INVESTIGATING THE POSTPRANDIAL GLYCEMIC RESPONSE TO WILD RICE CAKES USING A RANDOMIZED CROSSOVER CONTROLLED TRIAL

BY

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ABSTRACT

Background: Diabetes is a leading cause of mortality worldwide, with a prevalence of ~11% in Canada, linked to poor diets and an increasingly sedentary lifestyle. Therefore, designing novel ready-to-eat foods that could manage blood glucose (BG) concentrations is of interest. Wild rice (WR), a traditional staple food for many Indigenous people in North America, contains higher levels of antioxidants, fibre, and protein, and lower fat and calorie content compared to white or brown rice, which may contribute to its satiety and potential for regulating BG. However, no study has investigated the effects of WR cakes on post-prandial BG and satiety responses in humans.

Design: In a crossover trial, 18 healthy adults (9 males, 9 females) consumed test products. BG was measured at 0-120 min post-consumption. A dosing error in the white bread control led to a non-randomized fourth period with corrected carbohydrate matching (30 g). Test products included: 32 g white bread (15 g carb), 40 g BR cakes (100% BR), 40 g WR blend (25% WR, 75% BR), and 66 g white bread (30 g carb). All were consumed with 250 ml of water. Appetite and palatability were rated via visual analog scales.

Results: Effects of time, treatment, and time-by-treatment interactions on blood glucose over 120 minutes ($p \leq 0.05$) were observed for both the analysis with the white bread at 32 g and 66g. No differences in BG response were observed between the rice cakes ($p > 0.05$). White bread consumption (32 g) resulted in lower BG at 30, 45, 60, and 90 minutes, as well as a lower blood glucose iAUC and appetite compared to the rice cakes ($p < 0.05$). White bread consumption (66g) resulted in lower BG at 30 minutes only, and there were no differences in glucose iAUC, appetite or palatability between the treatments ($p > 0.05$). The blood glucose iAUC after bread was very

linear in terms of carbohydrate content with the 32 g serving having approximately half the iAUC of the 66 g serving.

Conclusion: Inclusion of 25% wild rice into a brown rice cake did not meaningfully impact blood glucose response.

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LIST OF ABBREVIATION

Abbreviation	Term
BR	Brown rice
CD	City diet
CGM	Continuous glucose monitoring
FBG	Fasting blood glucose
GI	Glycemic index
HBA1C	Haemoglobin A1c
HFC	High fat and cholesterol diet
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
Iauc	Incremental area under the curve
TFC	Total flavonoid content
TPC	Total phenolic content
VAS	Visual analog scale
WhR	White rice
WR	Wild rice

CHAPTER 1: INTRODUCTION

Diabetes mellitus is a metabolic chronic condition. Worldwide prevalence of diabetes, according to the International Diabetes Federation, is approximately 451 million people aged 18 to 99 years. This review was carried out in 2017 and it projected that by 2045 this number would rise to 693 million [1]. Diabetes (type 1 and type 2) is increasing in Canada. In 2017-2018, this metabolic condition affected approximately 3.4 million Canadians (8.1%) compared to 1.3 million Canadians (4.7%) in 2000-2001, with a 3.3% yearly mean increase in prevalence over that time [2]. Diabetes is a condition when blood glucose concentrations are consistently high (hyperglycemia), which results from defects in the secretion and/or the action of insulin, which affects macronutrient metabolism such as fats, protein, and carbohydrates [1]. Type 1 diabetes is an autoimmune disorder. It occurs when there is insufficient production of the hormone insulin which is needed for blood glucose regulation, due to the pancreatic beta cells being destroyed [4]. Approximately 5-10% of people living with diabetes have type 1 diabetes [3]. Type 2 diabetes is elevated blood glucose concentration due to insulin resistance. Insulin resistance is the body's resistance to the action of insulin, causing impaired blood glucose control [4], which may be influenced by lifestyle factors [5]. Approximately 90 -95% of people with diabetes live with type 2 diabetes [6].

The increasing prevalence of type 2 diabetes and its complications in Canada could potentially be linked to the increased consumption of ultra-processed foods and ready-to-eat foods, [7] and an increasingly sedentary lifestyle. Having elevated blood glucose concentrations due to diabetes can lead to various health complications, which include retinopathy, neuropathy, and nephropathy [8]. Complications of diabetes can lead to premature death. Therefore, people living with diabetes and

their caregivers often pay more attention to how food can influence glycemic control [9-11]. As people become more concerned with the health impact of the food they consume; more attention is paid to designing novel food products that have defined health benefits [12]. Research on rat and mouse models suggests that wild rice, a cereal grain, offers numerous potential health benefits. Evidence indicates that incorporating wild rice into the diet of animal models may result in decreased insulin resistance, atherosclerosis, and lipotoxicity, as well as reduced inflammation, allergies, and blood pressure [13-15]. These findings suggest wild rice may be a health-promoting food. When compared to white rice, wild rice's nutrient composition is superior, in terms of higher quality of protein, greater antioxidants, and fiber, as well as increased vitamin contents (riboflavin and thiamine). Consuming wild rice shows promise in reducing fasting blood glucose concentrations in rats and mouse models [14, 16]. Although a study by Moghadasian et. al [17] reported a 60% increase in fasting blood glucose concentrations after administering wild rice powder to rats.

The glycemic index (GI) of Asian wild rice *(Zizania latifolia)* has been estimated at 53.72, which is considered a low-GI food [18]. It has been recommended that the consumption of foods with low GI could help to improve blood glucose variability by lowering postprandial glycemia [11]. The postprandial glycemic response is used to describe blood glucose concentrations measured after an individual has consumed a meal [19]. Some of the interest in the potential of wild rice to reduce postprandial blood glucose is due to its high dietary fiber content (3.3 % and 0.8% insoluble fiber and soluble fiber by weight respectively) [20]. Soluble fiber in particular has been shown to help prevent rapid spikes in blood glucose after the consumption of a meal [21]. It is known for its viscosity and gelling properties, associated with its capability to dissolve in water, which helps to

slow down digestion and food absorption. This can lead to stable blood glucose because glucose release into the bloodstream is slower [21]. Additionally, dietary fiber has been linked to feeling full and satisfied, which may result in consuming less food during a meal and potentially lead to a lower glycemic response because the total amount of carbohydrates consumed is reduced [22, 23]. Therefore, there is a potential for wild rice to enhance satiety. Satiety is the sensation of being full after eating which stops you from eating more [24]. Satiety can help control appetite by sending signals to the brain after eating enough food. Very little research has been done on how wild rice or wild rice food products affects blood glucose concentrations or satiety in people. To our knowledge there are no studies that have explored how the incorporation of wild rice into rice cakes impact postprandial blood glucose concentration, appetite and the palatability of rice cakes. This study aims to fill these existing research gaps by examining how wild rice cakes affect postprandial blood glucose, palatability, and appetite in a randomized controlled trial.

CHAPTER 2: LITERATURE REVIEW

2.0 INTRODUCTION

This literature review provides an in-depth review of wild rice and brown rice, focusing on their nutritional composition and their effects on postprandial blood glucose response, fasting blood glucose, and satiety. By exploring existing research, the review aims to understand their responses associated with the consumption of these types of rice and their potential implications for managing blood glucose and promoting satiety.

2.1 Blood glucose, postprandial blood glucose, glycemic response, glycemic index, and glycemic load

Blood glucose is defined as the glucose concentration present in the bloodstream [25]. Carbohydrates play the most significant role in blood glucose concentrations, as they are broken down into glucose after ingestion. While other macronutrients such as protein and fats also influence blood glucose concentrations indirectly, the ingestion of food high in protein is associated with increased satiety, potentially reducing calorie intake [26]. Also, dietary fats can slow down gastric emptying and delay the absorption of glucose after food ingestion, which can help manage postprandial blood glucose concentrations [27, 28]. Blood glucose is regulated by various mechanisms and hormones, the key ones being insulin, which promotes glucose uptake by cells, and glucagon which prompts glucose release into the bloodstream when glucose concentrations are low [29, 30]. Postprandial glycemic response is defined as blood glucose measured after meal consumption, this shows a change in blood concentration, after consuming food. This change may be affected by the type of carbohydrates, food format, the amount of food consumed, and the nutrients contained [31].

Glycemic index (GI) is a scale that estimates the effects of food or beverages that contain carbohydrates on blood glucose after consumption [32]. GI is calculated by measuring the incremental area under the curve (iAUC) for glucose which can be calculated from the blood glucose sampled at baseline (0 min) and various time points (e.g. 15, 30, 45, 60, 90, and 120 minutes) over the period following the test food consumption [47]. GI is determined by measuring the iAUC of about 10 human participants who have fasted for a period of 12 hours. The participants consume a test food containing 50 g of available carbohydrates. The blood glucose of the participants is taken and monitored over 2 hours at regular intervals. From this, the iAUC is calculated. A reference food typically glucose or white bread, which also contains the same amount of available carbohydrates is used in a repeated process. The GI is then calculated using the formula: *"GI = iAUC of test food divided by iAUC for reference food multiplied by 100"*. The GI of the food is then ranked and compared on how they affect blood glucose concentrations [33]. Research has observed that consuming foods with a high GI cause faster larger spikes in blood glucose concentrations, compared to a slower and lower increases associated with lower GI foods [34]. It is thought that consuming food with a lower GI can lead to a reduction of approximately 30% in both glucose and insulin concentrations compared to a high GI food [35]. Low GI foods have a GI value lower than 55, while foods with a medium GI fall within the range of 56-69, and foods classified as high GI have a value of 70 or more [36]. Glycemic load (GL) considers both carbohydrates quantity and quality in a food. (GL = GI x dietary carbohydrate content/100g), it effectively reflects how the food affects blood glucose concentrations [37]. It has a scale that resembles that of GI, < 10 = low GL, > 20 = high GL. [38] Foods that have low GI and GL have

been recommended to reduce the risk of diseases such as diabetes, obesity, and cardiovascular diseases [39, 40].

2.2 How to measure postprandial blood glucose

Blood glucose concentrations are influenced by different factors, such as the amount of food consumed, the consumption time, the composition of the meal (type and amount of carbohydrates consumed), and other parameters [41]. It is recommended to test blood samples in the morning because they have been found to exhibit greater stability compared to samples collected in the evening [41]. There are various ways to measure postprandial blood glucose response in humans, which include finger stick blood collection and a glucometer or continuous glucose monitoring (CGM) which use subcutaneous calibrated sensors [42]. CGM is a device that is used to measure real time glucose concentrations using a electrochemical sensor electrode inserted under the skin, it is connected to a transmitter which sends glucose information to a detector like a smartphone or any compatible device, which can be used to monitor blood glucose continuously [43]. However, the use of a glucometer to measure blood glucose is the most common method [44]. Glucometers measure glucose using the glucose oxidase methodology, where glucose oxidase, an enzyme catalyst, causes the gradual hydrolysis of glucose, resulting in gluconic acid and hydrogen peroxide. An electrochemical detection system measures the generated electric current, correlating it with glucose concentration and the glucometer displays a digital glucose reading based on this measurement [45]. Blood samples are most often collected using the “finger stick” method where an individual's fingers are punctured with a lancet, and blood is collected on the meter test strip. The meter detects the reaction between the test strip and blood and displays glucose concentrations in mg/dL or mmol/L units [46]. The use of a glucometer has advantages such as cost-effectiveness,

ease of use, and quick results. Before measuring postprandial blood glucose, participants are recommended to fast overnight which could range from ten to twelve hours, so that fasting blood glucose can be taken at the start of the test (0 min) and participants can consume a test food and iAUC can be measured.

2.3 Glucose metabolism and satiety regulation

Glucose is the body's main energy source; it plays a vital role in regulating hunger and feelings of fullness through hormonal interactions [48]. Satiety is the feeling of fullness after eating, which stops you from eating more, this includes a decrease in appetite [24]. Insulin, a peptide hormone synthesized and released by the pancreas's beta cells, primarily manages blood glucose concentrations [49]. Insulin has an anorexigenic effect, having the ability to reduce appetite and induce satiety sensation. This effect plays a central role in promoting a sense of fullness after food consumption and curbing food intake [50, 51]. Additionally, insulin lowers blood glucose concentrations by facilitating glucose absorption into cells [52]. Nutrients, especially dietary fiber potentially influence satiety by modifying insulin and blood glucose response [53].

There are various other hormones related to glucose metabolism and satiety including ghrelin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide tyrosine [PYY] [54]. These hormones help control how our digestive system functions, regulate our appetite and food intake, and manage blood glucose concentrations after eating. They interact with receptors in the gastrointestinal tract and brain, giving fullness sensations [55]. Several hormones like ghrelin, CCK, GLP-1, and PYY regulate appetite, digestion, and post-meal blood glucose by signaling between the gut and brain [56]. They are classified into two distinct types "orexigenic" and

"anorexigenic". Orexigenic hormones are the hormones that trigger appetite which in turn increases food consumption, such as ghrelin [57]. Ghrelin is also known as the “hunger hormone”, it is produced in the stomach and stimulates food intake through various mechanisms [58]. It promotes the desire to eat and stimulates appetite, leading to increased food intake, when the hormone ghrelin levels are high, it might be associated with obesity due to increased food consumption [59-61]. Although research shows that ghrelin can affect blood glucose regulation, low levels of ghrelin are linked with high insulin levels which could lead to improved blood glucose regulation [62, 63]. Anorexigenic hormones are the opposite of orexigenic, they help to reduce food consumption by reducing appetite by increasing fullness sensation, and aid in inhibiting appetite such as peptide YY, cholecystokinin, and glucagon-like-petide-1. They work hand in hand to manage weight and regulate energy balance [64]. Research has shown that administering PYY, CCK, and GLP-1 in both humans and rodents help suppress appetite and promote satiety, which could be beneficial in preventing obesity and some chronic diseases [65, 66]. Cholecystokinin is a peptide hormone that plays an important role in digestion, although it does not have direct effect on blood glucose regulation, it helps in the digestion and absorption of nutrients and could stimulate the release of insulin [67-68]. Gastric emptying is associated with blood glucose regulation because slower gastric emptying leads to a more gradual release of nutrients into the small intestine which prevents rapid increase of blood glucose concentrations [70].

