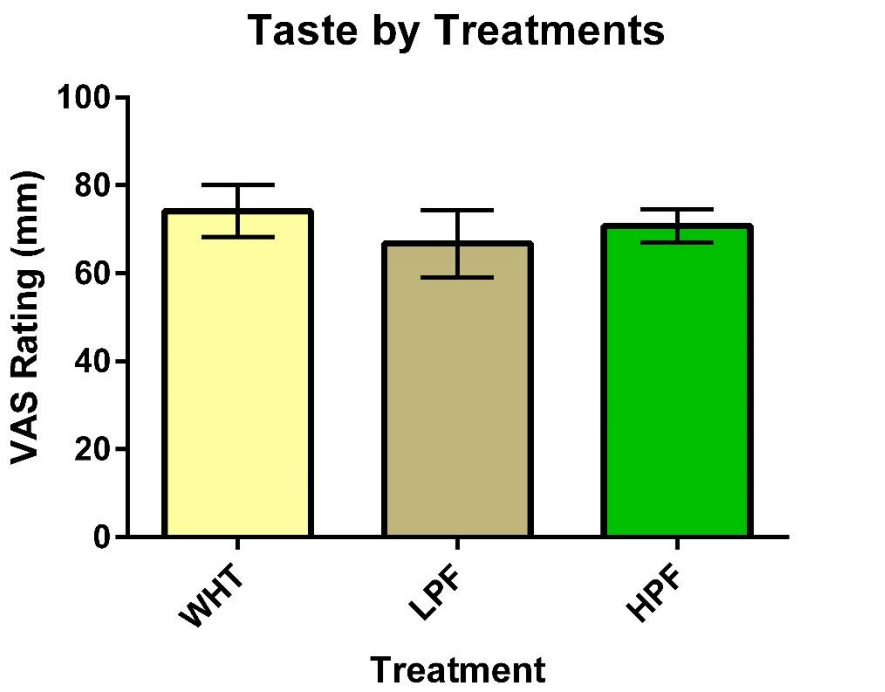


Figure 4 Subjective ratings of taste of different caloric treatments

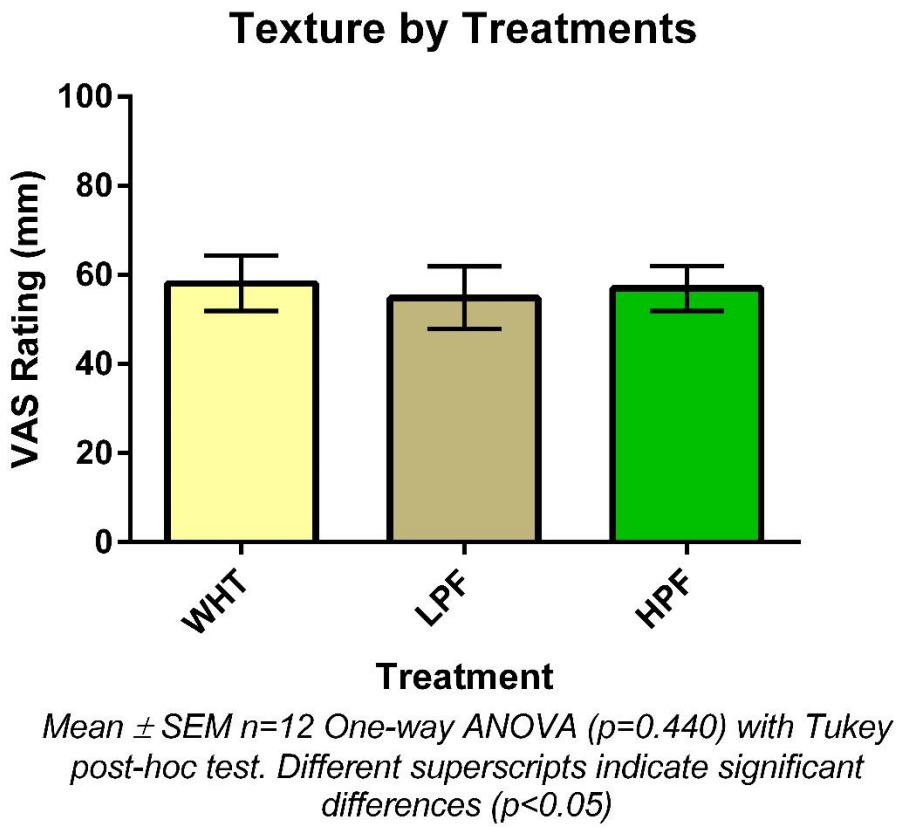


Figure 5 Subjective ratings of texture of different caloric treatments

Motivation to Eat Questionnaire by Treatment

The results from the motivation-to-eat VAS questionnaire were calculated from the 4 questions contained in the booklet. An appetite score for each timepoint (0,15,30, 45, 60, 75, 90, 105, 120 min) was calculated for each treatment, and column statistics were performed to assess normality using mean, mode, skewness, kurtosis, and a histogram. The data was assumed to be normally distributed and a one-way ANOVA was conducted at each time point to determine significance of treatment. No significant differences were noted overall, and there were no significant differences in appetite score between any of the four treatments. The results are summarized below in Figure 6.

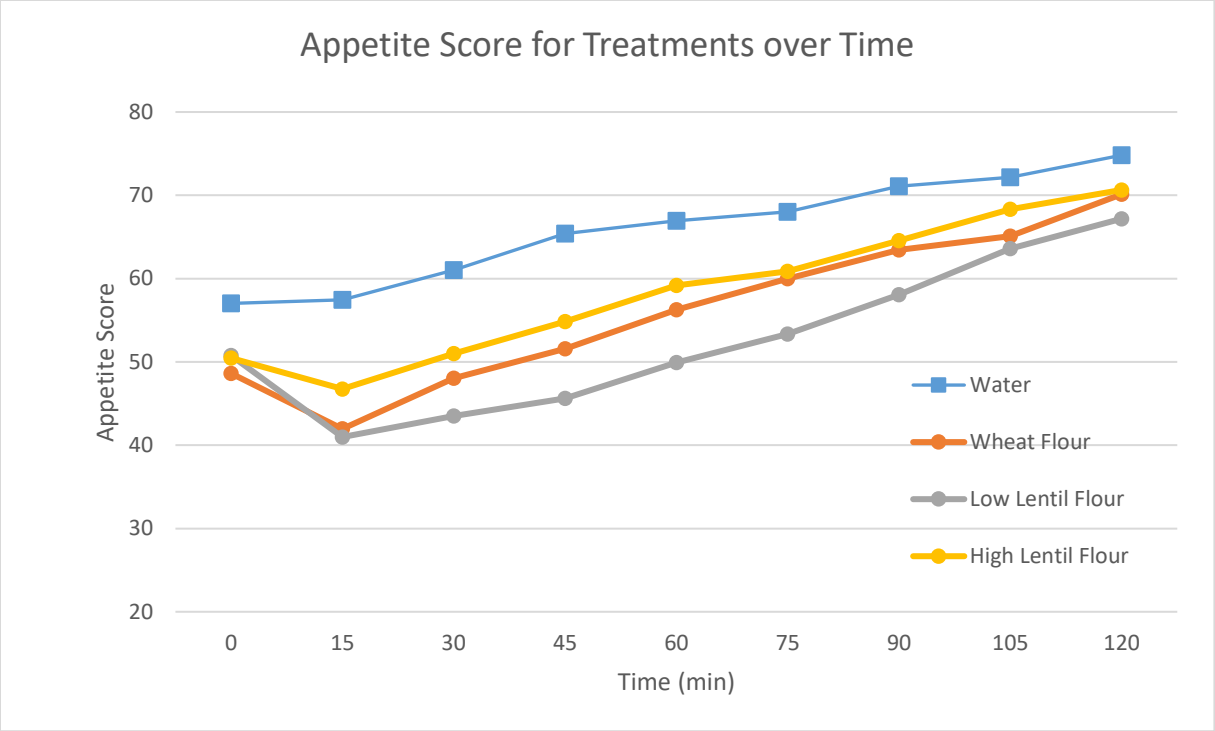


Figure 6 Average appetite score for each time point between treatments

A 2-way ANOVA was conducted to evaluate effect of time ($p<0.001$) treatment ($p<0.001$) and a time by treatment interaction ($p<0.001$). Overall, there was a significant effect of time on appetite score within each treatment ($p<0.001$), with significant changes in appetite rating noted at times 105 min ($p<0.0001$) and 120 min ($p<0.0001$) from baseline, and at 60 min ($p=0.018$), 75 min ($p=0.001$), 90 min ($p<0.0001$), 105 min ($p<0.0001$), and 120 min ($p<0.0001$) from 15 min after consuming the treatment. A significant effect was also observed at 30 min and 45 min after consuming the treatments, compared to appetite scores at 105 min ($p<0.0001$) and 120 min ($p<0.0001$). For the wheat flour treatment, the return to baseline appetite score was seen at the 45 min mark post-prandial. For the high pulse flour treatment, a return to baseline was seen at 30 min post-prandial. The low pulse flour treatment showed the longest suppression of subjective appetite score, with a return to baseline appetite score seen at 60 min.

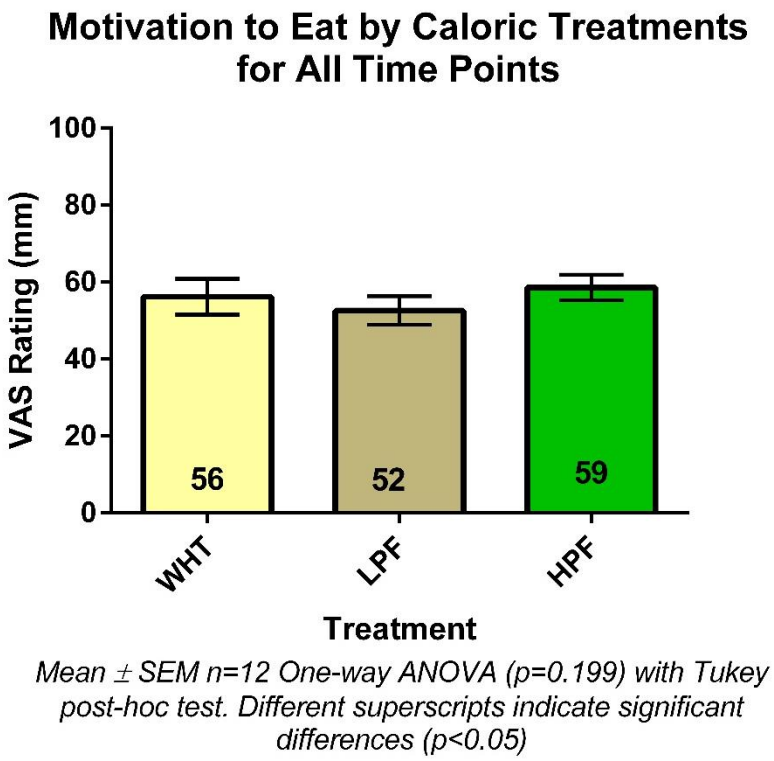


Figure 7 Motivation to eat over 2 hours between caloric treatments

Subjective Feelings of Wellness by Treatment

VAS for subjective comfort and gastrointestinal wellness were analyzed for differences between treatments. The data was treated assuming a normal distribution after generating mean, median, skewness, kurtosis, and histograms.

The results were tested for significant differences between treatments by one-way ANOVA at time 0, 60, and 120min, with no significant differences seen between treatments for thirst, tiredness, nausea, stomach pain, wellness, gas, and diarrhea. The results are summarized in Figures 8-14.