2.3 How to measure palatability and appetite

Visual analog scales (VAS) are used to measure participants' appetite and palatability before and after food consumption. They are scales that are often 100 mm in length and are labeled,

participants select a point on the scale to express their feelings after consuming a meal. Such as from “not hungry at all” to “extremely hungry” in the case of appetite. The scale can be adjusted to various directions based on their appetite and palatability [71]. The use of this scale has been reproduced and validated in both adults and children [72, 73].

2.4 Wild Rice: History and Habitat

Wild rice (*Zizania* sp.) also called manoomin in Ojibwe, which means “the good berry”, as well as Canadian rice, water oats, and Indian rice [74-75]. There are four primary species: *Zizania palutris, Zizania aquatica, Zizania texana*, *and Zizania latifolia,* while *Zizania latifolia* is native to Asia, the other three varieties can be found within the region of North America [75]. Wild rice species are grass indigenous to North America and have been widely cultivated and consumed by Indigenous People in the region [13]. It thrives in isolated lakes and slow-moving streams, spanning a geographical range from southern Canada to the Rocky Mountains, and the Gulf of Mexico [76]. For several centuries, the Indigenous Peoples within the area now called the United States and Canada have been involved in the practice of cultivating and harvesting wild rice [77]. It is currently in the consumer marketplace as a delicacy, owing to its unique flavor, color, texture, and taste [78]. Wild rice is mostly cultivated in North America, India, China, and specific parts of Nigeria, particularly in the northern regions [79]. According to Xu et al., wild rice might have originated in North America, before being distributed to eastern Asia via the Bering land bridge [80]. In North America, there are three species of wild rice indigenous to this area which includes (*Z. aquatica, Z. palustris, and Z. texana),* while there is another species *(Z. latifolia)* peculiar to eastern Asia [20]. Chinese wild rice *(Z. latifolia)* has been shown to have a low-GI and is a source of resistant starches [18]. Resistant starches are a type of carbohydrate that shows resistance to

digestion in the small intestine, allowing them to remain intact until they reach the large intestine [81]. Once in the large intestine, they undergo fermentation by the resident gut bacteria, which leads to short-chain fatty acid synthesis, sensitivity, increased satiation, and reduced postprandial blood glucose. They slow down the carbohydrate digestion in the meal, resulting in a slower and gradual release of glucose into the bloodstream. Resistant starches can help prevent rapid increase in blood glucose and reduce the possibility of developing type 2 diabetes [81-83]. As shown in (Table 1)**,** the nutrient composition of wild rice is reported.

Table 1 Reported Nutrient Composition of Wild Rice [84-86]

Nutrient Components	Concentration
Total carbohydrate	72.3-75.3%
Protein	10-18%
Albumins	10%
Globulins	10%
Prolamins	1%
Glutelin	79%
Fat/lipid	0.5-0.8%
Ash	1.2-1.4%
Moisture	7.9-11.2%
Crude fiber	0.6-1.1%
Starch	69.3%
Rapidly digestible starch	60.1%
Slowly digestible starch	4.0%
Resistant starch	5.2%
Thiamine (vitamin B_1)	0.30–0.63 mg/100 g
Riboflavin (vitamin B_2)	0.07–0.60 mg/100 g
Niacin (vitamin B_3)	4.60–10.3 mg/100 g
Tocopherols (vitamin E)	0.20–0.50 mg/100 g

According to Qiu et al., [87] wild rice contains a significantly higher concentration of phenolic compounds and exhibits antioxidant activity approximately ten times greater than that of white rice. The researchers focused on analyzing the antioxidants, specifically the total phenolic content (TPC). The results show that wild rice had higher TPC levels (419-588 mg GAE/kg) compared to white rice, which only contained 46 mg GAE/kg. Since wild rice likely has a lower GI, higher fiber and protein content as well higher in resistant starch, relative to white rice, it has the potential to be used to develop food products with low GL which could be beneficial for people in the management of their postprandial blood glucose response.

2.5 Wild rice: Glycemic response

A literature search of google scholar, Web of Science, UM library and PubMed was conducted, and studies focused on the effect of wild rice on glycemic response in both rats and humans [14, 16, 17]. In 2013, researchers in China conducted a study using rats to investigate the impact of substituting dietary carbohydrates with wild rice on insulin resistance [16]. The male Sprague-Dawley rats aged ten weeks were administered a diet rich in fat and cholesterol for the study. After an acclimatization period, the rats were randomly allocated to four groups of ten random. The groups were fed a city diet, a wild rice diet, a high fat/cholesterol diet (model), and a low-fat diet serving as the negative control, respectively. The city diet in this study was a diet high in cholesterol and fat and replicated the diet of the people living in modern Asian societies. Throughout 8 weeks, the rats were provided with diets based on their assigned groups, while their body weight and food consumption were consistently observed and documented. After analysis of the data collected, it showed that rats fed with a wild rice diet had a notable reduction in fasting blood glucose. Homeostatic model assessment for insulin resistance (HOMA-IR) index showed

that the wild rice diet had positive effects on glucose tolerance and insulin sensitivity. Findings from the studies suggest that wild rice could potentially be a good dietary option in the prevention of diet-related chronic diseases [16]. In 2015, Zhang and colleagues measured the GI of Chinese wild rice *(Z. latifolia)* and assessed its impact on blood glucose concentrations [14]. Eight healthy adults were administered sixty-seven grams of wild rice and fifty grams of glucose as a control. The Jenkins and Wolever formula were used to calculate a GI of 53.72 for the Chinese wild rice. Additionally, fifty male Sprague-Dawley rats were randomly allocated to diet groups including a negative control, an insulin resistance (IR) model, a high-dose wild rice, a low-dose wild rice, and white rice flour. It was concluded that Chinese wild rice can improve insulin resistance caused by a high-fat diet in rats [14]. Lastly, in contrast to the study above, a study carried out in low-density lipoprotein (LDL) receptor knockout rats concluded that long-term consumption (twenty-four weeks) of wild rice (*Z. palustris)* powder (60%) within an atherogenic diet led to a 60% rise in fasting glucose compared to the control group which was an atherogenic diet without the wild rice powder. The atherogenic rat diet consists of 9% fat which was supplemented with 0.06% cholesterol which made the diet atherogenic. Fasting blood glucose was collected from the jugular vein under anesthesia and reasons for the increase in blood glucose were not given [17]. The studies investigated the effects of wild rice on glucose metabolism, two of the studies found that wild rice improved sensitivity to insulin and reduced fasting blood glucose in rats and had a moderate GI in humans [16, 88]. However, one study reported that long-term consumption of wild rice within an atherogenic diet unexpectedly increased fasting glucose in LDL receptor knockout rats [17] (Table 2)

Table 2. Tabulated Literature review on glycemic response to wild rice [14, 16, 17]

Title	"Effects of Dietary Carbohydrate Replaced with Wild Rice (Zizania latifolia (Griseb) Turcz) on Insulin Resistance in Rats Fed with a High-Fat/Cholesterol Diet."	"Determining glycemic index of wild rice and effects on insulin resistance in rats".	"Anti-Atherosclerotic Properties of Wild Rice in Low-Density Lipoprotein Receptor Knockout Mice: The Gut Microbiome, Cytokines, and Metabolomics Study"
Author, Journal year, and references	*Han et al., 2013* [16]	*Zhang et al., 2015* [14]	Moghadasian et al.,2019 [17]
Objectives	To improve resistance to insulin in rats fed with a high-fat or high-cholesterol diet (HFC)	To determine the glycemic index of wild rice and its effects on insulin resistance after inducing rats with a high-fat diet.	Anti-atherosclerotic properties of Wild Rice
Intervention	Wild rice diet	Human: 67g wild rice Rats – 1) IR model group, 2) high-dose of wild rice group, 3) low-dose of wild rice group, 4) white rice-flour group	60% wild rice powder plus an atherogenic diet
Comparator	Low fat diet on the AIN-76A formulation HFC City diet	50 g glucose -humans negative control group-rats	Atherogenic diet, no wild rice powder
Study design and Participants	40 ten-week-old male SD rats fed for 8 weeks	8 adults 50 Sprague-Dawley rats fed for 8 weeks	16 males, 4-week-old LDL-r-KO mice
Outcome	Chinese wild rice prevents insulin resistance and decreases chronic metabolic syndrome in wild rice-fed rats.	Asian wild rice has a low glycemic index of 53.72. It may improve insulin resistance in rats.	Anti-atherogenic property was seen in the wild rice fed mice (higher interleukin-10 and erythropoietin in the plasma) but a 60% increase in the plasma glucose level was observed

2.6 Brown Rice: Nutrients and chemical composition

Brown rice is a type of unpolished whole grain, which is often developed by removing the outermost layer of the seed, also known as the husk or hull. Since it's only the outermost layer of the rice that is removed, the rice retains most of the nutrients which is beneficial compared to white rice that has both its bran and hull removed [89]. Brown rice includes its germ and bran layers, responsible for the brown color and nutty flavor characteristics [90]. There are numerous nutritional and bio-functional properties such as dietary fiber, vitamins, and y-oryzanol contained in brown rice because of the bran and germ present on the rice [91] (Table 3)

Table 3 Nutrients and chemical properties of brown rice per 100g [89]

Nutrients components	Concentration
Protein	4.88g
Carbohydrates	49.7g
Fat	1.17g
Dietary Fiber	3.32 g
Thiamin (B1)	0.223mg
Riboflavin (B2)	0.039mg
Vitamin B6	0.294mg
Niacin B3	2.730mg
Folacin	10mcg
Vitamin E	1.4mg
Phosphorous	142mg
Selenium	26mg
Potassium	137mg
Zinc	1.05mg
Magnesium	72.2mg

2.7.1 Brown Rice: Bio-active components and glycemic response.

Brown rice has bioactive compounds that may contribute to the nutritional value of this rice, such as dietary fiber. The consumption of foods with high dietary fiber has been linked with lowering blood glucose and cholesterol concentrations, and the prevention of gastrointestinal and heart diseases. Brown rice bran contains about 1.4-3.3% dietary fiber [92]. Foods with high dietary fiber have been shown to have reduced postprandial glycemic response and to be linked to a decreased likelihood of developing type 2 diabetes [93]. This link is thought to be due to increased viscosity of contents in the small intestine, reduction, and slowing down of glucose diffusion, and inhibition of a-amylase activity. Brown rice exhibits greater antioxidant properties, including phenolic compounds, either soluble or insoluble in comparison to white rice [51]. The most important types of soluble phenolic compounds it contains are "6′-O-(E)-Feruloyl sucrose (1.09 mg/100 g of flour) and 6′-O-(E)-sinapoylsucrose (0.41 mg/ 100 g of flour)" [94]. Some phenolic compounds are associated with antidiabetic properties, which might regulate blood sugar by improving insulin sensitivity and other mechanisms [95]. Research shows that in comparison to white rice, brown rice can help lower blood glucose which could be due to abundant dietary fibers and polyphenols. [96]. Aside from its antidiabetic properties, brown rice contains other phytochemicals and bioactive components that are beneficial to humans which might help reduce cholesterol levels, promote weight loss, prevent gallstones, inhibit mineral levels absorption, and prevent cancer [90].

2.7.2 Brown Rice: A literature review on the effect on glycemic response

In a clinical trial from 2014, 15 participants were overweight and had a body mass index greater than 23kg/m^2 [97]. They were fed the same meals for five days in a row, with different rice types

and legumes (50 g/day), their blood glucose responses were measured, and fasting insulin concentrations were taken before and at the end phase of each meal. The trial results showed that brown rice consumption, instead of white rice, lowered blood glucose response and concentrations of fasting serum insulin in people who were overweight [97]. In another study in 2006 carried out in vitro, and in humans, brown rice had a 23.7% reduction in the amount of blood glucose released compared to white rice. This study included 10 healthy individuals and 9 participants living with type 2 diabetes. It was observed that the GI was 12.1% lower in healthy individuals and decreased by 35.6% in individuals living with diabetes after the consumption of brown rice compared to white rice. It also suggested that brown rice is a preferable choice for individuals with diabetes compared to white rice [98]. In a meta-analysis of 19 randomized controlled trials and cohort studies which included studies that compared brown rice with white rice on cardiometabolic parameters, the evidence suggests that the likelihood of type 2 diabetes incidence is reduced when brown rice is incorporated into one's diet rather than white rice [99] (Table 4).

Table 4 Tabulated Literature review on glycemic response to Brown rice [97, 98]

Title	"Effects of Brown rice, White rice and Brown rice with legumes on blood glucose and Insulin responses in overweight Asian Indians"	"Blood glucose lowering effects in normal and diabetic patients"
Author, Journal year and references	*Mohan et al., 2014* [97]	*Panlasagui et al., 2015* [98]
Objectives	Assess the effects of brown rice (BR), wild rice (WR), and brown rice with legumes (BRL) diets on 24-hour glycemic and insulinemic responses in overweight Asian Indians.	Determine digestion rate in vitro and randomized crossover trials.
Study design and participants	15 overweight Asian adults (body mass index, ≥23 kg/m^2)	10 healthy individuals and 9 type 2 DM people.
Outcomes	Substituting brown rice (BR) for wild rice (WR) consumption may aid in lowering blood glucose and insulin reactions in overweight individuals.	Brown rice is a better option for diabetic patients than white rice.

2.7 Comparison of Wild rice to Brown rice and White rice

While comparing wild rice to white rice and brown rice, the macronutrient content of each rice per 100 grams is key to understanding the differences between the grains. The values in the table below may vary depending on the cooking methods (Table 5).

Table 5 Tabulated comparison between wild, brown, and white rice in raw form [100]

Nutrient	Wild Rice	Brown Rice	White Rice
Crude protein content (%)	12.8	8.0	7.8
Carbohydrates content (%)	76.1	76.8	80.5
Total dietary fibre (%)	4.4	3.0	0.9
Total fat (%)	0.9	2.7	0.9
Moisture content (%)	8.6	11.3	10.0
Total sugar content (g/100 g)	1.2	0.5	0.4
Energy (Cal/100 g)	359	355	349
Crude ash content (%)	1.6	1.2	0.8

Based on the provided table, wild rice has fewer calories than other rice types. A research article from 2009 in the "Journal of Agricultural and Food Chemistry" evaluated and compared the antioxidant levels across different rice varieties. The study revealed that wild rice showed the most antioxidant activity, primarily attributed to anthocyanins present in it. These attributes are believed to contribute to the health-enhancing characteristics of wild rice [87]. The nutrient composition of wild rice may contribute to the development of healthier food products.

CHAPTER 3: RATIONALE, OBJECTIVES AND HYPOTHESES

3.0 INTRODUCTION

This study aims to assess the blood glucose response to food products containing wild rice by investigating the postprandial glycemic response to rice cakes made with wild rice through a randomized controlled crossover trial. The trial is part of a larger project titled *"An Indigenous Strategy for Re-Energizing Traditional Wild Rice: Indigenous Wealth Creation"* and is funded by Protein Industries Canada (PIC). It is led by Myera Group, a Manitoba-based Métis company aimed to developing healthier and more culturally tailored Indigenous food products.

3.1 Study Rationale

The literature review reveals that wild rice has the potential to create novel products that may positively impact postprandial blood glucose concentrations in humans following consumption. Wild rice is rich in phenolic compounds and antioxidants, which are associated with improved glycemic control, reduced insulin resistance, and decreased inflammation in animal studies. Despite these findings, there is a knowledge gap in research regarding the impact of wild rice-containing products on human blood glucose concentrations. Addressing this gap is important for understanding wild rice's potential health benefits in managing human blood glucose concentrations. To bridge this knowledge gap, a first study on wild rice was carried out in our laboratory by Chukwu et al., [100] who investigated the effects of cooked Canadian wild rice and wild rice blend on postprandial appetite and blood glucose response compared to white and brown rice, evaluated the nutritional factors influencing these differences, and examined the impact of stovetop versus microwave cooking methods on these outcomes.

This second study aimed to investigate the postprandial glycemic response to wild rice cakes through a randomized controlled crossover trial. In this study, the carbohydrate content of wild rice cakes (25% wild rice and 75% brown rice) and brown rice cakes (100% brown rice) was matched with the carbohydrate control, white bread. By focusing on the postprandial glycemic response, this study aimed to provide information about the potential health benefits of incorporating wild rice into ready to eat food products. Additionally, the study evaluates the palatability and appetite associated with wild rice cakes. This research is significant as it addresses a critical knowledge gap by exploring the effects of wild rice-based snacks on postprandial blood glucose concentrations. While existing studies have examined the impact of various foods on blood glucose, to our knowledge no other study has specifically focused on wild rice-containing ready to eat products. By conducting this study, we aim to determine if wild rice cakes are beneficial in regulating blood glucose compared to traditional rice cakes. The findings will contribute to understanding of how consuming wild rice products impact health and may lead to the development and marketing of more food products made with wild rice.

3.2 Hypothesis

1) The incorporation of wild rice into rice cakes will reduce the postprandial glycemic response after consumption compared to existing conventional rice cakes and white bread.

2) Based on the nutrient content of wild rice, wild rice cakes will reduce appetite after consumption compared to brown rice cakes.

3.3 Objectives

The overall objective of this study is to determine whether consuming rice cakes containing wild rice can reduce postprandial blood glucose concentrations compared to rice cakes without wild rice in a randomized controlled trial.

The specific objectives include.

1. To examine the effects of consuming rice cakes containing wild rice on postprandial glycemic response, compared to traditional rice cakes made from brown rice and white bread currently on the market.
2. To evaluate the palatability and postprandial appetite of wild rice cakes following consumption compared to traditional rice cakes made from brown rice and white bread currently on the market.

CHAPTER 4: METHODOLOGY

4.0 METHODOLOGY

Health Canada guidelines for postprandial glycemic response and satiety guidance documents were followed in this research. All study activities were carried out at the Richardson Centre for Food Technology and Research (RCFTR) on the University of Manitoba Fort Garry campus.

4.1 Sample Size

For a three-period crossover design, a sample size of 18 (3 per sequence) is predicted to have .945 power to detect a difference of 22 (mmol/L) x minutes in the iAUC plasma glucose decrease (two-sided). The 22 mmol/L was chosen to represent a 20% decrease in iAUC, based on the minimal important difference proposed by Health Canada to substantiate claims of a reduction in postprandial blood glucose response. The estimated intra-participant standard deviation of 16% (18 mmol/L) [101].

4.2 Ethics Approval and Informed Consent

This research project was approved by the University of Manitoba Health Research Ethics Board (Ethics # HS25900; B2023:033). Informed consent was obtained from all participants prior to their involvement in the clinical trial. The trial was carried out in accordance with the Declaration of Helsinki, applicable regulations, and Good Clinical Practices.

4.3 Participants Recruitment, Consent and Screening

Participants for the trial in Winnipeg, Manitoba were recruited through various advertisements, including posters, emails within the University of Manitoba, and Facebook. Those expressing

interest were contacted to assess their eligibility and scheduled for an appointment at the Richardson Centre for Food Technology and Research, located at the University of Manitoba. During the appointment, participants reviewed the informed consent form and were given the opportunity to ask any questions regarding the trial and then the consent form was signed. Screening involved measuring participants' weight and height to calculate their body mass index, and blood glucose concentration (mmol/L) was assessed using a Stat Strip® glucometer (Nova Biomedical, Mississauga, ON, Canada).

4.4 Inclusion and Exclusion Criteria

A total of 18 participants (9 males and 9 females) were recruited between the ages of 18 and 50 years with a BMI ranging from 18.9 to 29.9 kg/m², fasting blood glucose concentrations below 5.6 mmol/L, and who typically consumed breakfast as part of their daily dietary routine. Participants were excluded if their fasting blood glucose was ≥ 5.6 mmol/L or < 3.5 mmol/L; if they were pregnant, planning to become pregnant during the trial, or breastfeeding; if they were taking medications or dietary supplements that could interfere with glucose metabolism; or if they had a history of clinically significant endocrine disorders (including type 1 or type 2 diabetes mellitus), AIDS, hepatitis, cardiovascular, pulmonary, biliary, or gastrointestinal disorders, or allergies to rice. Other exclusion criteria included specific dietary practices, excessive alcohol intake (>14 drinks per week), recent cancer history, recent substantial weight changes, and participation in another research project involving an intervention within the previous 12 weeks. The trial adhered to Good Clinical Practice (GCP) standards, as well as all regional and federal regulations.

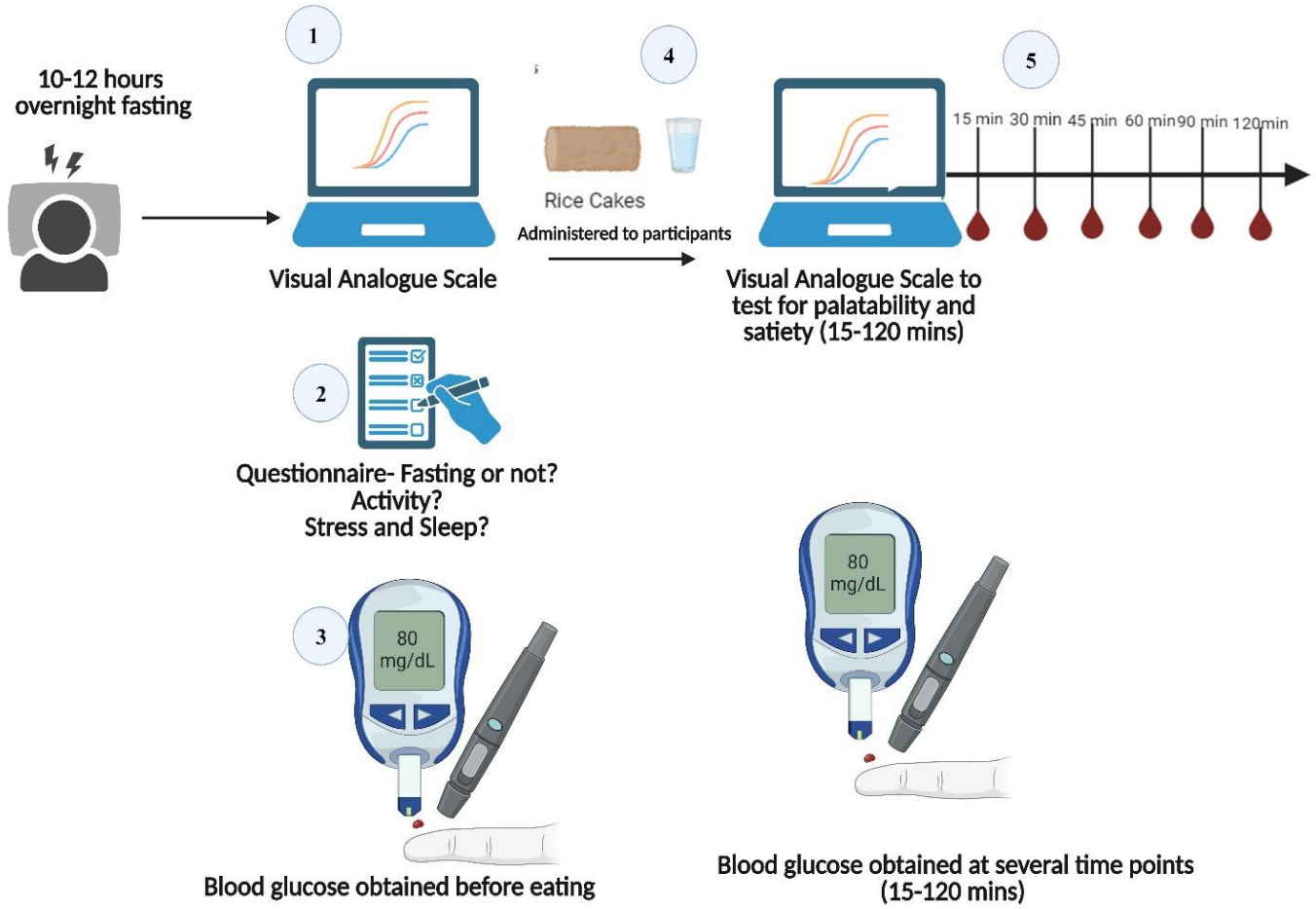
4.5 Trial procedure

After screening, 17 of the 18 participants completed all four trial sessions, each lasting approximately 2.5 hours, while one participant missed a single session. The first three sessions were scheduled with a minimum of 3 days between them and a maximum interval of 8 weeks. An additional fourth session was added following an ethics amendment, which was required due to an error identified in the amount of carbohydrates provided during the white bread control session. Participants had initially been given 32 grams of white bread as the control, which was erroneously calculated to provide the necessary matching carbohydrate due to the use of a value for carbohydrate content that was calculated from a freeze-dried sample which was not corrected for the freeze drying. This error resulted in a control which provided only half of the intended carbohydrate content. After the error was identified, all participants were contacted, and 17 out of 18 returned for an additional fourth session, during which they were provided with 66 grams of white bread, which matched the required 30 grams of available carbohydrates. During each session, participants consumed one of the test products along with 250 ml of water. The test products consisted of 32 g of white bread (15 g of carbohydrates), 40 g of brown rice (BR) cakes (100% BR), 40 g of wild rice (WR) blend cakes (25% WR and 75% BR), and 66 g of white bread (30 g of carbohydrates). The order of the first three test products was randomized, with each treatment consumed within 10 minutes. The final session was not randomized, and each participant had it as their last session and consumed within 10 min.

Participants who menstruate were scheduled for sessions during the follicular phase of their menstrual cycle, as literature shows resistance to insulin during the luteal phase [102]. Research visits required participants to arrive between 7:00 AM and 11:00 AM after fasting for 10–12 hours,

with a 24-hour alcohol restriction. Blood glucose concentrations were measured using a Stat Strip® glucometer (Nova Biomedical, Mississauga, ON, Canada) at 0, 15, 30, 45, 60, 90, and 120 minutes after the first bite, marking the start of the treatment. Appetite and palatability were assessed using VAS, AUC for appetite was calculated and incremental area under the curve (iAUC) was calculated for blood glucose (Figure 1).

Figure 1 Trial protocol



4.6 Rice cakes production procedure

Wild rice can be incorporated into various products, including rice cakes, through a process called puffing. Puffing is a food processing method that exerts high temperatures for a short time on parboiled grains, which apply pressure and heat, resulting in adhesion development among rice grains [103].