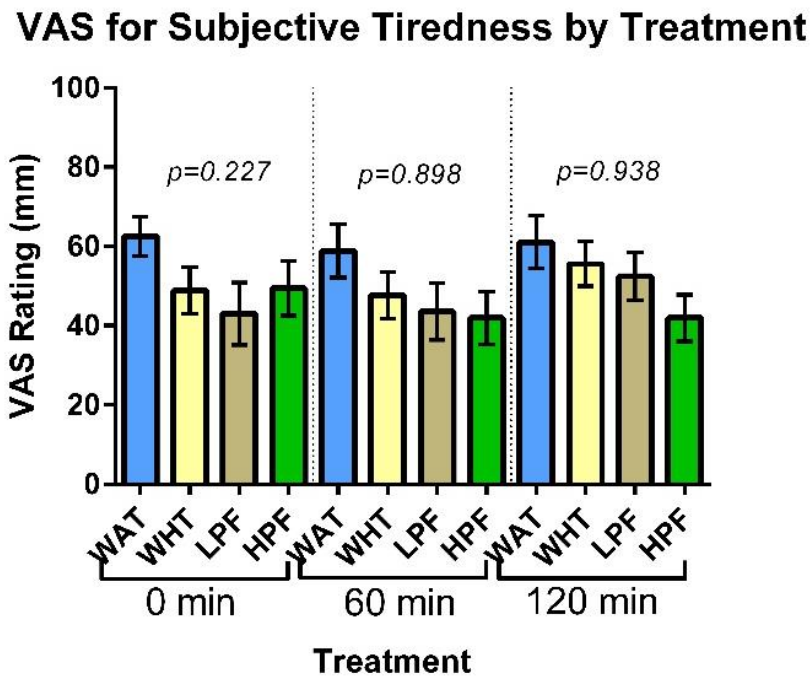


Figure 8 VAS for subjective tiredness

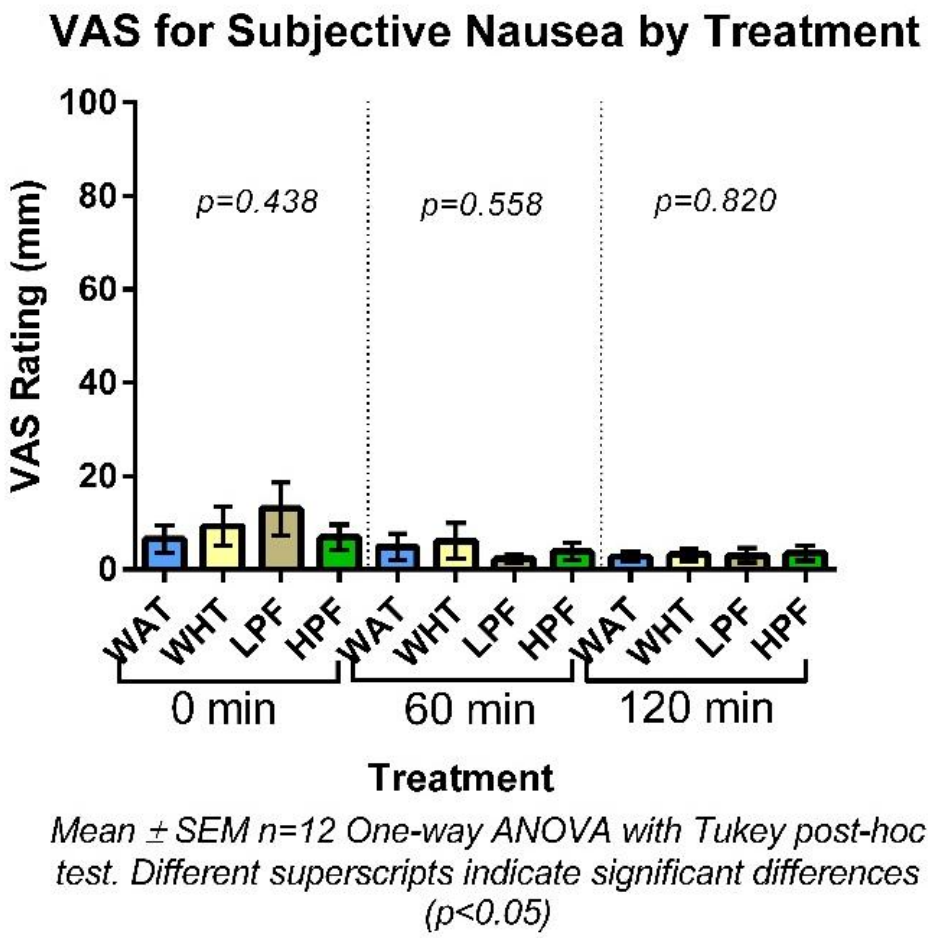


Figure 9 VAS for subjective nausea

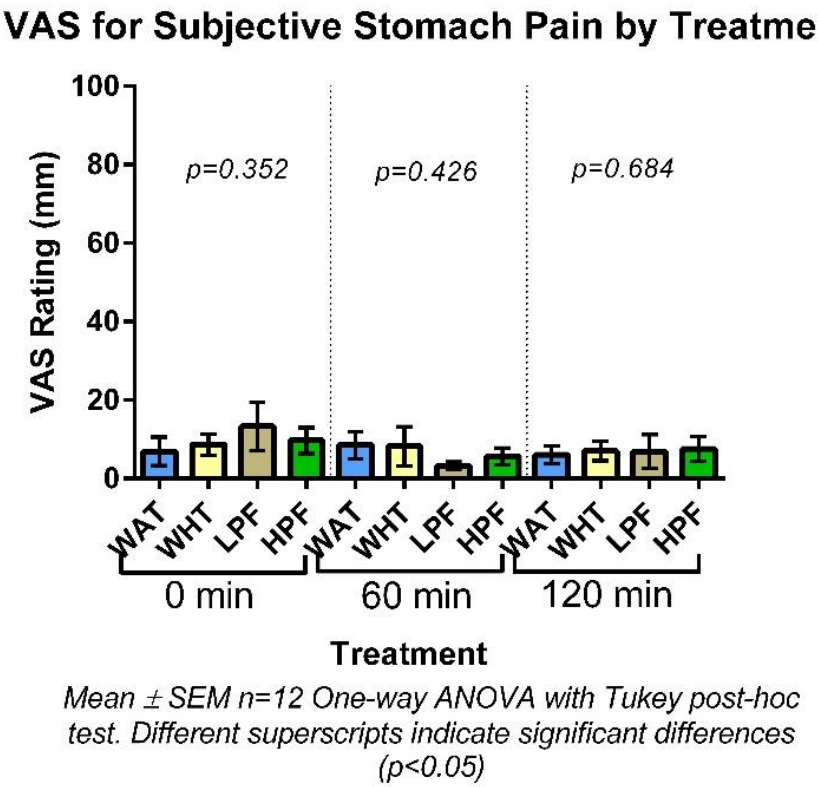


Figure 10 VAS for subjective stomach pain

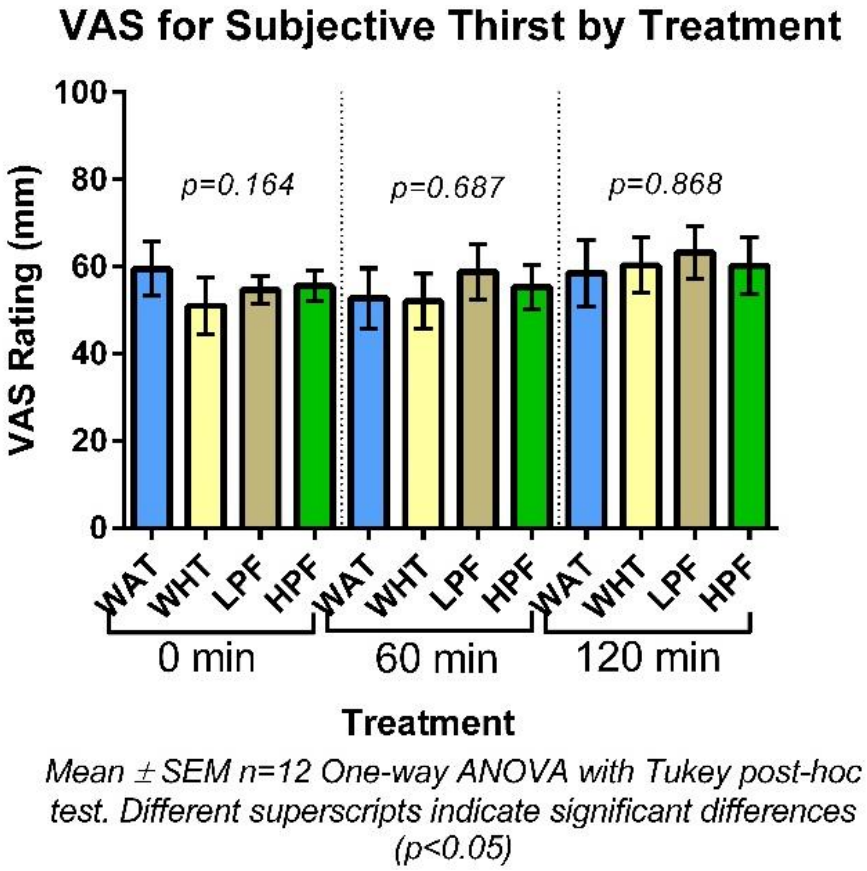


Figure 11 VAS for subjective thirst

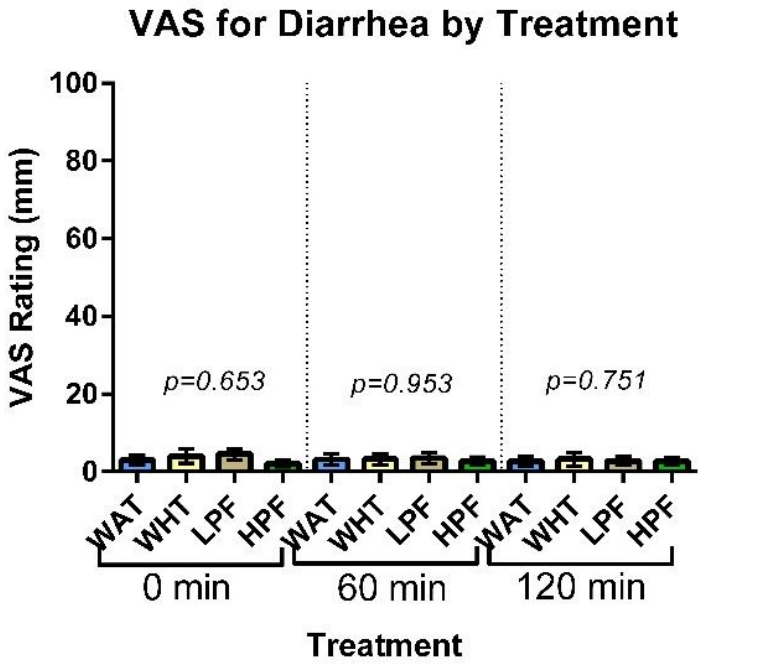


Figure 12 VAS for subjective diarrhea

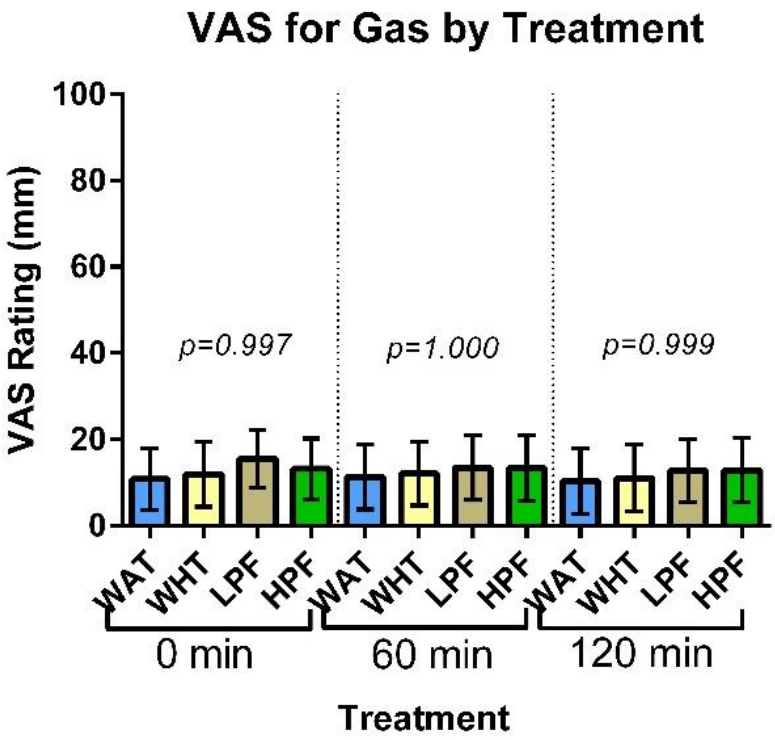


Figure 13 VAS for subjective feelings of gas

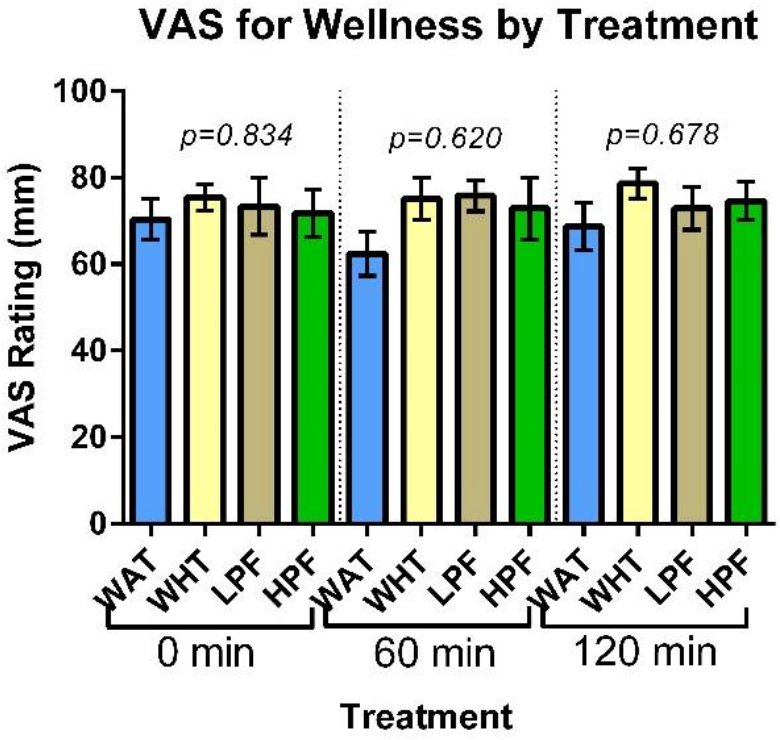
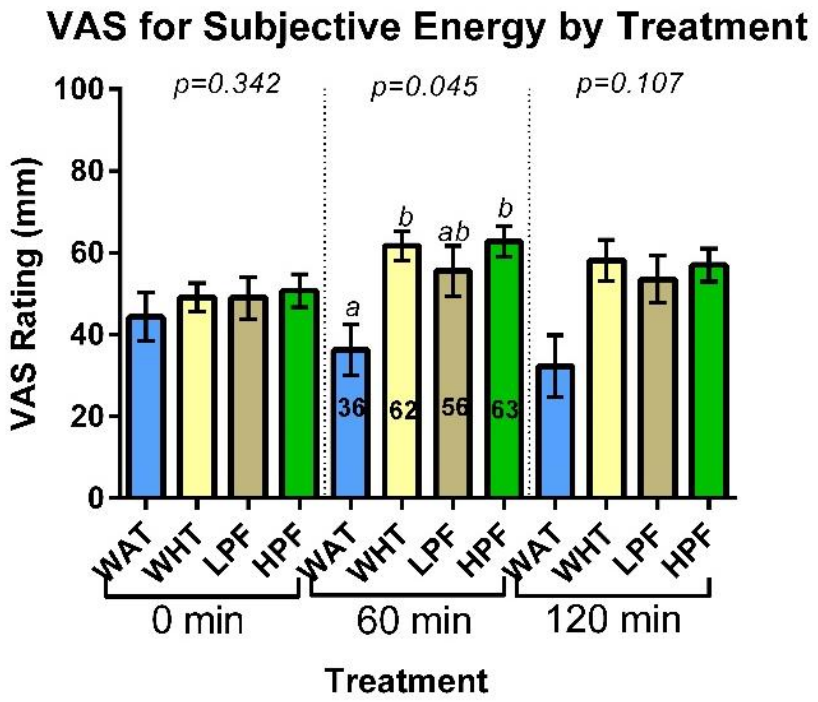


Figure 14 VAS for subjective wellness

While there was no significant difference for feelings of energy between treatments at time 0 and 120 min (p=0.799 and p=0.107, respectively), there was a significant difference noted at 60 min (p=0.045). A Tukey post-hoc analysis revealed a significant treatment interaction in subjective feelings of energy between the HPF and WAT treatments (p=0.020), and between WHT and WAT treatments (p=0.013). While not significant, a strong trend was noted for LPF and WAT (p=0.055). The mean VAS score for energy for WAT (32 ± 26.0) was lower than the mean scores for WHT (58 ± 17.5), LPF (54 ± 19.7), and HPF (57 ± 14.1) at 60 min. The difference in energy between WAT treatment and all other treatments was more robustly noted following a 2-way ANOVA to evaluate time by treatment interactions, with no significant differences seen within each treatment over time for energy (P=0.286), suggesting the difference seen are between treatments alone, and not within each treatment. The results are summarized below in Figure 15.