Wild rice cakes were made by a process called puffing. A mixture of 25% wild rice and 75% brown rice (for the wild rice blend cake) or 100% brown rice (for the control brown rice cake) was soaked in water for an hour, with salt added to batches of 5 kg. This combined rice and salt mixture was transferred into hoppers of puffing machines. The hoppers released the rice into individual molds, where it was cooked for 7 seconds at temperatures between 270-290°C. The resulting rice cakes were then packaged.

4.7 Data collection: Proximate analysis of study test products

Study test products were analyzed to determine various nutrients that could influence postprandial glycemic response after consuming the test products, including carbohydrates, protein, ash content, fats, and in vitro digestion.

4.7.1 Carbohydrates

The carbohydrate content in this study was calculated by difference. Specifically, it was determined by subtracting the sum of protein, fat, ash, and moisture contents from the total grams of the study treatments. This indirect method is commonly used when direct carbohydrate analysis is not conducted. [104]

4.7.2 Starch damage

The procedure to determine starch damage used Megazymes starch damage procedure which used AACC Methods 76-31 as a blueprint [106].

Sample preparation and analysis:

100mg of the samples were weighed into a glass KIMAX tube (15ml), and the samples were at the bottom of the tube, samples were placed into a water bath set to 40°C to equilibrate. Alpha-amylase was prepared and heated to 40°C by placing the enzyme into a centrifuge and carefully placed into a water bath to rest. 1.0mL of the enzyme solution (50U/mL) was vortexed for five seconds and incubated for ten minutes at 40°C. For five seconds 8.0mL of 0.2%v/v sulphuric acid was also vortexed to eliminate the reaction and centrifuged at 1000 x g for ten minutes. 1100uL of the centrifuged samples was pipetted into a culture tube, in duplicate, and 100uL of amyloglucosidase solution (2U) was added to each tube and vortexed for five seconds.

Two reagents are prepared reagent blank (200uL sodium acetate buffer (100 mM, pH 5.0 + 5nM calcium chloride) and glucose standards = 1100uL sodium acetate buffer (100mM, pH 5.0 + 5mM sodium chloride) + 100uL glucose standard (150uL/ 100uL) and the tubes are covered with an aluminum foil to prevent incubation and incubated at 40°C for ten minutes. In each tube, 4.0ml GOPOD is added and vortexed for five seconds, then incubated at twenty minutes at 40°C, the absorbance is read at 510nm [106].

The formula $SD\%, as\ is = E \times F/W \times 8.1$

Where E = absorbance read against reagent blank, W – weight, mg, F= 150mg/ (Absorbance of 150ug of glucose), and 8.1 =conversion factor.

4.7.3 Protein

Dumas's method AOAC 990.03 method was used to analyze the protein content of test samples [107]. This method determines the protein content of food by measuring nitrogen content.

Sample preparation and analysis:

200 to 250 mg of test samples were weighed in duplicate, sealed, and placed into an N-analyzer to ensure the conversion of nitrogen oxides into nitrogen gas. Nitrogen content was determined by combustion of the sample at approximately 900–950°C, converting nitrogen into nitrogen oxide gas, which was then reduced to elemental nitrogen (N_2) and quantified using thermal conductivity. During the combustion process, the oxidation of the test sample occurred, and finely ground particles were removed by passing the gas through an additional furnace at 850°C. Water and carbon dioxide were removed using sodium hydroxide and magnesium perchlorate, respectively. The purified nitrogen gas was then passed through a thermal conductivity cell, which emitted an electrical signal proportional to the nitrogen content. Calibration was performed using a nitrogen standard with ethylenediaminetetraacetic acid (EDTA), allowing the nitrogen detected to be directly related to the protein content of the test sample. After a few hours, the protein content results were displayed. The nitrogen content was converted into protein content using a standard conversion factor, typically 6.25 (assuming proteins contain an average of 16% nitrogen) [107].

4.7.4 Crude fat

The method used to analyze crude fat was the Soxhlet method. The test sample was dried to remove moisture that can interfere with extraction if it contains more than 10% of water, this was done by placing the test sample into an oven (95- 100°C) under pressure (< 100 mmHg) for five hours until a constant weight is attained. (AOAC 934.01). 2 g of finely ground sample was placed into a pre-dried extraction thimble and covered with glass wool. The thimble was weighed, and an anhydrous ether is placed in it. In 5-6 drops per second, the sample was concentrated by heating the extraction solvent in the flask for approximately four hours and dried with fat extracted at a temperature of 100°C for thirty minutes and placed in a desiccator to cool and the flask was weighed to know the extracted fat formula. Crude fat was then be measured using the recommended formula [108].

"% Fat on dry weight = (g of fat in sample / g of dried sample) x 100".

4.7.5 Ash content

Samples were weighed in duplicates 3.00 ± 0.01 g into the dish and placed in an oven tray. The oven temperature was set to 575°C, the ash oven was switched on, and samples are placed in it overnight. After this process, the oven was left to cool down to 130°C, and samples are taken out. The samples are placed in a desiccator to cool for an hour, and the ash content was reweighed and recorded to four decimal places [109].

The ash content was calculated using the formula

% Ash = weight of ash residue/sample weight x 100

4.7.6 Moisture content

The official method AOAC Method 2001.12 was used to determine the moisture content of study test products, this method involved an oven, and test samples were placed in an aluminum dish, and put into a preheated oven at a certain temperature. After the test samples are dried, the dishes were placed in a desiccator for about thirty minutes to cool and prevent air moisture absorption. Moisture content was determined by weighing the test samples before and following the experiment using a 0.05 accuracy (AB 104-S, Mettler-Toledo, Inc.) [110].

4.7.7 Total Phenolic Content (TPC) Sample extraction:

To determine the total phenolic content and total flavonoid content, 500 mg of each sample was extracted in 5 mL of 80% methanol [111].

Determination of TPC Total phenolic content:

The Folic-Ciocalteu method is described by Apea-Bah, Drawbridge, & Beta (2022). The results are expressed as milligram gallic acid equivalent per 100 grams (mg GAE/100g). The assay was conducted in triplicate for each sample [111]. 500 mg of milled sample was extracted in 5 mL of 80% methanol. The dry matter content was determined by setting the oven to 130 °C and drying the silica gel for thirty minutes to one hour. After this process, it was transferred into a desiccator, and 2 g of each sample is weighed into aluminum cans and dried at 130 °C for one hour. The dry matter content was calculated using the formula.

% Dry matter content of sample = weight of dried sample / original weight of sample x 100%

To extract the phenolic acid, 100mg of the sample was weighed into a colored 2 mL microcentrifuge tube (amber), and 1 mL of 80% aqueous HPLC-grade methanol was added and sonicated for 60 minutes under light protection. The mixture was centrifuged at 20,000 × g for 5 minutes to separate the supernatant and filtered using a 0.22 µm syringe filter into HPLC vials with inserts and stored at -20 °C for subsequent analysis. Phenolic acid standards are prepared at 1,000 µg/mL and mixed. A serial dilution down to 3.125 µg/mL was prepared and the column temperature set to 35 °C, and the sample oven at 15 °C. Mobile phases are prepared: **Phase A**: 0.1% aqueous formic acid and **Phase B**: 0.1% formic acid in methanol. In the HPLC column,10 µL of extracts and standards are injected and extracted with a linear gradient program (25 minutes). The retention times of chromatographic peaks are compared with standards at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids), and calibration curves of standard concentrations are used against the peak areas to quantify phenolic compounds. TPC was determined by preparing the ferulic and gallic acid standards within 0.025–0.150 mg/mL and extracts and standards are diluted in 50% aqueous methanol and added to a 96-well microplate: **18.2 µL** of extract or standard, **36.4 µL** of 10% Folin-Ciocalteu reagent, **145.4 µL** of 700 mM sodium carbonate. The extract was incubated in the dark at room temperature for two hours and the absorbance measured at 750 nm using a microplate reader, calibration curves are plotted to estimate total phenolic content, and the results were expressed in milligrams of gallic acid equivalents per gram of sample (mg GAE/g), on a dry weight basis [111].

4.7.8 Determination of Total flavonoid content (TFC):

TFC was determined following the method described by Souza et al in 2014 [112]. with slight modifications, and rutin was used as the standard flavonoid. The results are expressed as milligram rutin equivalent per 100 grams of sample (mg RE/100g). The assay was conducted in triplicate for each sample. Samples are prepared and 1 mL rutin is pipetted into a 10 mL volumetric flask, 4 mL of distilled water is added to the flask and mixed thoroughly, 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ solution is added to the mixture. The mixture was then let to stand for about 6 minutes to allow for the reaction. 2mL of 1 M NaOH is added and distilled water was also added to bring the total volume to 10 mL; the solution was mixed thoroughly. Absorbance measured by preparing a blank reagent following the same procedure but without the extract or standard solution and the spectrometer wavelength is set to 510 nm. The absorbance of the prepared solutions was measured against the blank reagent (Table 6).

4.8 Treatment Products

Rice cakes were provided by Myera group (refer to section ***4.6 Rice cakes production procedure*** for details on how they were produced, the brown rice cakes were also provided by Myera Group) and white bread control was Dempster's White Bread (Dempster's Bread-Division of Canada Bread Company Limited, Sudbury, ON).

Study test products include:

- White bread (32 g, 15 g of carbohydrates)
- Wild rice blend cakes = 75% WR and 25% BR

- Brown rice cakes = 100% BR
- White bread (adjusted to fit the required carbohydrate content of rice cakes) (66 g, 30 g of carbohydrates)

Table 6 Table showing the nutrients content of the study test products

Parameters	White bread (32 g)	White bread (66 g)	Brown rice cakes (40 g)	Wild rice cakes (40 g)
Carbohydrates(g)	14.42	31.12	31.60	31.41
Protein (g)	2.67	4.99	3.43	3.89
Crude Fat (g)	0.80	1.65	2.51	2.80
Moisture content (g)	13.26	26.48	1.58	1.19
Ash content (g)	0.85	1.76	0.88	0.71
Starch damage (%)	41.11	41.11	69.53	67.00
Amylose (%, w/w)	38.22	38.22	23.57	24.87
TDF (%, db)	3.40	3.40	3.35	3.36
TPC (mg GAE/100g)	69.22 ± 4.22	69.22 ± 4.22	50.45 ± 6.34	59.61 ± 1.65
TFC (mg RE/100g)	234.5 ± 4.32	234.5 ± 4.32	99.22 ± 0.79	117.00 ± 0.79

Note: The nutrient composition of the study test products is determined using the methods in section *4.7 Data collection: Proximate analysis of study test products* . The table represents the number of grams of test products consumed by participants. Bolded items to emphasis large differences. The carbohydrate content in this study was calculated by difference. Specifically, it was determined by subtracting the sum of protein, fat, ash, and moisture contents from the total gram of the study treatments. This indirect method is commonly used when direct carbohydrate analysis is not conducted.

4.9 Randomization procedure, allocation concealment, and blinding

Randomization was conducted by the Data Sciences platform located at the Centre for Healthcare Innovation in Winnipeg, Manitoba. A randomization schedule, created by the Data Sciences platform, was transferred into the Redcap platform, where allocation was managed by trial staff. The allocation of the intervention order was concealed, ensuring that both the investigators and participants were unaware of the treatment order before randomization. The test products were assigned pseudonyms, denoted as A, B, or C to blind the test products for data analysis. While it was acknowledged that study coordinators and participants might be able to determine the treatment based on its visual appearance, participants were not informed of the specific test products. The primary outcome analysis was conducted by an analyst who remained blinded to the test products. In addition to the randomized sessions, a fourth session was added and administered uniformly to all participants; this session was not randomized.

4.9.1 Statistical analysis

The trapezoid rule was used to calculate the incremental area under the curve (iAUC) for blood glucose over a 0–120-minute period, as well as the total area under the curve (AUC) for appetite and palatability. Total AUC was calculated using the trapezoidal rule, which estimates the area under a curve formed by plotting a variable time. Statistical analyses were performed using SAS 9.4 software. These analyses aimed to examine factors affecting postprandial blood glucose, with session and sex included as fixed factors in the model to assess potential session-by-treatment or sex-by-treatment interactions. Although, session was not included second set of analyses which used the adjusted bread dose, since it was not randomized.

The iAUC was used to quantify postprandial responses, to assess differences in blood glucose levels across the study test products, a linear mixed model was applied using PROC mixed in SAS. To evaluate the effects of test products, time, and time-by-treatment interactions on blood glucose, as well as the variability in baseline blood glucose and responses over time was analyzed a linear mixed-effects model in SAS (PROC MIXED). If the time by treatment interaction was significant then each timepoint was analyzed separately linear mixed-effects model. Palatability and appetite were assessed using the Visual Analog Scale (VAS), with differences between the test meals analyzed through a linear mixed-effects model in SAS (PROC MIXED). Tukey-Kramer post-hoc test was used to determine mean differences among the study test products. The graph and bar chart were created using R software version 4.5.0.