Mean ± SEM n=12 One-way ANOVA with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

Figure 15 Subjective Feelings of Energy

A two-way ANOVA was conducted to test for any significant time by treatment interactions. There were no significant differences over time within each treatment noted for feelings of energy ($p=0.286$), tiredness ($p=0.562$), thirst ($p=0.304$), stomach comfort ($p=0.377$), wellness ($p=0.820$), gas ($p=0.977$), diarrhea ($p=0.836$), and nausea ($p=0.077$).

Blood Glucose Concentration

Blood glucose concentrations were measured by comparing serum to a standard of known glucose concentration, as described in the methods.

All participants arrived to the lab after consuming a standardized breakfast at least 2 hours prior to the start of each of four treatment sessions. Baseline blood glucose was checked using a hand-held glucometer (accu-check compact plus) with new test strips (lot # 20815744, exp. 2018-07-31), and participants were allowed to participate in each session if their baseline blood glucose was measured below 6.0 mmol/L. A one-way ANOVA was performed on all baseline blood glucose measures for all participants, and, while there was a slightly lower mean at the baseline for WAT (4.54 mmol/L ± 0.73 S.D.) and WHT (4.55 mmol/L ± 0.54 S.D.) treatments compared to LPF (4.93 mmol/L ± 0.78 S.D.) and HPF (5.02mmol/L ± 0.73 S.D.), this difference was not significant ($p=0.214$), with no significant interactions observed between treatments. These results are illustrated in Figure 16 below.

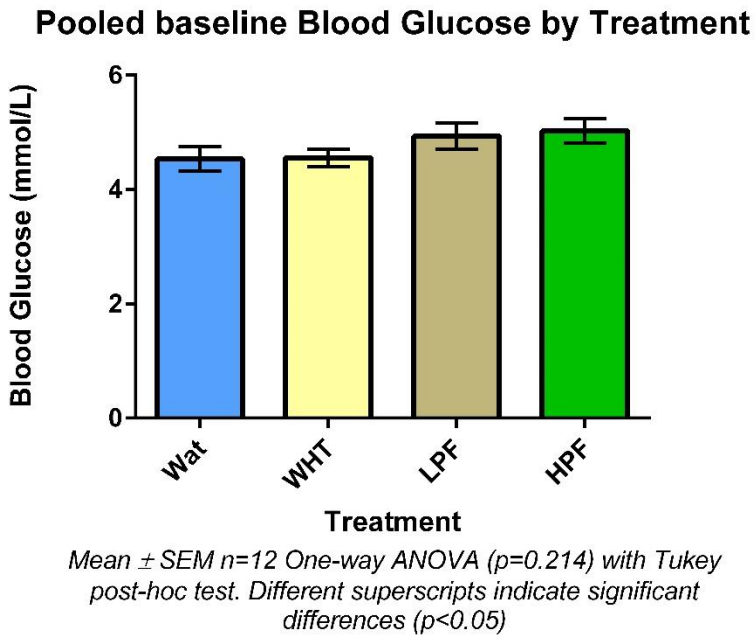


Figure 16 Baseline blood glucose concentrations

Figure 17 shows the mean blood glucose concentrations over time, with samples collected at 0, 10, 20, 30, 60, 90, and 120 minutes for each treatment.

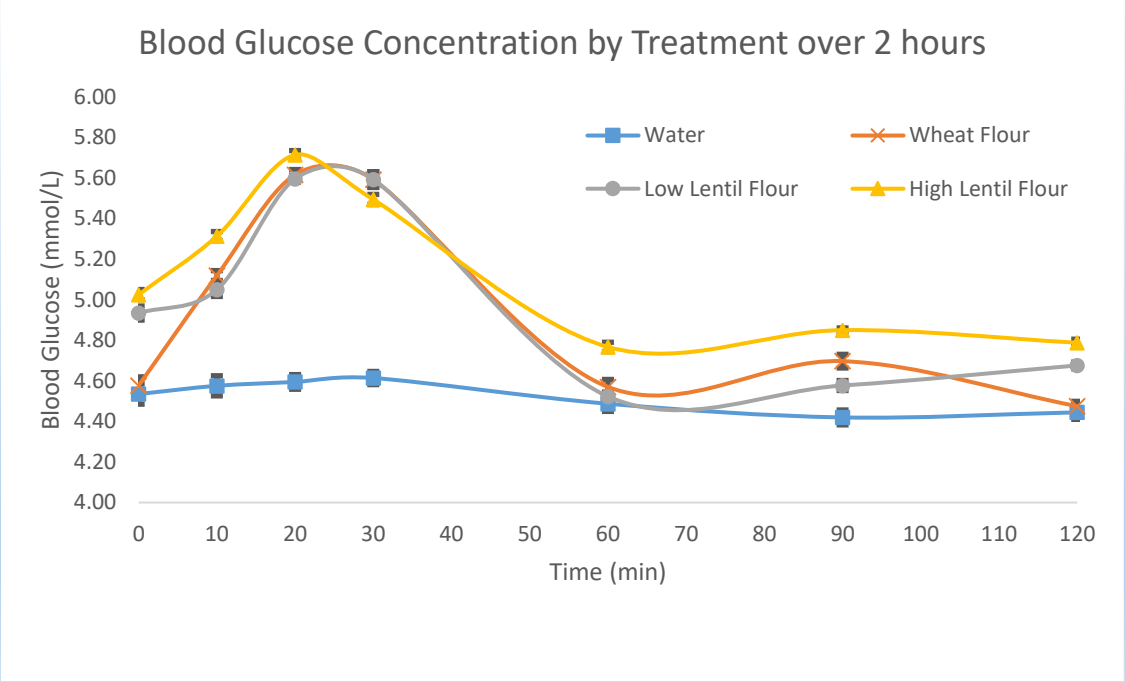


Figure 17 Average blood glucose concentrations for each time point for all treatments over 2 hours

Blood glucose concentrations were used to calculate four different types of area under the curve at 60, 90, and 120 min. These included totalAUC, iAUC, and netAUC. Briefly, totalAUC is the total area calculated from peaks above and below baseline. iAUC is the total area calculated from peaks above baseline, ignoring any area below the baseline. netAUC is the subtraction of the area from any peaks seen below baseline from those peaks above baseline (113).

A one-way ANOVA for totalAUC showed no significant differences at 120 min ($p=0.839$), 90 min ($p=0.764$), and 60 min ($p=0.811$) showed no significant differences overall. A two-way ANOVA revealed a significant time by treatment interaction ($p<0.001$), showing a significant change of AUC over time. The results of each time point, along with treatment interactions (Tukey's post-hoc analysis) between the caloric free WAT treatment, and the calorie containing WHT, LPF, and HPF treatments are illustrated below in Figures 18-20.

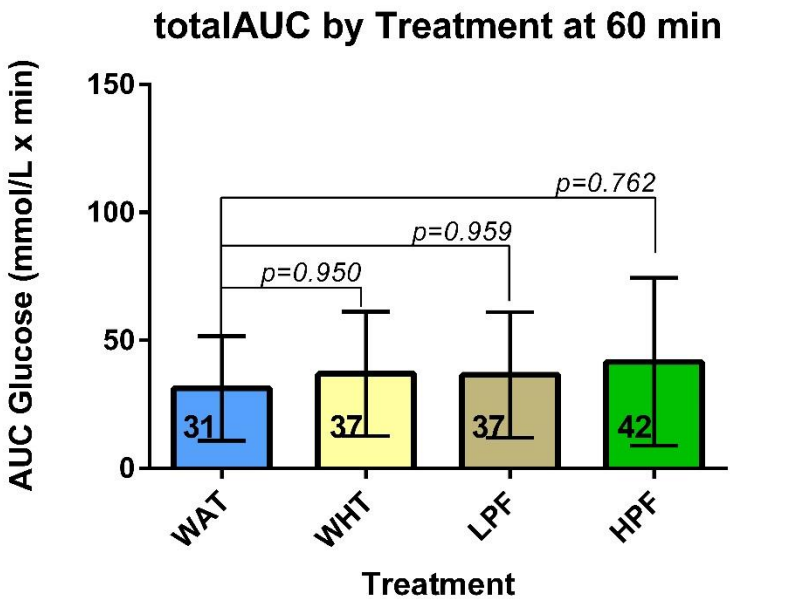


Figure 18 totalAUC at 60 min by treatment

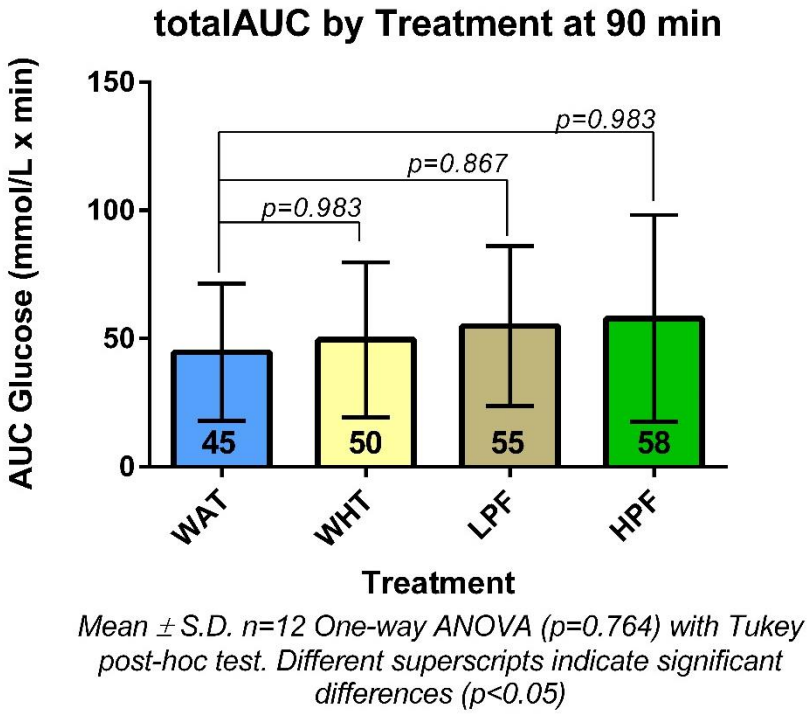


Figure 19 totalAUC at 90 min by treatment

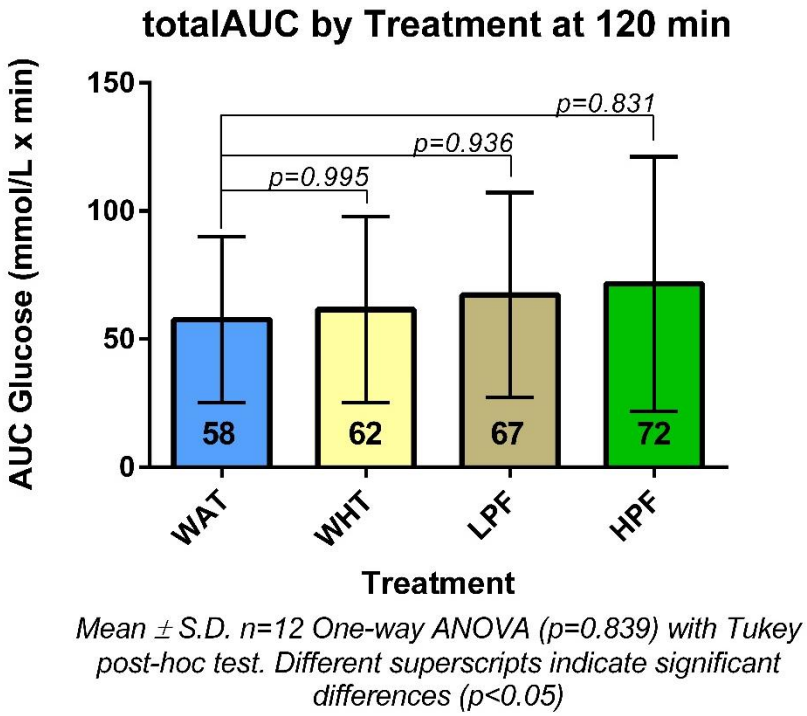
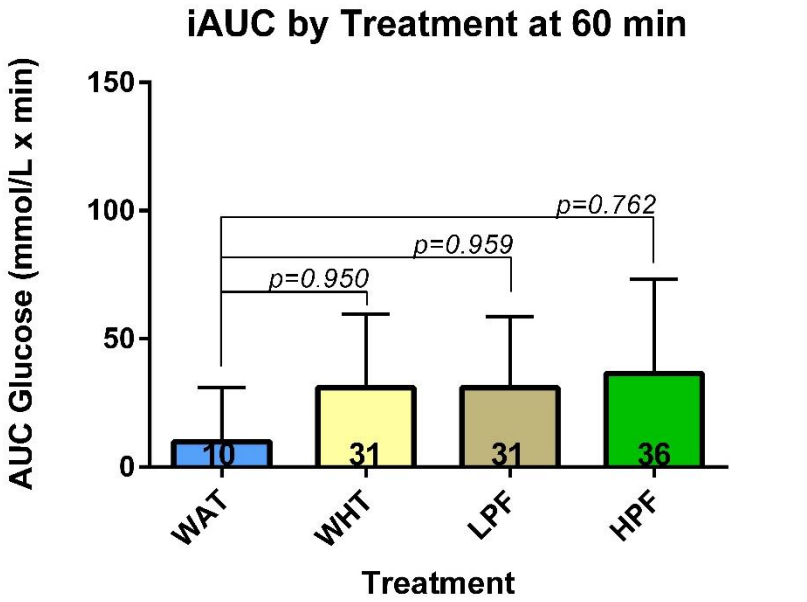


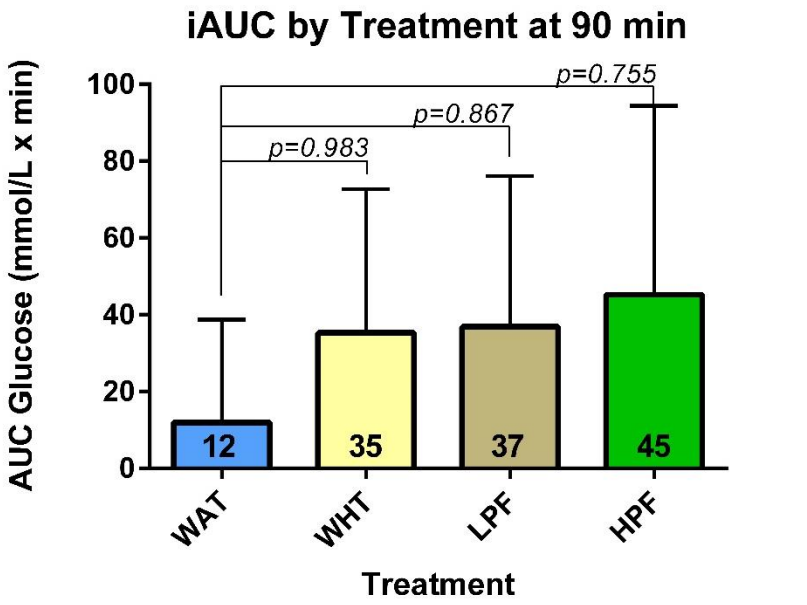
Figure 20 totalAUC at 120 min by treatment

Similarly, a one-way ANOVA for iAUC showed no significant differences at 120 min ($p=0.259$), 90 min ($p=0.200$), and 60 min ($p=0.136$) showed no significant differences overall. A two-way ANOVA revealed a significant time by treatment interaction ($p<0.001$), showing a significant change of AUC over time. The results of each time point, along with treatment interactions (Tukey's post-hoc analysis) between the caloric free WAT treatment, and the calorie containing WHT, LPF, and HPF treatments are illustrated below in Figures 21-23.