CHAPTER 5: RESULTS

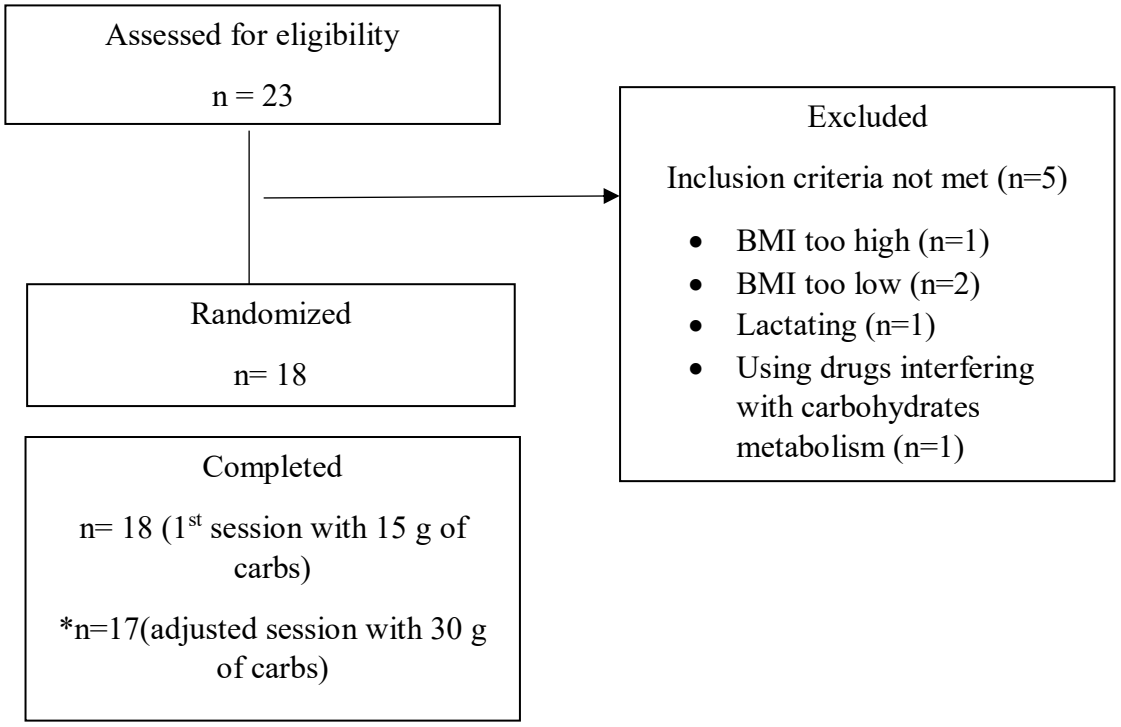
5.0 Result

A total of 18 participants completed the first session with 15 g of carbohydrates; this trial was randomized. Meanwhile, 17 participants completed the adjusted session with 30 g of carbohydrates, which was added to the end of the trial and in a randomized order.

5.1 Participants

Eighteen participants, male (n=9; 33.22 ± 5.98 years; 23.79 ± 2.00 kg/m²; 5.1 ± 0.28 mmol/L) and female (n=9; 30.33 ± 4.69 years; 24.75 ± 2.82 kg/m²; 4.98 ± 0.29 mmol/L) were recruited for the trial. (Figure 2)

Figure 2 Figure showing participants recruitment

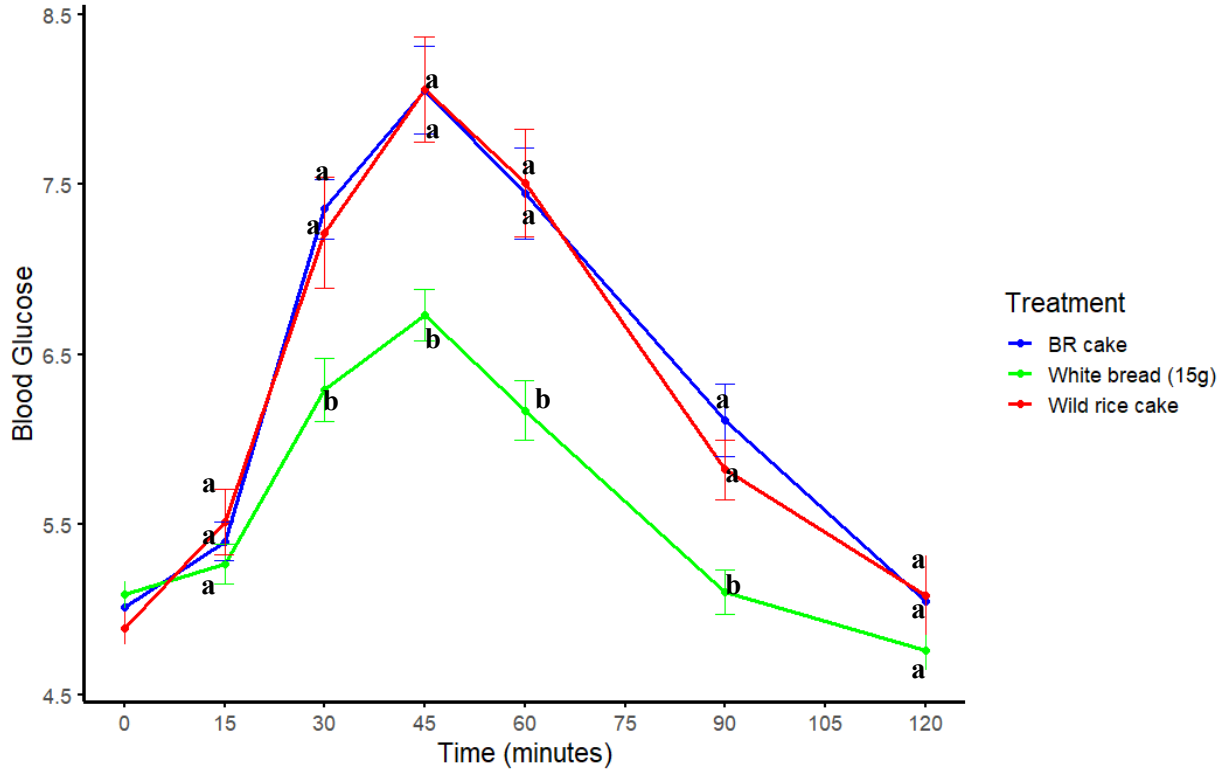


5.2 Blood glucose

In the first session where participants were fed 32 g of bread which provided participants with 15 g of carbohydrates. Over 120 minutes, there was an effect of time ($p < 0.05$), treatment ($p < 0.05$), and time-by-treatment interaction ($p < 0.05$) on postprandial blood glucose. However, no effect of sex was observed ($p = 0.3826$). Session effects were significant ($p = 0.0415$), indicating that there was an effect across the three sessions. Blood glucose differed at time points 30, 45, 60, and 90 minutes ($p < 0.05$), with white bread showing the lowest blood glucose compared to BR and WR cakes, Blood glucose peaked at 45 minutes across all test products and was lowest at 120 minutes. No differences were found between test products at 15 and 120 minutes. For the incremental area under the curve (iAUC), differences were observed between white bread and BR cakes ($p < 0.05$) and white bread and WR cakes ($p < 0.05$), but no difference was found between BR and WR cakes ($p > 0.05$) (Figure 3a).

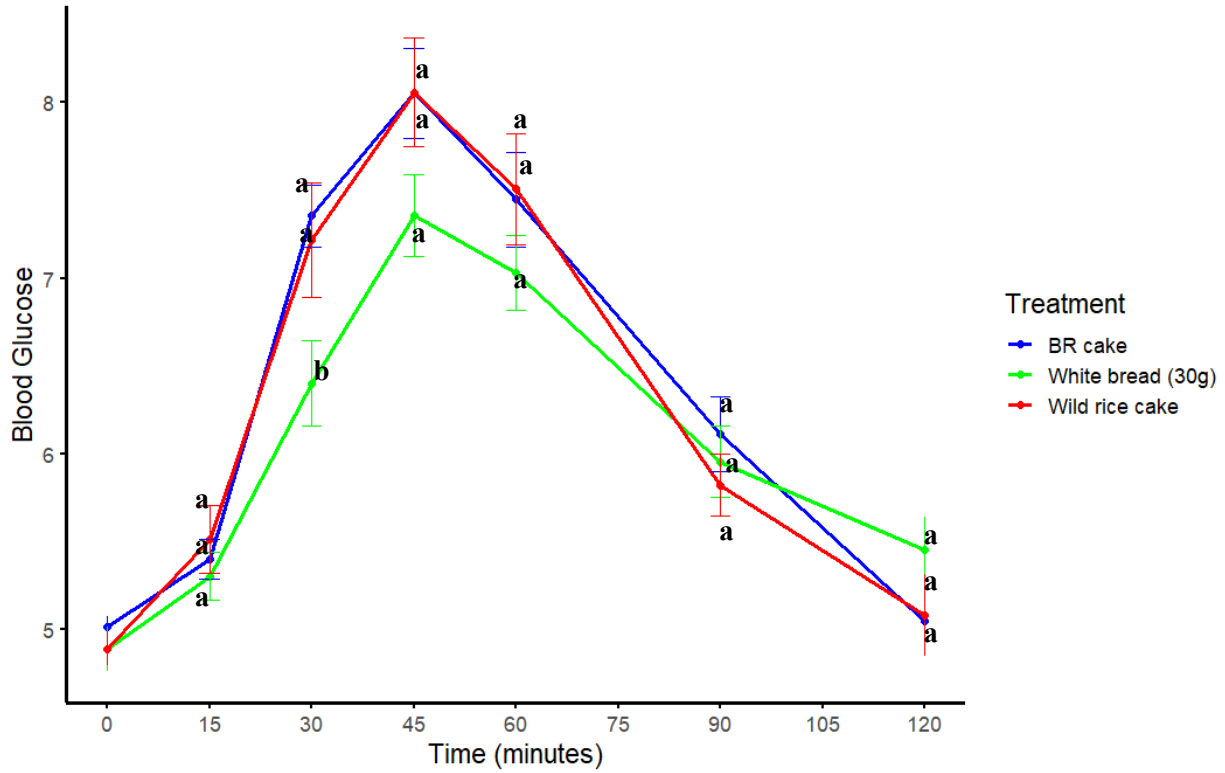
During the adjusted session, participants were fed 30 g of carbohydrates from white bread. Over 120 minutes, there was an effect of time ($p < 0.05$) and treatment ($p < 0.05$), on postprandial blood glucose. However, session effects were not taken into consideration when the data were analyzed, since the additional session was not randomized. The result of this re-analysis differed from the first one, with differences of blood glucose only at time points 30 minutes ($p < 0.05$), but a similar blood glucose peak at 45 minutes across all test products, there was also no effect on sex observed ($p = 0.5559$) (Figure 3b). For the incremental area under the curve (iAUC) for adjusted session when participants were fed 30 g of carbohydrate from white bread, no differences were observed between the treatments ($p > 0.05$) *(*Table 7)

3a Blood glucose response to study test products over 120 minutes (original white bread session)



Note: iAUCs were calculated using the trapezoid rule, and differences in postprandial responses were analyzed using linear mixed models (PROC MIXED, SAS 9.4), with Tukey-Kramer post-hoc tests; graphs were created in R (v4.5.0). In the initial session, participants were fed 15 g of carbohydrates from white bread, 30 g from the rice cakes. All values are presented as means ± SE (n = 18). No difference was observed at 15 and 120 minutes ($p > 0.05$). a and b denote significant differences between test products at 30, 45, 60, and 90 minutes ($p < 0.05$).

Figure 3b Blood glucose response to study test products over 120 minutes (adjusted white bread control session)



Note: iAUCs were calculated using the trapezoid rule, and differences in postprandial responses were analyzed using linear mixed models (PROC MIXED, SAS 9.4), with Tukey-Kramer post-hoc tests; graphs were created in R (v4.5.0). In the adjusted session where participants were fed 30 g of carbohydrates for all treatments. All values are presented as means ± SE (n = 17). No significant difference was observed at 15, 45, 60, 90 and 120 minutes ($p > 0.05$), a and b denote significant differences between test products at 30 minutes ($p < 0.05$).

5.3 Appetite

In the first session, participants consumed 32 g of bread, providing 15 g of carbohydrates. Over 120 minutes, there were significant effects of time ($p < 0.05$), treatment ($p < 0.05$), and time by treatment interaction ($p < 0.05$) on appetite. The total area under the curve (tAUC) for appetite was significant ($p < 0.05$). However, no differences were observed between sexes ($p > 0.05$). When comparing test products, no significant difference was found between white bread and brown rice ($p = 0.2152$), or between brown rice and wild rice ($p = 0.1206$). Appetite ratings differed significantly at 15, 30, 45, 90, and 120 minutes ($p < 0.05$), with wild rice eliciting the lowest appetite ratings compared to brown rice and white bread. Additionally, a significant difference was observed between white bread and wild rice cakes ($p < 0.05$), where wild rice resulted in the lowest appetite and white bread the highest, indicating a stronger urge to eat after consuming white bread (Figure 4a).