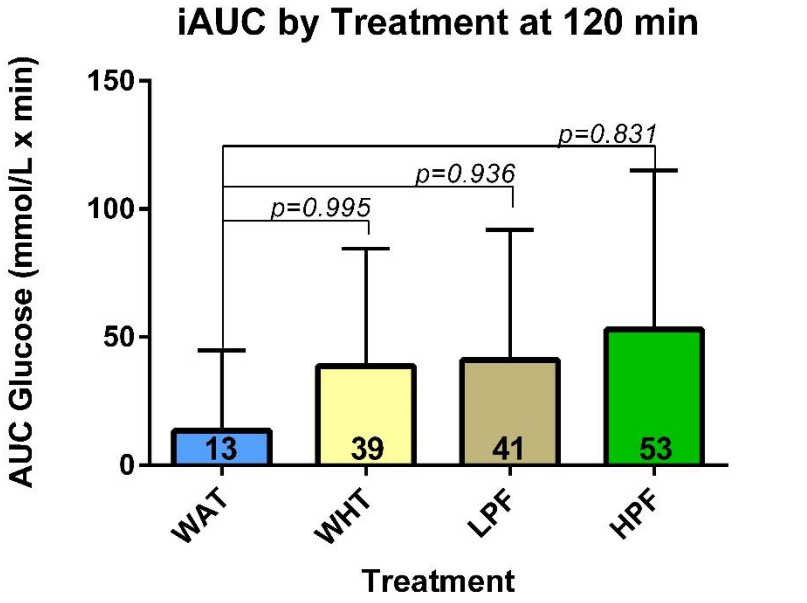
Mean ± S.D. n=12 One-way ANOVA (p=0.136) with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

Figure 21 incrementalAUC at 60 min by treatment



Mean ± S.D. n=12 One-way ANOVA (p=0.200) with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

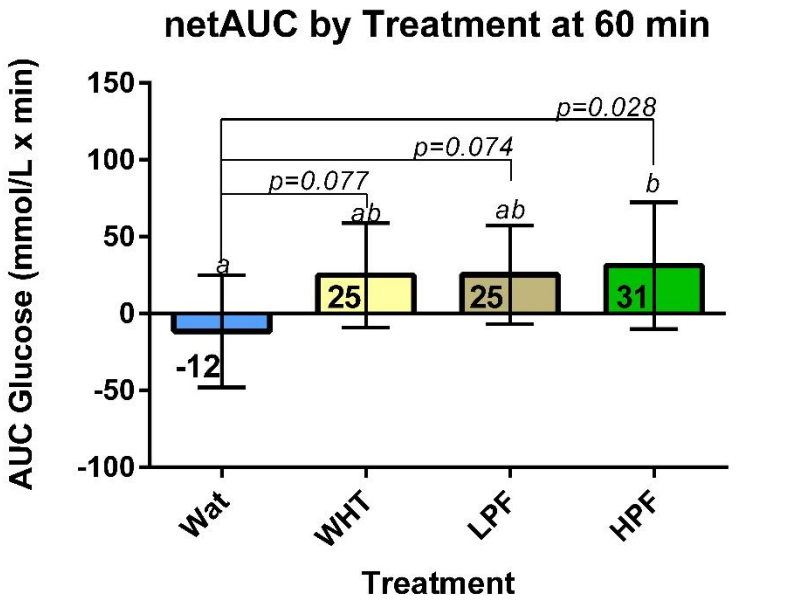
Figure 22 incrementalAUC at 90 min by treatment



Mean ± S.D. n=12 One-way ANOVA (p=0.259) with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

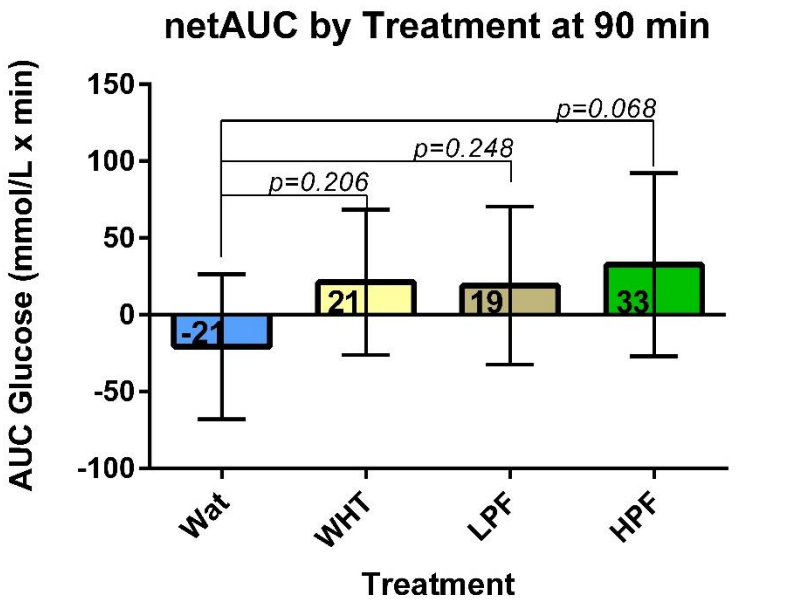
Figure 23 incrementalAUC at 120 min by treatment

A one-way ANOVA for netAUC showed no significant differences at 120 min ($p=0.106$) and at 90 min ($p=0.076$). However, a significant difference was seen for netAUC at the 60 min timepoint ($p=0.022$). A Tukey post-hoc analysis revealed a significant difference between netAUC for the WAT and HPF treatments ($p=0.028$). The results of each time point, along with treatment interactions (Tukey's post-hoc analysis) between the caloric free WAT treatment, and the calorie containing WHT, LPF, and HPF treatments are illustrated below in Figures 24-26. The Tukey's post-hoc analysis showed no significant interactions between all caloric containing treatments, with p values for each interaction equal to or greater than 0.97. A two-way ANOVA revealed a significant time by treatment interaction ($p<0.001$), showing a significant change of AUC over time.



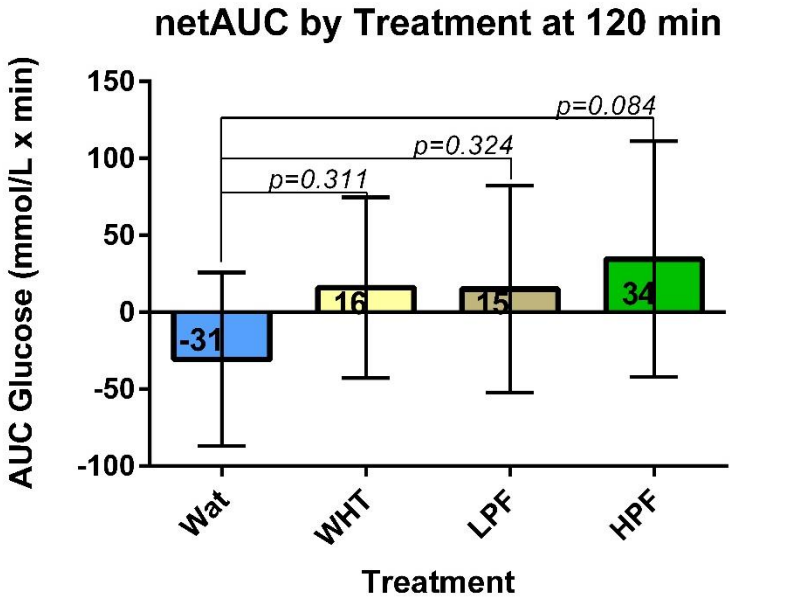
Mean ± S.D. n=12 One-way ANOVA (p=0.022) with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

Figure 24 netAUC at 60 min by treatment



Mean ± S.D. n=12 One-way ANOVA (p=0.076) with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

Figure 25 netAUC at 90 min by treatment



Mean ± S.D. n=12 One-way ANOVA (p=0.106) with Tukey post-hoc test. Different superscripts indicate significant differences (p<0.05)

Figure 26 netAUC at 120 min by treatment

Discussion

While adherence to recommended dietary guidelines has been shown to positively impact weight management, existing survey data on diet quality in Canada indicates that less than half of Canadians meet recommended guidelines on diet (114, 115). The inclusion of pulses as a regular part of a varied diet has been linked to improved health outcomes (116). Processed foods with added pulse ingredients can be found throughout consumer retail locations, marketed with the apparent perception that consumption of these products will offer the same benefits as the consumption of whole pulses. Although Canada does not require core nutrient components on foods that carry a specific health claim, the proposed changes from Health Canada on front of package labelling will help consumers determine more readily foods that are linked to poorer health outcomes, versus those they should consider more often (117). Purchasing trends indicate that Canadians continue to rely increasingly on ultra-processed foods to make up a large portion of their diet, so there exists a need to marry healthy, evidence based foods and food ingredients with processed foods that are preferred and accessible to the average Canadian consumer (42).

This research project sought to create a snack bar with added pulse ingredients, and evaluate the bar based on consumer preference and potential health impacts on post-prandial glycaemia. There haven't been investigations to date on the amount of processed lentil flour that is required to notice a positive impact on post-prandial glycaemia and short term food intake.

This investigation recruited young men with normal BMI. While an abnormal BMI, and specifically a BMI greater than 24.9kg/m^2 is not a marker of poor health or abnormal glucose metabolism, at the population level, a BMI considered overweight or obese is considered a risk factor for developing NCD, many of which do affect glucose metabolism, such as DM (19). Participants completed a baseline health screening questionnaire, which asked to identify any

known health conditions. To help eliminate the possibility of unknown or undiagnosed health conditions affecting the outcomes of this study, a normal BMI was selected as an inclusion criteria. While this may strengthen the results, it is important to recognize the limitation that this places on the investigation. Just over 50% of the Canadian male population is considered overweight or obese, so an investigation selecting only healthy weight males is not representative of the population of Canada (19). However, as this investigation was exploratory in nature, it was important to reduce the potential confounders that may affect post-prandial glycemia to investigate the effect, if any, of lentil flours. Further investigations may use the results from this study to inform a design that is more representative of the Canadian population.

Similarly, only men were included in this study. While this was due to logistical and time constraints, it is not representative of the population, and the results are not generalizable across female populations, or the population of Canada as a whole, due to this limitation.

Recipe Formulation and Sensory Results

There were three different bars formulated for use as treatments during this investigation. The control bar had 100% of the flour portion in the recipe as all-purpose white flour. The low pulse flour bar replaced half of this flour by weight with the milled lentil flour. The high pulse flour bar replaced 100% of the wheat flour by weight with milled lentil flour. A previous sensory investigation at Mount Saint Vincent University by honours student Allison Barnett conducted in 2015 with 28 participants (male and female), found that 45% replacement by weight of traditional whole wheat flour with a milled lentil flour had no significant effect on overall acceptability of a formulated snack bar. This builds on previously published results, showing a replacement of traditional ingredients with processed pulse ingredients in baked goods of 24-37% resulted in acceptable products for consumer preferences (90, 91). The recipe

developed by Allison Barnett, 2015, was used as a base recipe to inform the recipe used in this investigation. Some adaptations were made, including standardizing the recipe to yield 50g final baked weight per bar to meet serving size recommendations, and a replacement of non-traditional, expensive ingredients, such as coconut oil, with ingredients that are more cost effective and available for commercial reproduction (104). Whole wheat flour was also replaced with wheat flour. A preliminary taste test also showed that the 100% lentil flour bar was not as palatable as the other two treatments, so all three treatment recipes were adapted to increase the amount of cinnamon and vanilla extract in the recipes, as well as increase the baking time. This yielded a much more acceptable tasting bar based during an informal sampling among faculty and students at MSVU.

Participants (n=12) rated the sensory acceptability of the bars based on three parameters: pleasantness, taste, and texture. There was no significant differences noted between any of the treatments for all three parameters. Further to this, the participants generally rated the treatments as acceptable. For pleasantness, mean scores ± SEM were 70 ± 4.2, 66 ± 6.9, and 71 ± 3.3 for wheat, low pulse flour, and high pulse flour treatments respectively. For taste, mean scores ± SEM were 74 ± 5.9, 67 ± 7.6, and 71 ± 3.8 for wheat, low pulse flour, and high pulse flour treatments respectively. And finally, for texture, mean scores ± SEM were 58 ± 6.2, 55 ± 7.0, and 57 ± 5.0 for wheat, low pulse flour, and high pulse flour treatments respectively. These scores are similar to those seen during Allison Barnett's honours project in 2015 using the original bar recipe with whole wheat and lentil flour. The results of that Honours project produced scores for pleasantness and taste of 60-80, using the same VAS scale to measure participants responses as was used during this investigation. Although the results are not directly comparable, as the bar formulation between the two studies was different, and the participants in

the honours project were mostly female, compared to the all male participants in this study, the similarity in results to lend further weight that this style of bar with a filling is generally seen as acceptable amongst different types of consumers. Further to this, it shows that the addition of a lentil based flour into a snack bar formulation does not adversely affect preference over bars formulated with more traditional wheat flour ingredients.

Serving Size and Caloric Content of the Bars

Great care was taken to ensure that the snack bars baked for this sample had a final cooked weight of 49.3 ± 0.5g. Although this was based from reference serving sizes published by the Canadian Food Inspection Agency, it is important to note that there appears to be great variability among the serving sizes of snack bars in the Canadian marketplace (104). An unofficial survey of some snack bars available at grocery stores showed serving sizes varied greatly, with a Fibre One, Nutri-Grain, Natures Bakery fig, and Cliff bars having serving sizes of 25g, 37g, 57g, and 68g per bar, respectively. This survey included bars both with and without filling, which have the same serving size recommendation range of 20-50g (104). When the caloric information per serving, available on the Nutrition Facts Table from each product, was standardized for a 50g serving size, it was found that calories per 50g serving for a Fibre One, Nutri-Grain, Natures Bakery fig, and Cliff bars were 187 kcal, 183kcal, 191kcal, and 191kcal per bar respectively. This is compared to a caloric content of ~142 kcal for the bars formulated for this study, showing that the bar for this recipe appears to be much lower than similar bars available on the marketplace.