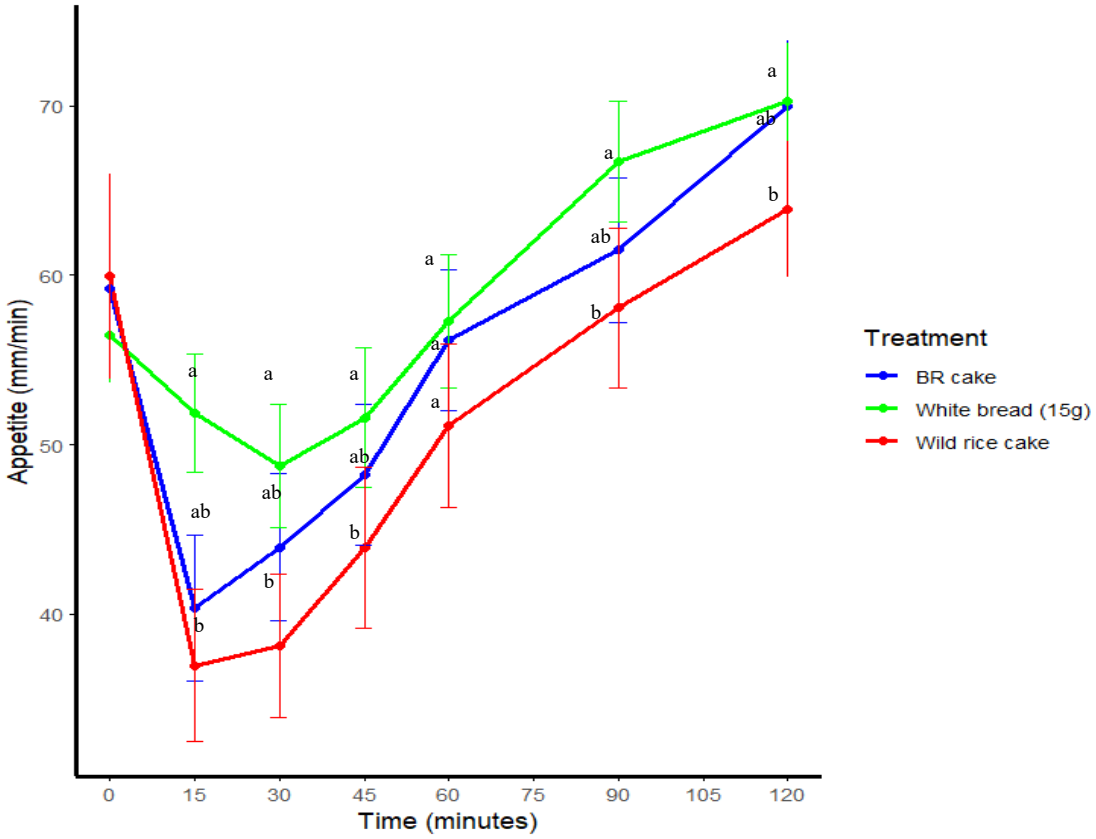
In the adjusted session, participants consumed 66 g of white bread, which provided 30 g of carbohydrates. Over 120 minutes, there were effects of treatment ($p < 0.05$) and time ($p < 0.05$) on appetite, but no time by treatment effect was observed ($p > 0.05$). The mean appetite values were 54.78, 53.24, and 50.31 for the white bread, brown rice cake and wild rice cake respectively. There was a difference between white bread and the wild rice cake ($p> 0.0279$), but no difference between rice cakes, or white bread and brown rice ($p > 0.05$ for both). There were no differences observed between the three treatments for the area under the curve (tAUC) for appetite ($p > 0.05$) and no effect of sex was observed ($p > 0.05$). This adjusted session was not randomized, so there was no session term in the analysis. (Figure 4b)

Table 7: Blood glucose incremental area under the curve, total appetite area under the curve and palatability

Test products	BGR (iAUC) (mmol.min/L)	Appetite (tAUC) (mm/min)	Palatability (mm)
White bread (15 g carbs)	75.46 ± 6.17[a]	6786.65 ± 452.47[a]	69.70 ± 4.64[a]
Brown rice cakes	172.80 ± 12.24[b]	6215.39 ± 453.21[b]	66.91 ± 4.56[a]
Wild rice blend cakes	185.25 ± 13.16[b]	5550.14 ± 453.57[b]	64.43 ± 4.57[a]
White bread (30 g carbs)	149.10 ± 17.32 [b]	6363.71 ± 478.26 [b]	70.59 ± 4.47[a]

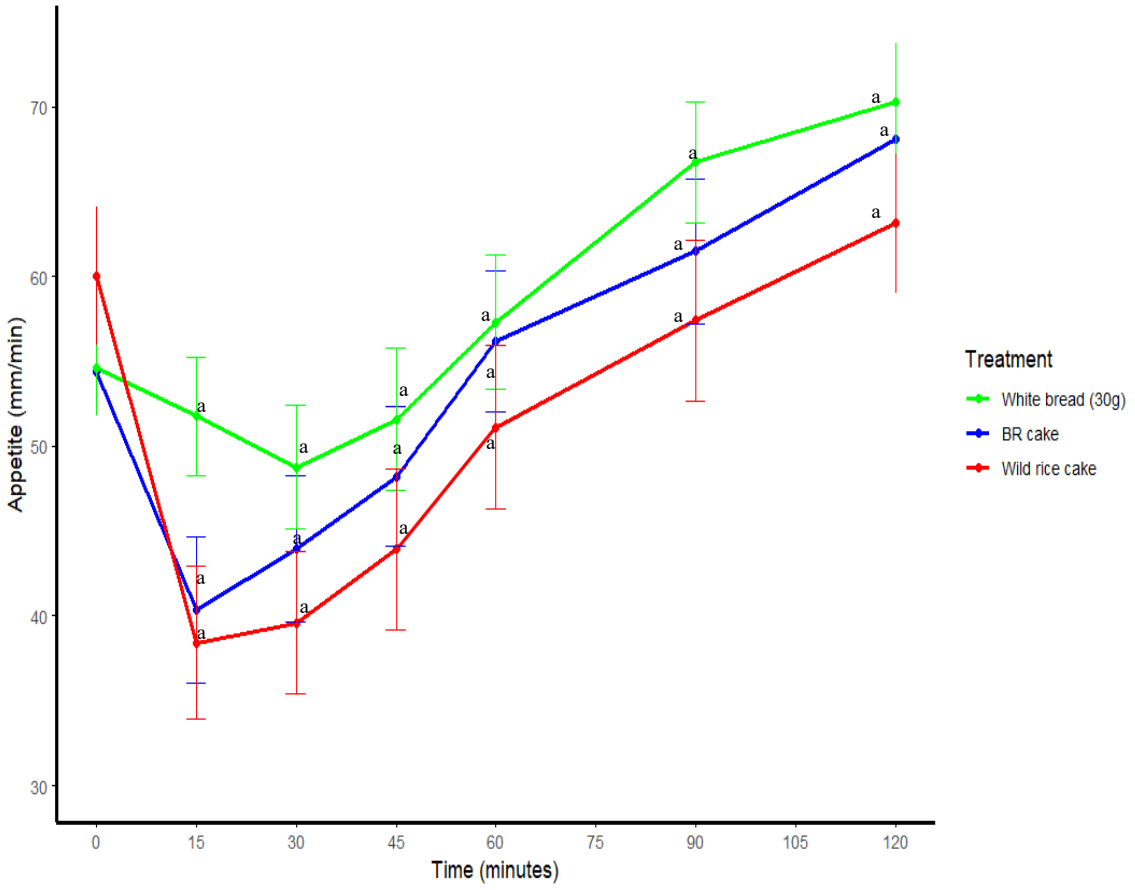
Note: In the white bread initial session (n =18), participants were fed 15 g of carbohydrates, while in the white bread adjusted session (n=17), they were fed 30 g of carbohydrates. Letter a and b denotes differences. The two bread sessions were not compared to each other. All values are mean ± SE. BGR= Blood glucose response, iAUC = incremental area under the curve from 0 – 120 minutes, tAUC = total area under the curve from 0 – 120 minutes.

Figure 4a Appetite response to study test products over 120 minutes (original white bread session)



Note: In the initial session where participants were fed 15 g of carbohydrates. All values are presented as the area under the curve. No difference was observed at 60 minutes ($p > 0.05$). a and b denote significant differences between test products at 15, 30, 45, 90, and 120 minutes ($p < 0.05$) (n =18). Participants filled out VAS questionnaires after treatment consumption to determine appetite. tAUC were calculated using the trapezoid rule, and differences in postprandial responses were analyzed using linear mixed models (PROC MIXED, SAS 9.4), with Tukey-Kramer post-hoc tests; graphs were created in R (v4.5.0).

Figure 4b Appetite response to study test products over 120 minutes (adjusted white bread control session)



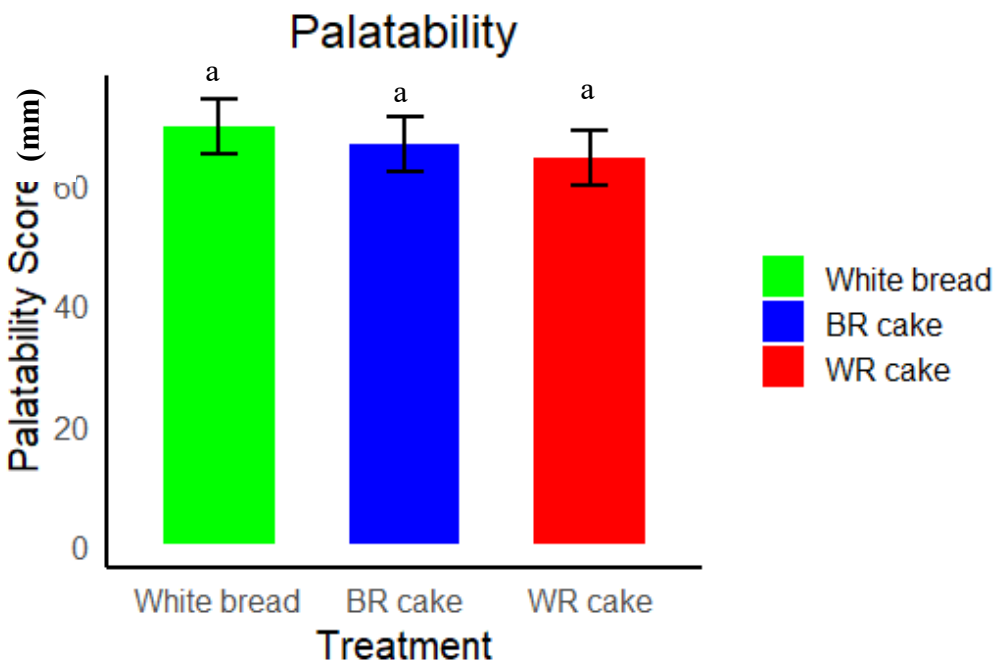
Note: In the adjusted session where participants were fed 30 g of carbohydrates. "a" indicates no significant difference. All values are presented as the area under the curve. No difference was observed ($p > 0.05$) ($n = 17$). Participants filled out VAS questionnaires after treatment consumption to determine appetite. tAUC were calculated using the trapezoid rule, and differences in postprandial responses were analyzed using linear mixed models (PROC MIXED, SAS 9.4), with Tukey-Kramer post-hoc tests; graphs were created in R (v4.5.0).

5.4 Palatability

In the first session, participants consumed 32 g of bread, providing 15 g of carbohydrates. No effect of treatment on palatability was found ($p > 0.05$), either before or after the Tukey-Kramer adjustment ($p > 0.05$). Additionally, no differences were observed between sessions ($p > 0.05$) or between sexes ($p = 0.5603$) (Figure 5a).

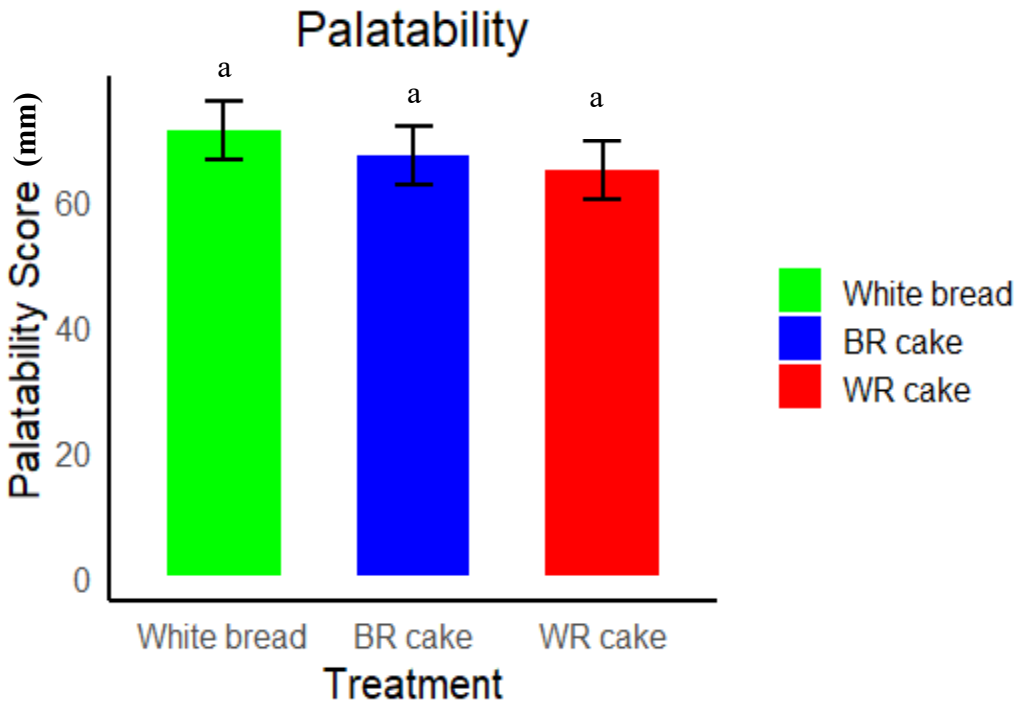
In the adjusted session, where they were fed 30 g of carbohydrates, similarly, there was no significant effect of treatment on palatability ($p > 0.05$). Since this session was not randomized, session was not included in the analysis. Additionally, no differences were observed between sexes ($p > 0.05$) (Figure 5b).

Figure 5a Histogram showing study test products' effects on palatability (mm) (original white bread control session)



Note: In the initial session where participants were fed 15 g of carbohydrates. All values are presented as means ± SE (n = 18). "a" indicates no significant difference. There were no differences between the three treatments ($p > 0.05$). Participants filled out VAS questionnaires after treatment consumption to determine palatability. Palatability scores was calculated using the trapezoidal rule. Differences in postprandial palatability responses were analyzed using linear mixed models (PROC MIXED, SAS 9.4), with Tukey-Kramer adjustments for multiple comparisons. All graphical representations were generated using R (v4.5.0)

Figure 5b Histogram showing study test products' effects on palatability (mm) adjusted white bread session)



Note: In the adjusted session where participants were fed 30 g of carbohydrates. All values are presented as means ± SE (n = 18). "a" indicates no significant difference. There were no differences between the three treatments ($p > 0.05$). Participants filled out VAS questionnaires after treatment consumption to determine palatability. Palatability scores were calculated using the trapezoidal rule. Differences in postprandial palatability responses were analyzed using linear mixed models (PROC MIXED, SAS 9.4), with Tukey-Kramer adjustments for multiple comparisons. All graphical representations were generated using R (v4.5.0)

CHAPTER 6: DISCUSSION, CONCLUSION AND FUTURE RESEARCH

6.0 Discussion

This study aimed to investigate the postprandial glycemic responses, appetite responses, and palatability in a randomized controlled crossover trial. This trial compared wild rice blend cakes with brown rice cakes and white bread. The results showed that incorporating 25% wild rice into cakes did not lower blood glucose levels at all measured time points. Furthermore, when the overall glycemic effect was summarized using the incremental area under the curve (iAUC), no differences were found between wild rice cakes and white bread, indicating that wild rice cakes had a comparable overall glycemic impact.

As expected, when participants consumed 15 g of carbohydrates from white bread compared to 30 g of carbohydrates from the rice cakes, differences in blood glucose concentrations were observed at 30, 45, 60, and 90 minutes. At these time points, white bread resulted in lower glycemic responses compared to both the brown rice and wild rice cakes. However, no significant differences were observed between the treatments at 15 and 120 minutes. When postprandial glycemic response was analyzed using the incremental area under the curve (iAUC, 0–120 minutes), a difference was found between wild rice cakes and white bread ($p < 0.05$).

Although when participants were fed 30 g of carbohydrates across each treatment, a significant difference in blood glucose concentrations was observed only at the 30-minute time point. At time points 15, 45, 60, 90, and 120 minutes, there were no differences between the test products. The analysis of the incremental area under the curve (iAUC, 0–120 minutes) showed no differences among the treatments ($p > 0.05$). While wild rice cakes led to higher blood glucose than white

bread at 30 minutes ($p < 0.05$), the overall glycemic response, as measured by iAUC, was not different. Suggesting the overall impact of wild rice cake on blood glucose over 2 hours is like that of white bread.

These findings expand upon prior research conducted by our group, which was the first clinical trial to examine the effects of wild rice consumption on postprandial metabolism. In this study, Chukwu et al. [100] assessed the acute postprandial glycemic and appetite responses of 20 participants to five test products, stovetop-cooked white rice, brown rice, wild rice, a microwaved wild rice blend, and a stovetop-cooked wild rice blend (15% wild rice, 85% brown rice). Results showed that 140 g of stovetop-cooked wild rice led to a 32.7% higher glycemic response than parboiled white rice, despite similar carbohydrate content and a more favorable nutrient profile. Appetite responses were not different between test products; however, wild rice and its blends were rated less palatable than white and brown rice. Cooking method (stovetop vs. microwave) had no effect on glycemic response or appetite, although microwave wild rice blends had 50% more carbohydrate. The findings suggest that parboiling may improve wild rice's nutritional quality and reduce starch digestibility, potentially aiding blood glucose management and developing food products made with wild rice [100].

The study did not find a clear explanation for how the nutritional composition of the products influenced the postprandial glycemic response observed. It is suggested that the lack of parboiling wild rice compared to the other rice varieties could have contributed to the higher blood glucose response to the wild rice. This is the only study to date that has determined the effects of wild rice on postprandial blood glucose and appetite in humans. In comparison to the earlier study, our findings revealed that wild rice blend cakes elicited a higher postprandial glucose response at

specific timepoints than white bread. However, there was no difference in glycemic response between wild rice and brown rice cakes. This lack of difference is not unexpected, given their nearly identical macronutrient profiles. Both rice cake types contain similar amounts of carbohydrates (~ 30 g) and comparable protein levels (brown rice cakes- 3.43 g and wild rice blend cakes 3.89 g, respectively). Since carbohydrate quantity is the primary driver of postprandial glycemic response, the protein differences were minimal; this nutritional similarity likely contributed to their comparable effects on blood glucose.

Although white bread led to lower blood glucose levels at specific timepoints, the incremental area under the curve (iAUC) showed that its overall postprandial glycemic response was not different from that of the rice cakes. This indicates that while white bread led to the lowest BG at 30 minutes, the cumulative glycemic effect over the two hours was comparable across treatments. These results highlight the importance of using measures such as iAUC to capture the total postprandial response, rather than concluding individual time-point differences alone.

The nutritional composition of the test products may provide important insights into these outcomes, which are associated with their higher moisture content of 26.48 g, as compared to the brown rice and wild rice cakes, which have much lower moisture levels (1.58 g, 1.19 g) (Table 6). Increased moisture content in foods like white bread can impact the rate of gastric emptying and digestion. Higher moisture slows gastric emptying because the larger volume and liquid composition delay the passage of stomach contents into the small intestine, thereby slowing the overall digestion and absorption of carbohydrates. This slower gastric emptying rate can lead to a more gradual release of glucose into the bloodstream, resulting in a lower glycemic response at 30

minutes compared to drier foods such as rice cakes, which empty from the stomach more rapidly and are digested faster. In addition to moisture, another important factor is starch damage, which refers to the extent to which starch granules are broken down during food processing, which can be associated with the rice cakes being puffed compared to white bread. Also, foods with higher starch damage are more rapidly digested, leading to faster glucose absorption. In this study, white bread had a starch damage value of 41 %, while brown and wild rice cakes had significantly higher values 66.91 % and 64.06 %, respectively (Table 6). This suggests that the starches in the rice cakes could be more readily digestible, leading to a more rapid glycemic response. Taken together, these findings indicate that despite their whole-grain composition, the structural and nutritional differences in rice cakes, particularly higher starch damage and carbohydrate content, may have contributed to their higher glycemic impact at 30 minutes compared to white bread, although the overall glycemic response was not different over the 2-hour period.

Collectively, these findings reveal the important role of the food matrix in nutritional composition. The food matrix is defined as the combination of food components including chemical, physical, and molecular elements that influence how food is digested and metabolized [113]. The matrix of food breaks down during digestion and could influence the absorption of nutrients which could impact the blood glucose of the study test products [114]. Food processing techniques plays a role in food digestibility and absorption, the rice cakes were produced by a process called puffing; puffing is defined as a food processing method that exerts high temperatures for a short time on parboiled grains, which apply pressure and heat, resulting in adhesion development among rice grains [103]. The method of preparing the rice cakes ‘puffing’ could be explored, as this could also influence blood glucose levels, because compared to whole grains, puffed rice has shown a higher

glycemic response in humans [115]. This can be due to starch gelatinization which increases starch surface area and can increase absorption of carbohydrates during digestion which can raise blood glucose[116]. This shows how food matrix could influence the blood glucose response, in our study, we used puffed rice cakes and compared it against sliced white bread.

Another factor to consider is the proportion of wild rice in the rice cakes, which was only 25%. This amount was too low to have an impact on the glycemic response. Increasing the wild rice content in the cakes may lead to a reduction in the blood glucose response. More research is needed to understand how the nutritional composition of wild rice affects glycemic response and how different harvesting, and cultivation methods may influence its effects on blood glucose.

In addition to glycemic outcomes, differences in appetite responses were also observed. When participants consumed 15 g of carbohydrates, wild rice cakes were associated with significantly lower appetite ratings compared to white bread ($p \leq 0.05$). This effect, however, was not evident when the carbohydrate dose was increased to 30 g, no differences were detected between treatments ($p > 0.05$). This is not unexpected as you would expect less food to be less filling.

Finally, no significant differences in palatability were reported among the three test products, indicating that participants accepted all treatments well. This is an important finding, as it demonstrates that the incorporation of wild rice into rice cakes did not compromise sensory appeal, which shows a good indication of acceptability in the market. Since both white bread and brown rice cakes are already widely available and commercially successful, the comparable palatability of wild rice cakes suggests potential for their successful introduction into the market. Ensuring consumer acceptability alongside nutritional benefits will be crucial for the development of food

products that incorporate wild rice as a functional ingredient This is because the white bread and brown rice cakes are commercialized and available in the markets.

6.1 Strengths and Limitations

The strengths of this study include its randomized crossover design, inclusion of both male and female participants, and consideration of hormonal influences in females by conducting the study only during the follicular phase of menstruating participants, and adherence to a standardized method using Health Canada's guidelines for measuring postprandial glycemic and satiety responses. Blood glucose was measured at multiple time points, allowing for both time-point comparisons and calculation of the incremental area under the curve (iAUC), therefore providing a comprehensive assessment of glycemic response. Assessing both appetite and palatability offered valuable insights into participants' subjective experiences with the different rice cake test products, which is important for real-world dietary recommendations. Additionally, multiple nutritional factors that could affect postprandial appetite and glycemic response were evaluated across the study test products.

However, the study had some limitations. Initially, an error occurred where participants were given fewer carbohydrates than intended in the white bread control, so this session had to be repeated at the end, which removed the randomized order of test products related to correct dose of control. Although, the glucose response to the repeated bread session was very linear in terms of carbohydrate content, suggesting the impact of the order on glucose response was minimal.

6.2 Conclusion

In conclusion, rice cakes containing 25% wild rice did not lead to lower postprandial blood glucose concentrations compared to white bread at specific timepoints, and no differences were observed between the wild rice blend cakes and brown rice cakes. Although the iAUC showed no difference when fed 30 g of carbohydrates across treatments, indicating that the overall glycemic response was comparable across treatments. These results suggest that a higher proportion of wild rice may be necessary to achieve more effective reductions in glycemic responses across timepoints. To our knowledge, this is the first study to examine blood glucose response to rice cakes incorporating wild rice, providing valuable insights for developing healthier ready-to-eat food options aimed at better blood glucose management. Building on these findings, Myera group plans to continue exploring the inclusion of other Indigenous foods and ready-to-eat food products such as rice cakes, with the goal of supporting blood glucose management and metabolic health for both Indigenous and non-Indigenous communities.

6.3 Future directions

Based on this trial, future research into understanding the impact of wild rice cake consumption on glycemic response should investigate cakes that incorporate more than 25% of wild rice. It may also be of interest to measure hormones, including insulin. Insulin is a hormone which assists in regulating blood glucose by increasing glucose uptake into the cells to provide energy. Measuring insulin and postprandial blood glucose might offer comprehensive insights to understand how the body manage blood glucose and food influences in regulating blood glucose and overall metabolic health. Carrying out future research measuring insulin will provide a better insight on the effect of

wild rice in controlling blood glucose, insulin resistance and metabolic health, even when the blood glucose concentrations might seem normal. Other hormones such as glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), leptin, ghrelin, C-peptide, peptide tyrosine (PYY), and cholecystokinin (CCK) which might help explain the reason for the differences in glucose response related to the white bread control, despite matched carbohydrate content. These hormones could also play a role in the digestion and absorption of the test products, and it needs to be further explored. To measure these hormones, an intravenous line (IV) is used for blood sampling to obtain the required blood volume for analysis. Conducting this research in other population including those living with diabetes may provide more insights into understanding wild rice consumption on satiety and its effect on blood glucose. This is because people living with diabetes have impaired insulin response, so this effect may have an improved effect on their postprandial glucose and insulin sensitivity. They also have differences in glucose response, which makes them more sensitive to changes in the quality of carbohydrates content present in food, since there is an instability in their glucose levels, an improvement shows up more clearly and has greater clinical relevance compared to healthy individuals. Exploring ways to cultivate wild rice or selection of wild rice with specific strains could also play a role in research as this could help farmers grow wild rice in ways that can help reduce blood glucose by improving the nutritional qualities of the rice which could affect glycemic response. The exploration of strain development that would reduce the carbohydrate content, increase the protein content, and increase fiber can be developed by plant breeders to use selective breeding methods to reduce the glycemic response and appetite. Additionally, adjusting the growing conditions may influence the starch composition and fibre content naturally. Certain improvements in strain development can be made, potentially leading to

a better blood glucose response. These directions could be a significant step to understanding wild rice effectively and its nutrient impact on glycemic response and satiety in humans.

CHAPTER 7: REFERENCES

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CHAPTER 8: APPENDICES

Appendix 1: Participants recruitment poster

University of Manitoba

Chronic Disease Innovation Centre

Wild Rice Products Nutrition Study

Research Participants Needed

- Are you 18 years or older and have normal blood glucose?
- Do you like rice cakes and rice cereal?

If so, you may be eligible to participate in a nutrition study at the University of Manitoba looking at the effects of wild rice cakes and wild rice cereals on blood sugar.

Send an email: wildricestudy@umanitoba.ca

Principal Investigator: Dr. Dylan Mackay

Version 2 November 15th 2023

Appendices 2: Research Participant Consent Form (Version 6, February 25, 2025)

Title of the study: Investigating the Postprandial Glycemic Response to Wild Rice Cakes Using Randomized Crossover Controlled Trials

Principal Investigators: **Dr. Dylan MacKay PhD**

Department of Food and Human Nutritional Sciences,

University of Manitoba

196 Innovation drive

Winnipeg, Manitoba, R3T 2N2

Dr. Rebecca Mollard PhD

Seven Oaks General Hospital

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2LB19-2300 McPhillips Street

Winnipeg, Manitoba R2V 3M3

Study Physician: Dr. Navdeep Tangri

Seven Oaks General Hospital

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2LB19-2300 McPhillips Street

Winnipeg, Manitoba R2V 3M3

Study Sponsors: University of Manitoba

Study Funders: Protein Industries Canada

Mitacs

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it

with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand. Participation in this study is voluntary.