While the Canadian Food Inspection Agency (CFIA) doesn't differentiate between sex, body composition, or life stage, outside of products for babies and toddlers, when determining reference calorie requirements for product labelling, it is important to consider this snack bar in

relation to snacking patterns (118). The CFIA format the nutrition facts table based on a 2000 kcal per day diet, which may meet the general needs of the majority of the population, but may not be applicable to all consumers (118). While this study was designed to specifically meet some of the requirements set forth in data collection for the proposed functional health claim on blood glucose, this came with specific limitations in design (53). The aim of this study was to see effects on blood glucose, food intake, and preference based on a standard serving size, in a normal, healthy population of young males. It is important to note that males tend to consume greater calories per day than females from snack foods, while females tend to snack more frequently (119, 120). Although this trend is generalizable across both normal and obese populations, with trends of proportion of calories from snacks and frequency of snacking similar by sex between BMI groups, the differences in patterns by sex may limit the generalizability of the results of this study to other populations (119). Further to this, caloric requirements differ by gender, age, body composition, and activity level (111). Although there may have been an effect from these differences on food intake during the pizza meal, and desire to eat scores during the treatment session due to different caloric needs between participants, each participant was exposed to all four treatments, which offers strength to the results as each participant was their own control. The purpose of this study was not to tailor a snack to different caloric needs, but to measure the effects of a snack that meets serving size recommendations on a representative sample of a defined population group. The variability in caloric requirements between participants was not measured but was also not a focus of this study.

Men in Canada are less likely to eat breakfast than other groups, and the most likely to consume more calories from snacks than from breakfast (120). This trend is seen strongest in the 19-30 age category, which is the age of participants recruited for this study (120). The total

calories for the breakfast was 350kcal (110 kcal from cheerios, 130 kcal from 2% milk, and 110 kcal from orange juice). This is much higher than the 142 kcal from the snack bar, suggesting that the design of this study may not fit into normal consumption patterns for males aged 19-30 in Canada, although there was no further dietary data collected from recruits for this study (120). In contrast to this, to measure the effects of the intake of a snack bar, it was important to feed the bars at a time and between meals where a snack may typically be consumed. A morning snack time was chosen, as it presented less challenges in controlling for an overnight fast, as participants were instructed to begin fasting after 9pm the night before study, and reminded via e-mail, call, or text message. It also gave control over standardized breakfast consumed, and allowing for a pizza meal to measure food intake following each treatment. Therefore, although the design may not be representative of normal dietary patterns for young males, it was designed to test the snack bar which was consumed as a snack between two meals (breakfast and lunch), both of which were prepared by the study organizers and controlled for, with less opportunity for confounding variables from intake from previous meals. This may have resulted in more reproducible results for blood glucose, food intake, and desire to eat, which were main objectives of this study. The time of eating the snack, and the inclusion of breakfast, may affect preference for the bar more than the other variables measured, and, although it isn't possible to measure any effect of timing on preference, the similar sensory scores to past sensory studies with this bar suggest that the design did not affect preference.

Motivation to Eat and Subjective Feelings of Wellness

After consuming the treatment, participants conducted a series of four VAS questions used to measure their motivation to eat which resulted in a calculated appetite score. Appetite scores were calculated using the equation described in the methods, and was the same technique

used in similar studies with comparable population groups of young men (106, 121-123). As can be seen in Figure 6, while there was a trend showing a higher appetite score for the water, reflecting a higher desire to eat, compared to the three calorie containing treatments, there was no significant different noted between all four treatments. The similar studies noted had participant numbers ranging from 8 participants, to 16 and above, and the results of the appetite score ratings were variable, with no significant differences in appetite ratings between treatments seen frequently (106, 121-123). It is important to note that, although the participant numbers for this study (n=12) were comparable to others that have used appetite rating, these other studies had differences in treatment composition and study design (106, 121-123). The investigation by Tan et al (2017) had treatment caloric contents of 216-416 kcal, which was higher than the 142 kcal present in the snack bars used in this study, and all treatments were not equal in calorie content (123). The method of treatment delivery also varied, where Anderson et al (2002) gave treatments via a liquid beverage (106). This study was also the only one using a standardized breakfast, while the other two studies utilized an overnight fast followed by the treatment (106, 123). Due to these differences, the comparison of results between studies on appetite rating may not be appropriate, however it is important to note similar non-significant trends in similar population groups and sample sizes.

Further to this, although a significant difference may be expected between the calorie containing treatments (WHT, LPF, and HPF) compared to the WAT treatment, especially at the time points immediately following consumption of the treatments (15min and 30min), the lack of significant results may be due in part to the trends noted earlier. These trends, including atypical consumption patterns for breakfast and snack sizes for young men, may have had an effect on subjective feelings of appetite (120). While not significant, Figures 8-9 for feelings of stomach

pain and nausea show a higher score for the LPF treatment at time 0. The higher S.E.M. of 6.2 and 5.6 at time zero for stomach pain and nausea respectively, both within the LPF, was due to two participants having much higher scores than the rest of the study group at this time point for these treatments. While not part of the study results, and speculative, several participants noted that the early morning, combined with restriction on coffee intake, led to increased feelings of discomfort. This may lend further evidence to the disordered eating pattern of this study design, compared to eating habits of young men. In addition to this, two of the twelve participants regularly experience presyncope during the blood collection procedure, having to lie down during blood collection, which may have also resulted in the increase in these scores at time 0, compared to later time points.

Despite no difference being seen between treatments, a 2-way ANOVA measuring the effect of treatment on time did show a significant effect ($p<0.001$) of time within each treatment. This suggests that the tool was working as intended, as it indicates the appetite score was sensitive enough to detect a change in appetite over the 2-hour period between the treatment consumption and pizza meal.

Not surprisingly, the calorie free WAT treatment resulted in a continuous rise from baseline in motivation to eat score. Conversely, all three calorie containing treatments resulted in a noticeable reduction in desire to eat following their consumption. However, as noted in the results, there were differences seen in the return to baseline of feelings of subjective motivation to eat between the three calorie containing treatments. The return to baseline was much quicker for the HPF treatment (30min), compared to the WHT treatment (45min) and the LPF treatment (60min). While the fiber content of these treatments would not be expected to play a role on satiety in such a short period of time (within 30-60 min), the protein content, which promotes

short-term satiety, may be affecting these outcomes (124, 125). The inclusion of protein in a meal, as well as the type of protein consumed, have been shown to play a role in satiety and food intake (125). However, there is some variability in the understanding of the role of protein on satiety. Effects have been shown with amount of protein as a percentage of a total meal, g/kg of body mass, as well as type and completeness of protein (125). Veldhorst et al. (2008) found that incomplete proteins, or food sources that do not provide all essential amino acids, seemed to induce higher levels of satiety compared to complete proteins, which would be counterintuitive to the results seen here with motivation to eat (126). The inclusion of both lentils and wheat together, each incomplete sources of proteins, are complimentary, and result in a full profile of essential amino acids (127). This would be expected in the LPF treatment, however, contrary to what was seen in Veldhorst et al. (2008), the higher amount of complete protein in the LPF compared to the WHT and HPF treatments did not result in reduced satiety (126). Rather, LPF decreased motivation to eat scores, a positive effect, with a return to baseline delayed to 60 min. Similarly, higher amount of protein tends to increase satiety over lower protein intake, again running counterintuitive to the results shown here, with the HPF treatment having the shortest time of supressing motivation to eat (30min), compared to both the WHT and LPF treatment, despite having the highest proportion of protein (9.3g compared to 5.0g and 7.1g) (126). There is a lack of research assessing processed lentil ingredients specifically, and their role on satiety, however, the consumption of whole lentils has been shown to have a positive impact on satiety within 2 hours (128). Again, this runs counterintuitive to the results seen here, as the HPF treatment, containing the highest proportion of pulse flours per bar, showed the shortest suppression of motivation to eat. The role of protein source and amount, and its effect on satiety and motivation to eat isn't fully understood, however there is some evidence to suggest that

protein may play a role in cholecystokinine (CCK) response, which may also influence satiety (126). While there is a lack of research and consensus on understanding the role of protein on satiety, the protein fraction may have played a role in the differences seen between the suppression of motivation to eat.

While there were no significant differences between any of the 4 treatments on desire to eat across all time points (0,15,30,45,60,75,90,105, and 120min), there was a noticeable effect of the LPF treatment on suppressing the motivation to eat below baseline, beyond the time seen with HPF and WHT treatments. This may suggest some promise in the use of lentil flour in suppressing appetite, meeting another draft health claim on satiety from Health Canada, which requires a 10% reduction of appetite of a test food or ingredient over a reference food (129). The mean VAS score from the motivation to eat questionnaire pooled from each time point over the 2 hour period was 56 ± 4.6 SEM, 52 ± 3.7 SEM, and 59 ± 3.3 SEM for WHT, LPF, and HPF respectively, showing a reduction in motivation to eat of approximately 10% between LPF and WHT, and LPF and HPF treatments. A one-way ANOVA showed no significant differences between the mean values over 2 hours ($p=0.199$). However, using the r-squared value of 0.034 ($r = 0.184$; Cohen's $d = 0.374$), and power of 0.8 with significance set at $p \leq 0.05$ as set out in the draft guidelines for satiety claim, a sample size of 58 participants would be sufficiently powered to assess motivation to eat between the three treatments over a time of 2 hours (129). The results of this study may indicate the opportunity for further investigation into the potential satiating effects of a mixture of lentil and wheat flour, compared to a wheat control.

Blood Glucose Response

One of the main outcomes of interest for this investigation was the potential effect of the snack bar on post-prandial blood glucose response. As the design of this study was set out with

the draft guidance document on functional claims based on post-prandial glycaemia as a guide, normal consumption patterns for snacks, including reference information from CFIA, were used in formulating the treatments (53, 104, 120). The draft guidance document from Health Canada points out that food products aiming to meet a reduction of 20% in post-prandial glycaemia should be tested under normal consumption conditions (53, 130). While the potential limitations in serving size, especially among young men, have already been discussed, it is important to also consider the limitations that serving size places on available carbohydrates. The amount of available carbohydrates for the pulse free control, low pulse flour, and high pulse flour treatments respectively were 28.55g, 26.42g, and 24.29g, as seen in Table 1-2. This is considerably lower than the 50g of available carbohydrates contained in the test meals used as a reference for area under the curve of blood glucose response used to guide the creation of the draft guidance document from Health Canada (53, 130, 131). It was previously noted that the bars used in this study contained between 74% and 77% of the calories in similar bars on the market when standardized to a serving size of 50g, so similar bars could contain up to 10-12g of carbohydrates more per serving, assuming the difference would be made up of only carbohydrates. However, even with the addition of 12g of carbohydrates per treatment, this would still bring the highest available carbohydrates per treatment to 40g, still well below the reference 50g value (131).

Although low glucose amounts have given significant results in detectable changes in blood glucose concentrations, there are many different factors that effect glucose metabolism (111). This may be in part why 50g of carbohydrates is used as a reference for the draft guidelines, as a higher amount of glucose would reduce the noticeable effects on variations in

glucose metabolism of potential confounding factors, but also do to the bulk of research that uses 50g of carbohydrates as a standard (53, 130, 131).

Perhaps not surprisingly, for pooled totalAUC, iAUC, and netAUC over the 120min, there were no significant difference between all treatments, including the caloric free water treatment, as shown in Figures 18-26. A minimum of three data points should be included to calculate reliable AUC values, with intervals of at most 15 minutes according to the draft guidance document on post-prandial glycaemia, so iAUC was also calculated for 60 min (10,20,30, and 60 min included), and 90 min (10,20,30, 60, and 90 min included), with the 0 timepoint used as the baseline value (53, 132). Although 60 min and 90 min are under the recommended 120 min measurement period suggested in the draft guidance document, this method is similar to that used by Anderson et al. (2002) to measure effects of different treatments on post-prandial blood glucose over shorter time periods (53, 106). As can be seen in Figure 24, the only significant results across the three time points was at 60min for netAUC. A post-hoc analysis showed a significant difference between the water treatment and the high pulse flour treatment, however, no other significant differences, or strong trends, were noted. These results may be somewhat misleading, as netAUC, being a subtraction of area under the curve below baseline from area under the curve above baseline, may have a value below zero. In this investigation, blood glucose concentrations continued to fall gradually over time from baseline for the WAT treatment, compared to the WHT, LPF, and HPF treatments, which all showed positive changes above baseline. WAT was the only treatment to show a negative value, suggesting that, over the course of 2 hours, the WHT, LPF, and HPF treatments all had a net positive effect on blood glucose concentrations.

For totalAUC, iAUC, and netAUC at 120, the HPF treatment had the greatest effect on blood glucose levels, with mean values of 72, 53, and 34 respectively. This is compared with the LPF (mean of 67, 41, 15), WHT (mean of 62, 39, and 16) and WAT (mean of 58, 13, and -51) treatments. As can be seen in Figure 17, the WHT treatment resulted in the greatest difference between baseline blood glucose and peak blood glucose concentrations at 20 min of 1.04mmol/L, compared to a change of 0.66mmol/L for the LPF treatment and 0.69mmol/L for the HPF treatment. Despite having the greatest increase in blood glucose concentrations, blood glucose fell much faster for the WHT treatment compared to the LPF and HPF treatments, resulting in a quicker return to baseline, and therefore a much smaller iAUC value. Although not significant, these results do suggest a more prolonged response in blood glucose concentrations with the addition of lentil flour into the treatment formulation. While there is limited research that has been conducted on processed lentil ingredients, lentils have been shown to delay post-prandial response compared to a wheat control (133). The results of this study suggest that there may be a partial retention of this characteristic when lentils are processed into a flour, and baked into a snack bar, even with a minimal inclusion amount of only 12g of lentil flour in the LPF treatment. However, with a low effect size (r-squared=0.015; r=0.122; Cohen's d=0.246) between the caloric treatments, a total of 134 participants would be required to detect significant differences in iAUC over 2 hours ($p \leq 0.05$ and power of 0.8).

While the lack of significant differences may be due in part to a small serving size, it was also noted that three of the participants had results that were not within the normal curves seen among the other nine participants. These abnormal responses to caloric containing treatments were characterized by almost no change in blood glucose after consuming the treatment from

baseline within 30 min, and very little observed change over the 2 hour time period on blood glucose.

Although the reason for the lack of response is unknown, there was a measure of error in the consumption of breakfast that may have been reduced. All participants were asked when entering the lab for each screening session when they had consumed the breakfast, and were not allowed to continue with that session if it had been within the past 2 hours, however, there was no way of verifying the accuracy of this. With the exception of one participant at one session, all other participants reported consuming the standardized breakfast at least 2 hours prior to arriving to the lab.