Purpose of project

Wild rice is a native grain in North America and has been used for food among the indigenous communities for thousands of years. It is a good source of protein, minerals, vitamins and phenolic compounds that elicit antioxidant activity. Due to its nutritious and potential health effects, it shows promise for development into high value products. This protocol outlines two clinical trials focused on wild rice products, including wild rice cakes. These trials area a part of a larger project titled "An Indigenous Wealth Creation" funded through Protein Innovation Canada (PIC). This PIC project is led by the Myera Group, which is a Manitoba based Metis company that is developing novel, sustainable biotechnology for producing high-value products that will have a positive impact on human health.

Trial 1 Test products

1. Whitebread
2. Brown rice cake (100% Brown rice)
3. Wild rice blend cake (25% Wild rice and 75% Brown rice)
4. **White bread (66 g)**

Study Procedure

You will attend one screening visit. In the screening visit, after we obtain your informed consent form, we will review the inclusion and exclusion criteria with you including measurement of fasting blood glucose (by finger prick) and body mass index (BMI) to determine your eligibility to this study. If you are eligible, you will be booked for your first study session. For this trial, you will complete four study sessions. During the study sessions you will consume different test products each session. Study sessions will take place at Richardson Centre for Food Technology and Research (RCFTR) in the University of Manitoba Fort Garry campus and each visit will last about 2.5 hours. Women will be scheduled during the follicular phase of their menstrual cycle (the 2 weeks following menstruation). The break between visits will be a minimum of 3 days. Before each study session, we will ask you to fast for about 10 - 12 hours, no food or drink will be allowed except for water. Additionally, no alcohol consumption is allowed 24hours before your session. At each session, you will be asked to eat a treatment and give finger stick blood samples. The order of the test products you will receive will be assigned by chance, but you will receive all of them by the end of the study. Finger stick blood samples will be taken to measure blood glucose using a portable glucose monitor. We plan to take 7 finger sticks during each session. The finger stick will be performed by qualified study personnel, who are trained by a registered nurse or physician in proper blood sampling procedure. There is a chance that additional finger stick samples will be taken if there is an issue with the blood glucose reading, however this is rare. Each session will last up to 2.5 hours. You may be asked to return to the RCFTR to repeat a session if any issues arise during the session i.e., feeling dizzy, problems with getting blood glucose readings, etc. You will be asked to arrive in RCFTR between 7am- 11am for the study sessions. At each session, we will start with measuring your fasting blood glucose by sticking your finger and measuring the

glucose value in a drop of blood using a glucometer. If your blood glucose value is over 5.6 mmol/L or less than 3.5, we will have you rest for 10 min before taking another blood glucose measurement. If your blood glucose value is still above 5.6 mmol/L or less than 3.5, you will be rescheduled to come back another day. If your blood glucose is below 5.6 mmol/L and greater or equal to 3.5, we will ask you to consume one of the test products with a glass of water. You will fill out a palatability questionnaire following consumption of the treatment. After that, we will measure blood glucose at 15, 30, 45, 60, 90 and 120 minutes after your first bite of the product. You will be asked to stay in the clinical area in Richardson Centre for Food Technology and Research (RCFTR) throughout the entire session. You will complete a motivation-to-eat visual analogue scale (VAS) to measure subjective appetite after each blood glucose measure. The questionnaires consist of 100 mm lines with opposing descriptions at either end for each question. Participants mark an "X" on the line to depict their feelings at each time point. You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff first.

Trial 2

1. Kellogg's - rice krispies (control)

2. Kellogg's - rice krispies (control) + 125ml milk

3. Novel cereal (40% Canadian wild rice, 60% fava bean blend) + 125 ml milk

4. Novel cereal (40% Canadian wild rice, 60% fava bean blend)

5. Novel cereal (40% Canadian wild rice, 50% fava bean blend) + 10% purple powder (sweet potato)

6. Novel cereal (40% Canadian wild rice, 50% fava bean blend) + 10% purple powder (sweet potato) + 125 ml milk

Risks and Discomforts

As with any study, there may be some risks associated with taking part. You may feel dizzy following the overnight fast, but this is rare. If this happens, you should feel better once you eat the wild rice provided to you. You may have an allergic reaction to the treatment; however, it only contains wild rice, so the risk is low. There is risk and discomfort associated with blood sampling procedure. Great care will be taken when taking your finger stick blood samples. The staff will help you. To make sure that you are not exposed to another person's needle, we will ask you to sit away from other study participants. We will use disposable finger stick lancets (needles) before taking each blood sample and then put them into the safety container as soon as we are done. There is very little risk of infection from finger stick blood sampling. We will clean your finger with a new alcohol swab before and after each finger stick and will use a new sterile lancet each time. Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is stuck and blood glucose is measured. If you have fasting glucose higher than 5.6mmol/L in two consecutive visits during screening or study visits, we will have to discontinue you from the study. Additionally, if during the study visits, your blood glucose is greater than or equal to 10.0mmol/L or at less than 3.5 mmol/L at 2 hours post product consumption, the PI will review the case with the study physician to discuss the appropriate actions for you.

Benefits

There is no direct medical benefit to you from participating in this study. However, your participation provides valuable information to researchers about the effects of wild rice on blood glucose control.

Costs

There will be no cost to participate in this study.

Reimbursements

You will be compensated for participating in the study, $40 for completing each study session, making a total of $120 by the end of the third study session.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information (such as address and email) will not be used or revealed in the publications or presentations. Your personal information and personal health information is being collected under the authority of The University of Manitoba Act. The information you provide will be used for the purpose of this research project. Your personal information and personal health information will not be used or disclosed for other purposes, unless permitted by The Personal Health Information Act (PHIA) or The Freedom of Information and Protection of Privacy Act (FIPPA). If you have any questions about the collection of your personal information or personal health information, contact the Access & Privacy Office (tel. 204-474-9462), 233 Elizabeth Dafoe Library, University of Manitoba, Winnipeg, MB, R3T 2N2.

Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. The electronic consent form will be signed and stored through an online platform (RedCap) which is managed by the Centre for Healthcare Innovation at the University of Manitoba. PDF copies of your consent will be stored at CDIC or RCFTR either on a password protected shared drive/computer and/or in a locked cabinet. We will employ a unique numerical coding system that will not contain any direct identifiers, such as your name or initials to de-identify the data collected in this study. All research data collected throughout the study will be inputted into the study database using this unique code. A master list record that will link your name, home and email addresses, phone and participant code will be retained in a locked cabinetin CDIC and/or on a password protected CIDC shared drive. Communication with study personal may happen through email or phone for the scheduling of study visits, study reminders, and any other study related communications. The study data will not be stored for longer than 7 years following the end of the research project. After this time, all study data will be destroyed. The paper documents will be destroyed using a secure document destruction company and electronic files will be permanently deleted. Representatives from the University of Manitoba Research Ethic Board may access your confidential records for quality assurance purposes; however, they are committed to confidentiality as well. In addition to the U of M Research Ethics Board, authorized representatives of Agriculture and Agri-Food Canada may inspect and/or copy de-identified research records for quality assurance and data analysis. The data collected from you during this study may be shared in a de-identified form to academic journals for publication purposes, or academic researchers for the purpose of meta-analysis and further data analysis. The data may also be stored by the academic journal or other open-access repositories

under an open access policy in which case it may be used by other researchers for further data analysis and research purposes. All physical records will be kept in a locked secure area in Chronic Disease Innovation Center (CDIC), Seven Oaks Hospital or RCFTR) and only those persons on the research team or identified will have access to these records. The paper file that links your personal and contact information to numerical coding system will be stored in a separate locked cabinet in CDIC. Your name and address will be used during the trial to deliver the trial interventions, remuneration cheques and material to your house, both by study staff and/or contracted delivery personnel. Electronic records of signed consent forms as well as your email address will be stored on REDCap, as well as stored in a password protected secure computer/shared drive at CDIC. No personal identifying information such as your name, address, or contact information will leave Chronic Disease Innovation Center, except as describe above.

This clinical trial is registered on a publicly available registry databank: Clinicaltrials.gov. This is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://clinicaltrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results which have been de-identified. You can review this website at any time.

Voluntary Participation/ Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate, or you may withdraw from the study at any time. If the Principal Investigator feels that it is in your best interest to withdraw you from the study, the Principal Investigator can remove you without your consent. We will tell you about any new information that may affect your health, welfare, or willingness to

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

Principal Investigator: Dr. Dylan MacKay dylan.mackay@umanitoba.ca

Principal Investigator: Rebecca Mollard rmollard@sogh.mb.ca

Study physician: Dr. Navdeep Tangri ntangri@sogh.mb.ca

Research Coordinator: Dianna Dandeneau ddandeneau@sogh.mb.ca

Foluke Oyekale: oyekalef@myumanitoba.ca

For questions about your rights as a research participant, you may contact The University of Manitoba Biomedical Research Ethics Board at (204) 789-3389. Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all questions.

Statement of Consent

1. I have read this consent form. I have had the opportunity to discuss this research study with Dylan MacKay and/or his study staff

2. I have had my questions answered by them in language I understand.

3. The risks and benefits have been explained to me.

4. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements.

5. I have no relationship (such as employee, student or family member) with the study team.

6. I understand that I will be given a copy of this consent form after signing it.

7. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time.

8. I freely agree to participate in this research study.

9. I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed.

10. I hereby consent to undergo all necessary assessments that may be required during this study.

11. I authorize the inspection of my research records by the University of Manitoba Research Ethics Board.

12. I consent to my address being used and provided to a delivery/courier service for the delivery of the study related materials and remuneration cheques.

Please check which trial you will be participating in:

Trial 1 ☐

Trial 2 ☐

Trial 1- 4th session extension (Choose this if you have previously signed a consent for trial 1, but are coming back for the 4th session that was added to correct for a mistake in the dose of the original bread session in trial 1)

Consent to future contact about other nutrition clinical trials (please check)

Yes ☐

No ☐

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature_____

Date _____(day/month/year)

Participant printed name: _____ I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent Printed Name: _____ Date _____ (day/month/year) Signature: _____

Role in the study: _____

Appendices 3: Screening Assessment:

1. Screening ID:
2. Date of screening:
3. Age:
4. Sex at birth:
5. Height:
6. Weight:
7. BMI(kg/m):
8. Fasting blood glucose:

Appendices 4: Eligibility criteria

Date form completed:

Completed by (Staff name):

Date (day/month/year) Section header: Inclusion criteria to meet inclusion criteria, ALL items must be “YES”

1. Aged 18 – 50 years
2. Able to give written informed consent and able to speak/read English?
3. BMI range between 18.9 - 29.9kg/m2
4. Fasting blood glucose ≤ 5.6 mmol/L
5. Participant usually eats breakfast? yes/no

Section Header: Exclusion criteria to meet exclusion criteria, ALL items must be “NO”

1. Participant has an existing relationship with research team such as supervisory relationship (student, employee) or familiar relationship (child, spouse etc)? yes/no

2. Participants who indicate that they could not finish study treatment within 10 minutes? yes/no

3. Fasting blood glucose ≥ 5.6 mmol/L? yes/no

4. Female participant who is pregnant, lactating or planning pregnancy during the course of the trial? yes/no

5. History of AIDs, hepatitis, a history of clinically important endocrine (including Type I and II diabetes mellitus) cardiovascular (including but not limited to atherosclerotic disease, history of myocardial infarction, peripheral arteria disease, stroke), pulmonary, biliary or Gi disorders? yes/no

6. Use of medication to influence carbohydrate metabolism, including not limited to adrenergic blockers, diuretics, thiazolidinediones, metformin and systemic corticosteroids within 4 weeks of screening visit? yes/no

7. Intolerance or allergic reaction to study test products? yes/no

8. Extreme dietary habits (Atkins diet, very high protein diet etc)? yes/no

9. History of hypertension yes/no

10. History of cancer for the last two years (except for non-melanoma skin cancer)? yes/no

11. Participating in another interventional trial that can influence the intervention or outcome of this trial? yes/no

12. Recent history (within 12 months of screening) or strong potential for alcohol or substance abuse. Alcohol abuse is defined as > 14 drinks per week, (1 drink = 12oz of beer. 5oz of wine or 1.5oz distilled spirits)? yes/no

13. Body weight change over 3.5kg in the past 3 months? yes/no

14. Have diabetes or thyroid problems or other major diseases that may affect glucose response? yes/no

15. Have major trauma or surgical event within 3 months of screening? yes/no

16. Participant is eligible for the trial? yes/no

17. Please comment on why participant is not eligible:

Date screening form completed:

Completed by (staff name):

PI signature:

Appendices 5: Presession checklist

1. Date of visit:
2. Is participant repeating a treatment in this visit? Select which treatment is being repeated
3. Fasted over the last 12 hours? Participant is not fasted. Please DO NOT proceed with this visit
4. Had alcohol in the last 24 hours?
5. Had a normal/typical night sleep?
6. Any unusual stress?
7. Participated in any vigorous activity this morning?
8. Any changes to medications since last visit/last recorded?
9. Can participant proceed with this visit?

Appendices 6: VAS Appetite

Time survey started:

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?
5. How thirty do you feel?

6. Complete?

Appendix 7: VAS Palatability

1. How pleasant have you found the beverage/food?
2. How tasty have you found the treatment?
3. How did you like the texture of the treatment? Complete? yes/no

0mm (very weak) to 100mm (very strong)