After consuming a carbohydrate containing meal, post-prandial glycaemic response is a measure of how quickly carbohydrates are digested and enter the bloodstream, and subsequently, the rate at which this glucose is then taken up by cells (33-35). The presence of glucose in the blood activates glucose regulation mechanisms, such as the release of insulin, which can stay in circulation for a period of time after consuming a carbohydrate containing meal (33-35). As insulin may affect the post-prandial glycaemic response, had participants consumed the breakfast within the 2 hour time frame before consuming the treatment, there may have been higher levels of insulin in their blood that would have blunted the post-prandial response after consuming the treatment (33-35). Compounding this, the treatments contained low levels of available carbohydrates, which may have resulted in abnormal blood glucose response curve seen in these few samples. Although the baseline blood glucose level was higher for these participants at the start, it was within the normal cut off range set for participants to be allowed to continue with the treatment session (4.0-6.0mmol/L). This was a limitation for this investigation, as there was no within subject control for baseline blood glucose level, rather just the cut off set at the population

level. With the limited sample size of 12 participants, the results appear to be skewed with a higher baseline for the two treatments containing the lentil flour. While this may have been reduced given a greater sample size, as there were only two participants responsible for the apparent skewed result, it was a limitation to not have as strict controls on confirming protocol was followed for fasting and breakfast consumption and meal time.

As the potential causes of these abnormal data sets may only be speculated at, the results of the original analysis using all 12 participants still point to the overall outcome of the study. Despite the slight changes in differences between mean values using the smaller sample size, the trends are similar. While no significant differences between WHT and the HPF treatments does suggest that consumption of 24g of processed pulse flour doesn't significantly reduce post-prandial glycaemic response over a wheat control, there were clear trends suggesting a delay in post-prandial response with the addition of lentil flour. This was further evidenced by the increased totalAUC, iAUC, and netAUC of the LPF and HPF treatments, versus the WAT and WHT treatments. While not significant, this does suggest that a snack meal containing as little as 12g of lentil flour, with 26g of available carbohydrates, and total caloric content of 142kcal, may have an impact on post-prandial glycaemic response. When considered in context with the other results, the higher protein content of the LPF and HPF treatments may also play a role in suppressing appetite, and the LPF treatment was shown to reduce food intake at a subsequent meal by 167kcal over a lentil free control.

Food Intake

The effect of the treatment on food intake at a subsequent meal was measured by feeding participants a pizza meal at the end of each treatment session. Overall, there was a significant effect of a treatment on food intake in two hours (P=0.049). While Figure 2 shows that a

Tukey's post-hoc analysis revealed no significant differences between treatments, there was a very strong relationship with calories consumed between the WAT and LPF treatments ($p=0.051$). These results follow the trend seen with the results of the motivation to eat scores, as the LPF treatment had the greatest effect on suppressing motivation to eat, compared to the other treatments. This effect seemed to translate into significant differences in pizza intake, when measured by total calories consumed. Although the samples size ($n=12$) may be too small to detect a significant interaction between treatments, the results of the calorie consumption of pizza meals was surprising, when viewed in contrast with the other results (134). While Anderson et al. (2002) detected a significant difference between calories consumed via an *ad libitum* pizza meal following their treatments with a small sample size ($n=8$), the time between participants receiving the treatment was 60 min, as opposed to 120 min used in this investigation (106). This difference is of importance, given the results of the 2-way ANOVA from the desire to eat questionnaire. While this trend continued to the end of the study, perhaps providing the pizza meal after only 60 min would have resulted in more significant results in food intake (106). Further to this, Anderson et al. (2002) fed participants a beverage containing 300kcal after an overnight fast, and, while there may have been some effect due to the texture of the treatment (beverage versus solid food matrix), it is important to note that the snack bar used in this study contained much less energy, at 143kcal per bar, less than half of what was used by Anderson et al (2002) (106, 135). However, as this study was not designed to mimic all normal dietary patterns among young men, and followed a different protocol than Anderson et al (2002), it is difficult to speculate as to the differences seen in this study compared to those of similar designs (106). Using the r-squared value of 0.162 ($r=0.402$; Cohen's $d=0.880$) of caloric intake from this study as the effect size, with a power of 0.8 and significance of $p\leq0.05$, a total of 13 subjects

would be required to see a significant effect on food intake, with a difference of 230kcal, or ~25%, intake seen between the WAT treatment, and the LPF treatment. Similarly, there was a difference of 122kcal or 15% of food intake between the LPF group, and the WHT group, although this was not significant (p=0.497). A total of 21 participants would be needed to see a significant effect between the LPF and WHT group. While a reduction of 122 kcal may seem small, this is the difference of approximately 3500kcal per month, or the energy equivalent of 1 pound. Considering this in context with the increasing obesity levels in Canada, while seemingly a small difference in calorie content, small differences can equate to long term weight gain, contributing to the overall obesity numbers in Canada (19).

It is important to note as well that VAS questionnaires were also conducted to measure feelings of gastrointestinal wellness. There were no significant differences seen between any of the treatments, which would not be expected, as moderate pulse intake is not associated with increased feelings of gastrointestinal discomfort (136). There was a significant difference seen in energy level at 60min, where energy levels were significantly lower for the WAT treatment (mean VAS score of 36), compared to the WHT (mean VAS score of 62) and HPF (mean VAS score of 63) treatments. The lower energy level at 60 min may be due to the effect seen on suppressing motivation to eat as late as 60 min for the caloric treatments compared to the WAT treatment. The lack of food intake with the WAT treatment may explain this difference.

While there was a significant difference in caloric intake between treatments, specifically with those containing calories versus the caloric free control, it is interesting that appetite score did not seem to reflect this trend. As can be seen in Figure 6, appetite score was suppressed at most for 60 min with the LPF treatment, and only 30 min for the WHT and HPF treatments. The lack of significant difference between VAS scores for subjective appetite may be due in part to

the length of the study, but also the small snack size of only 142kcal. This may not have been enough to significantly reduce appetite over a 2 hour period for healthy young males, as indicated by such small differences in final VAS scores between all treatments at time 120min. Conversely, the consumption of any calorie containing treatment reduced intake at the subsequent ad libitum pizza meal, independent of the appetite score.

Pulse Ingredient Selection

The lentil flour used in this investigation was commercially milled from a single crop yield, in a single batch, by Best Cooking Pulses, Winnipeg, MB. The particle size of the commercial flour was similar to one of the particle sizes used in a previous investigation conducted at Mount Saint Vincent University by the same research team. In brief, raw and cooked lentil flours were digested in test tubes using a method that has been shown to mimic the glycaemic response (66). The raw flour samples were added directly to the test tube, while the cooked samples were treated similarly to the cooking method used for the bars in this investigation, controlling for baking time, temperature, and moisture content. Figure 1 shows the results of the raw lentil flour, which demonstrates more starch is available for digestion as the particle size decreases. The same trend was seen in the cooked samples, as demonstrated by Figure 29 below. There was a strong and significant negative correlation for both raw and cooked lentil flours, showing an increase in area under the curve for glucose concentration over time inversely correlated with the particle size of the flour being investigated (raw: $R = -0.925$; $p<0.001$, cooked: $R = -0.91$; $p<0.001$). These results suggest an effect on carbohydrate digestibility based on the particle size distribution of the milled flour. Although there are distinct differences between the starch found in lentils compared to starch found in wheat, an analysis of particle size conducted by CIGI showed that the lentil flour used in this investigation was similar in particle size compared to the all purpose wheat flour used in this investigation, with mean

particle sizes of 94.2µm and 79.9µm respectively. The commercially milled flour sample used to create the bars was screened through a 40 mesh (400nm) screen, and included the whole hull of the lentil flour. Figure 29 below shows that the 40mesh commercially milled lentil flour followed a similar in vitro digestion trend as the course milled lentil flour provided by CIGI. While the starch granule storage molecules in wheat is different than seen in pulses, and while this difference does have a potential impact in the digestibility of the starch from wheat versus pulse sources, there is also an effect on the particle size within pulse flours on digestibility of the starch fraction (64, 88, 137). By standardizing the particle sizes of the wheat and lentil flours, the potential impact of particle size alone on the starch fraction availability for digestion has been controlled for.

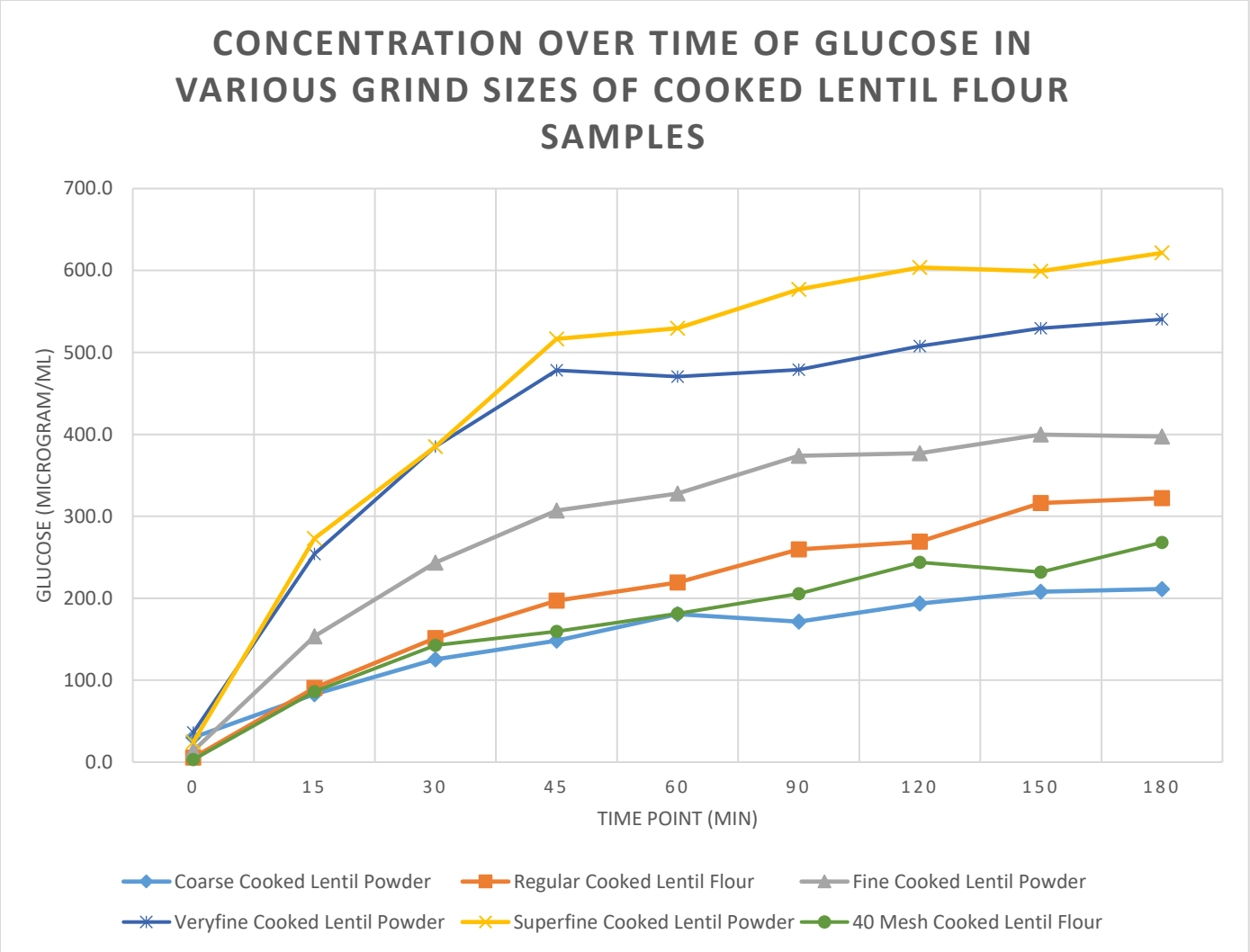


Figure 27 Cooked Lentil Flour Glucose Concentrations over 3 hours of in vitro digestion

Although the manufacturer for the lentil flour was the same for this study, and for the sensory study conducted by Barnett (2015), the average particle size of the pulse flour used in this investigation was reported by the manufacturer to be smaller than the particle size used in the Honours project with Allison Barnett due to improved processing methods and better screening of ingredients. Specifically, the mesh size that the lentil flour used in the investigation by Barnett (2015) was screened through a 20 mesh (841nm) screen, while the lentil flour used in this investigation was screened through a 40 mesh (400nm) screen. Despite this difference, and differences in the recipes between the investigation by Barnett (2015) and the bar used in this investigation, the results for the preference of the bars was similar between the two studies.

Further to this, there is evidence to suggest that increasing the particle size may improve sensory properties of bars, as Borsuk et al. (2012) found that increasing the particle size of lentil flour added to pita breads improved the sensory properties (138). CIGI has found similar acceptability among baked products made with different types and particle sizes of lentil flours, although their results have not been published through peer-reviewed process (139, 140).

In addition to similar sensory acceptability when replacing traditional wheat flours with lentil flours, there are other functional benefits being studied for the use of lentil flour in food product development. For example, Kucukcetin et al. (2012) found adding lentil flour to stirred yoghurt reduced syneresis during shelf storage, which may lead to improved shelf life and consumer perception of yoghurts using lentil flours (141). Similarly, Zhen et al. (2013) found the addition of lentil flour, especially when given a heat treatment, enhanced viscosity and consistency of salad dressings (142). Baugreet et al. (2016) found that the addition of lentil flours to frozen beef patties increased the protein content, while retaining acceptable sensory properties (143). While this is not a comprehensive review of the potential benefits of utilizing

lentil flour in food product development, it does highlight the variability in opportunities and the growing field of interest.

Despite the potential for functional benefits, it is important to note that many investigations, including the examples just given, that utilize lentil flours for functional benefits in food product development quote the health benefits of consuming pulses as rationale. While there are benefits to consuming pulses, and there have been some investigations showing a retention of these benefits when pulses are processed into ingredients, this project was the first that our research team is aware of that specifically sought to determine the dose-response amount of adding pulse ingredients to food products for post-prandial glycaemic control and short term food intake. Although the results were not as strong as predicted, this project does suggest a minimum of 24g of whole laird lentil flour with a mean particle size around 1042.2um does not have an effect on post-prandial glycaemia, when controlling for moisture, baking time, and baking temperature, in snacks containing less than 30g of available carbohydrates. The products reviewed above had a maximum inclusion of 5.25g of lentil flour per serving across all three projects (141-143). Similarly, the methods of processing of the lentil flour, including thermal processing, grinding, and sieving, were not controlled or comparable across these investigations (141-143). It important to point out the inconsistencies from other investigations on controlling for the amount, and processing of, lentil flours, especially in consideration towards potential health benefits.

The recent investigation utilizing an in vitro digestion model of both raw and cooked lentil flours processed to different particle sizes saw a significant effect of processing on the availability of the carbohydrate fraction of the flour for digestion. Building on this, the current project evaluated the effective dose of pulse flours needed to objectively measure an outcome

when controlling for processing method. The results of this study should inform the claims of future investigations using lentil flour, as it builds on the existing literature by demonstrating that both processing method and amount are important considerations when pointing to the health potential of processed pulse ingredients.

Conclusion

The partial or total replacement of all purpose wheat flour with a lentil flour of similar particle size did not negatively affect the sensory acceptability of a snack bar for taste, texture, and pleasantness. Three 50g snack bars were formulated, which contained either 24g of all purpose wheat flour, 12g all purpose wheat flour and 12g lentil flour, or 24g of lentil flours. The consumption of the bars containing lentil flours 2 hours after consuming a standardized breakfast did not lead to a significant difference in post-prandial blood glucose response, subjective feelings of appetite, or short term food intake, over a 2 hour post-prandial study period involving young males. While not significant, there was a noticeable decrease in calories consumed from an *ad libitum* pizza meal after consuming the lentil containing bars of approximately 100kcal compared to the wheat flour control. Similarly, while not significant, the bar formulated with 12g of lentil flour and 12g of wheat flour had an effect on supressing subjective feelings of appetite below baseline for 1 hour, compared to a shorter duration of only 30 minutes with the 100% wheat flour and 100% lentil flour bars. There were no measurable effects on measures of comfort and gastrointestinal wellness over the 2 hour study period after consuming bars formulated with lentil flour compared to bars formulated without lentil flour.

Overall, there was virtually no change in totalAUC, iAUC, and netAUC over 120 min, however, the inclusion of 12g and 24g of lentil flour into the treatments appeared to delay the post-prandial glycaemic response, compared to a lentil free wheat control. Further to this, 12g of lentil flour in combination with wheat flour resulted in a ~10% reduction in subjective feelings of appetite compared to a wheat free control. While not significant, the delay in post-prandial glycaemia, combined with the suppression of appetite, was hypothesized to have an impact on food intake. A significant effect was seen on food intake between the treatment made with 12g

of lentil flour, and a caloric free treatment. Further to this, the treatment formulated with 12g of lentil flour reduced food intake by 122kcal compared to a wheat free control. While the results of this study were not significant due to a low sample size (n=12), the design of this study was very robust in obtaining results that are relevant to normal snack patterns. As was discussed, the protocol of this study was based on reference serving sizes from the CFIA, which resulted in much less available carbohydrates per treatment, 24g – 29g, than used to guide the creation of the draft guidance for post-prandial glycaemic response of 50g (53, 104).

Although there were no statistically significant effects seen between treatments on desire to eat, and post-prandial glycaemia, the results of this study are still important in informing the overall conversation of the use of pulse products in formulated foods. Past investigations have noticed a retention of physiological properties of processed pulse ingredients, and the regular inclusion of pulses into the diet have been shown to improve markers associated with chronic health outcomes (70, 76). However, the effective dose to observe acute impacts of pulse consumption, when controlling for processing method, have, to date, not been investigated. The results of this study suggest that there is an observable effect on blood glucose concentrations after consuming 12-24g of lentil flour, compared to a lentil free control, in a healthy population of men aged 19-30.

Functional food use continues to grow in Canada, and there is a market to explore new ingredients for their functional properties. As Canadians continue to consume convenience processed foods as a major part of their diet, sensory acceptability needs to be considered. This investigation showed no detrimental outcomes in the partial or total replacement of wheat flour with lentil flour. While this study showed no statistically significant benefit of adding lentil flour to a snack bar of 50g on short term food intake, subjective hunger, or post-prandial glycaemic

response, other investigations have shown a benefit on post-prandial glycaemic response when comparing lentil products to other sources of carbohydrates (80, 144).

The results of this investigation may also suggests that the draft guidelines on functional claims based on post-prandial glycaemia may not account the reference amounts and serving sizes of those food products that have less than 50g of available CHO per serving. Future investigations could incorporate pulse flours at the dose used here, with higher doses used, into meals containing at least 50g of available carbohydrates, while also increasing sample size. Canada's guidelines on nutrition labelling are changing, and serving sizes are potentially changing to be more representative of actual consumption (27). If recommended serving sizes do change, it would be beneficial to investigate at what dose of lentil flour produces a detectable change of 20% in post-prandial glycaemia. While this recipe will fit well within the new healthy eating guidelines, and avoid the proposed front of package labelling requirements for products high in salt, fat, and sugar, it is important to note that this recipe was based on a previously developed recipe to reduce the sugar content, and include healthy, local, and affordable ingredients (27). However, this investigation was designed to evaluate the effect of lentil flour as a replacement, and so the results of the total bar recipe were of less import than the effects of the dose of lentil flour.

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Sleep Habits and Stress Factors Questionnaire

The Effect of Pulse Flours on Blood Glucose, Satiety and Food Intake

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?
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:
_____
_____

9. Have you had anything to eat or drink, other than water and provided breakfast, for the past 11-12 hours?
Yes_____ No_____
If yes, please describe briefly:
_____

Recent Food Intake and Activity Questionnaire

The Effect of Pulse Flours on Blood Glucose, Satiety and Food Intake

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:

Arrived to the lab at: _____ Baseline blood glucose _____ (mmol/L)

Treatment started at: _____

Comments/Notes:

_____

Visual Analogue Scale: Motivation to Eat

The Effect of Pulse Flour on Blood Glucose, Satiety and Food Intake

Time point: X MIN

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

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

   VERY weak _____ VERY strong

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

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

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

   NOT full at all _____ VERY full

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

   NOTHING at all _____ A LARGE amount

5. **How thirsty do you feel?**

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

Visual Analogue Scale: Energy and Fatigue

The Effect of Pulse Flour on Blood Glucose, Satiety and Food Intake

Time point: X MIN

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

1. How energetic do you feel right now?

NOT at all _____ VERY energetic

2. How tired do you feel right now?

NOT at all _____ VERY tired

Visual Analogue Scale: Physical Comfort

The Effect of Pulse Flour on Blood Glucose, Satiety and Food Intake

Time point: X MIN

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

1. **Do you feel nauseous?**

   NOT at all _____ VERY much

2. **Does your stomach hurt?**

   NOT at all _____ VERY much

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

   NOT well at all _____ VERY well

4. **Do you feel like you have gas?**

   NOT at all _____ VERY much

5. **Do you feel like you have diarrhea?**

   NOT at all _____ VERY much

Visual Analogue Scale: Treatment Palatability

The Effect of Pulse Flour on Blood Glucose, Satiety and Food Intake

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

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

NOT at all pleasant _____ VERY pleasant

2. How tasty have you found the treatment?

NOT at all tasty _____ VERY tasty

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

NOT at all _____ VERY much

Visual Analogue Scale: Pizza Palatability

The Effect of Pulse Flour on Blood Glucose, Satiety and Food Intake

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 pizza meal?

NOT at all pleasant	_____	VERY pleasant

Appendix 2 – Blood Collection Procedure

Blood Collection Procedure – Nurse:

Upon arriving to the lab, participants will complete baseline questionnaires, and have an IV catheter inserted into their arm for blood collection over the following 2 hours. Blood samples will be collected into 1 discard tube followed by 2 gold topped SST tubes, at 7 different time points: 0 min, 10min, 20min, 30min, 60min, 90min, and 120min. Nurses may work with up to 3 participants per session. **Please note:** Our lab policy allows nurses to attempt an IV up to 3 times per participant, per session.

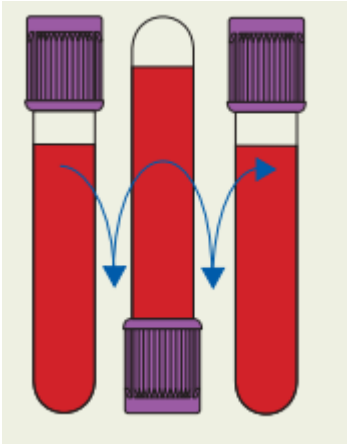
At the start of the clinical session:

1. Initiate the IV Catheter line and take discard tube of blood
2. **DO NOT DISCARD TUBE –** Give prefilled discard tube to blood analysis lab technicians for initial blood glucose reading
3. The participant will then be ready to start the study

Lab technicians will provide the nurse will pre-packaged supplies needed for each participant, along with pre-labelled tubes presented in order. Participants will keep an ID tag with their unique participant ID with them.

For each time point blood sample collection:

1. Ensure the Participant ID tag and time point match the label on the appropriate tubes
2. Flush the IV catheter with the syringe of saline solution
3. Draw enough volume of blood into the discard tube to completely replace any blood and saline solution in the IV catheter, discard. **Please note: the discard tube will be clearly labelled as a discard tube, but may be either red or gold topped!**
4. Fill two 5mL Gold Topped SST Tubes
   a. **It is very important that you ensure each tube is filled completely to ensure the proper ratio of blood to clotting mixture -** the vacuum will draw the correct amount of blood into the tube
   b. After the tube is filled, **invert 5 times**


This is 1 inversion

5. Give pre-filled tubes to the lab technician for processing
6. Make note of any important information in the chart form provided

3 Cheese Pizza

Ingredients:

CRUST: Enriched wheat flour, water, yeast, sugar, soya oil, salt, soy lecithin

CHEESES: Mozzarella, Cheddar, Parmesan Cheeses (milk ingredients, modified milk ingredients, bacterial culture, salt, calcium chloride, microbial enzyme, colour, lipase), corn starch

SIAUCE: Water, tomato paste, sugar, salt, corn starch, spices (contains mustard), garlic powder, wheat flour, natural flavour

Nutrition Facts
Valeur nutritive
Per 1 pizza (82 g) / par 1 pizza (82 g)

Amount Teneur	% Daily Value % valeur quotidienne
Calories / Calories 190	
Fat / Lipides 6 g	**9** %
Saturated / saturés 3 g + Trans / trans 0.2 g	**16** %
Cholesterol / Cholestérol 15 mg	
Sodium / Sodium 430 mg	**18** %
Carbohydrate / Glucides 24 g	**8** %
Fibre / Fibres 1 g	**4** %
Sugars / Sucres 2 g	
Protein / Protéines 9 g	
Vitamin A / Vitamine A	6 %
Vitamin C / Vitamine C	6 %
Calcium / Calcium	15 %
Iron / Fer	10 %

Pepperoni Pizza

Ingredients:

CRUST: Enriched wheat flour, water, yeast, sugar, soya oil, salt, soy lecithin

CHEESES: Mozzarella cheese (milk ingredients, modified milk ingredients, bacterial culture, salt, calcium chloride, microbial enzyme), corn starch

SIAUCE: Water, tomato paste, sugar, salt, corn starch, spices (contains mustard), garlic powder, wheat flour, natural flavour

TOPPINGS: Pepperoni (pork, salt, spices, dextrose, water, onion powder, spice extracts, garlic powder, ascorbic acid, sodium nitrite, bacterial culture, sodium ascorbate, smoke)

Nutrition Facts
Valeur nutritive
Per 1 pizza (87 g) / par 1 pizza (87 g)

Amount Teneur	% Daily Value % valeur quotidienne
Calories / Calories 190	
Fat / Lipides 6 g	**9** %
Saturated / saturés 3 g + Trans / trans 0.2 g	**16** %
Cholesterol / Cholestérol 15 mg	
Sodium / Sodium 490 mg	**20** %
Carbohydrate / Glucides 24 g	**8** %
Fibre / Fibres 1 g	**4** %
Sugars / Sucres 2 g	
Protein / Protéines 9 g	
Vitamin A / Vitamine A	4 %
Vitamin C / Vitamine C	6 %
Calcium / Calcium	10 %
Iron / Fer	10 %

Deluxe Pizza

Ingredients:

CRUST: Enriched wheat flour, water, yeast, sugar, soya oil, salt, soy lecithin
CHEESES: Mozzarella cheese (milk ingredients, modified milk ingredients, bacterial culture, salt, calcium chloride, microbial enzyme), corn starch
SIAUCE: Water, tomato paste, sugar, salt, corn starch, spices (contains mustard), garlic powder, wheat flour, natural flavour
TOPPINGS: Pepperoni (pork, salt, spices, dextrose, water, onion powder, spice extracts, garlic powder, ascorbic acid, sodium nitrite, bacterial culture, sodium ascorbate, smoke), onions, red peppers, green peppers

Appendix 3 – Nutrient Content of Pizza and Breakfast

Nutrition Facts
Valeur nutritive
Per 1 pizza (92 g) / par 1 pizza (92 g)

Amount Teneur	% Daily Value % valeur quotidienne
Calories / Calories 190	
Fat / Lipides 6 g	**9** %
Saturated / saturés 3 g + Trans / trans 0.1 g	**16** %
Cholesterol / Cholestérol 15 mg	
Sodium / Sodium 470 mg	**20** %
Carbohydrate / Glucides 25 g	**8** %
Fibre / Fibres 2 g	**8** %
Sugars / Sucres 2 g	
Protein / Protéines 8 g	
Vitamin A / Vitamine A	15 %
Vitamin C / Vitamine C	20 %
Calcium / Calcium	8 %
Iron / Fer	10 %

Honey Nut Cheerios:

Ingredients:

Whole Grain Oats, Sugar And/Or Golden Sugar, Oat Bran, Corn Starch, Honey, Salt, Refiner'S Syrup, Calcium Carbonate, High Monounsaturated Canola Oil, Trisodium Phosphate, Monoglycerides, Tocopherols, Natural Almond Flavour Vitamins & Minerals: Iron, Folic Acid, Niacinamide (Niacin), Calcium Pantothenate, Pyridoxine Hydrochloride (Vitamin B6).

Nutrition Facts
Valeur nutritive
Per 3/4 cup (29 g)

Amount / Teneur	Cereal Only	Plus 125 mL 2% p.s. Milk
Calories / Calories	110	170
	%Daily Value / % valeur quotidienne	
Fat / Lipides 1.5g *	2%	6%
Saturated / saturés 0.3g + Trans / trans 0g	2%	9%
Cholesterol / Cholestérol 0mg		
Sodium / Sodium 160mg	7%	9%
Carbohydrate / Glucides 23g	8%	10%
Fibre / Fibres 2g	8%	8%
Sugars / Sucres 9g		
Protein / Protéines 2g		
Vitamin A / Vitamine A	0%	6%
Vitamin C / Vitamine C	0%	0%
Calcium / Calcium	10%	25%
Iron / Fer	30%	30%
Vitamin D / Vitamine D	0%	25%
Niacin / Niacine	6%	15%
Vitamin B6 / Vitamine B6	10%	15%
Folate / Folate	8%	10%
Pantothenate / Pantothénate	6%	10%
Phosphorus / Phosphore	8%	20%
Magnesium / Magnésium	10%	20%
Zinc / Zinc	6%	10%

*Amount in cereal

2% Milk

Ingredients:

Partly Skimmed Milk. Vitamin A Palmitate, Vitamin D_3

Nutrition Facts
Valeur nutritive
Per 1 cup (250 mL) / par 1 tasse (250 mL)

Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 130	
Fat / Lipides 5 g	**8** %
Saturated / saturés 3 g + Trans / trans 0.1 g	**16** %
Cholesterol / Cholestérol 20 mg	
Sodium / Sodium 120 mg	**5** %
Potassium / Potassium 400 mg	**11** %
Carbohydrate / Glucides 12 g	**4** %
Fibre / Fibres 0 g	**0** %
Sugars / Sucres 12 g	
Protein / Protéines 9 g	
Vitamin A / Vitamine A	10 %
Vitamin C / Vitamine C	0 %
Calcium / Calcium	30 %
Iron / Fer	0 %
Vitamin D / Vitamine D	45 %

Orange Juice

Ingredients:

Orange Juice

Nutrition Facts
Valeur nutritive
Per 250 mL / par 250 mL

Amount / Teneur	% Daily Value / % valeur quotidienne
Calories / Calories 110	
Fat / Lipides 0 g	**0** %
Potassium / Potassium 470 mg	**13** %
Carbohydrate / Glucides 27 g	**9** %
Sugars / Sucres 23 g	
Protein / Protéines 2 g	
Vitamin C / Vitamine C	**120** %
Calcium / Calcium	**2** %
Folate / Folate	**25** %

Not a significant source of saturated fat, trans fat, cholesterol, sodium, fibre, vitamin A or iron.

Source négligeable de lipides saturés, lipides trans, cholestérol, sodium, fibres, vitamine A et fer.

Appendix 4 – Session Check List

Treatment Session Check List (To be used throughout treatment session):

- ☐ Collect empty milk, orange juice, and bag from cheerios from participant to nurse participant has consumed standard breakfast
- ☐ Administer baseline questionnaires (Sleep Habits and Stress Factors Questionnaire & Recent Food Intake and Activity Questionnaire)
- ☐ Have nurse initiate IV catheter and have lab blood technician check baseline blood glucose – if under 6mmol/L, proceed.
- ☐ Have the nurse draw the baseline blood tubes. Have participant fill out VAS forms for time zero
- ☐ Warm the treatment in the microwave for 35 seconds, and allow to cool for 2 min before providing to the participant. Measure out 250 mL of water to provide to the participant along with the bar, or just the 250mL of water, if it is the caloric free control session
- ☐ Take the participant to the prepared sensory booth and provide them with the treatment and the acceptability VAS scale form. Instruct them to consume the treatment, then consume water, then immediately fill out the VAS form. Start the timer as soon as they take the first bite.
- ☐ Take the participant back to the lab for time 10' blood sample draw, with further draws at 20', 30', 60', 90', and 120' min.
- ☐ Have the participant complete the VAS forms every 15 minutes – DOUBLE CHECK THE TIME POINT, PARTICIPANT ID, AND TX SESSION
- ☐ At 100 minutes, begin baking the pizza for the participant
- ☐ At the end of 2 hours, the IV catheter is removed, and the participant is brought to the sensory booth to consume pizza meal and water. Pre-weigh the pizza and water, and weigh each plate as it comes out. Record on the appropriate sheet for the file to the nearest .01 gram.
- ☐ Replace baked pizza with fresh pizza every 10 minutes
- ☐ Once the pizza meal is finished, take the participant back to the lab to complete the final VAS forms
- ☐ Ensure all forms are labelled and put into the participant file once the participant leaves.
- ☐ Clean up any dishes or mess left behind

Standard Operating Procedures – Stadiometer

Stadiometer: A stadiometer is a piece of medical equipment used to measure height (1).

All measured heights should be documented in centimetres (cm). Please refer to the labeled image on **page 2** when referring to the instructions below.

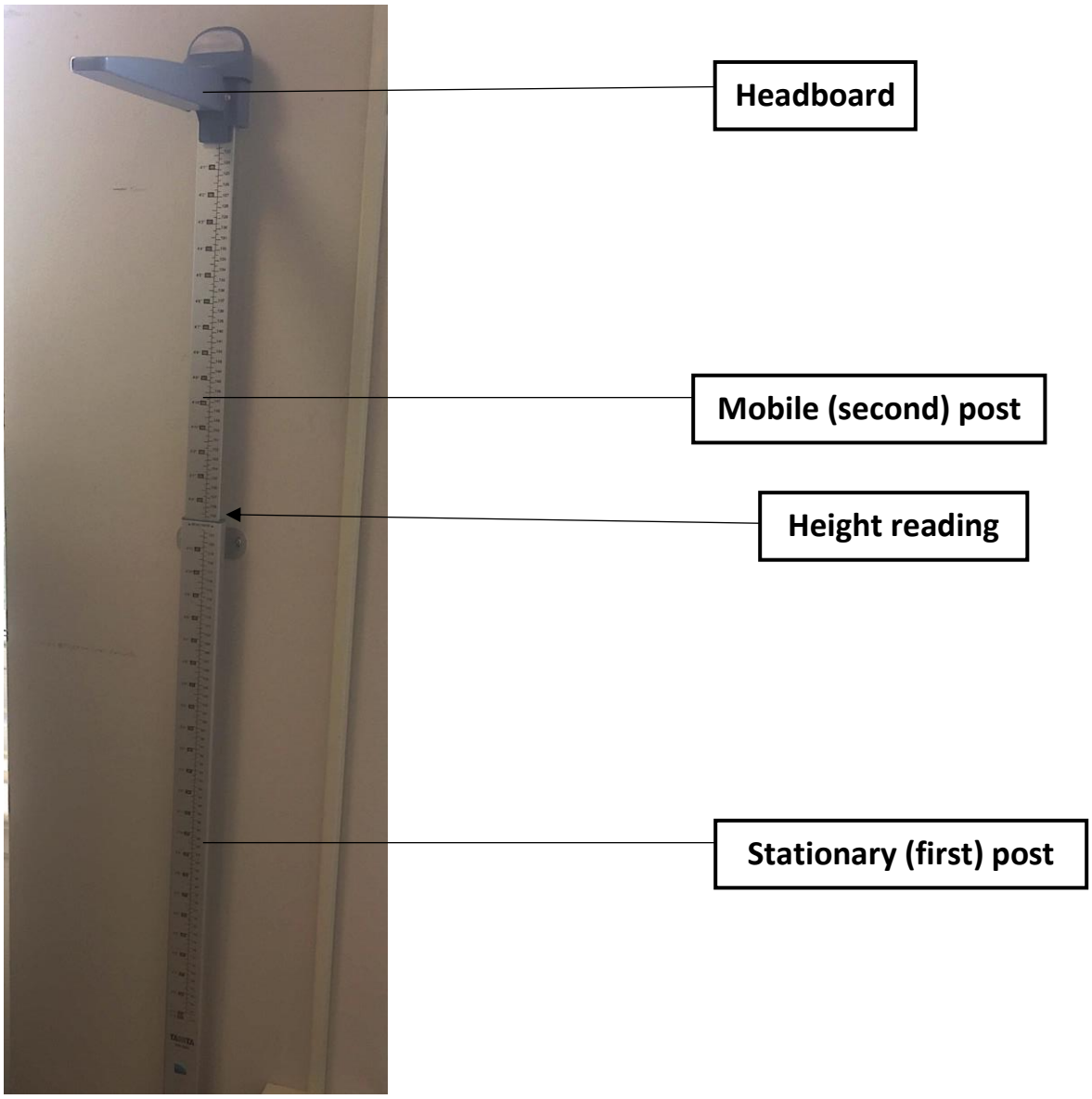
The standard procedures for using the stadiometer can be summarized in four main steps:

1) **Set up the stadiometer**
    a. Ensure that the headboard is at the very top of the post. It should remain at the top of the post at all times during the height measurement.
2) **Have the participant remove their shoes and anything on their head which may lead to an inaccurate height measurement.**
3) **Measure the participant's height.**
    a. Have the participant stand straight against the post with their face pointing straight forward and their chin parallel to the floor.
    b. Slowly lower the post and headboard together ensuring that the headboard remains at the top of the post.
    c. Once the headboard touches the top of their head (not just their hair), stop moving the post. The headboard should appear level at all times, at no time should it appear to be bent upward as this would mean that you have placed too much pressure on the participant's head have taken an inaccurate measurement.
4) **Record height.**
    a. Have the participant step away from the stadiometer and look at the reading on the post. The number you should be looking at is the number above the top portion of the stationary (first) post. This number will be read from the mobile (second) post (refer to **page 2** label "Height reading").
    b. Record the number that you have measured to the nearest 0.5 cm on the participant's **"Baseline Information Form – Part 2"** form and proceed to the "TANITA Body Composition Analyzer". You will need their height when using the "TANITA Body Composition Analyzer".

Appendix 5 – Stadiometer Use

1. stadiometer. (n.d.) *Medical Dictionary*. (2009). Retrieved July 12 2017 from http://medical-dictionary.thefreedictionary.com/stadiometer

STADIOMETER



Standard Operating Procedures – TANITA Body Composition Analysis

The standard procedures for using the "TANITA Body Composition Analyzer" can be summarized in two main steps:

5) Measure the participant's height.
    a. Before taking the participants BMI measurement on the "TANITA" machine, you must first accurately measure their height using the stadiometer. For detailed instructions on how to measure their height please refer to the "Standard Operating Procedures – Stadiometer Use" located beside the filing cabinets on the far wall in the lab.
    b. Record the participant's height in centimetres (cm) as you will need to record this on their "**Baseline Information Form – Part 2"** form and you will also need this number for the "TANITA" machine.

6) Guide the participant to the "TANITA" machine:
    a. Ensure the participant has their shoes off.
    b. Turn on the machine by pressing the power button.
    c. You will see a flashing arrow on the left-hand side of the screen. This arrow is pointing to "CLOTHES." Type in **1.2 for male** participants and **0.8 for female** participants (1).
    d. You will then see three flashing arrows bellow the "CLOTHES" option. Under the "BODY TYPE" section on the key pad, press "STANDARD" under the appropriate "MALE" or "FEMALE" subsection.
    e. You will now see a flashing arrow pointing at "AGE." Key in the participant's age in years.
    f. You will now see a flashing arrow pointing at "CM." Key in the participant's height in cm that you measured in step 1.
    g. You will then see "GOAL" flashing on the screen. Press 8 to bypass this step.
    h. You will now see that the flashing arrow is pointing at "STEP ON." Ensuring that the participant's shoes are off, direct them to step on the scale. Ensure they are not touching anything with their hands or any other body part.
    i. After a few seconds, a printout will be generated by the "TANITA" machine. Take this print out and use the data for weight (kg) and BMI and record it on their "**Baseline Information Form – Part 2"** form, before stapling it to this form.

1. Whigham LD, Schoeller DA, Johnson LK, Atkinson RL. Effect of clothing weight on body weight. Int J Obes. 2013;37(1):160–1.

Standard Operating Procedures – Telephone Use and Screening Questionnaire

During the recruitment phase of the clinical trial, we will be pre-screening potential participants by administering a telephone questionnaire. As the telephone is our first point of contact for many of the potential participants, it is important to gather the right information from them.

The standard procedures for telephone use encompass the following 4 main steps:

1) Upon arriving to the lab, ensure that the telephone is checked for any messages
    a. Record the information from the message in the log form, found in **page 4** of this booklet. **Please fill this out even if you immediately return the call.**
    b. If you are able, return the phone call for any potential participants and conduct the questionnaire, or leave a message.
        i. **Standard Message:**

    "Hello, this is (your name) returning your call from the Appetite Lab at MSVU. Thank you for your interest in participating in our study. Please call us back at your earliest convenience at (902) 457-6378. Once again, that number is 902-457-6378. Alternatively, feel free to contact us via email, at Appetite (dot) Study (at) MSVU (dot) ca. We look forward to connecting with you soon."

2) When the phone rings, or upon returning a call from a previous message:
    a. Fill out the information as you are talking to the potential participant found on **page 2** of this booklet.
    b. Use the below script to guide your conversation:
        i. **Standard Script:**

    "Hello, this is (your name). Thank you for your interest in participating in our study. In order to ensure that you are eligible to participate in the study, would you be able to complete a brief questionnaire with me over the phone, taking no more than 5 minutes?" (Yes) – If no, ask if there is a more convenient time to phone back, and put in **page 2**

    "Thank you. Before we begin, I want to outline what the study will entail. We will begin with this telephone screening questionnaire, followed by booking an in person screening session. During this session, we will explain the details about how you may participate in the study, as well as ask you to complete some questionnaires, forms, and body measurements.
    After this, we will schedule 4 separate morning sessions from 9:30am to 12:30pm, at least 1 week apart from each other. You will be asked to come to the lab, consume our test food product, and have blood samples taken over a 2 hour period. At the end of this time, you will be provided with a pizza meal. We will also provide you a breakfast of cheerios, milk, and juice, to consume the morning of the session, as well. If you are interested in participating, we will begin the phone questionnaire."
3) Complete the telephone screening questionnaire, found at the back of this booklet. Be sure to label it correctly, and after completing the phone call, begin a file for the participant, placing this form inside of it.
4) Book a time for an in-person screening session. Use the standardized script below as a guide. Fill in the information on **page 3** of this booklet.
        i. **Standardized Script**

Appendix 7 – Telephone Pre-screening Questionnaire and Script

"Thank you for completing the phone questionnaire. As previously mentioned, the last step is to book a convenient time for you to come in for an in person screening session. Is there a day and time in the near future that work well for you? " (Their response)

"Great, so I have you booked for (Date). We will touch base with you the day before to make sure this time is still convenient for you. Would you like us to also send you an e-mail reminder? (take email, and put into **page 3** of this booklet)"

The Effect of Pulse Flours on Blood Glucose, Satiety and Food Intake

Please print or circle the answer

Age _____

Height: _____ cm Weight: _____ kg

Have you lost or gained weight recently? Yes / No

Do you follow a special diet? Yes / No

Do you have any food allergies or food sensitivities? Yes / No

(If yes please explain: _____)

Health Problems? Yes / No (If yes please explain: _____

_____)

Medications?: Yes / No (If yes please explain: _____

_____)

Do you have any learning difficulties/problems? Yes / No

(If yes please explain: _____)

Do you have any behavioral or emotional problems? Yes / No
(If yes please explain:_____ _____)

Include in study? Yes / No

Appointment scheduled for: (date and time)

_____

Investigator/Date screened: