The Effect of Resistant Starch Bagels on Glycemic Response in Adults at Risk for Type 2 Diabetes

by

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ABSTRACT

The Effect of Resistant Starch Bagels on Glycemic Response in Adults at Risk for Type 2 Diabetes

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Acute and chronic resistant starch (RS) consumption has been shown to improve glycemia, however, more chronic interventions using high-RS foods are needed in individuals at increased risk of type 2 diabetes (T2D). A randomized, double-blind crossover study was conducted to examine the glycemic effects of 8-week consumption of high-RS bagels (25 g RS) or control bagels in high-T2D risk adults (n=24). Fasting and postprandial (with an oral glucose tolerance test) glucose, insulin and HbA1c were measured before and after each bagel treatment. Compared to the control bagel, the RS bagel did not change fasting or postprandial glucose or HbA1c but significantly reduced fasting insulin, 2- and 3-hour insulin iAUC, fasting insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B), and significantly increased fasting insulin sensitivity (HOMA-%S). These results demonstrated that high-RS bagel consumption improves fasting and postprandial insulin sensitivity and provide evidence for a feasible dietary strategy to reduce T2D risk.
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LIST of ABBREVIATIONS

2hPG = 2-hour plasm glucose
AIRg = First-phase insulin response
ALT = Alanine transaminase
ANCOVA = Analysis of covariance
ANOVA = Analysis of variance
AST = Aspartate transaminase
AUC = Area under the curve
BG = Beta-glucan
BMI = Body mass index
BPM = Beats per minute
CANRISK = Canadian Diabetes Risk Assessment Questionnaire
CDA = Canadian Diabetes Association
C_MAX = Maximum concentration
DP = Degrees of polymerization
EFSA = European Food Safety Authority
EGIR = European Group for the Study of Insulin Resistance
ELISA = Enzyme-linked immunosorbent assay
FFA = Free fatty acids
FPG = Fasting plasma glucose
FSANZ = Food Standards Australia New Zealand
FSIVGTT = Frequently-sampled intravenous glucose tolerance test
GI = Gastrointestinal
GIP = Glucose-dependant insulinotropic polypeptide
GLP-1 = Glucagon-like polypeptide-1
HAM = High-amyllose maize
HbA1c = Hemoglobin A1c, glycated hemoglobin
HNRU = Human Nutraceutical Research Unit
HOMA = Homeostasis model assessment
(-IR = of insulin resistance; -%S = of insulin sensitivity; -%B = of beta-cell function)
iAUC = Incremental area under the curve
ID = Identification number
IFG = Impaired fasting glucose
IGT = Impaired glucose tolerance
IOM = Institute of Medicine
iPeak = Incremental peak (of curve)
IV = Intravenous
IVGTT = Intravenous glucose tolerance test
M/I = mean glucose infusion rate / plasma insulin concentration (an insulin resistance index)
MINMOD = Minimal model calculation
MTT = Meal tolerance test
NHP = Natural health product
OGTT = Oral glucose tolerance test
PG = Plasma glucose
PHAC = Public Health Agency of Canada
RDS = Rapidly digestible starch
REB = Research ethics board
RS = Resistant starch
SD = Standard deviation (unless otherwise noted to be study day)
SDS = Slowly digestible starch
SE = Standard error
SST = Serum separator tube
T1D = Type 1 diabetes
T2D = Type 2 diabetes
TG = Triglycerides
TWW = Total wet weight
WHO = World Health Organization
I. INTRODUCTION

A. Type 2 Diabetes

A.i. Type 2 Diabetes is a Serious and Growing Concern

Type 2 diabetes (T2D) is a widespread chronic disease that ranks among the fastest growing chronic illnesses worldwide. In 2014, 387 million people were affected by diabetes globally, and this number is predicted to grow to 592 million by 2035 (1). While a large proportion of people living with diabetes are in low- and middle- income countries (1), there is a growing number of Canadians living with this disease. From 2003 to 2013 the prevalence of diabetes in Canada increased from 4.6 to 6.6% of the population aged 12 and older (2). This equates to nearly 2 million Canadians living with diabetes (2) of which 90-95% of cases are T2D (3).

T2D is one of several forms of diabetes, which are all characterized by inappropriate or insufficient glycemic control. Type 1 diabetes (T1D) occurs when the body’s immune system destroys its pancreatic insulin-producing beta cells, abolishing any insulin production and resulting in severe hyperglycemia (3). People with T1D are generally diagnosed at a very young age and are dependent on exogenous insulin administration to control their blood glucose levels. T2D is also characterized by hyperglycemia, which may be the result of relative insulin deficiency, insulin resistance, or a combination of both (3). T2D is the most prevalent form of diabetes, yet it is known that the onset of T2D in many individuals can be delayed or even prevented with basic diet modifications and regular physical exercise (3). While both a healthy diet and regular exercise are imperative in the prevention of T2D, simple dietary modifications are an approachable way to affect significant changes in T2D risk. T2D contributes to 41 500 deaths per year in Canada (3) and can greatly reduce the quality of life of those living with T2D;
these grave statistics emphasize the need for substantial scientific and consumer efforts into the prevention and management of T2D.

**A.ii. Risk factors for Type 2 Diabetes**

In order to develop T2D prevention strategies, an understanding of the multiple risk factors of T2D must be established. The etiology of T2D is multifactorial and highly individualized, but is generally the result of the accumulation of various genetic and lifestyle-related risk factors (4). Significant research has been undertaken to identify both modifiable and non-modifiable risk factors of this disease. Twin- and family-studies have revealed that there is a heritable component to T2D (5); the rate of concordance among monozygotic twins is much higher than that of dizygotic twins, and the overall risk of developing T2D during the lifetime is higher when one parent had the disease, and higher still if both parents had the disease (5). Further, as of 2011, 40 loci of genetic susceptibility to T2D have been identified (6). Ethnicity is also known to affect T2D susceptibility, as certain subpopulations have an increased risk of developing this disease (7). Further, age is another known risk factor for the development of this disease, as T2D prevalence generally increases with age, most noticeably after the age of 40 in Canada (3). While age is clearly non-modifiable, the increased risk that accompanies age may in part be due to the decline in physical activity and nutrition that may occur around the same time. Independent of age, some risk factors are lifestyle-dependent and modifiable including smoking status and being overweight or obese. Obesity, caused by inactivity and poor nutrition is likely the most well-known modifiable risk factor of T2D, with about 90% of T2D cases attributed to being overweight or obese (8). Obesity has several negative effects on health, particularly in the area of metabolic health. Being overweight, and specifically having increased visceral abdominal adiposity, has a detrimental effect on the glucose homeostatic mechanism (8,9).
Sustained visceral obesity over time and potentially in conjunction with pre-existing risk factors, contributes to relative insulin deficiency and insulin resistance, the cornerstones of T2D (10). Knowledge of the numerous risk factors of T2D is growing, but as T2D prevalence intensifies all around the world, continuous efforts are needed to inform the public of the role played by one’s dietary and lifestyle habits in their metabolic health and the prevention of T2D.

A.iii. Glycemic Control Overview

The regulation of glucose in the blood is a complex and synchronized process. In a metabolically healthy person, a minute change in the concentration of blood glucose will elicit a negative feedback response that elegantly coordinates various organs, tissues and hormones to restore safe blood glucose levels. For example, when the concentration of glucose in the blood increases after a meal or intravenous glucose infusion, the pancreatic beta-cells produce and secrete insulin, the primary glucoregulatory hormone, as well as amylin, which work to reduce blood glucose levels in a number of ways: insulin stimulates the uptake of glucose from the blood by tissue cells for glycolysis, or by the liver for storage as glycogen (10–12); insulin also suppresses the hormone glucagon in order to inhibit hepatic and renal glucose production (10–12); amylin works to reduce blood glucose by inhibiting glucagon also, though to a lesser extent than insulin (10–12). Conversely, if the concentration of glucose in the blood decreases, for example a few hours without caloric intake, insulin secretion is reduced as it is no longer stimulated by blood glucose. If the concentration of blood glucose continues to decrease, the pancreatic alpha-cells produce and secrete glucagon into the blood which stimulates hepatic glucose output via glycogenolysis in the liver, thereby increasing the concentration of glucose in the blood (10–12). Insulin plays a major role in the hormonal regulation of glycemia in concert with other supporting glucoregulatory hormones. However, this tightly regulated mechanism
may deteriorate due to a number of different factors, and if left unchecked, one may progress to develop T2D.

**A.iv. Development and Complications of Type 2 Diabetes**

The spectrum of glycemic control ranges from metabolically healthy at one end and outright T2D at the other, with a range of impaired glycemic control spanning the continuum. The course of development of T2D is unique to each individual but universally, progression to T2D is characterized by deteriorating insulin sensitivity and diminishing insulin secretion which leads to varying degrees of insulin resistance and deficiency.

One of the factors driving both insulin resistance and insulin deficiency stems from obesity and the consequent build-up of lipids in the liver, muscles and pancreas (9,13). When excess visceral adiposity, often a lifestyle-dependent risk factor, compounds with other risk factors such as underlying genetic risk factors, the glycemic control system is stressed intolerably. Initially the pancreas is able to compensate for the resulting hyperglycemia by increasing production and secretion of insulin and amylin from its beta-cells. However, when pancreatic beta-cells are continually exposed to the high levels of circulating free-fatty acids (FFA) and triglycerides (TG) associated with excess caloric intake, beta-cell function is significantly reduced (9), causing a relative insulin deficiency. Simultaneously, insulin resistance develops (potentially by a number of mechanisms) as the increased hepatic adiposity impedes insulin-mediated glucose uptake in muscle tissue (13), and insulin’s glucagon-inhibiting effect. This insulin resistance, coupled with the relative insulin deficiency, further aggravates hyperglycemia and promotes fat synthesis in the liver (9), completing the deleterious “Twin Cycle Hypothesis” of T2D development (9).
When a person with untreated T2D ingests a meal, glucose accumulates in the blood, either as a result of insufficient insulin secretion by defective beta-cells, or the resistance of target tissues to the insulin, and typically remains elevated for longer than in a metabolically healthy person. The resulting hyperglycemia must be managed effectively (often pharmacologically) to avoid short- and long-term complications. Short-term hyperglycemia can cause an increased risk of infections, diabetic ketoacidosis and other conditions (3). If glycemia is not closely managed, hypoglycemia may ensue which can lead to confusion, loss of consciousness, and if severe, may be fatal (3). Long-term complications of diabetes usually stem from the vascular damage caused by chronic hyperglycemia; over time, vascular damage will accumulate and lead to the deterioration of tissues and organ systems, in particular the heart, kidneys, eyes and nervous system (3). Among people who have T2D, the risk of cardiovascular disease is 2-4 times greater than the rest of the Canadian population, and the risk of hospitalization for renal disease is almost 6 times greater (3). Additionally, the risk of retinopathy and other eye diseases escalates as the duration of T2D increases (3). It is clear that proper glycemic control is vital to one’s health, but since this mechanism deteriorates slowly T2D is often not diagnosed until serious pathogenic processes have taken effect.

In the presence of a handful of non-modifiable risk factors and absence of a focussed effort to make appropriate lifestyle changes, the ability to regulate glycemia will deteriorate into varying degrees of insulin deficiency and resistance until the ability to regulate glucose homeostasis is lost and T2D ensues. If steps are not taken to control or reverse this condition, T2D can lead to a number of serious complications over time.
A.v. Screening and Biomarkers for Type 2 Diabetes

The timing of development of T2D is unique to each individual, but screening tools and the evaluation of clinical biomarkers can be used to determine where a person is balancing in the spectrum of glycemic control. The 2013 Canadian Diabetes Association (CDA) Clinical Practice Guidelines recommends T2D screening every three years in people 40 years of age or older, or younger if they are at high risk as determined by a risk assessment tool (14) such as the CANRISK diabetes risk assessment questionnaire (15). Screening should involve the evaluation of fasting plasma glucose (FPG) levels and/or glycated hemoglobin levels (HbA1c) (14). If an individual is believed to be at high risk for T2D, plasma glucose levels during a 2-hour 75g oral glucose tolerance test (OGTT) (2hPG) may also be evaluated to determine their level of glucose tolerance or diagnose T2D (14,16). Additionally, significantly elevated random plasma glucose (PG) may be evaluated regardless of time of previous meal may lead to a diagnosis of T2D (16). At least one of these four blood tests are required to diagnose T2D, and should be confirmed by either repeating the test, using an alternate test, or evaluating symptoms (16). The CDA’s recognized levels of FPG, 2hPG, random PG and HbA1c that represent prediabetes and T2D in adults are summarized in Table 1.

Table 1. Diagnostic indicators of prediabetes and type 2 diabetes as recognized by the Canadian Diabetes Association 2013 Clinical Practice Guidelines (14,16).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Prediabetes</th>
<th>Type 2 Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (FPG)</td>
<td>6.1-6.9 mmol/L Impaired Fasting Glucose (IFG)</td>
<td>≥7.0 mmol/L</td>
</tr>
<tr>
<td>2-hour Plasma Glucose (2hPG)</td>
<td>7.8-11.0 mmol/L Impaired Glucose Tolerance (IGT)</td>
<td>≥11.1 mmol/L</td>
</tr>
<tr>
<td>Random Plasma Glucose (PG)</td>
<td>-</td>
<td>≥11.1 mmol/L</td>
</tr>
<tr>
<td>Glycated Hemoglobin (HbA1c)</td>
<td>6.0-6.4 %</td>
<td>≥6.5 %</td>
</tr>
</tbody>
</table>
As T2D is a progressive loss of glycemic control, it is critical to identify diminished glycemic control as early as possible to allow for implementation of management and preventative procedures. With the right strategy that is fine-tuned to suit the patient, prediabetes and T2D may be reversed and the onset of this disease may be prevented or delayed significantly. Lifestyle-based interventions to reduce the risk of T2D generally require increased physical activity and significant improvements in dietary habits to effect a reduction of body weight (17). Increased exercise is imperative in all healthy lifestyles, and all efforts should be made to incorporate exercise into one’s daily routine, but a poor diet has the potential to blunt the effects of increased physical activity. There are a number of dietary changes one can make to improve their diet, but understanding and applying the nutritional guidelines can be challenging. Simple dietary substitutions with healthier foods are needed, and functional foods provide an attractive vehicle for bioactive ingredients that have been shown to reduce the risk of T2D. With the introduction of such functional foods into the marketplace, Canadians will have access to a simple and convenient dietary strategy help them reduce their risk of developing T2D.

A. vi. Diet as Prevention and Management of Type 2 Diabetes

The estimated 5.7 million Canadians that have prediabetes (18) and many more who have other risk factors of T2D stand to benefit substantially from making improvements to their diet and lifestyle, and may be able to prevent or delay the development of T2D and avoid its potential life-threatening complications. While increased exercise is imperative for all T2D interventions and prevention strategies, nutritional intervention is co-requisite to reduce the risk of developing T2D. A number of dietary strategies to improve glycemic control and reduce the risk of T2D have been studied. Dietary interventions aimed at modifying protein and fat intake show potential to improve glycemic health (19), but perhaps the most well-known and universally
recommended approach is to modify carbohydrate intake, specifically by increasing dietary fibre intake. The majority of Canadians do not consume their daily recommended adequate intake of dietary fibre each day (20), which illustrates the challenge of consuming enough dietary fibre every day. The rise of highly processed, often low-fibre convenience foods in recent decades may contribute to this problem, but food processing also has the capacity to improve the nutritional quality and fibre content of beloved convenience foods. Functional foods, which may be defined as foods that benefit health beyond providing basic nutrition (21), may be developed with this nutritional intervention in mind and have the potential to address the growing rates of prediabetes and T2D in Canada and worldwide (3). Fibre-fortified functional foods aimed at those who are at risk of the disease need to be developed and made widely available in order to maximize the effectiveness of nutrition-based approach to reduce the risk of T2D.

Meta-analyses and systematic reviews support the idea that consuming diets high in dietary fibre can reduce the risk of T2D (22–24), and even improve glycemic control in people who have T2D (25). Since functional foods have the potential to significantly increase fibre consumption it is important to identify a source of fibre that is effective at improving glycemic control and also a functional ingredient, well-suited to a variety of popular food applications. Resistant starch (RS) is a type of dietary fibre that has shown potential to accomplish both of these goals.

B. Resistant Starch

B.i. Starch

Starch is a granular polysaccharide produced in abundance by plants for energy storage (26,27). Many plants rich in starch (e.g. tubers, pulses, wheat and corn) are considered staple foods around the world and represent a major source of carbohydrate in the human diet (26,27).
There are two types of starch molecules: amylose and amylopectin, which are both composed of repeating glucose monomers linked together by linear α-D-(1-4) glycosidic bonds and/or branch-forming α-D-(1-6) glycosidic bonds (26,27). Amylose contains mostly α-D-(1-4) linked chains of glucose and occasional α-D-(1-6) bonds which create a branch point between glucose chains (26). Amylopectin is much more frequently branched, and also much larger than amylose, up to 2 million degrees of polymerization (DP), whereas amylose is often <6000 DP (26). Typically, the starch fraction is comprised of 75% amylopectin and 25% amylose (28).

Within amylopectin and amylose, glucose chains can spontaneously form single or double helices with themselves or others, which gives rise to higher-order structures. The organization of the higher order structures form the basis of the classification of starch as A-, B- or C-type starch structures (26–28). A-type starch tends to be more tightly packed and is characteristic of cereal starches, while B-type starch is more open-structured with room for water within its framework and is characteristic of tuber starches (26–29). C-type starch is a combination of the A- and B-type structures, and typically found in legumes (26–29). The various starch structures that are formed depend on the presence and expression of various starch synthase, starch branching, and starch de-branching enzymes, among others, that influence amylose and amylopectin content of the starch granule (28). The type of starch molecules and proportions in which they are present within the granule can affect the digestibility of starch once consumed.

In general, when starch is consumed it is hydrolysed by digestive enzymes α-amylase, glucoamylase and sucrose-isomaltase in the small intestine to yield free glucose which can then be absorbed through the walls of the small intestine and into the bloodstream (29). However, for various reasons, some starch is partially or entirely resistant to digestive enzymes and
consequently will pass into the large intestine where it may serve as a substrate for fermentation by the resident gut microflora (30). If not fermentable, some undigested starch may also be excreted in the feces. Early starch researchers Englyst et al. (1982) were able to mimic human digestion in vitro and while investigating methods of measuring of non-starch polysaccharides, they termed the portion of starch remaining after exhaustive digestion with amylolytic enzymes in vitro ‘resistant starch’ (RS) (31). Englyst and colleagues (1985) later confirmed that RS does in fact escape digestion in a physiological environment, as undigested starch was recovered from the effluent of healthy human ileostomy patients (30). Further revealing the timeline of starch digestion, Englyst et al. (1992) used digestive models in vitro to classify various starches as rapidly digestible starch (RDS), slowly digestible starch (SDS) or RS (32): RDS was completely hydrolyzed within 20 minutes of incubation with digestive enzymes; SDS was completely hydrolyzed within 20 – 120 minutes; and RS was the portion of starch that remained after 120 minutes of digestion (33). Physiologically speaking, when starch is consumed the RDS and SDS fractions are completely digested as they pass through the small intestine, but the RS fraction passes undigested through the small intestine and into the large intestine, where it serves as a substrate for fermentation by the resident gut microflora, or is excreted (30).

B.ii. Resistant Starch

RS is a class of starches that are resistant to digestion. RS is present as a natural component of some foods, for example some of the starch produced by plants such as corn, bananas and legumes is RS. RS may also be produced as a result of food processing, for example cooked and cooled potatoes (29). RS is present in some whole foods, and it can also be extracted from certain plants through milling, and used as an ingredient (29). It is estimated that the typical American diet provides 3-8g RS/day (34), and although Canadian RS intake estimates
are not available, it is likely to be similar. Intakes of RS are much higher (30-40g RS/day) in some developing nations with a greater dependency on starch in the diet (27). It is important to note, however, that the estimated RS content in certain foods may fluctuate with the various methods of analysis and RS and fibre definitions around the world.

When consumed, RS travels through the gastrointestinal tract with more similarities to dietary fibre than starch, and as a result, RS is generally considered its own class of fibre from a nutritional standpoint. Like other dietary fibres, all RS escapes digestion in the small intestine, but there are a number of factors that impart this resistance. As a result of these differences, four different subtypes of RS have been identified (Table 2).

**Table 2. Resistant starch (RS) subtypes (26,27,29).**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Description</th>
<th>Food Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS&lt;sub&gt;1&lt;/sub&gt;</td>
<td>• Starch is protected from digestive enzymes by cell walls or other non-digestible matrix</td>
<td>• Seeds, partially milled grains, some legumes</td>
</tr>
<tr>
<td>RS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>• Tightly packed molecular structure within the starch granule&lt;br&gt;• ‘Granular RS’</td>
<td>• Unripe banana starch, raw potatoes, high-amylose maize (corn)</td>
</tr>
<tr>
<td>RS&lt;sub&gt;3&lt;/sub&gt;</td>
<td>• Formed during processing, especially during moist heating then cooling&lt;br&gt;• ‘Retrograded RS’</td>
<td>• Cooked and cooled potatoes, bread, cornflakes</td>
</tr>
<tr>
<td>RS&lt;sub&gt;4&lt;/sub&gt;</td>
<td>• Created by cross-linking starch with chemical reagents&lt;br&gt;• ‘Chemically modified or cross-linked RS’</td>
<td>• Modified starches in some fortified foods i.e. certain enriched drinks or breads</td>
</tr>
</tbody>
</table>

For each subtype of RS, there are also factors that will make the starch more available to digestion, thus reducing the resistance of the starch. For example, RS<sub>1</sub> is susceptible to chewing and further milling which will expose the starch molecule (26,27,29). Alternatively, RS<sub>3</sub>, formed during retrogradation of starch, may be lost during further processing (26,27,29). Furthermore, some RS<sub>2</sub> is subject to food processing which may cause gelatinization (26,27,29). However,
some types of RS\textsubscript{2} such as the starch in high-amylose maize (HAM) can also resist gelatinization during cooking and processing (22), due to its much higher gelatinization temperature (26,27,29). The resilience of RS\textsubscript{2} makes this particular type of RS a good candidate for incorporation into functional foods that require some processing, such as baked goods.

Health and regulatory authorities in Canada and around the world now recognize the health benefits of consuming RS and accept RS as a healthy component of, or ingredient in, foods. The Institute of Medicine (IOM) considers naturally occurring RS to be dietary fibre, while isolated, extracted, or manufactured RS is considered functional fibre (35). Health Canada considers some types of RS to be a fibre as well, and classifies certain sources of fibre as ‘novel sources’ of fibre if they are manufactured or modified from their natural source (36). One example of a novel source of fibre is HAM, which Health Canada recently accepted for use as a source of dietary fibre (RS\textsubscript{2}) that may be used in foods in Canada (37), and contributes to the total dietary fibre declaration on a Nutrition Facts table (36). HAM is also recognized in Europe as a source of fibre (RS\textsubscript{2}) where the European Food Safety Authority (EFSA) has accepted certain health claims that link consumption of HAM-RS\textsubscript{2} to digestive health benefits, normal colonic metabolism and healthy blood glucose levels (38). With EFSA starting the trend to approve health claims for RS, and Health Canada’s acceptance of RS as novel sources of fibre, there are numerous opportunities for RS to be incorporated into novel functional food formulations.

**B.iii. Resistant Starch as a Functional Ingredient in Bread-Based Functional Foods**

RS\textsubscript{2} is available commercially as an ingredient called Hi-Maize\textsuperscript{®} 260, from Ingredion Inc. (Brampton, ON, Canada) (Appendix A). Hi-Maize\textsuperscript{®} 260 is pure cornstarch obtained from HAM (39). HAM is referring to selectively bread corn lines (i.e. amylose extender (ae-) maize)
that were found to exhibit less starch branching enzyme activity (specifically SBEIIb), and therefore, altered starch production (28). The amylopectin in ae- maize starch has fewer branching points and longer glucose chains, which give it “amylose-like properties” (28) and leads to the term ‘high-amyllose’ maize (40). The high ‘apparent’ amylose starch is tightly packed within the starch granules which imparts resistance to enzymatic digestion when consumed (29). The RS in HAM is classified as RS2, or HAM-RS2 (29).

Hi-Maize® 260 is a flour-like, white, odourless and bland-tasting powder that maintains its granular RS structure during food processing, and yields 60% RS2 (39). This ingredient can be incorporated into a number of food applications due to its mild sensory impact. Since Hi-Maize® 260 is a starch and has a flour-like texture and appearance, it lends itself particularly well to bread and bread-based products.

Bread products are commonly consumed in Canada, and the healthy bread market is extensive. The familiarity, utility, and popularity of bread products make them a prime target to advance the functional food market in Canada. Bagels epitomize this opportunity as they are versatile, easy to prepare, inexpensive, and a convenient food commonly enjoyed in Canada. The nutritional quality of bread products can vary greatly however: typical white bread products are a rich source of highly digestible carbohydrates (RDS) with very little fibre per serving; whole grain products often provide higher amounts of fibre though often at a cost to its palatability or sensory appeal.

Hi-Maize® 260 can replace a portion of the wheat flour in bread formulations with minimal impact on the texture and flavour of the product, and without detrimental effects on the structure of the resulting dough (39). By making this substitution, a portion of the highly digestible carbohydrates in bread is replaced with RS2, thereby increasing the dietary fibre
content and enhancing the nutritional quality of the product. Previous research indicates up to 60% of standard flour can be successfully replaced with Hi-Maize® 260 (41), whereas traditional fibres tend to be much more coarse and not conducive to dough development at this level of inclusion. For example, when wheat bran is added to dough, its sharp-edged particles can disrupt the critical networks of gluten that are formed and stretched during mixing and proofing, which will weaken the dough significantly. Furthermore, RS is well tolerated at high doses, with intakes around 60g RS$_2$ from HAM per day causing no adverse effects (42). This high-RS formulation strategy presents enormous opportunity to develop bread-based functional foods that provide significant amount of RS, and as a result, deliver fibre-related health benefits.

Bread formulations, including bagels, can benefit from the incorporation of RS, which has potential to earn them sought-after, fibre-related health claims. As previously mentioned, HAM-RS$_2$ has been accepted as a novel source of fibre and content claims are permitted if the food provides sufficient fibre from RS per serving. However, at present there are no permitted function claims that pertain to RS consumption. There is future potential for such RS function claims to be approved by Health Canada, as a Draft Guidance Document on Food Health Claims Related to the Reduction in Post-Prandial Glycemic Response was released in 2013 (43). Comments from the public were accepted until September 2013, though a final guidance document has not been released to date. In Europe, however, some RS and glycemic health claims have already been accepted. In 2011, EFSA published its scientific opinion that RS-specific health claims pertaining to its ability to the reduce postprandial glycemic response are substantiated in the current literature (38). Australia has also accepted RS-related health claims, and there are numerous products made with high-RS ingredients that are available to consumers in Australia (27). The food labeling regulatory body in Australia, Food Standards Australia New
Zealand (FSANZ), currently lists the RS-postprandial glycemia relationship as under systematic review for consideration of a potential ‘high-level health claims’ (similar to Canadian function claims) to be permitted in the future (44).

Fibre-enriched bread products are positioned well in the Canadian marketplace, as Canadians are becoming more aware of the benefits of higher-fibre diets (45). Given the insufficiency of dietary fibre in Canadian diets, the majority of Canadians would benefit from higher-fibre foods. Specifically, those at risk of, or who currently have T2D, stand to benefit the most from increasing their RS intake. High-fibre functional foods such as high-RS bagels would provide a nutritious and feasible strategy to increase RS intake among Canadians, with a view to reducing the risk of T2D. Research is needed, however, to support the consumption of RS as a strategy to reduce T2D before RS-centered functional foods are marketed to those at risk for the disease.

C. The Role of Resistant Starch in Improving the Glycemic Response

RS is a dietary fibre, a class of carbohydrates well studied for its effects on improving the glycemic response. A number of human clinical trials have been conducted to better understand the effects of both acute and chronic RS consumption on glycemic response. Studies looking at the acute effects of RS consumption show the direct effects of RS on the postprandial glycemic response to that specific meal, whereas chronic interventions reveal the potential of RS to improve both fasting and postprandial glycemic response. Researchers have also explored RS interventions in the form of supplements and RS-enhanced foods. RS supplement studies allow for the investigation of an isolated or concentrated compound, though supplements may face challenges related to acceptability into a typical diet. RS-enhanced foods are more easily incorporated into a typical diet, but face challenges in study design with controlling for
potentially confounding variables within the food matrix. Nonetheless, numerous human clinical trials of RS consumption have been conducted and overall, demonstrate the ability of RS to attenuate the glycemic response. The following is an organized review of the literature that looks at the effects of acute and chronic consumption of RS supplements and RS-enhanced foods on glycemia.

C.i. Acute Human Intervention Studies

In order to examine the immediate effects of RS consumption on glycemic response, a number of human intervention studies of acute RS consumption have been conducted. Over the past three decades researchers have examined RS consumption in both supplement- and food-form. Studies that examine RS supplements have precise control of the dose of RS, and by nature, reveal the effects of RS alone, minimizing any potentially confounding variables associated with a food matrix. Supplement studies do have a drawback however, as they may be perceived as a drug or medicine and face challenges being accepted into the diet. To overcome this challenge researchers also study foods that naturally contain RS, or incorporate high-RS ingredients into foods to investigate the effects of consuming an RS meal. Regardless of the supplement or food format used, RS studies are inconsistent with respect to the choice of control treatments which may be designed to match the total or available carbohydrates of the test product. When treatments are matched on the basis of total carbohydrates, the high-RS treatment will yield far fewer available carbohydrates, which in itself could cause a reduction in glycemic response. However, when treatments are matched in terms of available carbohydrates, the high-RS treatment would be larger in total serving size which could hamper the reduction in glycemic response, if present. While neither study design is perfect, both offer insight to the potential
benefits of RS consumption. Considered together, the many acute RS intervention studies demonstrate the ability of RS consumption to attenuate the postprandial glycemic response.

C.i.a. Acute Human Intervention Studies: Resistant Starch Supplements

(Summarized in Appendix B)

The acute glycemic effects of RS in supplement form was first examined by Raben et al. (published in 1994) (46) using a randomized crossover design. Ten healthy, normal-weight males consumed raw potato starch with or without added RS (27.1 g), matched for total carbohydrates and mixed into an artificially sweetened fruit syrup after which postprandial blood glucose and insulin were measured (46). The RS treatment was able to significantly reduce the peak 2- and 5-hour area under the curve (AUC) for both blood glucose and insulin compared with the control (46).

Using a similar acute study design Haub et al. (published in 2010) (47) also compared RS treatments that were matched for total carbohydrate content when they compared the effects of consuming 30 g RS₄ (from wheat), 30 g RS₂ (from HAM) or a dextrose control on postprandial glucose in 11 healthy adults. This study also found that the RS treatments significantly reduced glucose iAUC (incremental AUC) compared to control (47), which may be explained by the difference in available carbohydrates. Interestingly, the magnitude of reduction in glucose iAUC was significantly greater for the RS₄ relative to the RS₂ treatments (47), despite likely providing equal available carbohydrates.

In a different study designed to match treatments for available carbohydrate content published in 2010, Bodinham et al. (48) examined the acute effects of consuming 0 or 24 g RS₂ supplements mixed into a flavoured mousse at breakfast and lunch, in 20 healthy males. When matched for available carbohydrate content, the RS treatment in this two-meal study also had no
significant effect on postprandial glucose or postprandial insulin sensitivity (during a meal tolerance test (MTT) using the MINMOD calculation), but did significantly reduce the total (7-hour) postprandial insulin AUC when consumed for both meals (48). This may have been due to the longer test period and second meal study design which allowed time for the RS to significantly reduce the insulin response, as the insulin response to the first RS breakfast was not different from the control meal.

In another study designed to examine the effects of RS treatments matched for available carbohydrates, Haub et al. (published in 2012) (49) tested the acute consumption of 30 g RS$_4$ from two different raw potato starches in 10 healthy males. Each starch was mixed into a dextrose or a water beverage, with the control as the dextrose beverage without RS (49). After either RS-beverage consumption, there were no significant differences in postprandial glucose compared to control (49), consistent with the results of Bodinham et al. (48). This study’s results were limited however, as Haub et al. (49) did not measure postprandial insulin, which is an important endpoint that Bodinham et al. (48) showed could be modified by RS consumption.

While RS supplement studies that match for available carbohydrate do not seem to significantly reduce the postprandial glucose response, they have demonstrated an ability to reduce the insulin required to handle the glucose. Further, looking closely at the treatments, when matched for available carbohydrates, the high-RS treatments require a larger serving size, and provide more carbohydrates in total. The fact that the glycemic response is not different among supplements of with varying carbohydrate content is appreciable however, and this effect could be exploited by food, by substituting RS in place of regular starch as a means of reducing available carbohydrates.
C.i.b. Acute Human Intervention Studies: Resistant Starch Functional Foods

(Summarized in Appendix C)

It is of value to study RS in a food matrix to increase relevance of the results to the food industry, healthcare professionals and consumers. Due to the flour like texture of some commercially available RS sources, it has been incorporated into a diversity of foods including sliced bread, muffins, chip, pasta and pizza crusts, though other foods (e.g. rice) have also been modified in some way to contain higher levels of RS. When designing and testing a food that provides increased amount of RS, the nutritional composition of the food matrix must be considered. The non-RS portion of the food or meal will contribute additional macronutrient value, in particular available carbohydrates, which may interact with the effects of RS on postprandial glycemia. Despite the added complexity, humans consume food to a much greater extent than supplements, so it is important to determine if RS incorporated into a food or meal can benefit the postprandial glycemic response. The importance of this concept is clear, as evidenced by the abundance of clinical trials on RS-enhanced foods and glycemia.

Goddard et al. (published in 1984) (50) was the first study to examine the glycemic effects of RS in a food matrix by using starch composed of various ratios of amylose to amylopectin. RS was not routinely quantified from starch at the time, but it is now known that starch with higher amylose content is associated with higher RS content (29). Goddard et al. (50) performed a crossover study of 33 healthy adults who consumed 50 g of carbohydrates of 3 different rice varieties with 0%, 14-17%, or 23-24% amylose fractions, or a glucose tolerance beverage, and measured the acute postprandial glucose and insulin response over 3 hours (50). This study revealed that the highest amylose rice caused a significantly lower peak in glucose at 30 minutes and insulin at 30 and 60 minutes compared to the 0% amylose rice (50). While total
glucose AUC was not different among rice treatments, insulin AUC was significantly lower after the highest amylose rice compared with the 0 and 14-17% amylose rice (50).

Behall et al. (51) had similar results in their crossover study (published in 1988) of 25 healthy adults that studied the acute effects of consuming high (70%) or low (30%) amylose crackers on the postprandial glucose and insulin response. Compared to the low-amylose crackers, the high-amylose crackers (matched for total carbohydrate content) significantly reduced the glucose response at 30 minutes but then significantly increased the glucose response at 120 and 180 minutes, resulting in no significant difference in summed glucose levels above fasting (51). Furthermore, the high-amylose crackers significantly reduced the insulin response at 30 and 60 minutes as well as the summed insulin above fasting levels, compared to the low-amylose crackers (51). The results from Behall et al. (51) and Goddard et al. (50) reveal that the starches were equally digested by the end of the 3 hour postprandial period, however the high-amylose treatments caused a more level and sustained glucose response while reducing the amount of insulin required for glucose clearance from the blood.

Hospers et al. (52) also conducted a crossover study (published in 1994) on the acute effects of amylose-containing foods on postprandial glycemia, and modulated the amylose content of their study-treatments, this time in four variations of pasta made with: regular durum flour (24.5 % of carbohydrates from amylose); durum flour with regular cornstarch (25.9% of carbohydrates form amylose); durum flour with HAM flour (39.6% of carbohydrates from amylose); and durum flour with very high amylose cornstarch (41.9% of carbohydrates from amylose). Sixteen healthy males consumed each of the study pastas (matched for total carbohydrate content and served with tomato sauce, ground beef and cheese) and had their glucose and insulin response measured for 3 hours (52). There were no significant differences in
glucose 3-hour AUC when each pasta was considered individually, but total and net 3-hour glucose AUC of both high-amylose pastas combined was significantly reduced compared to the normal pasta (with or without cornstarch) combined, and the combined high-amylose pastas 1-hour total and net glucose AUC were significantly reduced when compared to the normal (with cornstarch) pasta only (52). The effects on the insulin response were more pronounced, as each of the high-amylose pastas individually significantly reduced the total 3-hour insulin AUC compared to both normal pastas individually, and the net glucose AUC compared to the normal (without cornstarch) pasta only (52). There were no significant differences between the high-amylose pastas on either glucose or insulin AUC, suggesting a plateau effect beyond a certain level of amylose inclusion, or more likely, that the difference between levels of amylose in the two high-amylose treatments was not sufficient to produce statistically significant differences in glycemic response (52). The results of this study agree with Goddard et al. (50) and Behall et al. (51) who demonstrated that total-carbohydrate matched, high-amylose meals significantly reduce postprandial insulin response compared to low-amylose meals, though in contrast to the same studies, the present study demonstrated the ability of high-amylose meals to modestly reduce the glucose AUC.

Achour et al. (published in 1997) (53) also examined the acute effects of consuming high-amylose starch based meals on postprandial glucose and insulin in their crossover study of 8 healthy adults. High-amylose starch or regular starch was mixed with sucrose and water to make a gel, and consumed with cheese and tea or coffee, with each meal providing equal amounts of total carbohydrates (53). Postprandial glucose and insulin were measured for 8 hours following consumption of breakfast test meals, and for an additional 3 hours the next day after consuming the same test meal for a late dinner (53). Postprandial glucose and insulin responses
were significantly reduced following the HAM-based breakfast compared to the regular cornstarch breakfast, but no significant differences in glycemic response were present during the 3-hour period the following day (53). The significant reduction of insulin AUC or summed insulin by high-amylose foods have been demonstrated previously by Goddard et al. (50), Behall et al. (51) and Hospers et al. (52), but this study also demonstrated that a high-amylose food, controlled for total carbohydrate content, caused a significant reduction in glucose AUC, in line with Hospers et al. (52), though more markedly and over an 8-hour period.

Behall and Hallfrisch (published in 2002) (54) also studied the acute effects of varying amylose content of foods on resulting glycemia in their crossover study of breads with 30, 40, 50, 60 and 70% amylose content in 25 healthy adults. This study demonstrated that among treatments with equivalent total carbohydrate content, increased amylose content (higher than the test meals in Hospers et al. (52)) reduced glucose excursion, as the 70% amylose bread significantly reduced the 2-hour glucose AUC compared to the 30-50% amylose breads (54). In addition, the glucose peak at 30 minutes was also significantly reduced following 50-70% amylose bread consumption, compared to 30-40% amylose breads (54). With respect to insulin response, the 60-70% amylose breads significantly reduced the 2-hour insulin AUC, 30 minute (70% amylose bread only) and 60-minute insulin compared to all other breads (54). Lastly, the glucagon response was also significantly reduced by all bread treatments compared to the glucose control, but no significant difference was found between bread treatments (54). van Amelsvoort and Westrate (published in 1992) (55) examined the acute effects of consuming total carbohydrate-matched mixed meals of ham and vegetables in tomato sauce, with either high-amylose rice and HAM starch or regular rice, and both fresh and after overnight storage and reheating. This crossover study of 22 healthy adult males revealed that the high-amylose meals
significantly reduced the glucose response during the first hour, but then significantly increased
the glucose response at 120, 240 and 360 minutes, resulting in an increased total and net glucose
AUC compared to low-amylose meals (55). The initial glucose-lowering effects are similar to
Behall and Hallfrisch (54), but the study by van Amelsvoort and Westrate (55) was the first to
show an increased glucose response to a high-amylose meal compared to low-amylose meal
when the extended 6-hour postprandial period is considered. This is inconsistent with Achour et
al. (53) who observed an overall reduction in glucose response to high-amylose food over an 8-
hour period. With respect to insulin, this study revealed that the high-amylose meals
significantly reduced the insulin response at 30, 60 and 240 minutes as well as total and net AUC
compared to low-amylose meals (55), consistent with previous studies that have demonstrated a
reduced insulin response to high-amylose foods (50–54). A novel aspect of this study’s design is
that it examined the effects of storing and reheating the high- and low-amylose meals before
consumption, although this process only significantly reduced the glucose response at 60
minutes with no effect on total or net glucose response unless combined with the effects of
amylose content and storage, when net glucose AUC was reduced compared to fresh, low-
amylose meals (55). The insulin response was also only significantly reduced after consuming
the stored and reheated high-amylose meals, but at 120 and 240 minutes, as well as after the
whole 6-hour total and net insulin AUCs (55). The results of this study revealed that high-
amylose meals reduced the glucose and insulin response during the first hour postprandially, then
proceeded to increase glucose while decreasing insulin several hours after the meal, and that this
effect may be increased in high-amylose meals after overnight storage and reheating (55).

Westrate and van Amelsvoort (published in 1993) (56) also studied the glycemic effects
of consuming high- and low-amylose foods, but this time also exploring a second-meal effect in
their crossover study design. This study provided 22 healthy adult males with high- and low-amylose starches in various foods in two meals (apple-filled breakfast bread and second meal pizza lunch), and found that the high-amylose breakfast significantly reduced the insulin AUC compared to low-amylose breakfast, though there were no significant differences in glucose AUC between breakfast treatments (56). After the second meal, the glucose AUC was significantly reduced after high-amylose second meal regardless of breakfast type, while the insulin AUC was significantly reduced only when both meals were high-amylose, compared to the low-amylose meals (56).

Krezowski et al. (published in 1987) (57) explored the acute effects of a high-amylose muffin compared with low-amylose muffin, cornflake, and glucose controls, on the glucose and insulin response. This study is unique in its participant sample of adult males diagnosed with T2D (n=9) (57). In contrast to previously mentioned studies (50,51,53–56), this study found that the high-amylose muffins significantly reduced the 3-hour glucose iAUC compared to all other treatments without significant differences in the insulin response between treatments (57). As both high- and low-amylose muffins were matched for total carbohydrate content, the reduction of available carbohydrates may account for the reduction of glucose response, as seen in some previously mentioned acute RS-supplement studies (46,47). Reader et al. (published in 2002) (58) also focused on participants with T2D (n=10) in their crossover study that measured the 5-hour postprandial glucose and insulin response to 3 different snack bars: RS-bar (7.25 g RS), an energy bar (no RS) and a Snickers Bar® (no RS). While each snack bar provided approximately 49 g of total carbohydrates, their macronutrient distribution varied greatly (58). Similar to Krezowski et al. (57), the RS-bar significantly reduced glucose iAUC (in addition to peak glucose at 90 minutes) compared to control treatments (58). The RS-bar in this study also
significantly reduced insulin iAUC and concentration at 90 minutes compared to the energy bar, but not compared to the Snickers Bar®, which the authors attributed to the difference in macronutrient composition between all treatments (58). When considering just the RS-bar and energy bar (more closely matched for lipid and protein content), the RS-bar did significantly reduce glucose and insulin iAUC compared to the energy bar (58). This study demonstrated the benefits of consuming RS in a food, while emphasizing the importance of the macronutrient distribution of the food.

Granfeldt et al. (published in 1995) (59) studied the acute effects high-RS arepas while controlling for both total and available carbohydrates, using HAM to increase RS$_2$ content in the study treatments. In addition to the control arepa (1.8 g RS), two test arepas were studied including a high-RS arepa (24.7 g RS) matched to the control arepa for available starch, and a mid-RS arepa (15.9 g RS) matched to the control for total starch (59). In the sample of 9 healthy adults, both test arepas significantly reduced glucose and insulin iAUCs compared to control arepa, with no significant differences between the two arepas despite one arepa providing a larger portion of available starch (59). The authors noted the lack of difference in responses between the high- and mid-RS arepas may be due to the relatively high amount of RS in both arepas, which might have overcome the 16 g difference in available carbohydrates (59). This study was among the first to identify an effective amount of RS in a food matrix to reduce the glycemic response compared to a similar food.

Li et al. (60) and Anderson et al. (61) (both published in 2010) also studied the effects of acute RS consumption when matched for total carbohydrate content on postprandial glucose (60, 61) and insulin (60) response in their crossover studies, each among 16 healthy adults. In the study by Li et al. (60) the treatments consisted of high-amylose rice (8 g RS), regular rice (1 g
RS), or a glucose control beverage. The high RS rice significantly reduced peak glucose and glucose iAUC compared with the regular rice for 4 hours postprandially (60). The RS rice also significantly reduced insulin iAUC compared to regular rice for 2 hours postprandially (60). In the study by Anderson et al. (61) treatments were tomato-based soup containing either 19 g RS from regular cornstarch, 23 g RS from HAM, 27 g RS from whole-grain HAM cornstarch (all high-RS soups); 6 g RS from maltodextrin (low RS-soup); or a starch-free soup. All three high-RS soups elicited glucose iAUCs significantly lower than the maltodextrin (6 g RS) control, and higher than the starch-free soup (61). Further, the whole grain (27 g RS) soup significantly reduced glucose iAUC compared to the regular cornstarch (19 g RS) soup (61), though is it not clear if this was due to the increased amount of RS in the whole-grain HAM soup, or because the whole-grain HAM RS treatment provided less rapidly and slowly digestible starch content (therefore less readily available starch). Regardless, all RS treatments in this study reduced postprandial glucose response compared to the maltodextrin control matched for total carbohydrates in healthy adults (61). The glucose-lowering results of the study by Anderson et al. (61) are consistent with the results of Li et al. (60), and Behall and Hallfrisch (54), who both demonstrated the ability of RS-enhanced food to reduce the postprandial glucose and insulin response among healthy adults when compared to control matched for total carbohydrates.

Behall et al. (published in 2006) (62) also controlled for total carbohydrates in their crossover study that examined the acute glycemic effect of muffins enhanced with three levels of RS$_2$ (0.9-6.5 g RS) as well as three levels of beta-glucan (BG) in 10 healthy-weight (BMI < 25 kg/m$^2$) women and 10 overweight (BMI > 25 kg/m$^2$) women without T2D. However, the results were not significantly different between normal weight and overweight groups so the groups were combined for the statistical analysis (62). This study determined that the high- and
medium-RS muffins (both with high BG content) significantly reduced glucose AUC compared to the glucose control and the low-RS/low-BG and low-RS/medium-BG muffins (62). In addition, the high-RS/high-BG muffin significantly reduced the insulin AUC compared to the glucose control and all medium- and low-RS muffins (62). The results of this study are consistent with previous research that demonstrates a reduction in postprandial glucose (54,60,61) and insulin (54,60) response following consumption of RS-functional foods, compared to total carbohydrate-matched controls.

Along the same lines as Behall et al. (62), Yamada et al. (published in 2005) (63) also explored acute RS consumption matched for total carbohydrates, though this time among both healthy (n=8) and “borderline glucose intolerant” individuals (with increased fasting glucose levels; n=12). Participants consumed sliced bread made with tapioca starch (6 g RS) or regular bread (0.9 g RS) and had their postprandial glucose and insulin response measured (63). This study revealed that sliced bread with 6 g RS significantly reduced the difference in glucose from baseline at 60 and 90 minutes, as well as both glucose and insulin AUCs compared to control bread, though only in the borderline glucose intolerant group (63). The healthy group of participants derived no significant benefits on glycemic response from RS bread consumption (63). The distinction of ‘borderline’ glucose tolerance in this study is based on fasting glucose levels ≥111 mg/dl (or approximately 6.2 mmol/L) which is slightly elevated but below the cut-off for diagnosis of T2D, according to current Canadian clinical practice guidelines (16), so the results cannot be compared to those of Krezowski et al. (57), who studied people with T2D. Since some of the participants in the study by Yamada et al. (63) were healthy while others met the CDA criteria for IFG (but did not have T2D), the improvement in postprandial glycemia was not significant when the entire sample was considered together, further indicating that those with
increased fasting glucose levels respond to RS consumption to a greater extent than ‘healthy’ people. This research also suggests that IFG status may be a more important indicator of potential RS-benefit than being overweight (defined by BMI >25 kg/m² (64)), since Behall et al. (62) separated participants based on elevated BMI but found no statistical significance between healthy and overweight participants.

To summarize at this point, acute consumption of RS-enhanced foods have been shown to reduce the postprandial glucose and/or insulin response in healthy people (50,51,54–56,59–62), people with borderline impaired glucose tolerance (63), and people with T2D (57). While this research does illustrate the potential of RS to modulate glycemic response, it is possible that these results are due to the reduced available carbohydrate that accompanies food with sizable RS content matched to a control for total carbohydrate. In effort to reduce variation in available carbohydrates as a potentially confounding variable, the following studies explored the acute consumption of RS compared to control foods matched for available carbohydrate content.

Ekstrom et al. (published in 2013) (65) and Hallstrom et al. (published in 2011) (66) both conducted crossover studies on the acute intakes of RS₂-enhanced sliced bread, with controls matched for available starch content, among healthy adults. These researchers found that bread with 6.7-9.1 g RS (65) and 7.7-11 g RS (66) per serving significantly reduced the glucose response (iPeak (65,66) and iAUC (66)) and the insulin response (iPeak (65)) compared to controls. In the Hallstrom et al. (66) study however, insulin iAUC was significantly increased from 60-180 minutes postprandially, with no significant difference in 2-hr insulin iAUC between treatments (66). The authors mention this reduction of insulin iAUC early on, followed by increased late-phase insulin may be explained by presence of some slowly digestible starch produced by the pumpernickel baking methods used in the study (66).
Al-Tamimi et al. (published in 2010) (67) and Klosterbuer et al. (published in 2012) (68) also both examined the effects of acute consumption of RS-enhanced food matched to controls for available carbohydrates on the postprandial glucose and insulin response among healthy adults. Al-Tamimi et al. (67) examined the effects of wheat-based snack bars with 0 or 14 g RS$_4$, or a glucose control beverage in their crossover study (n=13). Klosterbuer et al. (68) tested mixed meals (including a muffin, cereal and a beverage) with 25 g RS$_3$ or 25 g soluble corn fibre (SCF), each with and without 5 g pullulan, and a control meal with no added fibre in their crossover study (n=20). Glycemic response was measured as iAUC by Al-Tamimi et al. (67) and as AUC of increase from baseline by Klosterbuer et al. (68). Despite differences in the food forms used and the type and amount of RS in their respective treatments, the RS-foods in both studies significantly reduced the glucose and insulin response compared to control treatments (67,68). In the Klosterbuer et al. (68) study however, only the RS treatment combined with 5 g pullulan significantly reduced the insulin AUC compared to the control treatment, while both RS treatments (with and without pullulan) significantly reduced insulin AUC compared to SCF treatments. Together, these studies contribute to the growing body of evidence (56,61,62-glucose only) to support the ability of RS-foods to reduce the postprandial glucose and insulin response when matched for available carbohydrates, among healthy adults (67,68).

Behall et al. (published in 2006) (69) also controlled for available carbohydrates in a crossover study of acute consumption of three levels of RS$_2$ (0-9 g RS) incorporated into muffins with three levels of BG. This study is very similar to previous research among women from the same authors (62), but focussed on the postprandial glucose and insulin responses among men, 10 of whom were healthy-weight (BMI < 25 kg/m$^2$) and 10 were overweight (BMI > 25 kg/m$^2$) without T2D (69). The overweight men had significantly increased glucose and insulin
responses compared to healthy-weight men, but since there was no significant interaction between participant group and treatment the results were presented with both groups combined (69). The high- and medium-RS muffins in this study, when combined with high-BG, significantly reduced glucose AUC compared to medium-RS/medium-BG and low-RS/medium-BG muffins, which does not demonstrate a glucose lowering effect of RS on its own (69).

Similarly, all high-BG muffins (with all 3 levels of RS content) significantly reduced insulin AUC compared to the glucose control and all other muffins, indicating that the RS content did not affect postprandial insulin as much as BG did in this study (69). Behall et al. (69) pointed out that no participants had fasting glucose levels that would qualify them for the “borderline” group by Yamada et al. (63), which could help explain the lack of significant results due to RS treatment in the present study, the results are still not consistent with previously mentioned available starch-matched, acute RS studies in healthy participants (59,65–68).

Behall and Scholfield (published in 2005) (70) also explored if the postprandial response to acute RS consumption is related to the health status of the individual in their crossover study among 12 healthy and 12 hyperinsulinemic adults who consumed both corn chips and muffins made with either high-amylose corn starch (HAM-RS) or standard cornstarch. Though the actual amount of RS each serving provided is not clear, high- and low-amylose treatments were matched to provide 1 g available carbohydrate per kg body weight per person (70). This study found while the hyperinsulinemic participants had significantly higher glucose and insulin AUCs compared to control group within each treatment, both groups of participant had significantly reduced glucose and insulin AUC after high-amylose muffins foods compared to low-amylose foods (70). Interestingly, this study further explored the effects of food form (muffins and chips) and particle size (with and without corn meal) on glycemia and determined that the moist food
form (muffins) was more effective at reducing the glycemic response compared to increasing to particle size by adding cornmeal (70). The results of this study not only add to the growing evidence that acute RS-enhanced food consumption, when matched for available carbohydrates, reduces the postprandial glucose and insulin response, it contributes evidence that this effect may also apply to hyperinsulinemic adults (70).

MacNeil et al. (published in 2013) (41) extended this research by investigating acute RS-enhanced food consumption in a sample of adults with T2D. MacNeil et al. (41) incorporated HAM-RS$_2$ into a bagel and tested various combinations of RS-bagel and standard bagel to determine the effect of RS treatments matched to control for total and available carbohydrates. Treatments A (control bagel, 1 g RS) and B (22 g RS) were matched for total carbohydrates, but treatment B provided fewer available carbohydrates (41). When compared, treatment B significantly reduced the peak glucose and glucose at 60, 90, and 120 minutes postprandially (41). Also after treatment B, the 3-hour glucose iAUC was reduced compared to treatment A though did not reach statistical significance ($P=0.07$) (41). Similarly, treatment B significantly reduced peak insulin, insulin at 90 and 120 minutes, as well as 3-hour insulin iAUC compared to treatment A (41). These results demonstrate that RS-bagel can reduce the postprandial glucose and insulin response when matched to control for total carbohydrate in adults with T2D.

Treatment C (33 g RS) matched the control bagel for available carbohydrates and provided a greater amount of total carbohydrates (41). Treatment C did not significantly reduce the glucose or insulin response compared to treatment A, despite its high RS content, which indicates that acute RS consumption does not significantly improve glycemia when matched for available carbohydrates in adults with T2D (41). This study also examined the effect of RS consumption after a second meal, and found that treatment C significantly increased insulin iAUC compared
to treatment A, which the authors noted to be a favourable response among people with T2D, who often exhibit a blunted insulin response (41). The present study agrees with the limited research among individuals with T2D, as Krezowski et al. (57) also found that high-amylose muffins matched to control for total carbohydrate content significantly reduced the postprandial glucose response with limited effect on the postprandial insulin response. However, this is the only study known to have examined the acute effects of RS treatments matched for available carbohydrates in people with T2D. Since previous research in people with hyperinsulinaemia suggest such treatments do attenuate the postprandial response (70), it is possible that the amount of RS required by individuals with overt T2D is higher than those with hyperinsulinaemia, or that the advanced disease process of T2D is not modifiable through this dietary intervention.

C.ii. Chronic Human Intervention Studies

Since acute RS consumption demonstrates a promising ability to reduce postprandial glycemia, the logical progression is to investigate the effects of chronic RS consumption on glycemic handling of a standardized meal. Improvements in glycemic handling of a standard meal would contribute to a reduced risk of developing T2D. Many studies of chronic RS consumption have been designed to investigate such interventions in a variety of populations that range from people who exhibit normal glucose tolerance to those who have T2D. Such studies have looked at RS supplements as well as foods and face challenges parallel to those of acute interventions, though issues of acceptance and palatability pose a greater risk in chronic intervention studies which require longer-term compliance. The issue of matching control treatments for total or available carbohydrates that exists in acute RS studies is not as predominant in chronic intervention studies, since in chronic RS studies, participants consume the study treatments incorporated into their usual diet, which will vary in total and available
carbohydrate content regardless of the intervention. Furthermore, chronic RS studies examine overall glycemic handling by measuring the postprandial glycemic response to a standardized meal, as opposed to measuring the acute response to a RS-meal that is also affected by other aspects of the meal (e.g. changes in available or total carbohydrates). Chronic intervention studies have the potential to demonstrate the ability of including RS into a diet to improve glycemic handling, and further, to reduce the risk of T2D. Due to the substantial variation in the study designs, intervention types, and study populations employed, the literature to date provides inconsistent evidence to support this proposition. The following is an organized review of the literature that looks at the effects of acute and chronic consumption of RS supplements and RS-enhanced foods on glycemia.

C.ii.a. Chronic Human Intervention Studies: Resistant Starch Supplements

(Summarized in Appendix D)

Commercially available HAM-RS$_2$ has a fine, powder-like texture with minimal taste and colour, and is readily mixed into beverage or added to foods by a consumer (39). Such qualities make it an ideal supplement that is most frequently used in chronic RS supplement intervention studies.

Robertson et al. (published in 2003) (71) conducted a single-blind crossover study of the effects of consuming 60 g RS$_2$ (divided into 4 doses taken over the course of 1 day) on postprandial glycemic response in 10 healthy adults (slightly increased mean BMI=26.9 kg/m$^2$). The morning after the 1-day intervention, participants consumed a fibre-free standardized meal and completed a MTT (71). The RS treatment significantly reduced the glucose and insulin response to the MTT compared to the control treatment (71). The RS treatment also significantly
increased the ratio of C-peptide to insulin, as well as postprandial insulin sensitivity (during the MTT using the MINMOD calculation) (71).

Robertson et al. (published in 2005) (72) conducted a longer-term crossover study of daily consumption of 30 g RS₂ for 4 weeks in 10 healthy adults (mean BMI = 23.4 kg/m²). Participants in this study underwent a euglycemic-hyperinsulinemic clamp test at 3 weeks, and also completed a MTT following 4 weeks of each treatment (72). Following both tests researchers observed a significant increase in postprandial insulin sensitivity (using MINMOD from MTT data and using the M/I calculation from euglycemic-hyperinsulinemic clamp test data) compared to control treatment (72). During the MTT, the glucose responses were not significantly different between treatments, however, the insulin AUC was significantly reduced, indicating the insulin that was required for glucose clearance was lower following RS compared to control (72). The RS treatment also significantly improved the ratio of C-peptide to insulin during the MTT, similar to Robertson et al. (71) which the authors attributed to improved hepatic glucose clearance (72). Fasting insulin sensitivity (HOMA %S) and beta-cell function (HOMA %B) were not significantly improved following RS treatment (72). This study added evidence that a lower-dose but longer-term RS intervention improves postprandial insulin sensitivity but not fasting insulin sensitivity among healthy adults (72).

Postprandial insulin sensitivity was also improved following euglycemic-hyperinsulinemic clamp test (using the M/I calculation) at the end of a 12-week, parallel-arm intervention of 40 g RS supplement daily, conducted by Johnston et al. (published in 2010) (73). The participants in this study (n=20), however, were considered at risk for T2D with their insulin resistance (fasting insulin > 60 pmol/L) and overweight BMI (mean BMI > 30 kg/m²) (73). Consistent with Robertson et al. (72), fasting insulin sensitivity measures (HOMA-%S and
HOMA-%B) were not significantly improved following RS intervention, nor was the postprandial glucose response (73). While the amount of RS used in this intervention was greater and the treatment period was longer than Robertson et al. (72), this research demonstrated that the postprandial insulin sensitizing effects of RS supplements may be extended to insulin resistant individuals (73).

In another study that also examined chronic RS_{2} supplementation in participants with increased risk of T2D, Maki et al. (published in 2012) (74) included 33 adults with increased waist circumference (≥102.0 cm (males); ≥89.0 cm (females)) and BMI (mean BMI = 30.6 kg/m^{2}). Following 4 weeks of supplementation with either 0, 15, or 30 g RS_{2}/day participants completed an intravenous glucose tolerance test (IVGTT) to reveal the effects of each treatment on fasting and postprandial glycemic response (74). Consistent with previously mentioned studies (72,73) there was no significant improvement in fasting insulin sensitivity (HOMA-%S) or beta-cell function (HOMA-%B) following any study treatments (74). The RS treatments did, however, both significantly increase postprandial insulin sensitivity (during IVGTT using the MINMOD calculation) compared to the control treatment, though this effect was present in the male participant group only (n=11) (74). The authors noted it is unclear why this effect was significant in men only (74). Overall, although seemingly sex-specific, significant insulin-sensitizing results were present following supplementation as low as 15 g RS_{2}/ day for only 4 weeks (74).

Bodinham et al. (published in 2012) (75) also utilized a 4-week treatment period in their crossover study of consumption of a 40 g RS_{2} supplement daily in 12 participants with insulin resistance (mean fasting insulin=96 pmol/L). Following each intervention period participants underwent a frequently sampled-IVGTT (FSIVGTT) to determine their postprandial glucose and
insulin response to a standardized meal (75). Consistent with previous research (72,73), the RS intervention did not significantly reduce the postprandial glucose response (75). This intervention did not result in a significant increase in postprandial insulin sensitivity (during FSIVGTT using the MINMOD calculation) (75), despite previous research by Johnston et al. (73) who demonstrated an improvement in postprandial insulin sensitivity (during euglycemic-hyperinsulinemic clamp using the M/I calculation) among insulin resistant individuals following RS supplementation. This may be due to the longer treatment period utilized by Johnston et al. (73) (12 weeks), compared to 4 weeks in Bodinham et al. (75). However, Bodinham et al. (75) did show that the RS treatment significantly reduced fasting glucose, postprandial insulin and C-peptide concentrations, as well as the first-phase insulin response (AIRg). The authors mentioned that first-phase insulin response is limited in people with T2D (75). Though these participants did not have T2D, they were insulin resistant so it is possible that a loss of the first-phase insulin response was in progress, and that the RS intervention in this study improved this function among those at increased risk of T2D.

In another sample of participants with insulin resistance (n=15), defined by increased fasting insulin (60-156 pmol/L), adiposity (mean waist circumference=106.1 cm) and BMI (mean BMI=34 kg/m^2), Robertson et al. (published in 2012) (76) investigated the effects of 8 weeks of daily 40 g RS supplementation in their crossover study. Following each intervention, participants underwent a euglycemic-hyperinsulinemic clamp and MTT to measure their fasting and postprandial response (76). Similar to Bodinham et al. (75), daily 40g RS supplementation significantly reduced fasting glucose compared to control treatment (76). In addition, this study was the first to demonstrate the ability of chronic RS supplementation to significantly reduce fasting insulin resistance (HOMA-IR) (76), perhaps in part due to their strict inclusion criteria
pertaining to insulin resistance. The RS treatment in this study also significantly increased glucose uptake despite no significant change in insulin during the euglycemic-hyperinsulinemic clamp, which can be interpreted as an improvement in postprandial insulin sensitivity (76).

In the most recent chronic RS-supplement study, published in 2014, and the first of its kind to study participants with T2D, Bodinham et al. (77) examined the effect of consuming 40 g RS2 for 12 weeks. Participants (n=17) completed a euglycemic-hyperinsulinemic clamp test and MTT (on separate days) following each intervention (77). There were no significant improvements in fasting insulin sensitivity (HOMA-%S), beta-cell function (HOMA-%B), C-peptide or HbA1c (77). While the RS treatment significantly reduced postprandial glucose AUC during the 2-hour MTT, this did not translate to a reduction in postprandial insulin sensitivity (during MTT using the Matsuda index calculation) (77). The reduction of postprandial glucose during the MTT without improved glycemic handling during the clamp method suggests that RS consumption may act on gut-mediated factors, however, the intestinally-derived hormone GLP-1 did not significantly affect the insulin response in this study as noted by the authors (77). Previous chronic RS-supplement studies, for the most part, have not demonstrated a reduction in postprandial glucose as seen here, except for the 1-day, 60 g RS intervention in healthy participants by Robertson et al. (71). Similarities between these two studies include the MTT method and substantial amount of RS in each intervention. A clear difference between these two studies is that Robertson et al. (71) studied healthy participants while Bodinham et al. (77) focused on people with T2D. It is important to note, however, that these people had well-controlled T2D (mean HbA1c=6.4%) (77), with many taking oral hypoglycemic medications, potentially limiting the actual difference in baseline glycemic control.
Research to date on the effects of chronic consumption of RS supplements on postprandial glycemic response is consistent with respect to the use of HAM-RS$_2$ as the supplement, and for the use of control treatments matched to the available carbohydrate of the RS treatment (71–77). There is variation, however, in the participant characteristics, which ranged from healthy to T2D; the amount of RS in the supplements, that ranged from 15 to 60 g RS; and duration of treatment periods, that ranged from 1 day to 12 weeks. The only chronic RS supplement study among people with T2D to date demonstrated that RS significantly improved postprandial glucose AUC, however did not significantly improve fasting glucose or insulin, or postprandial insulin sensitivity (77). However, among people without T2D, a number of studies demonstrate the potential for RS supplementation to benefit the glycemic response, most often by improving insulin sensitivity (71–74,76), and predominantly in those with an increased risk of T2D (73,74,76).

**C.ii.b. Chronic Human Intervention Studies: Resistant Starch Functional Foods**

(Summarized in Appendix E)

Chronic RS-food intervention studies provide a more realistic test of plausibility, as the participants are usually asked to incorporate the intervention into their regular diet, and most often in a free-living environment. These studies also test efficacy of an intervention in a realistic setting as they modify a small component of an entire diet, which allows for interaction between the study treatment and background diet, personal preferences, and lifestyle. As the RS in these studies is incorporated in food products, there is more variation in design of study treatments compared to RS supplement studies. There is also more variation among chronic RS studies with respect to test methods, though they all provide information about the glycemic response following a specified period of daily RS-food consumption.
In an early crossover study published in 1989 by Behall et al. (78) 12 healthy males (described as “within 20% of desirable height/weight ratio”) consumed a controlled, rotating diet that provided 52% of energy from carbohydrates, with 66% of those carbohydrates from either 70% amylose/30% amylopectin (high-RS) starch, or 30% amylose/70% amylopectin (low-RS) starch. To accomplish this, the high- and low-RS starches were incorporated into muffins and puddings and consumed daily for 5 weeks (78). After 4 weeks of each diet participants completed an OGTT, and after 5 weeks of each diet participants completed a MTT where the meal was the high- or low-RS ingredients made into a cracker (78). The high-RS treatment did not result in any significant improvements in fasting or postprandial glucose or insulin during the OGTT after 4 weeks, but did result in significant glycemic improvements following the MTT after 5 weeks (78). Ingestion of the high-RS cracker for the MTT after the high-RS diet caused postprandial glucose to significantly decrease at 30 and 60 minutes and significantly increase at 180 minutes, compared to the low-RS cracker and diet (78). The high-RS cracker and diet also significantly reduced postprandial insulin at 30, 60 and 180 minutes, as well as overall (“summed insulin”) compared to low-RS cracker and diet (78). Lastly, the postprandial glucagon response was significantly reduced at 30, 60 and 120 minutes after high-RS cracker and diet compared to low-RS cracker and diet (78). While the MTT was completed after long-term consumption of a high-RS diet, the meal selected for the test was itself a high-RS meal, therefore does not differentiate if the reductions in glycemic response during the test was due to the acute or chronic RS consumption.

In another study of 9 healthy female participants (mean BMI=21.3 kg/m²) Weickert et al. (published in 2005) (79) explored the postprandial effects of consuming various fibre-enhanced breads over the course of 1 day, which included a test bread with 31.2 g RS (divided into three
10.4 g RS servings throughout the day) in a small sub-study. Following a MTT (to a standardized bread meal), the RS bread treatment did not significantly affect fasting or postprandial glucose or insulin compared to the control bread, although a trend toward reduced postprandial glucose was observed ($P=0.09$) (79). The authors suggested this might have been due to lack of power in the small RS sub-study (n=9) (79).

Another study among 25 healthy overweight adults (mean BMI=27.9 kg/m$^2$), Park et al. (published in 2004) (80) explored the effect of consuming soup with 0 or 24 g RS for a 3-week period on fasting glucose and insulin in their parallel-arm intervention. Although there were no significant differences in glucose or insulin between the RS and control soups, fasting glucose was significantly reduced from baseline within the RS treatment, which might be explained by the significantly higher baseline glucose in the RS relative to the control soup treatment (80). The results of this study are limited to the effects of RS on fasting glycemic response, as Park et al. (80) did not examine the postprandial glycemic response. These results are similar to Weickert et al. (79) who demonstrated that the RS intervention did not significantly improve glycemic response, though the sample size was small (n=9) and the intervention duration was only 1 day. This research is somewhat consistent with Behall et al. (78), who did not see significantly improved fasted glucose or insulin following RS intervention, though Behall et al. (78) did observe a significant improvement in insulin AUC after RS intervention.

In a sample of healthy (normal fasting insulin; n=10) and hyperinsulinemic (mean fasting insulin 167 pmol/L; n=14) adults Behall and Howe (published in 1995) (81) examined the effects of consuming high- and low-amylose diets on postprandial glycemic response. Participants completed MTTs at weeks 4, 8 and 13 of a 14-week diet (10 weeks of self-selected diet and 4 weeks of controlled diet that both provided starch-based foods made with 70% amylose (high-
RS) or 30% amylose (low-RS) starch) (81). Similar to the 1989 study by Behall et al. (78), the meals consumed in the MTT were high- and low-amylose study products (81). Within the hyperinsulinemic group, insulin concentrations were significantly reduced following bread MTT after the high-amylose diet at specific time points which resulted in a significantly reduced insulin AUC compared to the low amylose diet at weeks 8 and 13 (81). Insulin AUC was also significantly reduced following the high-amylose diet and bread at weeks 4 and 13 within the control group (81). Glucose AUCs were not significantly affected by starch type within either participant group or when participant groups were combined and neither were fasting values of glucose or insulin (81). While this study also utilized the study treatment in the MTT, the MTT was repeated over the course of the treatment period, helping to reveal the time-course of the effect of long-term consumption. Within the hyperinsulinemic group, the insulin AUC was not affected at week 4 but was significantly reduced at weeks 8 and 13 (81), suggesting the insulin response improved over time. Also, since a significant reduction in insulin AUC was present within the control group at week 4, but not until week 8 in the hyperinsulinemic group, this research suggests that the duration of intervention required to benefit from high-amylose treatment increases with the degree of hyperinsulinaemia.

Further exploring the effects of chronic RS-enhanced food on postprandial glucose and insulin among those at an increased risk of T2D, Noakes et al. (published in 1996) (82) conducted a crossover study among 23 overweight adults (mean BMI = 29 kg/m²) with mild hypertension (< 140/90 mmHg) or elevated circulating triglycerides (> 2.0 mmol/L). Participants consumed a high-amylose diet (17 g RS in women, 25 g RS in men), low-amylose diet (low-RS) and a high oat bran (high-fibre) diet by including study foods (bread, pasta and muffins) into their regular diet in a crossover design with no washout period in between
treatments (82). Fasting glucose and insulin measurements and a MTT (after consuming a muffin with the corresponding starch) were completed at the end (but not at the start) of each treatment period (82). Consistent with previous studies of long-term RS-enhanced food interventions (78–81), the high-amylose diet did not significantly improve fasting glucose or insulin concentrations compared to low-amylose (or control) diets (82). However, during the MTTs the high-amylose diet did significantly reduce postprandial glucose at 45 minutes compared to the low-amylose diet (82). Further, the high-amylose diet significantly reduced postprandial insulin at 75 minutes as well as the overall summed insulin response compared to the low-amylose diet and treatment (82). Perhaps due to similarities in study populations (both had risk factors or T2D), these results are similar to Behall and Howe (81), who also saw a significantly improved insulin response following a high-amylose diet and MTT compared to low-amylose diet. Noakes et al. (82) further demonstrated that a significant improvement in insulin response may occur after just 4 weeks, compared to 8 weeks in Behall and Howe (81).

Penn-Marshall et al. (published in 2010) (83) also focused on RS consumption in people with an increased risk of T2D, in their crossover study of 15 overweight (mean BMI = 37.7 kg/m²) African-American adults with a family history of T2D. Participants consumed sliced bread made with or without HAM-RS₂ (12 g RS) daily for 6 weeks, and had fasting glucose and insulin measured before and after each treatment period, as well as HbA1c measured before and after the entire study period (14 weeks including the 2-week washout period) (83). Consistent with previous studies of chronic RS-enhanced food interventions (78–82), the RS bread intervention did not significantly reduce fasting glucose and insulin compared to control bread treatment (83). HbA1c was also not significantly reduced over the course of the study (83), although without measuring this endpoint after each intervention, it would have been unclear
which treatment period caused the effect. Previous research has shown some improvement in
glycemic response without significant improvement in fasting glucose or insulin (78,81,82);
however, this study did not evaluate the postprandial glycemic response (83). Based on the lack
of significant results of this study the authors inferred that people with this degree of risk of
T2D may require higher amounts of RS each day to improve their postprandial glycemic control
(83).

In participants with impaired fasting glucose, fasting hyperinsulinemia or newly
diagnosed T2D (n=85), Kwak et al. (published in 2012) (84) conducted a parallel-arm study to
determine the effect of chronic RS-enhanced food consumption on postprandial glucose and
insulin response. Before and after 4 weeks of daily consumption of rice with 6.51 or 0 g RS,
participants consumed a standard meal and completed a MTT (84). Compared to the control
rice, RS-rice consumption significantly reduced postprandial glucose at 60 and 120 minutes
which resulted in a significant reduction in glucose AUC, and a significant increase in
postprandial insulin sensitivity (during MTT using the Gutt index calculation) (84). The RS-rice
treatment significantly reduced HOMA-IR, fasting insulin, and postprandial insulin AUC,
although these findings were no longer significant after adjustment for baseline values (84).
Despite being among the smallest amount of RS in an intervention, this study is the first to show
a significant reduction in insulin resistance (therefore increased insulin sensitivity) (using the
Gutt index calculation) and postprandial glucose AUC during a standardized MTT following
chronic RS-enhanced food consumption (84). While a reduction in postprandial glucose at
individual time points has been observed following RS-based MTTs by Noakes et al. (82) and
Behall et al. (78), those reductions were not enough to translate into a reduction in overall
glucose AUC.
There is significant variation in the study designs used to investigate chronic consumption of RS-enhanced foods. Studies in healthy people provide mixed results, and may be confounded by the limited room for improvement in those with healthy glycemic control. Studies in people with increased risk of T2D also show mixed results: some studies show RS-enhanced foods have improved postprandial glycemic response (most often a reduction in insulin AUC (81,82)), while no studies to date have demonstrated the ability of RS-enhanced food to significantly reduce fasting glucose or insulin compared to control, when adjusted for baseline. There are also inconsistencies in the methods used in these studies, as some researchers measure the postprandial response to a standardized MTT, while others measure the response to various RS-containing test foods, introducing new variables and limiting the conclusions that can be drawn from the results. Studies that measured glycemic response following a standardized test (standard MTT, IVGTT, euglycemic-hyperinsulinemic clamp methods) should be considered better indices, though still provide mixed results (RS did not improve insulin response following an OGTT (78); RS did increase insulin sensitivity during a standard MTT (84)). Considering the total body of human RS-intervention studies there does seem to be evidence for the potential of chronic RS consumption to improve various aspects of the glycemic response, however there is a lack of consensus among these RS-enhanced food interventions, despite this overall design of study being most relevant when considering strategies to reduce T2D risk.

C.iii Summary of Human Intervention Studies

There are a number of human intervention studies that investigate the effects of consuming RS supplements and food on the glycemic response. Based on the current literature, acute studies that match treatments with control for total carbohydrates tend to significantly reduce the postprandial glucose response in healthy people (50,52,54,57,59–62,69), those at risk
of T2D (63,70) or those who have T2D (41,57,58), often with a significant reduction in postprandial insulin response (50,52–54,58–60,62,63), while a few studies have demonstrated that RS significantly reduced postprandial insulin but not glucose in healthy people (51,55,56). While these studies are most common among acute interventions, the improved glycemic response may be affected by the reduced available carbohydrates of the RS treatments, potentially overshadowing a unique response to RS. The acute studies that minimize this variable by matching treatments with controls for available carbohydrates are less abundant, but do demonstrate beneficial effects of RS on glycemia. These studies demonstrate that RS significantly reduces postprandial glucose in healthy people (59,65–69) and those at risk of T2D (70), and sometimes with a significant reduction in postprandial insulin response (59,65,67,68,70), while one study determined the RS intervention did not significantly affect postprandial glucose or insulin in people with T2D (41). Replacing typical foods with foods or supplements that reduce the immediate postprandial glycemic response may be beneficial in the short term as a lower and slower rise in glucose (and consequently insulin) is less likely to lead to insulin resistance, T2D and other complications. Acute RS consumption appears to favourably impact immediate postprandial glycemic response in a variety of individuals.

What remains unclear is if chronic consumption of RS supplements and foods can affect an overall improvement of glycemic response, and reduce the risk of developing T2D. Chronic RS studies that demonstrate an improved fasting glucose over time are limited, and only do so in participants with insulin resistance (75,76). Some studies have demonstrated the ability of RS consumption to improve insulin sensitivity over time when consumed as a supplement (71–74,76) and as a food (84), in healthy people (71,72), insulin resistant or otherwise high T2D risk people (73,74,76), and people with prediabetes or diagnosed T2D (84), however there are also
studies that showed no significant improvement in insulin sensitivity following chronic RS consumption (75,77,83). Overall there is significant variation among the study designs used which could contribute to the mixed results. With a foundation of encouraging evidence from acute and chronic RS supplement studies, and a few optimistic chronic RS-enhanced food studies, further investigations into RS-food consumption on glycemic response that utilize standardized methods are warranted to clarify the true effects of consuming high-RS foods on risk of T2D.
II. STUDY RATIONALE, PURPOSE, OBJECTIVES and HYPOTHESES

A. Study Rationale

With the growing prevalence of T2D in Canada and around the world (1), strategies to improve lifestyle and dietary habits are needed to prevent and manage T2D. Although some risk factors for T2D are non-modifiable, various lifestyle modifications can reduce the risk of developing T2D. While exercise is paramount for reducing the risk of chronic diseases such as T2D, there is an enormous opportunity for dietary improvements to assist in the prevention and management of T2D. In particular, incorporating more dietary fibre into the diet can lead to improved glycemia and is often recommended as a key nutritional strategy for the prevention and management of T2D. Despite this, the majority of Canadians do not meet their recommended intake of dietary fibre each day (20). The general Canadian population, especially those with or at risk of T2D, stand to benefit from increased daily dietary fibre intake.

Given the abundance of bread- and starch-based foods consumed by Canadians, bread products are a suitable food matrix to enhance with dietary fibre in order to deliver substantial health benefits to consumers. While whole-wheat and whole-grain bread products generally contain more dietary fibre and have a healthier nutrient profile than white bread, it is the white bread products that are often preferred among the general population (85). Unfortunately, commonly consumed bread products are often highly refined and fibre-depleted, which is likely a contributing factor to why the majority of Canadian are not meeting their recommended daily intake of dietary fibre.

Functional foods such as bread products formulated to provide more fibre are a great strategy to increase fibre intake among consumers; however, the type of fibre selected must not significantly alter the sensory aspects, particularly the taste and appearance, of the final product.
Commercially available Hi-Maize® 260 is a source of granular RS (RS₂) that can withstand food processing (i.e. high temperatures during baking) and contributes dietary fibre to food. Hi-maize® 260 is white in appearance with a fine, flour-like texture (39), and can be incorporated into bread-based foods at a high level without detrimental effects associated with other high-fibre ingredients. This ingredient provides a unique opportunity to impart the health benefits of RS and fibre in an appealing white bread format (85).

Previous research on the acute effects of RS consumed in supplement and food forms demonstrate its ability to improve postprandial glycemic response in a variety of people from healthy individuals to those with T2D. Research that investigated the chronic effects of RS consumed in supplement and food forms has also demonstrated its ability to improve glycemia, although these studies are limited, and vary greatly in core study parameters such as participant characteristics, RS type and form, duration of intervention, and importantly, in the glycemic testing methods used. Well-designed clinical research studies that examine the effects of consumption of RS incorporated into a well-accepted food on overall glycemic response among people at increased risk of T2D are required to expand and clarify the promising effects of RS observed in the literature to date.

B. Study Purpose, Objectives and Hypotheses

The purpose of this study was to determine if 8 weeks of daily consumption of bagels high in RS would significantly improve markers of T2D, compared to control bagels (without added RS), among adults who are at an increased risk of T2D. In addition, this study sought to determine if the study bagels were palatable and tolerable to participants. The specific objectives of this research were:
1. To determine the effect of 8 weeks of daily RS bagel consumption on hemoglobin A1c, compared to control bagel consumption;

2. To determine the effect of 8 weeks of daily RS bagel consumption on fasting plasma glucose, serum insulin, and fasting insulin sensitivity compared to control bagel consumption;

3. To determine the effect of 8 weeks of daily RS bagel consumption on postprandial glucose and insulin response to a standard 75 g glucose beverage load, compared to control bagel consumption;

4. To explore the tolerability of RS and control bagels, as well as the sensory response to study bagels, in terms of appearance, aroma, flavour, taste, and texture.

It was hypothesized that daily consumption of a bagel that contains approximately 25 g RS for 8 weeks would significantly improve markers of T2D, in a sample of adults who are at an increased risk of developing T2D, compared to consumption of control bagels, made without added RS. With respect to each of the study objectives, it was hypothesized that:

1. The RS bagel treatment would significantly reduce hemoglobin A1c, compared to control bagel treatment;

2. The RS bagel treatment would significantly reduce fasting plasma glucose and serum insulin, and significantly increase fasting insulin sensitivity compared to control bagel treatment;

3. The RS bagel treatment would significantly improve the glucose and insulin response to a standard 75 g glucose beverage load, compared to control bagel treatment;

4. The RS and control bagels would be well tolerated, and found to be acceptable in terms of appearance, aroma, flavour, taste, and texture.
III. RESEARCH METHODS

A. Study Approvals

A.i. Research Ethics Board Approval

The research design, protocol and all associated study documents (i.e. recruitment flyer, participant questionnaires, consent forms) were submitted to the University of Guelph Research Ethics Board (REB) for a full board review. After a revision process, it was determined that this research complies with the University of Guelph’s ethical standards, consistent with those of the second edition of the Tri-Council Policy Statement (86). This research received REB approval (REB# 13MY041) on July 5th, 2013, for the period of one year. An extension of this approval was received July 8th, 2014, until July 5th, 2015 with eligibility for extension of approval time as needed (Appendix F).

A.ii. Biohazardous Materials Certification

An application describing the study protocol as well as the safe handling protocol of the biohazardous materials that were to be used or collected during this study was submitted to the Biosafety Committee in the Department of Environmental Health and Safety at the University of Guelph. The application was approved and this research received a Biohazard Permit (H254-10-15-06), valid from June 19, 2013 until June 30, 2015 and eligible for extension of approval as needed (Appendix G).

A.iii. Clinical Trials.gov Registration

A detailed description of the study (i.e. study design parameters, investigator contact information, study timeline, sponsors, and collaborators) was submitted to clinicaltrials.gov and the record was maintained and updated when study milestones were reached. The clinicaltrials.gov registration number was NCT ID: NCT02129946.
B. Study Participants

B.i. Participant Inclusionary and Exclusionary Criteria

The study participants recruited for this research were intended to represent the growing population of adults in Canada who are generally healthy, but at an increased risk T2D. The following inclusionary and exclusionary criteria were established (Table 3) in order to include adults of this description, but eliminate potential confounding variables where possible.

Table 3. Participant inclusionary and exclusionary criteria with rationale

<table>
<thead>
<tr>
<th>Participant Inclusion Criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult males and post-menopausal females</td>
<td>To limit the potential effects of the menstrual cycle on glycemia (87).</td>
</tr>
<tr>
<td>Age ≥40 years</td>
<td>Consistent with the Public Health Agency of Canada’s (PHAC) identification of increased age as a risk factor for T2D (88).</td>
</tr>
<tr>
<td>BMI ≥25 kg/m² and &lt;40 kg/m²</td>
<td>To include individuals at higher risk of disease, including T2D, but not with class III obesity (64,88,89).</td>
</tr>
<tr>
<td>Waist circumference ≥94 cm for men or ≥80 cm for women</td>
<td>Consistent with the WHO identification of individuals at increased risk of metabolic complications, including T2D (89).</td>
</tr>
<tr>
<td>CANRISK score ≥21</td>
<td>A CANRISK score ≥21 indicates an increased risk of diabetes considering multiple risk factors (15). The validated CANRISK questionnaire (Appendix H) (90) includes questions about age, BMI, waist circumference, exercise and dietary habits, medical and family history of diabetes, and ethnic background. The questionnaire assigns points to higher risk responses to provide a cumulative CANRISK score.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participant Exclusion Criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus (fasting blood glucose ≥7.0 mmol/L)</td>
<td>Preventative study warrants the exclusion of those with T2D.</td>
</tr>
<tr>
<td>Gastrointestinal conditions (Celiac’s disease, Crohn’s Disease, Ulcerative Colitis, Inflammatory Bowel Disease)</td>
<td>The presence of these conditions may alter study endpoints and the treatment bagels contain gluten which may not be tolerated by people with these conditions.</td>
</tr>
<tr>
<td>Participant Exclusion Criteria (continued)</td>
<td>Rationale</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Renal or hepatic conditions</td>
<td>Treatment bagels or study protocol may be contraindicated for people with such conditions and people with health complications may require medications that could alter study endpoints.</td>
</tr>
<tr>
<td>Surgery or major medical event within 3 months of starting the study (including severe vomiting or diarrhea)</td>
<td>Treatment bagels or study protocol may be contraindicated in people who have recently undergone surgery or a major medical event. Such events, their required medications, or associated complications may alter the study endpoints.</td>
</tr>
<tr>
<td>Select medication use (insulin, oral-hypoglycemic agents, cholesterol-lowering medications, antibiotics within 6 months, other medications known to influence study endpoints)</td>
<td>Use of medications may alter study endpoints, including another study endpoint (fecal bacteria) not included in this thesis.</td>
</tr>
<tr>
<td>Select natural health product (NHP) use (NHPs intended for glycemic or cholesterol control) or any other NHP not on a stable dose for 3 months</td>
<td>Use of NHPs may alter study endpoints.</td>
</tr>
<tr>
<td>Phytosterol use (NHP or functional food)</td>
<td>Use of phytosterols may alter study endpoints. Those intending to lower their cholesterol with these products may also try other methods to further lower their cholesterol that may affect study endpoints (dietary or behavioural).</td>
</tr>
<tr>
<td>Gluten allergy or intolerance</td>
<td>Treatment bagels contain gluten which may not be tolerated by people with these conditions.</td>
</tr>
<tr>
<td>Alcohol consumption &gt;15 drinks/week for men and &gt;10 drinks/week for women</td>
<td>Alcohol consumption above these levels increases the risk of long-term health complications and may interfere with study endpoints (91).</td>
</tr>
<tr>
<td>Significant international travel within 6 months or plan for significant international travel during the study</td>
<td>Significant international travel during this period may affect gut microbiome composition due to varying bacteria present in international water supplies. Travel during the study period may impact study visit scheduling.</td>
</tr>
<tr>
<td>Eating habits questionnaire score &gt;16 (top quartile of maximum score of 22 and indicative of restrained eating)</td>
<td>Scoring in the top quartile of the cognitive restraint scale of this questionnaire implies that one’s eating habits are affected by motives other than their hunger. Highly restrained eating habits may affect study endpoints (satiety portion of study not included in this thesis).</td>
</tr>
</tbody>
</table>
B.ii. Sample Size Calculation

In order to determine an appropriate sample size for this study, a sample size calculation was completed using an online sample size calculator (92). Using the primary endpoint of fasting blood glucose, the variables used included an alpha level of 0.05, a beta (power) level of 80%, a minimal detectable difference (effect size) of 0.529 mmol/L (which represents a 10% decrease in the average baseline fasting glucose among similar RS intervention studies (72,74,83)) and a standard deviation of 0.56 (as reflective of the variability in fasting blood glucose in similar RS intervention studies (72,74,83)). It was established that in order to detect statistically significant changes if they are present, a minimum of 20 participants were required to complete the study. To account for possible participant attrition, a total of 25 participants were recruited.

B.iii. Participant Recruitment

A variety of advertising methods were utilized between September 2013 and June 2014 to recruit 25 interested and committed participants for this study. Designing and executing these recruitment techniques required great attention to detail and strategy with respect to the wording and location of the various media. The REB-approved study recruitment poster (Appendix I) was a major recruitment tool, and was designed to be eye-catching, to deliver a brief summary of the study, and to describe the responsibilities of the study participants. Careful consideration was given to the wording of the posters in order to attract a broad range of potential participants, but also to be sufficiently detailed so that only those who fit the age requirements would proceed with contacting the study email or phone number. Approximately 250 recruitment posters were posted in Guelph and surrounding areas. To maximize the study’s exposure in Guelph and the local area, posters were displayed in grocery stores, convenience stores, community centres,
libraries, shopping malls and throughout the University of Guelph campus. Stacks of half-page versions of the recruitment poster were also distributed at diabetes-centred local events and waiting areas in community centres, as well as other community sites and events. Special care was taken to advertise the study in places that serve higher numbers of potentially eligible individuals. For example, posters were placed in various medical offices and clinics based on the logic that people who have perhaps just learned they have elevated fasting glucose, but not type 2 diabetes, may be interested in a nutrition prevention study pertaining to diabetes. To further advertise the study, classified advertisements were posted online (Kijiji.ca) and printed in local newspapers (e.g. Guelph Mercury, Guelph Tribune, Wellington Advertiser). A story about the study was also printed on the front page of the Guelph Mercury (March 18, 2014). A segment of the local TV station (Inside Guelph - Rogers TV) was dedicated to an interview with Dr. Alison Duncan and Sarah Dainty about the study (recorded April 1, 2014). Radio advertisements were played on local stations (CJOY AM1460 and The Grand FM92.9) and emails were sent to various departments at the University of Guelph. Electronic versions of the recruitment flyer were posted on social media and information was spread through word-of-mouth.

The effectiveness of each recruitment action, as indicated by the number of participants that were enrolled into the study, is summarized in Table 4. The most effective recruitment strategy as per the numbers of the participants enrolled in the study was newspaper advertising (Guelph Mercury or Guelph Tribune). This was followed by past positive experiences in research at the Human Nutraceutical Research Unit (HNRU) and word of mouth. Other recruitment methods for enrolled participants included word of mouth, online classifieds, recruitment posters and emails.
Table 4. Participant enrolment by recruitment method

<table>
<thead>
<tr>
<th>Method/location of advertisement</th>
<th>Enrolled participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newspaper advertisement and article – Guelph Mercury</td>
<td>6</td>
</tr>
<tr>
<td>Newspaper advertisement – Guelph Tribune</td>
<td>5</td>
</tr>
<tr>
<td>Previous participant in HNRU (flyer/email)</td>
<td>4</td>
</tr>
<tr>
<td>Word of mouth</td>
<td>4</td>
</tr>
<tr>
<td>Online classifieds</td>
<td>2</td>
</tr>
<tr>
<td>Recruitment flyer in community</td>
<td>2</td>
</tr>
<tr>
<td>Recruitment flyer on campus</td>
<td>1</td>
</tr>
<tr>
<td>Recruitment Email</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations used: HNRU = Human Nutraceutical Research Unit.

B.iv. Participant Screening

A 3-step screening process was used to determine participant eligibility based on the study’s inclusion and exclusion criteria. Screening-1 entailed a brief telephone questionnaire that was constructed to efficiently determine eligibility based on the simplest and highest priority inclusion and exclusion criteria (e.g. age, diagnosis of diabetes or other health conditions, food allergies) (Appendix J). If deemed eligible after the screening-1 questionnaire and interested in continuing, the potential participant was then invited to the HNRU for a screening-2 visit, which involved a consent form (Appendix K), completion of a detailed eligibility questionnaire (Appendix L) and a CANRISK diabetes risk assessment questionnaire (15) (Appendix H). In addition, body measurements were completed including height (measured without shoes using a stadiometer (SECA Portable Stadiometer 214, Hanover, MD, USA)), unfasted body weight (to calculate BMI), waist circumference and hip circumference. If the participant was found to be eligible at this point, he or she was invited back to the HNRU for a screening-3 visit. The screening-3 visit involved a consent form (Appendix M), a fasted blood sample (for measurement of glucose, liver function enzymes (aspartate transaminase (AST), alanine transaminase (ALT)) and kidney function (creatinine)), fasted body weight (to confirm BMI),
and blood pressure and heart rate measurements. At the end of this visit the participant was provided with a snack and a cookbook as compensation for the completion of the screening process.

**B.v. Study Orientation**

If the participant was deemed eligible to participate in the study from the screening process and they were still interested in participating in the study, they were invited to a study orientation session. The study orientation involved a detailed review of the study handbook (table of contents summarized in Appendix N) that contained everything the participant needed to know about the study and is summarized in Table 5. A study coordinator reviewed the handbook with each participant and provided detailed instructions on how to complete a 3-day food record. The participants were also provided with samples of each of the treatment bagels (served toasted with margarine) to help them decide if they could consume them for the entire study duration. After the detailed review of the study handbook and any remaining questions or concerns were addressed, the participants were provided with the study consent form to read (Appendix O). If they were still interested in participating, they signed two copies of the study consent form, one which they kept and one which was filed in their study binder. For all participants who started the study, a study binder was created that contained all of their completed screening questionnaires and laboratory results, signed consent forms, study flowsheets (details of every study visit) with body measurement data, completed study diaries, sensory questionnaires, food records and any adverse events forms, if warranted.
<table>
<thead>
<tr>
<th>Section of Handbook</th>
<th>Summary of Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Welcome</td>
<td>• An introductory welcome letter that briefly describes their contribution to the research, and the purpose of this study handbook.</td>
</tr>
<tr>
<td></td>
<td>• A quick welcome to interact with us at any time, and to thank them for their participation and commitment to the study.</td>
</tr>
<tr>
<td>Contact Information</td>
<td>• HNRU location and contact information.</td>
</tr>
<tr>
<td></td>
<td>• Study director and study coordinator contact information.</td>
</tr>
<tr>
<td>Research Summary</td>
<td>• An overview of the study purpose and design.</td>
</tr>
<tr>
<td></td>
<td>• A brief description of the study endpoints.</td>
</tr>
<tr>
<td></td>
<td>• An explanation of how their participation and commitment to the study contributes to the scientific literature.</td>
</tr>
<tr>
<td>Study Calendar and Activities</td>
<td>• An overview of the study timeline.</td>
</tr>
<tr>
<td></td>
<td>• A detailed study visit schedule that outlines what to expect at each study visit with space to record the dates and times of their study appointments.</td>
</tr>
<tr>
<td></td>
<td>• A detailed list of what is requested of the participant at every stage of the study, including how to prepare for study visits and lifestyle reminders for each stage of the study.</td>
</tr>
<tr>
<td>Study Treatment Bagels and Background Diet/Lifestyle</td>
<td>• A description of how to store, prepare and consume the treatment bagels.</td>
</tr>
<tr>
<td></td>
<td>• An ingredients list and nutrition facts table for each treatment bagel.</td>
</tr>
<tr>
<td></td>
<td>• Reminders of which foods, NHPs and medications to avoid during the study.</td>
</tr>
<tr>
<td></td>
<td>• Reminders to maintain their habitual diet and lifestyle.</td>
</tr>
<tr>
<td>Medications and NHPs</td>
<td>• A reminder to record any changes in the NHPs and medications they take and place to record when and how this changed during the course of this study.</td>
</tr>
<tr>
<td>Study Measurements</td>
<td>• A description of the various measurements to be taken during the study.</td>
</tr>
<tr>
<td></td>
<td>• Detailed instructions on how to complete weighted food records.</td>
</tr>
<tr>
<td></td>
<td>• Detailed instructions on how to collect fecal samples.</td>
</tr>
<tr>
<td>Food Records</td>
<td>• A detailed guide to completing 3-day food records, including a serving size reference diagram.</td>
</tr>
<tr>
<td></td>
<td>• Food record forms to be completed and submitted.</td>
</tr>
<tr>
<td>Study Diary</td>
<td>• Space to record study bagel consumption details (preparation methods, condiments or toppings, time of consumption, etc.) for every study day.</td>
</tr>
</tbody>
</table>
C. Study Design and Treatments

C.i. Study Design

This study employed a randomized, double-blind crossover design that consisted of two 8-week treatment periods separated by a 4-week washout period (Figure 1). Participants were enrolled in the study on an ongoing basis from November 25, 2013 to June 3, 2014. All study visits occurred at the HNRU between January 15, 2014 to October 31, 2014. All study visits were arranged to be efficient yet feasible for the HNRU space availability and phlebotomist schedules. A study email account (bagel@uoguelph.ca) was set up to facilitate and organize communications with the participants, schedule all study visits and e-mail reminders for the study visits (Appendix P), and to share study documents among the research team.

![Figure 1. Overview of study design](image)

C.ii. Study Treatments: Formulation and Production

The study treatments were bagels that were formulated by Canada Bread, Maple Leaf Foods and produced at their commercial bakery in Concord, ON. The study bagel recipes were based on a high-RS bagel previously formulated by Bruce McKeown (Product Development Manager, Canada Bread) and produced by Canada Bread for a previous University of Guelph
The communication between those involved in the study bagel formulation, production and analysis are detailed in Appendix Q.

In the formulation process it was determined that the most functional and palatable RS bagels had 60% of the standard wheat flour replaced with Hi-Maize® 260 (Ingredion, NJ, USA) (41,93). The control bagel was representative of a standard bagel, as it contained only hard wheat flour (Archer Daniels Midland Company, IL, USA) as the carbohydrate source. The RS and control bagel recipes were comparable in all other respects, except the RS bagels required the addition of gluten. Gluten is a network of protein in wheat that imparts extensibility and elasticity to dough. The right balance between these properties is needed in bread making, particularly for dough to maintain the characteristic bagel shape. Since the RS bagel dough had a large amount of the gluten-containing wheat flour replaced with gluten-deficient Hi-Maize® 260, gluten was added to this dough at a level comparable to what the wheat flour would have provided, in order to achieve a suitable dough quality. In addition to restoring the dough quality, the addition of gluten to the RS bagel recipe also reduced the difference in protein content between the two types of bagels.

During the previous high-RS bagel formulation process (93), it was also determined that the high level of Hi-Maize® 260 in the recipe imparted a slight bitter taste. To resolve this, a flavour modulator was used to mask this bitterness and improve palatability of the RS bagels. Unfortunately the flavour modulator that was used previously was no longer available at the time of production of the current study’s bagels, so a new flavouring product had to be tested. Bruce McKeown sourced four alternate flavouring products which were tested in small batches of RS buns at the Maple Leaf Foods Canada Bread ThinkFOOD! test kitchen in Mississauga, ON. The products used and the results of each batch is summarized in Table 6 below. Sensient®’s
Smoochenol® flavour modulator was found by all to yield the best tasting bagel, and was confirmed to be used in the recipes for both the RS and control treatment bagels.

Table 6. Results of flavour modulator testing for study bagel recipe

<table>
<thead>
<tr>
<th>Flavour</th>
<th>Observations</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no flavour added)</td>
<td>Slight bitter aftertaste</td>
<td>-</td>
</tr>
<tr>
<td>Kerry Natural Sugar Type FMT 30578489</td>
<td>Somewhat reduced bitter aftertaste</td>
<td>Will not be used</td>
</tr>
<tr>
<td>Kerry Natural Sugar reenf 30605070</td>
<td>Somewhat reduced bitter aftertaste (No discernible difference from above ‘sugar’ flavour)</td>
<td>Will not be used</td>
</tr>
<tr>
<td>Kerry Vanilla Natural reenf 30579093</td>
<td>Noticeable vanilla aroma and flavour with subtle sweetness</td>
<td>Will not be used</td>
</tr>
<tr>
<td>Sensient® Smooochenol® flavour modulator Sept-05-2014</td>
<td>Reduced bitter aftertaste, no additional flavours</td>
<td>Most preferred variation; will be used</td>
</tr>
</tbody>
</table>

1Tasting was performed by Bruce McKeown, Alison Duncan, Sarah Dainty and Brandon Guild (Canada Bread Summer Co-op Student, Product Development Technician).
2Kerry Ingredients & Flavours (Beloit, WI, USA).
3Sensient® Flavours Canada Inc. (Mississauga, ON).

After the flavour modulator testing was completed, the recipe was tested in the commercial bagel production line at the Canada Bread, Maple Leaf Foods frozen division in Concord, ON. As mentioned previously, it was critical that the doughs could be incorporated into the production line, as it is an automated system that needed to flow efficiently through the stages of bagel production without clogging or stopping the equipment. The process to test the bagel recipes to determine if these bagels would meet the practical production requirements as well as the study requirements was guided by Bruce McKeown, Product Development Manager of Canada Bread, on October 5, 2014. The recipes tested were found to be acceptable in terms of bagel formation and final product taste, texture and appearance. It was therefore decided that the recipes would not have to be changed, pending acceptable RS content and macronutrient distribution.
Once the test bagels were produced (October 5, 2014), samples from the beginning, middle and end of each bagel’s production line were analyzed for macronutrient distribution by Medallion Laboratories (Minneapolis, MN, USA) and dietary fibre and RS by Nutrition R&D (Bridgewater, NJ, USA), summarized in section C.iii. The macronutrient distribution and RS content of the bagels were found to be acceptable and the final batch of RS and control bagels were produced at the Maple Leaf Canada Bread frozen division in Concord, ON, on January 18, 2014. The ingredients and their proportions for the RS and control bagel recipes are detailed in Table 7.

### Table 7. Study bagel formulation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount of Ingredient (kg) in Batch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Bagel</td>
</tr>
<tr>
<td>Hi-Maize® 260&lt;sup&gt;TM1&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Hard wheat flour&lt;sup&gt;2&lt;/sup&gt;</td>
<td>280.0</td>
</tr>
<tr>
<td>Water</td>
<td>136.1</td>
</tr>
<tr>
<td>Gluten</td>
<td>0</td>
</tr>
<tr>
<td>Sugar</td>
<td>10.1</td>
</tr>
<tr>
<td>Salt</td>
<td>5.04</td>
</tr>
<tr>
<td>Yeast</td>
<td>4.20</td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>3.36</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>1.26</td>
</tr>
<tr>
<td>Smoothenol®</td>
<td>0.56</td>
</tr>
</tbody>
</table>

<sup>1</sup>Hi-Maize® 260 lot # FLI0011 – Ingredion Inc., NJ, USA.

<sup>2</sup>Hard wheat flour lot # 08NOV13D – Archer Daniels Midland Company, IL, USA.

### C.iii. Study Treatments: Nutritional Composition

Following the production of the treatment bagels, 6 samples from the middle of each batch for both the RS and control bagels were shipped to Ingredion Inc. to be analyzed again to confirm energy and macronutrients by Medallion Labs (Minneapolis, MN, USA), and dietary fibre and RS content by Nutrition R&D (Bridgewater, NJ, USA). To maintain blindness of researchers when receiving the nutritional analysis results, the A and B bagels were re-coded by a third party, and the lab results (indicating the RS content of each bagel) were not revealed to
the researchers until the un-blinding of the original bagel code after data collection and analysis was complete. The analytical methods used for the bagel compositional analyses are summarized in Table 8.

Table 8. Study bagel nutritional analysis and composition per bagel

<table>
<thead>
<tr>
<th></th>
<th>Analytical Method</th>
<th>Control Bagel (124.2 g)</th>
<th>RS Bagel (119.8 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>Calculation&lt;sup&gt;1&lt;/sup&gt;</td>
<td>314.2</td>
<td>228.2</td>
</tr>
<tr>
<td>Total Carbohydrates (g)</td>
<td>Proximate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>63.9</td>
<td>57.9</td>
</tr>
<tr>
<td>Total Dietary Fibre (g)</td>
<td>AOAC 991.43&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.61</td>
<td>25.6</td>
</tr>
<tr>
<td>Resistant Starch</td>
<td>Modified Englyst assay&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.83</td>
<td>25.4</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>Proximate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.99</td>
<td>1.44</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>Proximate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>11.4</td>
<td>8.75</td>
</tr>
</tbody>
</table>

<sup>1</sup>Energy calculation based on general Atwater factors (94).

<sup>2</sup>Proximate analyses performed by Medallion Laboratories, Minneapolis, MN, USA.

<sup>3</sup>Dietary fibre analysis and modified Englyst assay performed by Nutrition R&D, Bridgewater, NJ, USA.

<sup>4</sup>Average of three 15-bagel samples from the beginning, middle and end of each batch with CV of the three averages as 4.03% and 3.98% for the control and RS bagels, respectively.

C.iv. Resistant Starch Analysis

The RS analysis of the treatment bagels was performed by Nutrition R&D, the in-house laboratory for Ingredion Inc., who is also the manufacturer of the high-amylose maize (Hi-Maize® 260) used in the treatment bagels. This laboratory uses an in-house method of RS analysis that is a slightly modified Englyst assay, updated to use modern digestive enzymes and glucose oxidase detection (95). Careful thought was given to which method of RS analysis to use, as the various methods of RS analysis yield varying results from the same samples. The method selected should be best suited for the type of RS expected as well as the food matrix, if present. RS is often analyzed as total dietary fibre using AOAC 991.43 (48,62,68,71,73–75); however, since the RS in the study product is provided in a bread matrix that includes other dietary fibre-containing ingredients, a RS-specific method was required so the result was not inflated by other sources of dietary fibre.
Two of the common RS-specific assays currently used in the literature and industry are AOAC 2002.02 (41,63,83) and the modified Englyst RS method (32,46,67,96,97). The AOAC 2002.02 assay subjects the sample to harsh digestion (16 hours of enzymatic digestion) (95), which tends to underestimate RS$_2$ in Hi-Maize 260® products (95). In contrast, the Englyst RS method was designed to simulate human digestion by first digesting the sample with pepsin, then incubating the sample in amylase, invertase and amyloglucosidase for 2 hours at 37°C (32,95,98). After several communications with Dr. Christine Pelkman, Senior Nutrition Scientist and Clinical Research Manager of Ingredion Inc., the Englyst RS method was determined to be the most appropriate analysis for the treatments in the current study.

The study protocol required to participants to consume one bagel each day, which provided 25.4 and 6.83 g of RS in the RS and control bagel, respectively. The factors that contributed to this RS dose included: the limit to the amount of Hi-Maize® 260 that could be incorporated into a bagel without deleterious sensory effects; the ability for the production of the bagels to fit seamlessly into the production line at a large commercial bakery with a maximum bagel size limit; and the desire to incorporate the dose into one bagel per day to minimize participant attrition.

C.v. Study Treatments: Blinding, Coding and Delivery

To ensure the researchers were blinded to the RS and Control bagels, the bagels were coded A or B by Bruce McKeown on January 18, 2014. The code was documented in duplicate and sealed in 2 separate envelopes which were stored in Alison Duncan’s office and laboratory until data collection and analysis were complete. All RS and control bagels were produced in single batches at the Canada Bread, Maple Leaf Foods frozen division in Concord, ON. Bagels were frozen immediately after production and packed in bulk boxes, labeled Bagel A or Bagel B.
according to the pre-determined code. The frozen bagels were transported in a refrigerated vehicle to the Food Science building at University of Guelph. Researchers efficiently repackaged the bagels into clear plastic bagel sleeves provided by Canada Bread, Maple Leaf Foods (7-8 bagels/sleeve) and labeled with Bagel A or Bagel B. The repackaging process was completed quickly in order to avoid thawing of the bagels. Once bagels were packaged and labeled they were stored in sealed plastic bins in a -20°C freezer.

C.vi. Study Treatments: Randomization, Distribution and Consumption

As participants started the study (n=25) they were randomized to one of the two study treatments for the first treatment period, and then assigned to the alternate treatment for the second treatment period. Two-block randomization was completed by recording the two possible treatment orders (6 of A-B, 6 of B-A) for the first 12 people to start the study on small pieces of paper. These pieces of paper were folded in half identically, placed in a small box, and randomly drawn from the box without replacement. Once the first set was drawn, all pieces of paper were returned to the box and the procedure was repeated to determine the order of the next 12 participants. All pieces of paper were returned to the box and one was drawn to determine the treatment order of the 25th participant.

Treatment bagels were provided to participants in frozen, 2-week supplies on study days 1, 15, 29 and 43. Two sleeves of the assigned bagels were placed in an opaque outer bag labelled with participant identification number (ID), treatment period and study days to consume the bagels. Participants were always provided with 15 bagels per 2-week supply to ensure they had a replacement bagel if one was lost, dropped, burnt or otherwise spoiled. Participants were instructed to keep their bagels frozen and defrost their daily bagel just before consumption to ensure freshness. Of note is that participants were instructed to incorporate the bagels into their
daily diet by replacing another bread-based food with the study bagel each day. The purpose of this was to ensure that the participants did not increase their daily energy intake due to the treatment bagels and gain weight as a result. Participants also completed 3-day food records to reveal average energy intake mid-way through each treatment period.

In order to maximize compliance with consumption of the treatment bagels, participants were allowed to prepare and consume them according to their preferences. For example, the bagels could be fresh or toasted, consumed with any condiments or toppings, or incorporated into a sandwich. Further, the participants were allowed to consume their treatment bagels at any time of day, e.g. as breakfast one day, or as a snack another day. In addition to being accommodating, the allowance of a variety of bagel preparation methods represented how the bagels would be incorporated into a typical diet which would increase the applicability of the study treatment in extension of the study results. While the study bagel preparation and consumption instructions were flexible, the importance of regular daily bagel consumption was emphasized at study visits and in the study handbook. It was also stressed that participants should incorporate bagels into their diet by replacing some other bread or bread-based product from their diet. Participants were instructed to record how and when they prepared and consumed their treatment bagels in a daily study diary, which was reviewed at every study visit. Strategies such as regular communication between participants and researchers, instructions to return any uneaten bagels and packaging, occasional newsletters with study updates and bagel preparation ideas, and frequent conversation about how the participants were incorporating the bagels into their diets were employed to encourage participants to consume their bagels regularly.
D. Data Collection and Sample Analysis

D.i. Data Collection Schedule

Study visits occurred on study days 1, 15, 29, 43 and 57 of each treatment period at the HNRU with various activities occurring as summarized in Figure 2. The logistical details of each study visit were itemized in the study flowsheets (example provided in Appendix R). The study flowsheets were designed to guide the study coordinator through every participant interaction and study activity at each study visit. The study flowsheets included space to record any data collected and prompts for any reminders to be provided to the participant.

Figure 2. Data collection schedule for each treatment period. Circled days indicate a study visit to the HNRU. OGTT = oral glucose tolerance test; BodyWt = body weight; Bagels = 2 weeks of treatment bagels dispensed; SensoryQ = Sensory questionnaire; 3-DayFR = Three-day food record (study days 26-28); * = Pre-study 3-DayFR was required within 1 week prior to treatment period 1.

D.ii. Body Weight

Body weight was measured at each study visit, after a 12-hour fast on study days 1 and 57, and after avoiding food and beverages (except water) the morning of study days 15, 29 and 43. Participants stood without shoes or bulky clothing on an electronic scale (SVI-200F, Acculab Sartorius Group, Edgewood, NY) and body weight was measured to the nearest 0.1 kg. BMI was then calculated as body weight (kg)/height (m$^2$), using the height measurement from
screening-2. Body weight was monitored throughout the study to ensure no significant change trends occurred.

D.iii. Study Diaries

Participants completed a daily study diary (Appendix S) in which they were instructed to record how (preparation methods, spreads used, etc.) and when they consumed their daily treatment bagels; any sickness or discomfort felt related to study bagel consumption; any changes in their medication routine or any extra medications consumed; any changes in their exercise habits; and anything else they felt would be relevant.

Participants submitted their completed study diaries in 2-week time periods at study visits on days 15, 29, 43 and 57 of each treatment period. Study diaries were examined for bagel consumption and any patterns in study treatment bagel consumption. The number of treatment bagels not consumed was tallied to quantify self-reported compliance as a percentage of treatment bagels consumed for each participant. Study diaries were also reviewed for any changes in medications, any discomforts experienced that may be related to study bagel consumption, and any changes in exercise and lifestyle habits.

D.iv. Sensory Questionnaires

Participants completed a sensory questionnaire about their study bagel consumption over the previous 2-week period on study days 15, 29, 43 and 57 of each treatment period (Appendix T). The sensory questionnaire included a combination of closed- and open-ended questions which were designed to gather information about the acceptance of both types of treatment bagels with respect to specific attributes, overall impression of the bagels, the willingness to consume the bagels outside of a study, as well as information about any side effects felt in relation to consuming the treatment bagels, if present.
Participants were asked to describe how they typically prepared and consumed their
treatment bagels over the previous two weeks, as well as anything they liked and disliked about
the bagels. This information was used to generate conversation about bagel consumption to
encourage treatment compliance and also to gauge trends in bagel consumption.

Acceptance of the treatment bagels was assessed at all study visits using 9-point hedonic
scales that queried participants’ experiences in response to specific attributes and their overall
impressions of the treatment bagels, as well as the appearance, aroma, flavour, taste and texture
of their treatment bagels over the previous 2 weeks. The possible responses ranged from 1-
“Dislike Extremely” to 9-“Like Extremely”.

On study day 57 of each treatment period two additional questions were included as
another measure of acceptance of the treatment bagels. Participants were asked to rate their
willingness to consume that treatment period’s bagels if they were available after the study, and
again their willingness to consume those bagels if they bore a satiety-related health claim that
said these bagels would keep them fuller for longer. Participants were presented with a food
action (FACT) rating scale for each of these questions and asked to circle one of nine statements
that ranged from “I would eat this food every opportunity I had” to “I would eat this only if I
were forced to”, adapted from Schutz (99).

Lastly, participants were asked if they experienced any side effects related to their bagel
consumption by selecting from a list of possible side effects. As summarized in Appendix T, the
list of possible side effects focussed on gastrointestinal (GI) effects due to the high-fibre nature
of the RS treatment bagels, though there was an ‘other’ option for participants to record any
other side effect(s) they felt were due to the bagel consumption. Responses were reviewed by
researchers and participants were asked if they considered anything to be a serious adverse effect in which case a serious adverse event report would be completed and submitted to the REB.

Data from the completed sensory questionnaires were tabulated in Microsoft® Excel® spreadsheets and subjected to statistical analysis.

**D.v. Three-Day Food Records**

Participants completed three 3-day food records over the course of the study to determine average energy and macronutrient intake before (within one week of starting the study) and during (study days 26-28 of each treatment period) the study. All participants were trained on how to complete accurate food records during the study orientation, and provided with detailed instructions and examples of food records in their study handbooks. Participants were asked to record their entire food and beverage intake, including as many descriptive and quantitative details as possible over the course of three days within a specified time period. Participants were asked to choose three days that would reflect their typical daily intake on two weekdays and one weekend day. Completed 3-day food records were submitted at the subsequent study visit and promptly reviewed for completeness by a study coordinator in the presence of the participants so that any missing information could be filled in.

All food records were entered into ESHA the Food Processor Software version 10.13.1 (ESHA Research, Salem, OR, USA), and analyzed for 3-day average intakes of energy, macronutrients and dietary fibre. Data was used to characterize participants based on baseline energy and nutrient intakes and analyzed for any significant changes in energy and nutrient intakes between treatment bagel intervention periods.
D.vi. Blood Sample Collection, Processing and Analysis

Fasting and postprandial blood samples were collected during a 75 g-OGTT on study days 1 and 57 of each treatment period. On these study days, participants arrived at the HNRU after a 12-hour overnight fast and having avoided alcohol, uncharacteristic physical activity and over-the-counter medications for 24 hours prior. Upon arrival, participants were taken to the HNRU sampling bay, which is a private area designed for phlebotomy-related activities. A trained HNRU phlebotomist prepared the participant’s arm and inserted an intravenous (IV) catheter into a vein in the antecubital region of the prepared arm to facilitate repeated blood sampling. The IV catheter was kept patent with an attached sterile saline drip solution. Once the IV line was established and the participant was comfortable, a fasted baseline blood sample was collected. Immediately after the baseline blood sample, the participant consumed the standard OGTT beverage (75g glucose in 300mL beverage; Trutol™ 75g Orange flavour, Thermo Fisher Scientific, Waltham, MA, USA) within 5 minutes. The participant was then walked to a comfortable and quiet resting area of the HNRU called the postprandial lounge, which is equipped with a couch, chairs, a television, a coffee table and wireless internet. The participant was made comfortable again, and remained in the lounge area for the duration of the OGTT. Over the next 3 hours, blood samples were drawn via the IV line at 15, 30, 60, 90, 120 and 180 minutes after the baseline fasted blood sample. After collection of the blood sample at 180 minutes, the phlebotomist removed the catheter from the participant’s arm and bandaged the area. The participant was provided with a light lunch and remained at the HNRU until they felt ready to leave. The processing of all blood samples is summarized in Table 9, after which the samples were frozen at -80°C until analysis.
Table 9: Blood sample collection and processing details

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Time point</th>
<th>Type of Blood Collection Tube</th>
<th>Blood Sample Collection and Processing Instructions</th>
</tr>
</thead>
</table>
| Plasma Glucose | 0, 15, 30, 60, 90, 120, 180 minutes | 4.0 mL BD sodium heparin (green top) vacutainer | • Collect blood into tube and gently invert 8 times.  
• Store blood in refrigerator until centrifuge is available.  
• Centrifuge\(^2\) within 2 hours for 15 minutes at 3000 rpm and 4°C-8°C.  
• Aliquot plasma into two 1.0 mL cryogenic vials\(^3\) and store at -80°C until analysis. |
| Serum Insulin | 0, 15, 30, 60, 90, 120, 180 minutes | 5.0 mL BD Serum-Separator Tube (SST) (gold top) vacutainer | • Collect blood into tube and gently invert 5 times.  
• Allow blood to clot for 30 minutes at room temperature.  
• Centrifuge\(^2\) for 15 minutes at 3000 rpm and room temperature.  
• Aliquot serum into two 1.0 mL cryogenic vials\(^3\) and store at -80°C until analysis. |
| Whole blood HbA1c | 0 minutes | 4.0 mL BD K\(_2\)EDTA (lavender top) vacutainer | • Collect blood into tube and gently invert 8 times.  
• Store in refrigerator until pickup by courier for contracted lab analysis. |

\(^1\)BD Vacutainers: 5.0 mL Gold Top SST/ Green Top 4.0 mL Sodium Heparin / Lavender Top 4.0 mL K\(_2\)EDTA Tubes; Becton, Dickinson and Company (Franklin Lakes, NJ, USA).

\(^2\)Allegra TM X-22R Centrifuge; Beckman Coulter Incorporated (Fullerton, CA, USA).

\(^3\)Cryogenic vials: Corning, Incorporated (Acton, MA, USA).

Plasma glucose was analysed using an automated YSI 2300 STAT Plus Glucose Analyzer (YSI Life Sciences, Yellow Springs, OH, USA). All samples from each participant were analyzed in the same batch with assays of 1 or 2 participants completed. Inter- and intra-assay variation was 0.80% and 0.19%, respectively.

Glycated hemoglobin (HbA1c) was analyzed from whole blood collected into BD K\(_2\)EDTA (lavender top) vacutainers and stored at 4°C until same-day pick up by courier and
delivery to LifeLabs® Medical Laboratory Services (Kitchener, ON). Analysis was completed on whole blood using the COBAS Integra 800 A1c assay (Roche, Switzerland) for HbA1c measurement within 1 day of sample collection.

Serum insulin was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen™ Human Insulin ELISA kit #KAQ1251, Life Technologies, Carlsbad, CA, USA). All samples from each participant were analyzed in the same assay with assays of 1 or 3 participants completed. Inter- and intra-assay variation was 0.80% and 0.19%, respectively.

Postprandial OGTT plasma glucose and serum insulin time point curve data for each study day and treatment were Microsoft® Excel® spreadsheets and summarized by analysis for 2-hour and 3-hour incremental area under the curve (iAUC) using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA), using baseline as the glucose or insulin value at the 0 minute time point and ignoring area below baseline. The maximum concentration (C_MAX) for each time point curve was determined by visual inspection in Microsoft® Excel® spreadsheets.

Surrogate indices of fasting insulin resistance (HOMA-IR), beta-cell function (HOMA-%B) and insulin sensitivity (HOMA-%S) were calculated using fasting plasma glucose and serum insulin data and the homeostasis model assessment (100–102).

E. Statistical Analysis

All statistical analyses were performed using the Statistical Analysis System (version 9.3, Cary, NC, USA) with \( P \leq 0.05 \) considered statistically significant. Data were examined for normality using stem leaf diagrams, box plots and residual plots and it was determined that all insulin and HOMA data required log transformation. These data were therefore exponentiated and presented as geometric means following statistical analysis.
Participant characteristic data was averaged at baseline and body weight and BMI were compared between treatment bagels at each study day using repeated measures analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons and presented as means ± SE.

Sensory questionnaire data was compared between treatment bagels at each study day using repeated measures ANOVA and presented as means ± SE. Data from the question about willingness to consume the treatment bagels after the study if a satiety health claim were present were analysed for the RS treatment bagel only and compared with the data from without a health claim using frequency percentages. Treatment bagel side effects reported were tallied for each side effect and study day and expressed as number and percentage of participants.

Energy and nutrient intake data from pre-study and the midpoint of each treatment period were compared using repeated measures ANOVA, followed by the Tukey’s test for multiple comparisons and presented as means ± SE.

Fasting plasma glucose, HbA1c and natural log transformed serum insulin were compared between treatment bagels at study day 1 using repeated measures ANOVA and at study day 57 using repeated measures analysis of covariance (ANCOVA) including study day 1 values as a covariate, both followed by the Tukey’s test for multiple comparisons, and reported as least squares means and 95% confidence intervals.

Postprandial plasma glucose and serum insulin were compared among time points at study days 1 and 57 within the control and RS treatment bagels using repeated measures ANOVA, followed by the Tukey’s test for multiple comparisons and reported as least-squares means and 95% confidence intervals.
Postprandial plasma glucose and serum insulin OGTT 2-hour iAUC, 3-hour iAUC and $C_{\text{MAX}}$ as well as surrogate indices of fasting insulin sensitivity (HOMA-IR, HOMA-%B, HOMA-%S) were compared between treatment bagels at study day 1 using repeated measures ANOVA and at study day 57 using repeated measures ANCOVA including study day 1 values as a covariate, both followed by the Tukey’s test for multiple comparisons, and reported as least squares means and 95% confidence intervals.
IV. RESULTS

A. Participant Flow

Participant flow through screening-1, screening-2, study enrolment and study completion is summarized in Figure 3. A total of 144 interested individuals completed screening-1 of which 97 were not eligible to move on to screening-2 (reasons detailed in Figure 3). Of the 47 people who completed screening-2, 18 were excluded (reasons detailed in Figure 3). Of the 29 people who completed screening-3, 4 were excluded (reasons detailed in Figure 3) and 25 people were randomized to a treatment and started the study.

Of the 25 participants who started this study, two participants were either excluded or did not complete the study (Table 10). One participant was excluded on study day 15 of the first treatment period due to antibiotic use; this participant’s data was not included in the statistical analysis. Another participant dropped out of the study on study day 29 of the second treatment period due to relocation out of the country earlier than scheduled; this participant’s data was included in the statistical analysis. Data from 24 participants were included in the data and statistical analysis.
Figure 3. Participant flow diagram. Abbreviations used: BMI = body mass index; CANRISK = Canadian Diabetes Risk Questionnaire; GI = gastrointestinal; Scr = screening; TFEQ = three-factor eating questionnaire (for satiety component of study); TPI = treatment period one; TPII = treatment period two.

1 Participant excluded due to antibiotic use. 2 Participant moved out of country.
Table 1. Summary of participant attrition: exclusions and dropouts

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Time of Attrition</th>
<th>Dropout / Excluded</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>TPII – Day 29</td>
<td>Dropout</td>
<td>Family relocated out of country</td>
</tr>
<tr>
<td>19</td>
<td>TPI – Day 15</td>
<td>Excluded</td>
<td>Started antibiotic treatment</td>
</tr>
</tbody>
</table>

1Abbreviations used: ID = identification; TPI = treatment period one; TPII = treatment period two.

B. Participant Characteristics

Participant characteristics at baseline indicated that they were at high risk for diabetes (Table 11). Participants included more males (n=18) than females (n=8), likely due to the inclusionary criteria for post-menopausal women. Participants were on average, 55 years old with a BMI of 30.2 kg/m², and waist circumference of 106.3 cm. Their average CANRISK score of 31.3 is at the higher end of the ‘moderate risk’ range (scores 21-32), though participants did not have diabetes with their fasting plasma glucose below the level that the CDA identifies as the prediabetes range of 6.1 – 6.9 mmol/L, and their HbA1c below the 6.0 - 6.4% range that would indicate prediabetes according to the CDA 2013 Clinical Practice Guidelines (14).

Participant body weight and BMI during the study did not significantly differ between the treatment bagels at any of the study days (Table 12).
Table 11. Participant characteristics at baseline (n= 24)$^{1,2}$

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)</td>
<td>16 male / 8 female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.3 ± 1.59</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>30.2 ± 0.57</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>90.4 ± 2.25</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.02</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>106.3 ± 1.9</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>110.5 ± 1.37</td>
</tr>
<tr>
<td>CANRISK score</td>
<td>31.3 ± 0.96</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.41 ± 0.14</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.72 ± 0.04</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>132.8 ± 2.53</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>86.7 ± 2.11</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69.4 ± 2.59</td>
</tr>
<tr>
<td>Prescription medication use (n)</td>
<td>0.54 ± 0.24</td>
</tr>
<tr>
<td>Natural health product use (n)</td>
<td>1.25 ± 0.27</td>
</tr>
</tbody>
</table>

$^1$Data are means ± SE.

$^2$Abbreviations used: BMI = body mass index; CANRISK = Canadian diabetes risk assessment questionnaire; mm Hg = millimetres of mercury; bpm = beats per minute.
Table 12. Participant characteristics during the study$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>Control Bagel (n=24)</th>
<th>RS Bagel (n=24)$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 1</td>
<td>90.0 ± 2.28</td>
<td>90.4 ± 2.24</td>
</tr>
<tr>
<td>Study Day 15</td>
<td>89.8 ± 2.33</td>
<td>89.5 ± 2.37</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>89.8 ± 2.31</td>
<td>90.0 ± 2.27</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>88.9 ± 2.47</td>
<td>88.8 ± 2.65</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>90.3 ± 2.17</td>
<td>89.5 ± 2.50</td>
</tr>
<tr>
<td><strong>BMI (kg/m$^2$)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 1</td>
<td>30.0 ± 0.57</td>
<td>30.2 ± 0.57</td>
</tr>
<tr>
<td>Study Day 15</td>
<td>30.0 ± 0.59</td>
<td>30.2 ± 0.61</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>30.1 ± 0.58</td>
<td>30.1 ± 0.58</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>30.0 ± 0.64</td>
<td>30.1 ± 0.66</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>30.1 ± 0.57</td>
<td>30.0 ± 0.61</td>
</tr>
</tbody>
</table>

$^1$Data are means ± SE.
$^2$Abbreviations used: BMI = body mass index.
$^3$n=23 for day 57 of RS bagel treatment period due to 1 participant dropout.

C. Self-Reported Compliance, Treatment Tolerance and Side Effects

Self-reported compliance was high with both treatment bagels during the study. Self-reported compliance, quantified as number of treatment bagels not consumed throughout each treatment period, was 99.4 ± 0.3 % (mean ± SE) for the control bagel and 99.2 ± 0.4 % (mean ± SE) for the RS bagel treatment period. Self-reported compliance was high overall with 100% compliance reported for 38 out of 47 completed treatment periods. In addition, participants routinely returned their bagel packaging and uneaten extra bagel at study visits.

Treatment bagel tolerance as assessed by sensory questionnaires is summarized in Table 13. The control bagel was preferred, as indicated by significantly higher liking scores for overall
liking, and liking of appearance, aroma, flavour, taste and texture at each study visit, when compared to the RS bagel ($P<0.05$).

When participants were asked about their feelings on consuming the treatment bagels if they were available after the study on study day 57, the most common response selected for both the RS and control bagels was “I like this and would eat it now and then” (Figure 4). There were no significant differences between the RS and control bagels for frequencies of any of the other response selections (Figure 4).

Presence of a satiety-related health claim on the RS bagels did not significantly change the frequency of any of the response selections (Figure 5). The most common response selected was still ‘I like this and would eat it now and then’ with the frequency increasing from 6 to 9 participants (Figure 5).

Side effects, as reported in questionnaires completed at each study visit, are summarized in Table 14. The majority of participants reported that no side effects (‘none’) were experienced during both treatment periods, and those who did report side effects described them as minor and therefore did not warrant a serious adverse event report. Flatulence was the most frequently reported side effect during the RS bagel treatment period, and was reported by up to 29% participants on each questionnaire (Table 14).
Table 13. Reported responses to sensory liking attributes of study treatment bagels following each two-week period of consumption\textsuperscript{1,2,3}

<table>
<thead>
<tr>
<th>Liking Attribute</th>
<th>Control Bagel (n=24)</th>
<th>RS Bagel (n=24)\textsuperscript{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Liking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 15</td>
<td>7.50 ± 0.19\textsuperscript{a}</td>
<td>5.75 ± 0.39\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>7.25 ± 0.24\textsuperscript{a}</td>
<td>5.75 ± 0.35\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>7.13 ± 0.23\textsuperscript{a}</td>
<td>5.13 ± 0.41\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>7.08 ± 0.23\textsuperscript{a}</td>
<td>5.22 ± 0.36\textsuperscript{b}</td>
</tr>
<tr>
<td>Liking of Appearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 15</td>
<td>7.38 ± 0.22\textsuperscript{a}</td>
<td>4.67 ± 0.44\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>7.29 ± 0.21\textsuperscript{a}</td>
<td>4.88 ± 0.42\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>7.17 ± 0.22\textsuperscript{a}</td>
<td>4.75 ± 0.42\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>7.21 ± 0.22\textsuperscript{a}</td>
<td>4.78 ± 0.38\textsuperscript{b}</td>
</tr>
<tr>
<td>Liking of Aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 15</td>
<td>6.42 ± 0.25</td>
<td>6.04 ± 0.29</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>7.00 ± 0.22\textsuperscript{a}</td>
<td>5.33 ± 0.32\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>6.88 ± 0.25\textsuperscript{a}</td>
<td>5.33 ± 0.27\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>6.79 ± 0.24\textsuperscript{a}</td>
<td>5.48 ± 0.26\textsuperscript{b}</td>
</tr>
<tr>
<td>Liking of Flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 15</td>
<td>7.50 ± 0.20\textsuperscript{a}</td>
<td>5.75 ± 0.42\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>7.25 ± 0.23\textsuperscript{a}</td>
<td>5.26 ± 0.40\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>7.04 ± 0.24\textsuperscript{a}</td>
<td>5.29 ± 0.35\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>7.00 ± 0.24\textsuperscript{a}</td>
<td>5.30 ± 0.33\textsuperscript{b}</td>
</tr>
<tr>
<td>Liking of Taste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 15</td>
<td>7.54 ± 0.20\textsuperscript{a}</td>
<td>5.88 ± 0.39\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>7.25 ± 0.23\textsuperscript{a}</td>
<td>5.42 ± 0.39\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>7.10 ± 0.23\textsuperscript{a}</td>
<td>5.25 ± 0.38\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>7.04 ± 0.24\textsuperscript{a}</td>
<td>5.30 ± 0.34\textsuperscript{b}</td>
</tr>
<tr>
<td>Liking of Texture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Day 15</td>
<td>7.29 ± 0.19\textsuperscript{a}</td>
<td>5.46 ± 0.42\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 29</td>
<td>7.21 ± 0.21\textsuperscript{a}</td>
<td>4.83 ± 0.39\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 43</td>
<td>6.83 ± 0.30\textsuperscript{a}</td>
<td>4.83 ± 0.38\textsuperscript{b}</td>
</tr>
<tr>
<td>Study Day 57</td>
<td>7.13 ± 0.21\textsuperscript{a}</td>
<td>4.87 ± 0.34\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are means ± SE.
\textsuperscript{2}Values within a row with different subscripts are significantly different (\(P<0.05\)).
\textsuperscript{3}Participants rated each attribute on a 9-point hedonic scale (1-9), reflecting on the previous 2 weeks of treatment bagel consumption.
\textsuperscript{4}n=24 for all study days except for study day 57 in RS bagel treatment period due to 1 participant dropout.
Figure 4. Participants’ reported feelings on consuming the study treatment bagels if they were available after the study.
Participant response selections to the question ‘Please indicate which of the following statements best reflects your feelings on consuming these bagels if they were available after the study’ and list of responses in relation to consuming the control and RS bagels. The question with the list of responses was posed on the last study day (day 57) of each treatment period. N=23 for the RS bagel treatment period due to 1 participant dropout.
Figure 5. Participants’ reported feelings on consuming the RS bagels if they were available after the study, with and without a health claim. Participant response selections to the question ‘If a function claim that relates resistant starch to satiety appeared on the treatment bagels, how likely is it that you would choose these bagels based on the function claim?’ Participants were given examples of possible function claims, such as ‘A serving of a resistant starch bagel is more filling for longer than a serving of a non-resistant starch bagel’. The question with the list of responses was posed on the last study day (day 57) of both treatment periods and data were analyzed for the RS bagel only. N=23 during RS bagel treatment period due to 1 participant dropout. N=22 for question with health claim as one participant did not answer the question.
### Table 14. Number of reported side effects to consumption of the study treatment bagels

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Control Bagel (n=24)</th>
<th>RS Bagel (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD15</td>
<td>21 (88)</td>
<td>16 (67)</td>
</tr>
<tr>
<td>SD29</td>
<td>21 (88)</td>
<td>17 (71)</td>
</tr>
<tr>
<td>SD43</td>
<td>19 (79)</td>
<td>13 (54)</td>
</tr>
<tr>
<td>SD57</td>
<td>20 (83)</td>
<td>13 (57)</td>
</tr>
<tr>
<td>Abdominal bloating, swelling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD15</td>
<td>0 (0)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>SD29</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD43</td>
<td>0 (0)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>SD57</td>
<td>1 (4.2)</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Belching</td>
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<td></td>
</tr>
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<td>SD15</td>
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</tr>
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<td>SD29</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD43</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD57</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Choking or difficulty swallowing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD15</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD29</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD43</td>
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<td>1 (4.2)</td>
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<tr>
<td>SD57</td>
<td>1 (4.2)</td>
<td>0 (0)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Control Bagel (n=24)</th>
<th>RS Bagel (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD15</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>SD29</td>
<td>1 (4.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD43</td>
<td>1 (4.2)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>SD57</td>
<td>1 (4.2)</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD15</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD29</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SD43</td>
<td>2 (8.3)</td>
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</tr>
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<td>SD57</td>
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<td>2 (8.7)</td>
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<tr>
<td>Flatulence</td>
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</tr>
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<td>3 (13)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>SD29</td>
<td>2 (8.3)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>SD43</td>
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<td>7 (29)</td>
</tr>
<tr>
<td>SD57</td>
<td>2 (8.3)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Nausea</td>
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<td></td>
</tr>
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<td>0 (0)</td>
</tr>
<tr>
<td>SD29</td>
<td>0 (0)</td>
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<tr>
<td>SD43</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td>SD57</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stomach pain</td>
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</tr>
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<td>SD15</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>SD29</td>
<td>0 (0)</td>
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<td>SD43</td>
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</tr>
<tr>
<td>SD57</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>Vomiting</td>
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<td></td>
</tr>
<tr>
<td>SD15</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>SD29</td>
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<td>0 (0)</td>
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<tr>
<td>SD43</td>
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<td>0 (0)</td>
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<tr>
<td>SD57</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD15</td>
<td>18 (4.2)</td>
<td>24 (8.3)</td>
</tr>
<tr>
<td>SD29</td>
<td>18 (4.2)</td>
<td>16 (4.2)</td>
</tr>
<tr>
<td>SD43</td>
<td>0 (0)</td>
<td>17 (4.2)</td>
</tr>
<tr>
<td>SD57</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

1 Data are n (%).
2 Abbreviations used: SD = study day.
3 n=24 except for study day 57 of RS bagel treatment period due to 1 participant dropout.
4 Altered timing of bowel habits
5 More bowel movements.
6 Drier stool.
7 Heartburn.
8 Softer stool.
D. Energy, Macronutrient and Dietary Fibre Intakes

Intakes of energy, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, or carbohydrates did not significantly differ among pre-study, RS bagel or control bagel treatment periods (Table 15). As expected, dietary fibre intake was significantly higher by approximately 20 g during the RS bagel period compared to the pre-study and control bagel periods ($P<0.0001$) (Table 15).

Table 15. Energy, macronutrient and dietary fibre intakes before and during the study (n=24)$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>Pre-Study (n=24)</th>
<th>Control Bagel (n=24)</th>
<th>RS Bagel (n=23)$^{3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kilocalories)</td>
<td>2022 ± 106.5</td>
<td>2060 ± 109.3</td>
<td>2019 ± 112.3</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>86.0 ± 6.28</td>
<td>82.9 ± 4.54</td>
<td>87.1 ± 6.66</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>79.3 ± 5.40</td>
<td>75.1 ± 5.78</td>
<td>72.4 ± 5.95</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>25.4 ± 2.09</td>
<td>26.3 ± 2.52</td>
<td>24.1 ± 2.28</td>
</tr>
<tr>
<td>Monounsaturated Fat (g)</td>
<td>18.5 ± 1.94</td>
<td>16.0 ± 1.44</td>
<td>16.2 ± 1.95</td>
</tr>
<tr>
<td>Polyunsaturated Fat (g)</td>
<td>8.85 ± 1.26</td>
<td>7.20 ± 0.72</td>
<td>8.5 ± 1.05</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>237.6 ± 17.2</td>
<td>260.9 ± 15.12</td>
<td>261.5 ± 14.9</td>
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<tr>
<td>Dietary Fibre (g)</td>
<td>20.3$^a$ ± 1.50</td>
<td>20.2$^a$ ± 1.39</td>
<td>41.4$^b$ ± 1.57</td>
</tr>
</tbody>
</table>

$^1$Data are means ± SE.
$^2$Values within a row with different superscripts are significantly different ($P < 0.001$).
$^3$n=23 due to 1 participant dropout.
E. Glycemic Response Biomarkers

E.i. Fasting Plasma Glucose, HbA1c and Serum Insulin

Fasting plasma glucose (Table 16, Figure 6), HbA1c (Table 16) and serum insulin (Table 17, Figure 6) were not significantly different between the RS and control treatment bagels on study day 1, providing evidence that the washout period was sufficient. On study day 57, fasting plasma glucose (Table 16, Figure 6) and HbA1c (Table 16) did not significantly differ between the RS and control treatment bagels; however, fasting serum insulin (Table 17, Figure 6) was significantly decreased following consumption of the RS treatment bagel compared to the control treatment bagel ($P=0.04$).

E.ii. Postprandial Plasma Glucose and Serum Insulin

Postprandial plasma glucose (Figure 7) and serum insulin (Figure 8) time curves at study days 1 and 57 for both the RS and control treatment bagels revealed significant differences between time points as expected. Generally, glucose and insulin significantly increased from time point 0, peaked at time points 30-60 minutes and decreased back to baseline by time points 120-180 minutes.

At study day 1, postprandial plasma glucose (Table 16, Figures 9 and 11) and serum insulin (Table 17, Figures 9 and 11) peak concentrations ($C_{MAX}$), 2-hour iAUC and 3-hour iAUC did not significantly differ between the RS and control bagel treatments, providing evidence that the washout period was sufficient.

At study day 57, postprandial plasma glucose $C_{MAX}$, 2-hour and 3-hour iAUC also did not significantly differ between the RS and control treatment bagels (Table 16, Figures 10 and 11); however, there were some significant differences for postprandial serum insulin. Serum insulin 2-hour iAUC and 3-hour iAUC at study day 57 were significantly decreased following
consumption of the RS treatment bagel compared to the control treatment bagel ($P=0.008$ and $P=0.05$, respectively) (Table 17, Figure 12). Serum insulin C$_\text{MAX}$ at study day 57 was not significantly different between the RS and control treatment bagels (Table 17).

**E.iii. Fasting Insulin Resistance, Insulin Sensitivity and Beta-Cell Function**

At study day 1, surrogate indices of fasting insulin resistance (HOMA-IR), beta-cell function (HOMA-%B) and insulin sensitivity (HOMA-%S) did not significantly differ between the RS and control treatment bagels, providing evidence that the washout period was sufficient (Table 18, Figure 13).

At study day 57, HOMA-IR and HOMA-%B were significantly decreased following consumption of the RS treatment bagels compared to the control treatment bagels ($P=0.04$ and $P=0.009$, respectively) (Table 18, Figure 13). Conversely, study day 57 HOMA-%S was significantly increased following consumption of the RS treatment bagels compared to the control treatment bagels ($P=0.04$) (Table 18, Figure 13).
Table 16. Fasting and postprandial OGTT plasma glucose response and HbA1c before and after consumption of control and RS bagel treatments\(^1,2\)

<table>
<thead>
<tr>
<th></th>
<th>Control Bagel</th>
<th></th>
<th>RS Bagel</th>
<th></th>
<th>P value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study Day 1 (n=24)</td>
<td>Study Day 57 (n=24)</td>
<td>Study Day 1 (n=24)</td>
<td>Study Day 57 (n=23)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fasting glucose (mmol/L)</th>
<th>5.36 (5.13, 5.59)</th>
<th>5.31 (5.16, 5.46)</th>
<th>5.33 (5.10, 5.56)</th>
<th>5.28 (5.13, 5.43)</th>
<th>0.79</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Postprandial glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose C(_{\text{max}}) (mmol/L)</td>
<td>8.57 (7.95, 9.19)</td>
<td>9.09 (8.51, 9.67)</td>
<td>8.86 (8.24, 9.48)</td>
<td>8.54 (7.95, 9.13)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>2-Hour glucose iAUC (mmol/L x 120 minutes)</td>
<td>198.3 (152.2, 244.3)</td>
<td>245.2 (199.3, 291.1)</td>
<td>230.4 (184.4, 276.5)</td>
<td>207.4 (160.5, 254.3)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>3-Hour glucose iAUC (mmol/L x 180 minutes)</td>
<td>202.9 (150.1, 255.6)</td>
<td>261.9 (210.5, 313.3)</td>
<td>246.5 (193.8, 299.2)</td>
<td>217.8 (165.3, 270.3)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>HbA1c (%)</td>
<td>5.72 (5.64, 5.81)</td>
<td>5.66 (5.60, 5.73)</td>
<td>5.69 (5.61, 5.78)</td>
<td>5.71 (5.64, 5.78)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

\(^1\)Data are least squares means (95% confidence intervals).

\(^2\)Abbreviations used: C\(_{\text{max}}\)=maximum concentration of response; HbA1c=glycated hemoglobin; iAUC=incremental area under the curve; OGTT=oral glucose tolerance test.

\(^3\)P value from ANCOVA on study day 57 values including study day 1 values as a covariate followed by the Tukey’s test for multiple comparisons.
Table 17. Fasting and postprandial OGTT serum insulin response before and after consumption of control and RS bagel treatments

<table>
<thead>
<tr>
<th></th>
<th>Control Bagel</th>
<th>RS Bagel</th>
<th>P value&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study Day 1</td>
<td>Study Day 57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=24)</td>
<td>(n=24)</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>75.2 (62.0, 91.1)</td>
<td>88.2 (75.2, 103.5)</td>
<td>90.0 (74.3, 109.1)</td>
</tr>
<tr>
<td>Postprandial insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin C&lt;sub&gt;MAX&lt;/sub&gt; (pmol/L)</td>
<td>657.9 (536.5, 806.9)</td>
<td>693.6 (599.9, 802.0)</td>
<td>730.9 (596.0, 896.4)</td>
</tr>
<tr>
<td>2-Hour insulin iAUC (pmol/L x 120 minutes)</td>
<td>42100 (34031, 52083)</td>
<td>47794 (41690, 54792)</td>
<td>48627 (39307, 60157)</td>
</tr>
<tr>
<td>3-Hour insulin iAUC (pmol/L x 180 minutes)</td>
<td>49396 (39402, 61925)</td>
<td>55673 (47846, 64783)</td>
<td>55434 (44219, 69495)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are geometric least squares means (95% confidence intervals).
<sup>2</sup>Abbreviations used: C<sub>MAX</sub>=maximum concentration of response; iAUC=incremental area under the curve; OGTT=oral glucose tolerance test.
<sup>3</sup>P value from ANCOVA on study day 57 values including study day 1 values as a covariate followed by the Tukey’s test for multiple comparisons.
a. Fasting plasma glucose

![Graph showing fasting plasma glucose levels for control bagel and RS bagel treatments on study days 1 and 57.]

b. Fasting serum insulin

![Graph showing fasting serum insulin levels for control bagel and RS bagel treatments on study days 1 and 57.]

Figure 6. Fasting plasma glucose (a) and serum insulin (b), on study days 1 and 57 of the control and RS bagel treatments (n=24). Data are least squares means (glucose) and geometric least squares means (insulin) and 95% confidence intervals. n=23 on study day 57 of RS bagel treatment period due to 1 participant dropout. *P=0.04, ANCOVA on study day 57 values including study day 1 values as a covariate followed by the Tukey’s test for multiple comparisons.
Figure 7. Postprandial plasma glucose time point curves for the control (a and b) and RS (c and d) bagel treatments on study days 1 and 57 (n=24). Data are least squares means and 95% confidence intervals. n=23 on study day 57 of RS bagel treatment due to 1 participant dropout.
Figure 8. Postprandial serum insulin time point curves for the control (a and b) and RS (c and d) bagel treatment on study days 1 and 57 (n=24). Data are geometric least squares means and 95% confidence intervals. n=23 on study day 57 of RS bagel treatment due to 1 participant dropout.
a. Postprandial plasma glucose – study day 1

![Graph showing plasma glucose levels over time for control and RS bagel treatments.]

b. Postprandial serum insulin – study day 1

![Graph showing serum insulin levels over time for control and RS bagel treatments.]

Figure 9. Postprandial plasma glucose (a) and serum insulin (b) time point curves on study day 1 for the control and RS bagel treatments (n=24). Data are geometric least squares means and 95% confidence intervals.
a. Postprandial plasma glucose – study day 57

![Plasma Glucose Time Point Curves](image)

b. Postprandial serum insulin – study day 57

![Serum Insulin Time Point Curves](image)

Figure 10. Postprandial plasma glucose (a) and serum insulin (b) time point curves on study day 57 for the control and RS bagel treatments (n=24). Data are least squares means (glucose) and geometric least squares means (insulin) and 95% confidence intervals. N=23 on study day 57 of RS bagel treatment period due to 1 participant dropout.
a. Plasma glucose 2-hour iAUC

![Graph showing plasma glucose 2-hour iAUC for control and RS bagel treatments on study days 1 and 57.]

b. Plasma glucose 3-hour iAUC

![Graph showing plasma glucose 3-hour iAUC for control and RS bagel treatments on study days 1 and 57.]

Figure 11. Plasma glucose 2-hour (a) and 3-hour (b) iAUC on study days 1 and 57 of the control and RS bagel treatments (n=24). Data are least squares means and 95% confidence intervals. N=23 on study day 57 of RS bagel treatment period due to 1 participant dropout.
Figure 12. Serum insulin 2-hour (a) and 3-hour (b) iAUC on study days 1 and 57 of control and RS bagel treatment periods (n=24)\(^1\). Data are geometric least squares means and 95% confidence intervals. n=23 on study day 57 of RS bagel treatment period due to 1 participant dropout. * P≤0.05, ANCOVA on study day 57 values including study day 1 values as a covariate followed by the Tukey’s test for multiple comparisons.
Table 18. Surrogate indices of fasting insulin resistance, insulin sensitivity and beta-cell function before and after consumption of control and RS bagels\(^1\)\(^2\)

<table>
<thead>
<tr>
<th></th>
<th>Control Bagel</th>
<th></th>
<th>RS Bagel</th>
<th></th>
<th>P value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 (n=24)</td>
<td>Day 57 (n=24)</td>
<td>Day 1 (n=24)</td>
<td>Day 57 (n=23)</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.97 (2.40, 3.67)</td>
<td>3.47 (2.91, 4.13)</td>
<td>3.52 (2.85, 4.36)</td>
<td>2.67 (2.24, 3.19)</td>
<td>0.04</td>
</tr>
<tr>
<td>HOMA-%B</td>
<td>140.0 (119.3, 164.4)</td>
<td>169.6 (148.4, 193.9)</td>
<td>168.6 (143.6, 197.9)</td>
<td>130.8 (114.2, 150.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>HOMA-%S</td>
<td>33.7 (27.3, 41.7)</td>
<td>28.8 (24.2, 34.4)</td>
<td>28.4 (22.9, 35.1)</td>
<td>37.4 (31.3, 44.7)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\(^1\)Data are geometric least squares means (95% confidence intervals).
\(^2\)Abbreviations used: HOMA=homeostasis model assessment of (IR=insulin resistance; %B=beta-cell function; %S=insulin sensitivity).
\(^3\)P value from ANCOVA on study day 57 values including study day 1 values as a covariate followed by the Tukey’s test for multiple comparisons.
Figure 13. Surrogate indices of fasting insulin resistance (HOMA-IR; (a)), beta-cell function (HOMA-%B; (b)) and insulin sensitivity (HOMA-%S; (c)) on study days 1 and 57 of the control and RS bagel treatments (n=24). Data are geometric least squares means and 95% confidence intervals. n=23 on study day 57 of RS bagel treatment period due to 1 participant dropout. *P≤0.05, ANCOVA on study day 57 values including study day 1 values as a covariate followed by the Tukey’s test for multiple comparisons.
V. DISCUSSION

The primary purpose of this study was to determine if chronic consumption of bagels made from high-RS flour would improve markers of T2D in adults at increased risk of T2D. Participants completed a 3-step screening process to confirm they were at an increased risk of T2D, consumed a high-RS (25 g) and control bagel daily for 8-weeks separated by a 4-week washout period in a randomized crossover study design. This study adds to a limited number of RS-food intervention studies that have examined those at risk of T2D. Focusing on those at risk of T2D is the most pertinent strategy in order to determine the effects in those in which the development of T2D pathologies may have begun, and therefore, may stand to benefit from RS consumption in a measurable amount. This study also expands the literature with its focus on RS in a food matrix, an approachable form of intervention compared to supplements, and more relevant to food manufacturers, healthcare professionals and consumers. Finally, the study of postprandial glycemic effects of chronic RS consumption in response to an OGTT in this study is unique as some RS-food studies (78,81,82) include the RS treatment in the postprandial meal challenge, introducing new variables and potentially confounding the results. Other chronic RS studies (72–75,77) have used more standardized tests such as IVGTTs or euglycemic hyperinsulinemic clamp tests and are of value; however, bypass the effects of gut-secreted hormones which are stimulated by nutrient absorption in the intestinal tract. Alternatively, a standard OGTT measures the effects of chronic RS consumption on the overall glycemic response, incorporating the physiological effects of digestion, and without introducing the pre-test meal as an additional variable. Finally, the clinical relevance of an OGTT is high since they are routinely used in clinical practice to assess glycemic response.
A. Participant Flow

The present study experienced a relatively low 8% attrition rate (2 out of 25 participants), whereas previous RS-food intervention studies have seen higher attrition rates of approximately 12% (83), 15% (84), 17% (81) and 21% (82). The low rate of attrition may be due to numerous aspects of the present study including the 3-step screening process; the completion of a study orientation session to fully inform participant of the requirements of the study; the effort put forth by researchers to maintain a welcoming and safe atmosphere and good rapport with participants; and the popularity and ease of incorporating the treatment bagels into the participants’ lifestyles. Though the two participants who left the study did so during their RS-bagel treatment period, their reasons were unforeseen (antibiotic use and family relocation) and not related to the study treatments or protocol.

B. Participant Characteristics

The current study employed a thorough 3-step screening process to ensure a sample of adults at high risk for T2D. The key determining factor of T2D risk was the CANRISK questionnaire (Appendix H), which assessed a number of factors including age, BMI, waist circumference, previous medical and family history, aspects of diet and lifestyle, and socio-demographic factors (15). Increased age, BMI and waist circumference were considered in the CANRISK questionnaire, and were also individual specific inclusionary criteria as they are each considered risk factors for T2D (64,88,89). Fasting blood glucose was also measured at screening to exclude those who were likely to have T2D (16). The thorough screening process aided in the identification of a sample of participants reflective of those who stand to benefit from increased RS consumption and reduced T2D risk, but excluded those with T2D who may have disordered metabolic processes in place.
The strategy to focus on participants at increased risk of T2D has been used in previous research, for example Maki et al. (2012) (74) studied participants with increased BMI and waist circumference, and Johnston et al. (2010) (73), Bodinham et al. (2012) (75) and Robertson et al. (2012) (76) studied insulin resistant participants defined by increased fasting insulin, though these were chronic RS-supplement studies. Chronic RS-food studies among those at increased risk of T2D have been completed, though criteria to determine T2D risk have varied among studies: Penn-Marshall et al. (2012) (83) defined high-risk of T2D by increased BMI, family history and ethnicity, while Noakes et al. (1996) (82) defined T2D risk by increased BMI and the presence of hypertension or hypertriglyceridemia. Interestingly, these two studies demonstrate somewhat contrasting results, which may have been partly due to differences in participant characteristics. The present study is a valuable addition to the literature as it sought to determine the impact of chronic RS-food consumption among adults at increased risk of T2D as determined by a validated, multifaceted screening tool (90).

Participants in the current study had an average baseline body weight of 90.4 kg and BMI 30.2 kg/m² which contributes to their increased T2D risk. Importantly, these variables did not significantly change during the study which reduces the potential confounding effect on the study and gives strength to the study’s results as it demonstrates that the effects of RS consumption are not likely to be due to any changes in BMI over the course of each treatment period.

C. Self-Reported Compliance, Treatment Tolerance and Side Effects

Overall, self-reported compliance with each study treatment was high, as 100% compliance was achieved for 39 out of 48 treatment periods, with an overall mean compliance rate > 99%. Self-reported compliance was encouraged during the study through frequent
conversation between study coordinators and participants about how the bagels were incorporated into the participants’ diets, occasional newsletters with recipes and bagel preparation ideas, and by reviewing participant study diaries at bi-weekly study visits. The high rate of compliance during this study, though self-reported, is an indication that the treatment bagels are a feasible strategy to increase RS in the daily diet for at least 8 weeks.

As a measure of bagel palatability, another component of feasibility, participants completed bi-weekly sensory questionnaires throughout each treatment period, rating their liking of sensory qualities of each of the study bagels. The mean scores for how much the participants liked or disliked the bagels ‘Overall’ ranged from 5.13 to 5.75 (5=‘Neither like or dislike’ and 6=‘Like slightly’) for the RS bagels, and 7.13 to 7.5 (7=‘Like moderately’ and 8=‘Like very much’) for the control bagels, indicating that neither bagel was disliked. Results showed that participants preferred the control over the RS bagel as the mean scores for all sensory qualities (except aroma) of the control bagel were significantly higher than the RS bagel. Given that the Hi-Maize® 260 ingredient does not have a strong taste or aroma on its own and has a texture similar to wheat flour, it was expected that the final high-RS treatment bagels would not vary greatly from the control treatment bagels with respect to their taste, aroma, texture or appearance. It would appear, however, that the proportion of Hi-Maize® 260 used in the product formulation is an important factor in the extent of the sensory impact of the Hi-Maize® 260 on the final product. There is limited research that investigates the sensory impact of incorporating Hi-Maize® 260 into wheat bread-based foods published to date; one such study by Maziarz et al. (2013) (103) found that RS muffins scored higher (but not significantly) in ‘overall liking’ compared to the wheat muffin control among an untrained sensory panel. However, the level of inclusion of Hi-Maize® 260 in the muffin was only 4.73 g/100g (total wet weight (TWW) of
dough), compared to 30.2 g/100g TWW of dough in the present study’s RS treatment product (calculated from recipe formulation of 168 kg Hi-Maize® 260 in 556.5 kg total dough). When included at a higher level in focaccia bread in the same study by Maziarz et al. (10.17 g Hi-Maize® 260/100g TWW of dough), the overall liking of the RS focaccia bread was significantly lower than the wheat-focaccia control (103). These data suggest that low proportions of Hi-Maize® 260 incorporated into wheat bread-based applications may not have a significant effect on liking while higher proportions (> 10 g Hi-Maize® 260/100g TWW of dough) may detract from the overall liking of the final product. This has important implications for functional food product development, as the amount of RS/serving of a high-RS food may need to be higher than the RS muffin in the study by Maziarz et al. (103) in order to provide health benefits following chronic consumption. This also demonstrates that there is great opportunity for the improvement of high-RS ingredients and food formulations.

Common verbal feedback from participants throughout the study included the desire for other bagel flavours, which was not possible for this study due to the need to produce all control and RS bagels each in a single batch, and due to the goal of maximizing the versatility and universal acceptance of the study bagels. It is possible that adding flavours or ingredients that are common among commercially available bagels may improve the palatability of the RS treatment bagels, though efforts should be made to minimize the macronutrient contribution of any added flavouring ingredients so as not to disturb the macronutrient distribution found to be effective in the present study.

When participants were questioned on how likely they were to consume the bagels if they were available after the study, there were differences between the RS and control bagel. Most notably, 76% of participants within the control versus 39% of participants within the RS bagel
treatment responded with the statement “I like this and would eat it now and then” or a better statement, though this did not reach statistical significance. The chronic RS-food interventions reviewed so far have not reported posing this question to their participants, but the data from the current study indicate that the high-RS bagel formulation used in this study should be improved if made available for sale.

When participants were presented with a scenario in which the RS bagels would have a satiety-related health claim that said these bagels would keep them fuller for longer than non-resistant starch bagels, there was an increase in the percent of participants (64% vs 39%) that answered “I like this and would eat it now and then” or a better statement, though this did not reach statistical significance. It is intuitive to expect that the presence of a health claim on the RS bagels would increase the willingness to consume the RS bagels, but there are many factors that impact consumers’ willingness to consume functional foods, such as belief in the health claim, and one’s knowledge of food, nutrition and health as demonstrated in Belgian adults by Verbeke (104). Moreover, another study by Verbeke in 2006 (105) found that consumers were unlikely to sacrifice taste for the benefits of functional foods. The questionnaires used in this study did not attempt to ascertain the reason why participants were less likely to consume the treatment bagels after the study, but it is likely that the taste and overall liking of the RS treatment bagels played a role. In summary, it appears that though these results did not reach statistical significance, participants generally accepted the RS bagels while preferring the control bagels, and were more likely to consume the RS bagels if they carried a satiety function claim.

Participants in the current study reported only minor side effects from consuming both the RS and control bagels during the 8-week interventions. Though there were higher rates of flatulence, bloating and belching throughout the RS bagel treatment period than the control bagel
treatment period, no serious adverse events were reported during either treatment period. Flatulence was the most frequently reported side effect, which was reported by 21-29\% and 8-13\% of participants each 2-week period during the RS and control bagel treatment periods, respectively. No other side effect (belching, abdominal bloating, constipation, diarrhea, difficulty swallowing) was reported more than three times during either the RS or control bagel treatment periods. Previous studies document that reported side effects of RS consumption are generally related to the GI system, though their significance is inconsistent in chronic RS intervention studies to date. In an 8-week 40 g HAM-RS2 supplement study (n=15 insulin resistant adults) by Robertson et al. (2012) (76) there were no significant effects of RS on flatulence, stool frequency, abdominal pain or bloating. However, a small but significant increase in flatulence was previously described in a 12-week 40 g HAM-RS2 supplement study (n=17 adults with T2D) by Bodinham et al. (2014) (77). The low number of reports of GI-related side effects and inconsistently significant results are also reported in chronic RS-food studies. Park et al. (2004) (80) intervened with 24 g RS\(_3\) in a soup mix daily in 25 healthy adult women and found no significant increase in reports of bowel discomfort (including flatulence, nausea and distension) during the RS intervention, compared to control. Similarly, Klosterbuer et al. (2013) (106) who reported on the GI effects of the consumption of meals (muffin, hot cereal and beverage) with 25 g RS\(_3\) for 7 days, found no significant difference in GI score (mean score of bloating, flatulence, cramps and stomach noise; higher score = higher frequency) at the end of the treatment periods compared to control, however, consumption of similar meals with 25 g RS with the addition of 5 g pullulan fibre did significantly increase GI score compared to all other treatments. The low frequency of side effects in the current study of 25 g RS for 8 weeks are consistent with a review by Grabitske and Slavin (2009) that summarized that doses >50 g
RS for 4 weeks have been tolerated well with only mild side effects, most often pertaining to increased flatulence, bloating, fecal weight and consistency (107). The data from the current study provide further evidence that as much as 25 g of RS incorporated into a bagel can be consumed daily for up to 8 weeks, and that this is a feasible strategy to greatly increase RS intake since it did not result in any significant increases in GI-related side effects.

**D. Energy, Macronutrient and Dietary Fibre Intakes**

Three-day food records collected before the study and in the middle of each treatment period showed that energy and macronutrient intake did not significantly differ over the course of the study. However, dietary fibre intake was significantly higher by approximately 20 g during the RS bagel treatment period. This increase was expected and can be directly attributed to the incorporation of one RS bagel into the diet each day, which provided 25.6 g dietary fibre, compared to the control bagel which provided 2.6 g dietary fibre daily. The increase in daily fibre intake was slightly less than the difference in fibre content of each bagel, which may be due to the satiating effect of a high-fibre meal which could reduce the desire to eat other filling (e.g. high-fibre) food later in the day, although did not manifest in any energy intake differences. The lack of significant differences in energy intake between pre-study and the RS bagel treatment indicates that the RS bagels can be incorporated into a diet without altering a major contributing factor of body weight and BMI, increases in which lead to an increase in T2D risk (64,108). The consistent energy intake throughout the present study also provides evidence that the mechanism by which RS exerts its effects is independent of a reduction in overall energy intake, a strategy that itself improves markers of T2D if the caloric reduction is significant and maintained (9). The lack of any significant differences in energy and macronutrient distribution between the RS
and control bagel treatments reduces the potentially confounding effect these variables may have had on the study’s glycemic outcome measures.

E. Glycemic Response Biomarkers

E.i. Fasting Plasma Glucose, HbA1c and Serum Insulin

Fasting plasma glucose, HbA1c and serum insulin were not significantly different between the RS and control bagel treatments on study day 1, providing evidence that the participants began each treatment period in a similar state of glycemic control, and evidence that the 4-week washout period was sufficient.

Fasting plasma glucose was also not significantly different between the RS and control bagel treatments on study day 57 which is consistent with the majority of previous chronic RS-supplement and RS-food intervention studies (71,72,74,77–84). Chronic RS interventions by Bodinham et al. (75) and Robertson et al. (76) are the only studies to date that have reported a significant reduction in fasting glucose levels compared to control treatments, and both interventions were high-dose (40 g) RS in supplement form consumed for 4 weeks in 12 insulin resistant participants (75) and 8 weeks in 15 insulin resistant adults (76).

HbA1c was also not significantly different between the RS and control bagel treatments on study day 57, and while this endpoint is not often measured, all three chronic RS interventions that did measure HbA1c also found no significant effect of 12 weeks of 40 g RS supplements in 17 adults with T2D (77); 6 weeks of 12 g RS in bread in 15 obese African-American adults (83); or 4 weeks of 6.51 g RS in rice mix in 85 adults with prediabetes or T2D (84). It is possible that the lack of effects were due to insufficient treatment duration in the present study (8 weeks) since HbA1c typically represents the mean blood glucose concentration over the previous 12 weeks (109).
Fasting serum insulin was significantly decreased by approximately 24% by the RS bagel compared to the approximate 17% increase with the control bagel in the current study. This is comparable to a study that examined 4 weeks of a RS-rice mix in 85 adults with prediabetes or T2D, although the significant reduction in insulin was lost once it was adjusted for baseline values (84). Most other studies of chronic RS-food interventions have not detected a significant improvement in fasting insulin following consumption of muffins, pudding and crackers for 5 weeks in 12 healthy adults (78); breads, cereals, muffins and cheese puffs for 14 in weeks 24 adults (10 healthy and 14 with hyperinsulinemia) (81); breads, muffins, pasta and cereals for 4 weeks in 23 overweight adults (82); soup mix for 3 weeks in 25 healthy adults (80); or bread for 1 day in 9 healthy adults (79) and for 6 weeks 15 obese African-American adults (83). These results could be due to an insufficient treatment duration (e.g. 1 day (79)), a relatively low dose of RS in the study treatment (e.g. 6.51 g (84), 10.4 g (79) or 12 g (83)), an insufficient sample size (e.g. n=9 (79) or n=12 (78)), or possibly a small sample size (n=25) combined with a parallel-arm study design (80). In addition, the lack of effect on fasting insulin in some studies may be due to the fact that the some or all participants were healthy and did not have elevated fasting insulin at baseline (78,81), or possibly because the study had no inclusion criteria specific to current risk of T2D, insulin resistance or elevated fasting insulin (82).

Other studies of RS supplement interventions ranging from doses of 16 to 60 g RS/day for 24 hrs to 12 weeks (71,72,74,75,77) have largely found no significant changes in fasting serum insulin, although one study in 15 insulin resistant adults that used a 40 g RS supplement and measured insulin at the end of 8 weeks did find a significant decrease in fasting insulin by approximately 16% compared to control (76).
Fasting insulin concentration is relevant as elevated fasting insulin is an indicator of the development of insulin resistance and can often predict IFG and T2D development (110). The concentration of fasting insulin is closely related to that of fasting glucose in metabolically healthy individuals (111). However, as fasting glucose concentration rises (due to reduced glucose uptake in peripheral tissues, a feature of insulin deficiency and/or resistance, or due to unsuppressed hepatic glucose production, a feature of hepatic insulin resistance (12)), pancreatic beta-cells produce and secrete more insulin in attempt to maintain glucose homeostasis in the fasted state, leading to elevated fasting insulin concentrations (110). Diminished hepatic insulin extraction (clearance of circulating insulin) can also occur in individuals with obesity and/or insulin resistance, which may also contribute to increased fasting insulin levels (112). The presence of elevated fasting insulin with normal or slightly elevated glucose levels can indicate early hepatic insulin resistance (100), which is often an early indicator of prediabetes (10). Increased insulin secretion will compensate for elevated glucose at first, but with time increased insulin demand along with sustained hyperglycemia (and often concomitant dyslipidemia) will reduce the ability of beta-cells to compensate and exacerbate insulin resistance (108); over time the body’s insulin requirements will outweigh its provision, leading to hyperglycemia and shifting the individual towards development of T2D. However, if some lifestyle, dietary or pharmacological intervention is undertaken early enough in this pathogenesis, a reduction in fasting insulin concentration over time would indicate that the insulin sensitivity is being restored, which is the goal of many T2D prevention strategies. The present study demonstrated that a simple dietary intervention (such as the incorporation of 25 g RS in the form of a bagel each day for 8 weeks) can significantly reduce fasting insulin, a potential indicator of reduced insulin resistance, and therefore reduce the risk of developing T2D.
E.ii. Postprandial Plasma Glucose and Serum Insulin

The current study also examined postprandial glycemic response to a 3-hour OGTT before and after consumption of the study bagels. Following consumption of the glucose beverage plasma glucose rose predictably, peaking around 30 – 60 minutes, and returning to baseline around 180 minutes, followed closely by serum insulin which peaked slightly later, at around 60 minutes. These responses, which were similar for the RS and control bagel treatments before and after the intervention, are characteristic of the response of a non-diabetic to a standard OGTT (113).

The comparability of the postprandial glucose and insulin responses on study day 1 of each the RS and control bagel treatments, including the peak concentrations ($C_{\text{MAX}}$), 2-hour iAUC and 3-hr iAUC, provided evidence that the participants began each treatment period in a similar state of glycemic control, and that the 4-week washout period was sufficient.

Study day 57 postprandial glucose parameters ($C_{\text{MAX}}$, 2-hour iAUC and 3-hour iAUC) did not significantly differ between the RS and control bagel treatments, which is consistent with a previous chronic RS supplement studies that measured the postprandial glucose response to a standard (RS-free) MTT following consumption of 30 g RS for 4 weeks in 10 healthy adults (72). Postprandial glucose measured during a treatment tolerance test (test meal included the RS treatment) were also not significantly reduced following chronic consumption of high-RS interventions such as high-amylose pudding, muffins and crackers (RS dose not reported) for 5 weeks in 12 healthy adults (78); high-amylose breads, cereals, muffins and cheese puffs (RS dose not reported) for 14 weeks in 10 healthy and 14 hyperinsulinemic adults (81); 17-25 g RS in breads, cereals pasta and muffins for 4 weeks in 23 overweight adults (82); and 30 g RS in bread for 1 day in 9 healthy adults (79). However, one study did find a significant reduction in glucose
AUC in 85 adults with prediabetes or T2D following consumption of a RS-rice mix for 4 weeks (84). The severity of insulin resistance of the participants (indicated by the presence of prediabetes of T2D) may have increased their likelihood of response to the RS, in contrast to the current study’s participants who likely did not have a high degree of insulin resistance as they did not have prediabetes or T2D.

Study day 57 postprandial serum insulin parameters (2-hour and 3-hour iAUC) were significantly decreased with consumption of the RS compared to the control bagel treatment, and there was a non-significant ($P=0.07$) trend toward a decreased peak insulin in the current study. These results are consistent with previous chronic RS-food studies which have demonstrated significantly reduced postprandial insulin AUC (or ‘summed insulin’ - the sum of insulin levels at each timepoint; AUC not calculated) following 5 weeks of high-amylose pudding muffins and crackers in 12 healthy males (RS dose not reported) (78); 14 weeks of high-amylose bread, muffins, cereals and cheese puffs (RS dose not reported) in 10 healthy and 14 hyperinsulinemic adults (81); and 4 weeks of 17-25 g RS in bread, cereal, pasta and muffins in 23 overweight adults (82). An important distinction, however, is that these studies included RS as part of the meal tolerance test (rather than using a standardized OGTT as in the current study). The RS in the meal challenge would contribute to the postprandial response and therefore the results cannot be attributed to the chronic RS consumption alone. Kwak et al. (84) did utilize a standardized, RS-free meal tolerance test in 85 adults with prediabetes and T2D after 4 weeks of consumption of 6.51 g RS in a RS-rice mix, and also found that insulin AUC was significantly reduced, however the significance was lost when the data were adjusted for variation in baseline values.

Postprandial insulin iAUC has also been significantly reduced in previous chronic RS-supplement studies that have examined a standardized (RS-free) tolerance test after RS exposure.
for 1 day in 10 healthy adults (71) and 4 weeks in 10 healthy adults (72). However another study did not observe a significant change in postprandial insulin iAUC response to a standardized test following 12 weeks of a 40 g RS supplement in 17 adults with T2D (77), possibly due to the presence of T2D as the loss of beta-cell function is likely to be more advanced in these individuals (12) making them incomparable to healthy individuals.

The present study is the first to demonstrate that chronic consumption of a RS-enhanced food significantly reduces postprandial serum insulin in response to a standardized oral glucose challenge (OGTT), among adults at an increased risk of T2D. This result is important as the use of an OGTT to evaluate glycemic response is clinically relevant. In addition, by using the standardized OGTT to measure the postprandial response before and after the intervention, any effects on the postprandial response can be directly attributed to the intervention. Furthermore, the only studies that demonstrated the benefit of chronic RS consumption on postprandial glycemia following a standardized (RS-free) tolerance test in adults without prediabetes or T2D (with significant results) were RS-supplement studies (71,72), therefore this study extends the literature by demonstrating the benefits of chronic RS consumption when incorporated into a food in adults at an increased risk for T2D. This provides great opportunity for food manufacturers, consumers and healthcare providers to benefit from the development and consumption of RS-enhanced foods in the future.

This study demonstrated that while the postprandial glucose response was not significantly reduced, the postprandial insulin response required to manage the same concentration of glucose was significantly reduced following the 8-week RS treatment bagel intervention, which can be collectively interpreted as improved postprandial insulin sensitivity. Postprandial insulin sensitivity was not measured in the current study. It is possible to calculate
indices of postprandial insulin sensitivity from methods other than the OGTT used in the present study, such as an intravenous glucose tolerance test (IVGTT) using the minimal model analysis (MINMOD) (74,75), or the highly regarded euglycemic-hyperinsulinemic clamp test using M/I (rate of glucose infusion divided by plasma insulin concentration over 30 minutes at steady-state (72,73)). Previous chronic RS interventions that calculated such indices of postprandial insulin sensitivity following a euglycemic-hyperinsulinemic clamp (72,73) and a IVGTT (74,75), have demonstrated the ability of RS supplements (30 g RS for 4 weeks in 10 healthy adults (72); 40 g RS for 12 weeks in 20 insulin resistant adults (73); and 15 and 30 g RS for 4 weeks in 11 overweight and obese males (74)) to increase postprandial insulin sensitivity, while one 4-week, 40 g RS supplement intervention in 12 insulin resistant adults did not improve insulin sensitivity (75). While IVGTT and euglycemic-hyperinsulinemic clamp methods are highly regarded, they are also costly, labour intensive, and less physiological than an OGTT which incorporates the effects of digestion since it does not bypass the gastrointestinal tract (100). Although the present study did not calculate an index of postprandial insulin sensitivity, this research extends the literature by providing evidence that chronic RS-food consumption significantly reduces the postprandial insulin response to manage a constant glucose concentration, and thereby improves postprandial insulin sensitivity among adults at risk of T2D, as measured during a relatively simple and accessible OGTT.

E.iii. Fasting Insulin Resistance, Insulin Sensitivity and Beta-Cell Function

Although postprandial insulin sensitivity was not measured in the current study, indices of fasting insulin resistance, insulin sensitivity and beta-cell function (HOMA-IR, HOMA-%S, HOMA-%B) were calculated from fasting glucose and insulin values on study days 1 and 57 of each treatment period. Study day 1 values of HOMA-IR, HOMA-%S or HOMA-%B were not
different between the RS and control bagel treatments, providing further evidence of similar starting glycemic control and indicating a sufficient washout period.

Study day 57 values of HOMA-IR, HOMA-%S and HOMA-%B revealed that the RS bagel treatment significantly reduced HOMA-IR (fasting insulin resistance) and increased HOMA-%S (fasting insulin sensitivity) while significantly reducing HOMA-%B (beta-cell function). The simultaneous reduction of insulin resistance and beta-cell function illustrates the concept of improved insulin sensitivity, where less insulin is secreted in order to effectively manage a constant glucose challenge.

These results are consistent with Robertson et al. (76) who found significantly reduced fasting insulin resistance (or increased fasting insulin sensitivity) following 8 weeks of a 40 g HAM-RS2 supplement in 15 adults with insulin resistance. Participants had elevated abdominal adiposity, BMI and fasting insulin (76) which is comparable to the current study, although the participants in the study by Robertson et al. (76) fulfilled the European Group for the Study of Insulin Resistance (EGIR) diagnostic criteria of insulin resistance, which the authors noted as the “most discriminating” insulin resistance criteria. Looking closely at studies that focussed on adults with established insulin resistance highlights the inconsistency of results in chronic RS research, as a very similar study to Robertson et al. (76), (by Johnston et al. (73) who studied supplementation of 40 g RS for 12 weeks in 20 adults with insulin resistance) found that the RS supplement did not improve HOMA-%S or HOMA-%B. Nevertheless, as the present study’s participants did not satisfy all EGIR criteria for insulin resistance, this research extends the literature in that a similar result was present (significantly improved fasting insulin resistance (HOMA-IR)) among adults without a diagnosis of insulin resistance, but with elevated risk of T2D. Further, this effect was achieved using a lower-dose of RS (25 g RS compared to 40 g RS
in Robertson et al. (76)), to a greater extent (present study reduced HOMA-IR by approximately 24%, compared to approximately 14% in Robertson et al. (76)), and additionally, in the form of popular and convenient a bread-based functional food (compared to a supplement in Robertson et al. (76)).

Despite the significant findings by Robertson et al. (76) and the present study, the majority of chronic RS interventions that have examined fasting insulin sensitivity have not found an improvement following RS consumption in the form of either a RS-supplement (60 g RS for 1 day in 10 healthy adults (71); 30 g RS for 4 weeks in 10 healthy adults (72); 15 and 30 g RS/day for 4 weeks in 33 overweight and obese adults (74); 40 g RS for 12 weeks in 17 adults with T2D (77); and 40 g RS for 12 weeks in 20 adults with insulin resistance (73)) or a RS-food (12 g RS in bread for 6 weeks in 15 obese African-American adults (83)). Complete comparison of the current study’s fasting insulin sensitivity, resistance and beta-cell function results with the literature is limited since many other chronic RS food studies did not report these measures (75,78–82). Given the positive results seen in the fasting HOMA indices in the current study, the high feasibility of their calculation, and their validation against other methods (e.g. clamp methods) (101), it is worthwhile for future RS intervention studies to include them.

E.iv. Glycemic Response Biomarkers Summary

Overall the glycemic response biomarker results of this study are consistent with previous research in healthy or at-risk of T2D participants that demonstrate no significant effect of chronic RS consumption on fasting glucose (RS supplements (71–74), high-RS food (78–83)) and postprandial glucose (RS supplements (72,75), high-RS food (78,79,81,82)). Conversely, this research is the first chronic RS intervention to demonstrate a significant reduction in fasting insulin, HOMA-IR and 2- and 3-hour insulin iAUC following an OGTT, and as a result,
improving fasting insulin sensitivity and glycemic efficiency following a RS-food intervention. It is possible that previous RS-food interventions did not observe significant reductions in fasting or postprandial insulin sensitivity as a result of certain aspects of their study design, for example an inadequate dose of RS (79,83,84), an insufficient treatment period duration (79,80), or the fact that participants had healthy insulin sensitivity at baseline (78,79). Careful thought was given to these aspects of study design when planning the present study, which may have contributed to its likelihood to detect significant improvements in glycemic efficiency and fasting insulin sensitivity in adults at risk of developing T2D.

**F. Study Strengths**

There are several strengths of this research related to the overall study design, study treatments, participants data collection and study endpoints, all of which contribute to the validity of the results. Firstly, this study used a randomized, double-blind crossover design that consisted of two 8-week treatment periods separated by a 4-week washout period. The randomized and double-blinded qualities reduced bias towards a treatment by either participants or researchers. The crossover study design allowed each participant to serve as their own control which minimized inter-individual variation due to inherent differences between participants. The 8-week treatment period duration was not too long to increase participant attrition or reduce compliance but proved sufficient to detect some significant results. While a longer treatment period duration was originally considered, participant burden and therefore compliance were important concerns. The washout period allowed for any residual effects of either treatment to return to pre-study conditions, as demonstrated by a lack of significant differences in glycemic endpoints on study day 1 of each treatment period. Another strength of this study is its statistical power due to the pre-study sample size calculation that utilized a power of 80% and was based
on the variation and effect size of fasting blood glucose data in the relevant literature. This clinical trial was thoughtfully designed to investigate the chronic effects of RS consumption while reducing biases in a well-controlled and scientifically justified and feasible manner.

The study treatment bagels and their instructions for consumption also add strength to the study design. Fundamentally, the bagels themselves are a relevant food to use in a study that required daily treatment consumption due to their versatility and popularity and as a both a meal and a snack food among Canadians. Additionally, the RS treatment bagels were plain in flavour to maximize their utility as a bread product, and participants were allowed to consume their treatment bagels however they liked (toasted or not, on their own or as part of a meal), at any time of day and with any toppings, as long as one whole study bagel was consumed each day of the treatment period. Participants could also prepare their treatment bagel differently each day in order to incorporate more variety into their bagel-meals, if desired, and avoid feeling fatigued or bored of the treatment bagels. This flexibility allowed for the participants freedom to incorporate the treatment bagels into their unique lifestyle and preferences, which likely contributed to the high treatment compliance, and also maximized the ecological validity of this study. The fact that that the participants were consuming the bagels as they would be consuming them outside of a clinical study supports the extension of these results to a setting where the bagels could be made commercially available.

The screening methods used and the resulting participant characteristics also add strength to this study. Participants were screened using a thorough 3-step screening process that allowed ample time to understand the study and extended the opportunity to raise any questions they had about their participation in the study, or the study in general, helping to minimize attrition during the study. Additionally, this screening process included a validated diabetes risk assessment
questionnaire that assessed socio-demographic, anthropometric, and lifestyle-related risk factors of T2D. Participants with elevated risk of T2D are a logical subset of the population to study due to their ability to benefit from T2D risk-reducing strategies. Since participants were not required to meet specific criteria for pre-diabetes, insulin resistance or impaired glucose tolerance, the sample is representative of a larger portion of the Canadian population who might meet these criteria, but also including those with only partially diminished glycemic control who may be shy of these criteria. Additionally, using a diabetes risk-assessment questionnaire that considered a wide range of risk factors of T2D was beneficial. Since the etiology of T2D is complex and unique to each individual, utilizing a risk assessment tool that is based on a wide range of risk factors may increase the variation of the disordered mechanisms or other contributing factors that are present in the participant sample, achieving a better representation of the population as a whole. The screening methods used allowed the inclusion of a range of participants that were representative of the many Canadians who are poised to benefit from a chronic RS-food intervention.

Another strength of this study is that the treatment bagels altered only one component of the participants’ dietary intake. The analysis of 3-day food records revealed that there were no significant differences in energy intake and macronutrient distribution between the pre-study period and each treatment period, with the exception of dietary fibre which was significantly increased in the RS bagel treatment period by approximately the same amount of fibre in the RS treatment bagel. Participants were instructed to maintain their usual daily lifestyle and dietary habits throughout the study with the exception of replacing other bread products in their diet with the treatment bagels; however, this was a free-living study which always raises the risk of non-compliance. Regardless, the only significant change in nutritional composition of the diets
between the RS and control treatment period was an increase in dietary fibre due to the substantial amount of RS in the RS treatment bagels. This is a clear strength of the study as it excludes the ability to conclude the results of the intervention were due to other changes in the participants’ diets, and significantly increases the internal validity of this research.

A further strength of this study is the utilization of a standard OGTT to measure glycemic control, the primary study endpoint. Previous studies have included the treatment and control ingredients in a meal tolerance test after the respective treatment period (78,81,82); however, this approach cannot separate the acute and chronic effects of the RS treatment. Additionally, there would have been inherent differences in the meals used in the meal tolerance tests, making the results of different studies incomparable. The use of a standard and clinically relevant oral glucose challenge following each treatment period instead of including the treatment (and control) in the challenge is therefore a favourable strategy that reduces confounding variables and increases the internal validity of the study. Using the OGTT is also beneficial as it simulates the physiological effects of eating more closely than a euglycemic-hyperinsulinemic clamp (used by Johnston et al. (73) and Robertson et al. (72)) or a FSIVGTT (used by Maki et al. (74), Bodinham et al. (75) and Robertson et al. (76)), methods which infuse glucose intravenously. Administration of glucose by IV bypasses, and therefore limits the effects of the digestive system, such as the release of incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) which are stimulated by consumption of glucose or a meal and play a role in insulin secretion (10). Furthermore, the OGTTs were completed before and after each treatment period, as opposed to only at the end of each treatment period as in some studies (71,72,74–78,81,82). This strategy allowed for a more sophisticated statistical analysis as the baseline measurements of each treatment period could be considered as a
covariate, thereby accounting for any difference present in baseline values at the beginning of each treatment period. The use of standard OGTTs at the beginning and end of each treatment period to measure glycemic control gives strength to the study as it reduces confounding variables, mimics the physiological response of eating, and permitted a thorough statistical analysis.

Other strengths of this study pertain to the consistency and thoroughness of data collection and verification techniques employed throughout the trial. A small team of researchers who were trained and practiced together completed the data collection for the entire study. Furthermore, researchers closely followed the established detailed protocols for participant interactions and laboratory procedures. For example, the study flowsheets detailed each participant interaction at each study visit to ensure completion of all questionnaires and measurements with spaces to record the data and any notes or concerns. In addition, detailed standard operating procedures were developed for the laboratory procedures used in the study and posted in the laboratory for reference as needed. The analysis of glucose and insulin were performed by one researcher and with complete participant sample sets in single batches in order to minimize variation wherever and whenever possible. Finally, after data was entered into excel files, all data was verified by a different researcher for accuracy and completion, and any discrepancies were identified for review. The time and effort put forth to ensure accurate and precise data collection and sample analysis ensured high-quality data and adds strength to the results of the study.

A final strength of this study worth mentioning is the significant effort that was put forth into developing relationships with the participants and ensuring a safe and welcoming environment for the participants. Participants were asked about any questions or concerns at
each study visit and encouraged to contact the study team by calling the lab or emailing the study email account at any time. Regular email and telephone reminders of appointments and study activities added to the positive rapport and assisted with overall study compliance. Periodic study newsletters with information about the researchers, study updates, and bagel preparation ideas and recipes were mailed to the participants in effort to encourage participant connection with the study while at home. It is likely that this attention to participant relationships, comfort and enjoyment of the study played a role in minimizing missed appointments and achieving >99% self-reported compliance during each treatment period, further adding strength to this study.

In summary, there are numerous factors that give this study strength, including its overall study design; the study treatment bagels; the screening methods and resulting participant characteristics; the successful manipulation of a single dietary variable throughout the study; the consistency and thoroughness of sample analysis; the utilization of standard OGTT to measure the glycemic response; thorough data collection and verification methods; and the development of participant relationships and communication.

G. Study Limitations

While there are a number of strengths of this study, there are also limitations that should be mentioned with respect to some aspects of the study treatment bagels and also the participant characteristics.

The first limitation of this study pertains to the study treatments, in that bagels in general are typically processed, white-bread refined products which might be contraindicated for those at increased risk T2D due to the rapid digestibility and high glycemic load typically associated with bagels. Furthermore, the bagels were fairly large at 120-125 g per bagel, which is approximately
three servings of bread according to Canada’s Food Guide that documents a serving of bread as 35 - 45 g (114). This large serving size was required in order to provide 25 g RS/ bagel since the maximum proportion of Hi-Maize® 260 flour was 60% of the total flour to produce an acceptable end product. Despite the fact that avoiding or reducing consumption of refined bread and other high-carbohydrate, low-fibre products is often a strategy to improve one’s diet, the reality is that bread products are a staple in the Canadian diet and its popularity and versatility make it unlikely to disappear. Further, the use of white bread as the basis of the bagels was warranted in order to reduce any bioactives that might be present in healthier whole-grain or high-fibre breads which would introduce more variables and confound the results of the study.

Given that the results of the study on fasting and postprandial insulin were positive despite the ‘unhealthy’ white bread matrix demonstrates the potential of RS as a bioactive in a food matrix, which could be further enhanced by utilizing a healthier food matrix in the future. Despite the fact that the large size and the white-bread nature of the food matrix used in this study was justified by the need for a high-RS product devoid of other bioactive ingredients, it remains a limitation of the study since it is not an ideal food matrix for those at risk of T2D.

Another limitation with respect to the treatment bagels is the slight difference in nutritional composition between the RS and control treatment bagels. Ideally, the RS bagel and control bagel would have precisely the same macronutrient distribution and vary only in RS (and dietary fibre, as a result). However, to achieve the high RS content of the RS-treatment bagel, a large portion of the hard wheat flour in the control bagel was replaced with Hi-Maize® 260 flour, which has slightly different characteristics and provides a different nutrient profile than the standard hard wheat flour. As a result there were some differences between the RS- and control doughs and their final products. There were slight differences in fat and protein between the
control and RS-treatment bagels due to the difference in nutrient content of the Hi-Maize® 260 flour and the hard wheat flour. Differences in water absorbing capacity of the flours meant that more water was required in the high-RS dough. Although the bagels were portioned in the same manner before baking, moisture loss during the baking process was greater in the RS- than the control bagel dough, which caused slight differences in the total weight of the baked bagels. The treatment bagels also differed in dietary fibre which was a result of the high RS content of the RS-treatment bagel, since the method of analysis of dietary fibre used (AOAC 991.43) includes some RS as dietary fibre. The higher fibre content of the RS treatment bagel, though similar in total carbohydrates, means the RS treatment bagel provides less available carbohydrates and subsequently less energy than the control bagel, since dietary fibre provides approximately 2 kcal/g while available carbohydrates provide 4 kcal/g (94). This difference in dietary fibre was unavoidable since fortifying the control bagel with an alternate fibre in effort to reduce the difference was likely to introduce other bioactives to the control and confound the results of the study. Despite the fact that the differences in nutritional composition of the RS and control treatment bagels were unavoidable given the food matrix, this remains a limitation of the study since the study treatments varied in more than one component. It is important to note however, that the differences in energy and macronutrient distribution of the RS and control bagels were a small part of the participant diet as a whole and ultimately did not manifest as significant differences in energy and nutrient intake in the overall participant diets, assessed by 3-day food records.

Another study limitation worth mention is the 3-day food records used to estimate dietary intake during each treatment period. The 3-day food records are self-reported in nature which increases the likelihood of response bias, as participants may intentionally or unintentionally
provide more desirable information or leave out less desirable information to either simplify the work or impress researchers. Self-reported food records also rely on accuracy of estimated portion sizes, the varied perception of which presents inter-participant variation, though this was potentially controlled for by the crossover design of the study. Lastly, these food records are subject to the limitations of using food record analysis software which is a tedious process that may introduce entry errors, and also depends on an imperfect nutrient database due to the ever-changing landscape of commercially available foods. Unfortunately, more reliable methods of dietary assessment such as weighted food records would have been too burdensome on the participants given the requirement of three separate 3-day food records over the course of the study. In effort to maximize the reliability of this tool the participants were trained on completing accurate and detailed food records and given written instructions and reference guides to use, and the food records were thoroughly reviewed by researchers with the participants at the time of submission. Ultimately, however, 3-day food records provide only an estimate of mean dietary intake and should be interpreted with caution.

One final limitation of the study is that the participant sample was largely Caucasian which does not accurately reflect the multicultural nature of the Canadian population, limiting the ability to extend the results to non-Caucasian population. Despite the fact that a multifactorial questionnaire was used to assess risk of T2D that included a section on ethnicity that would have increased the likelihood of including more ethnic groups in the study due to the increased risk of T2D among people of Asian, Aboriginal and African (and other) descent (7), the population of Guelph and surrounding area yielded a high number of Caucasian respondents to recruitment ads and posters. As a result, the highly Caucasian participant sample is a limitation
of this study as it does not represent the greater Canadian population who may have increased risk of T2D due to genetic predispositions linked to their ethnicity.

Overall the limitations of this study were related to some aspects of the study bagels, the 3-day food record analysis, and high proportion of Caucasians included in the participant sample. These limitations, however, were largely unavoidable given the resources of the study, and do not invalidate this research.

**H. Future Research**

There are number of areas of this research that necessitate further investigation in order to fully understand the effects of chronic RS consumption on markers of T2D. These areas include expanded mechanistic studies and additional RS-food effectiveness studies to determine the optimal intervention strategy to reduce the risk of developing T2D, or possibly as a treatment strategy. In addition, further attention to the food product development of more palatable bagels with high RS is warranted.

The present study was focused on determining the efficacy of the RS bagel treatment and therefore the endpoints do not provide evidence of a mechanism that is responsible for the improvement in fasting and postprandial insulin response. Previous studies have attempted to determine a mechanism by which insulin sensitivity is improved by measuring endpoints such as the expression of genes involved in lipid uptake and metabolism in specific tissues (72,76), or production (72,83,106,115) and uptake (72) of short-chain fatty acids as a result of colonic fermentation of RS, although they do not completely define the mechanism by which RS exerts its benefits on insulin sensitivity. Future research should aim to continue to uncover the processes and mechanisms involved in improving insulin sensitivity in order to refine potential T2D prevention and potentially treatment strategies.
The literature should also be expanded through further chronic RS-food studies that seek to determine if chronic RS-food consumption is able to improve insulin sensitivity when consumed in alternate food matrices, lower daily doses, or perhaps less frequent consumption. It would also be worthwhile to investigate these possibilities in subsets of adults with specific risk factors of T2D, and in those who have T2D as these people are more likely to be aware of the concept of controlling glycemia and therefore potentially more receptive to the idea of utilizing functional foods designed to improve insulin sensitivity. Lastly, if such studies do support the efficacy of RS-foods at improving insulin sensitivity in adults with T2D, investigating the effectiveness of such interventions in conjunction with lifestyle and pharmacological interventions would be prudent, given that such interventions are likely to be prescribed simultaneously (if not before) recommending a functional food-based approach, in order to observe the relationships and any interaction between interventions. Future research that further develops and refines possible RS-food interventions, in participants with increased risk of T2D and who have T2D, who may be simultaneously undergoing lifestyle or pharmacological interventions would be of value. Such research would help to identify the optimal combinations of RS-food form, dose and duration of intervention and target population to maximize the benefits of chronic RS-food consumption.

Another area of future research relates to RS-food product development. While the present study demonstrated there are significant benefits of consuming the RS treatment bagels, it was apparent from the sensory questionnaires that there is room for improvement with respect to the overall liking and liking of individual sensory attributes. While self-reported treatment compliance was high throughout the study, an increase in overall liking of the RS bagels would likely improve consumption of the bagels as a dietary intervention beyond a clinical trial, where
the desire to consume the bagels is likely heightened among participants committed to a clinical trial. For the purposes of the present study the treatment bagels were based on a plain bagel formula, but efforts into research and development could potentially lead to more favourable formulas that include various flavours, fruit, seeds, or the inclusion of other ingredients such as whole grain and whole wheat flours which may also appeal to additional potential consumers. Of course, it would need to be determined that the bagels maintain their efficacy at improving insulin sensitivity and are still well tolerated following daily consumption. Evidently, more research is needed to develop RS bagels with more desirable flavours and other sensory attributes, as well as to ensure the RS bagel is still an effective intervention to reduce the risk of developing T2D.

Overall, there is justification to conduct additional research in the realm of chronic RS consumption, including mechanistic research, follow up clinical trials to determine optimal dose, duration and participant characteristics to derive a significant benefit from RS-food consumption, and also to develop more favourable RS-bagel formulas to increase their overall likability.
VI. SUMMARY and CONCLUSIONS

This research generated evidence about the effects of chronic consumption of high-RS bagels on glycemic markers of T2D in adults at increased risk of T2D. Through the utilization of a randomized, double-blind crossover study consisting of two 8-week interventions separated by a 4-week washout, this study provides data which indicates that chronic consumption RS-bagels can improve some glycemic markers of T2D.

The first hypothesis of this research was rejected, as the RS bagel treatment did not significantly improve HbA1c compared to control bagel treatment.

The second hypothesis of this research was that the RS-bagel treatment would significantly reduce fasting glucose and insulin and significantly increase fasting insulin sensitivity compared to control. This hypothesis was partly accepted and partly rejected, since fasting insulin, but not glucose, was significantly reduced following the RS bagel treatment compared to the control bagel treatment. Even without a significant reduction of fasting glucose, calculated indices of fasting insulin resistance, beta-cell function and insulin sensitivity (HOMA-IR, HOMA-%B and HOMA-%S) were all significantly improved following the RS compared to the control bagel treatment, providing further support for a reduction in risk of T2D.

The third hypothesis of this research was that the RS bagel treatment would significantly reduce postprandial glucose and insulin response to a standard 75 g glucose beverage (OGTT), was also partly accepted and partly rejected. Postprandial glucose excursion following the 2- or 3-hour OGTT was not significantly different between bagel treatments while postprandial insulin response (2- and 3-hour iAUC) was significantly reduced following the RS bagel compared to the control bagel treatment. This result, along with the improved fasting insulin sensitivity and
resistance results (HOMA indices), may be interpreted as improved glycemic efficiency, since the body’s tissues were more sensitive to a smaller amount of insulin.

Finally, the last hypothesis of this research was accepted, as the RS bagels were well tolerated and generally considered palatable. However, participants did prefer the control bagels over the RS bagels, which highlights the opportunity for further research to improve high-RS food formulations in order to increase overall liking and therefore feasibility of a high-RS bagel as a strategy for reducing risk of T2D.

Overall, this research supports the use of RS-enhanced foods to help improve glycemic control, and therefore reduce the risk of developing T2D, in adults with an increased risk of T2D. Further research is needed to clearly define the mechanisms by which chronic RS consumption improves insulin sensitivity, and further product development efforts are warranted to improve the overall liking of high-RS bagels. Nevertheless, this study provides substantiation of the incorporation of high-RS food into the diet as a feasible strategy to improve both fasting and postprandial insulin response as well as insulin sensitivity, and therefore, to reduce the risk of developing T2D.
VII. REFERENCES


2. Statistics Canada. Table 105-0501 - Health indicator profile, annual estimates, by age group and sex, Canada, provinces, territories, health regions (2013 boundaries) and peer groups, occasional [Internet]. 2014 [cited 2014 Dec 30]. Available from: http://www5.statcan.gc.ca/cansim/a26


38. European Food Safety Authority. Scientific Opinion on the substantiation of health claims related to resistant starch and reduction of post-prandial glycaemic responses (ID 681), “digestive health benefits” (ID 682) and “favours a normal colon metabolism” (ID 783) pursuant to Article 13. EFSA J. 2011;9:2024 [17 pp.].


92. Schoenfeld DA. Statistical considerations for clinical trials and scientific experiments, Massachusetts General Hospital Biostatistics Centre [Internet]. 2010 [cited 2012 Jan 1]. Available from: http://hedwig.mgh.harvard.edu/sample_size/size.html


VIII. APPENDICES
Appendix A. Hi-Maize® 260 Technical Details

HI-MAIZE® 260 RESISTANT STARCH

Hi-MAIZE 260 resistant starch is a natural food starch that resists digestion in the small intestine and behaves as dietary fiber in the large intestine. It resists dietary fiber for food labeling purposes according to official AOAC methods 985.29 and 991.43. With a range of health benefits, HI-MAIZE 260 is a vital ingredient for formulating great tasting, high quality, “better for you” foods.

PHYSICAL PROPERTIES

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>White/Off-white</td>
</tr>
<tr>
<td>Form</td>
<td>Fine Powder</td>
</tr>
<tr>
<td>Taste</td>
<td>Bland</td>
</tr>
<tr>
<td>pH</td>
<td>4.5 – 7.5</td>
</tr>
<tr>
<td>Particle Size</td>
<td>10 – 15 microns</td>
</tr>
<tr>
<td>Moisture</td>
<td>10 – 14%</td>
</tr>
<tr>
<td>Total Dietary Fiber</td>
<td>60% min. (DSB)</td>
</tr>
<tr>
<td>Calories</td>
<td>1.6 Kcal/gram</td>
</tr>
</tbody>
</table>

FEATURES AND BENEFITS

Hi-maize 260 offers numerous substantiated health benefits to consumers including:

- Reduces calories (when substituted for digestible carbohydrates)
- Reduces glycemic response of foods (when substituted for digestible carbohydrates)
- Increases insulin sensitivity
- Increases satiety short term (post-meal) and long term (24 hours)
- Prebiotic fiber
- Improves digestive health (bowel/colon)
- Contributes to regularity

Hi-maize 260 is organoleptically invisible in most applications due to a small particle size, white appearance and bland flavor. Therefore, it will not compromise taste texture or appearance of food products.

GENERAL:

Hi-MAIZE 260 is Kosher certified. It is derived from high amylose cornstarch, a natural food source.

REFERENCES:

- AOAC Method 991.43
- Based on insoluble fiber being non-caloric
### Appendix B. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS Supplements

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raben A et al. &lt;br&gt;Resistant starch: the effect postprandial glycemia, hormonal response, and satiety. Am J Clin Nutr. 1994;60:544-51. (46)</td>
<td>Crossover  &lt;br&gt;Randomization not described  &lt;br&gt;2 treatments  &lt;br&gt;6-week washout period  &lt;br&gt;Blinding not described</td>
<td>n=10  &lt;br&gt;Healthy males  &lt;br&gt;Ages 20-31 years  &lt;br&gt;Normal weight</td>
<td>Raw potato starch (27.1 g RS)  &lt;br&gt;Pre-gelatinized potato starch (0 g RS) mixed with 500 mL artificially sweetened fruit syrup  &lt;br&gt;Matched for total potato starch</td>
<td>Postprandial glucose and insulin</td>
<td>Reduced postprandial glucose (reduced peak glucose, 2- and 5-hour AUC)  &lt;br&gt;Reduced postprandial insulin (reduced peak value, 2- and 5-hour AUC)</td>
</tr>
<tr>
<td>Bodinham CL et al. &lt;br&gt;Acute ingestion of resistant starch reduces food intake in health adults. Br J Nutr. 2010;103:917-922. (48)</td>
<td>Crossover  &lt;br&gt;Randomized  &lt;br&gt;2 treatments  &lt;br&gt;1-week washout period  &lt;br&gt;Single-blind</td>
<td>n=20  &lt;br&gt;Healthy males  &lt;br&gt;Mean age of 25.8 years  &lt;br&gt;Mean BMI of 23.2 kg/m²</td>
<td>24 g RS added to mousse (to be consumed with a meal at both breakfast and lunch on same day  &lt;br&gt;Starch-free mousse  &lt;br&gt;Matched for available carbohydrate and energy content</td>
<td>Postprandial glucose  &lt;br&gt;Postprandial insulin  &lt;br&gt;C-peptide  &lt;br&gt;Calculated postprandial insulin sensitivity (SISO) using the minimal model (MINMOD) calculation with meal tolerance test (MTT) data</td>
<td>RS for both meals reduced postprandial insulin response over the two-meal (7 hour) MTT</td>
</tr>
</tbody>
</table>

**RS form:**
- **Supplement (gel)**
- **Supplement (mousse)**

**Abbreviations used:** AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
### Appendix B. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS Supplements - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| **Haub MD et al.** Different resistant starch elicit different glucose responses in humans. J Nutr Metab. 2010; ID 230501. (47) | - Crossover  
- Randomized  
- 3 treatments  
- 2-day washout periods  
- Blinding not described | n=11  
- Healthy adults  
- Mean age 24 years  
- Mean BMI 23 kg/m² | 30 g HAM (<30 g RS₂) in water  
30 g resistant wheat starch (cross-linked RS₄) in water  
30 g dextrose solution  
Matched for total carbohydrate content | Fasting and postprandial glucose | Both RS treatments reduced glucose (iAUC) compared to dextrose  
RS₄ reduced glucose iAUC compared to dextrose and RS₂ treatment |

**RS form:**  
Supplement (powder)  

**RS type & source:**  
RS₂ from HAM RS₄ from wheat

| **Haub MD et al.** Novel resistant potato starches on glycemia and satiety in humans. J Nutr Metab. 2012; ID 478043. (49) | - Crossover  
- Randomized  
- 5 treatments  
- Washout period not described  
- Single-blind | n=10  
- Healthy adults  
- Mean age not provided  
- Mean BMI 20-30 kg/m² | 30 g RS₄ from two different potato starch sources, mixed into dextrose solution or water  
Dextrose control  
Matched for available carbohydrate content | Postprandial glucose | None |

**RS form:**  
Supplement (beverage powder)  

**RS type & source:**  
RS₄ from potato starch

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Abbreviations used: BMI=body mass index; HAM=high-amylose maize; iAUC=incremental area under the curve.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goddard MS et al.</td>
<td>Crossover</td>
<td>n=33</td>
<td>23-35% amylose rice</td>
<td>Fasting and postprandial glucose and insulin response to study treatments</td>
<td>All 3 rice treatments increased glucose AUC compared to glucose treatment</td>
</tr>
<tr>
<td></td>
<td>Randomized</td>
<td>Healthy adults</td>
<td>14-17% amylose rice</td>
<td></td>
<td>High-amylose rice reduced insulin AUC compared to all other treatments</td>
</tr>
<tr>
<td></td>
<td>4 treatments</td>
<td>Mean age 62 years</td>
<td>0% amylose rice</td>
<td></td>
<td>High-amylose rice reduced peak glucose at 30 min compared to the 0% amylose rice and glucose treatments</td>
</tr>
<tr>
<td></td>
<td>1-2 week washout periods</td>
<td>Within 20% of ideal body weight</td>
<td>50 g glucose beverage (control beverage)</td>
<td></td>
<td>High- and medium-amylose rice increased glucose at 180 min compared to 0% amylose rice and glucose treatments</td>
</tr>
<tr>
<td></td>
<td>Blinding not described</td>
<td></td>
<td>Treatments were matched for total carbohydrate content (50 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>RS form:</strong> Food (rice)**</td>
<td>RS type &amp; source: Starch from high-amylose rice</td>
<td>RS not quantified</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>RS type &amp; source:</strong> Starch from high-amylose rice</td>
<td>(RS type not specified, deduced as RS$_3$)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: AUC = area under the curve.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krezowski PA et al.</td>
<td>Crossover Randomized 4 treatments Washout period not described Blinding not described</td>
<td>n=9 Adult males Mean age 62 years Mean 124% of ideal body weight Diagnosed with T2D</td>
<td>Muffin made with low-amylose maize Muffin made from HAM Cornflakes Glucose beverage Treatments matched for total carbohydrates (50 g) RS not quantified</td>
<td>Fasting and postprandial glucose and insulin response to study treatments</td>
<td>High-amylose muffins reduced peak glucose rise from baseline, and net glucose AUC compared to all other treatments</td>
</tr>
<tr>
<td></td>
<td>RS form: Food (muffins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behall KM et al.</td>
<td>Crossover Randomization not described 2 treatments 2-week washout period Blinding not described</td>
<td>n=25 Healthy adults Ages 28-58 years Within 20% ideal body weight</td>
<td>Crackers made with high-amylose (70% amylose) corn starch (source of RS) or high-amylopectin (70% amylopectin) cornstarch Portions of crackers provided 1 g carbohydrate/kg body weight RS not quantified</td>
<td>Fasting and postprandial blood glucose and insulin response to study treatments</td>
<td>Glucose lower at 30 min and higher at 120 and 180 min after high-amylose crackers Insulin lower at 30 and 60 min, and ‘summed insulin’ after high-amylose crackers</td>
</tr>
<tr>
<td></td>
<td>RS form: Food (crackers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; HAM=high-amylose maize.
## Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
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<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Amelsvoort JMM &amp; Westrate JA.</td>
<td>Crossover, Randomized, 4 treatments, 1-week washout period, Blinding not described</td>
<td>n=22, Healthy males, Mean age 40 years, Mean BMI 24 kg/m²</td>
<td>High- and low-amylose meals served both fresh, and after storage, Meals matched for total carbohydrate content, RS not quantified</td>
<td>Fasting and postprandial glucose and insulin response to study treatments</td>
<td>High-amylose meals reduced postprandial glucose at 30 and 60 min, but increased glucose at 120, 240 360 min, resulting in an increased total and net glucose AUC compared to low-amylose meals</td>
</tr>
<tr>
<td></td>
<td>RS form: Food (mixed meal)</td>
<td></td>
<td></td>
<td></td>
<td>High-amylose meals reduced postprandial insulin at 30, 60 and 240 min, as well as total and net insulin AUC compared to low-amylose meals</td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
## Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| Westrate JA & van Amelsvoort JMM. Effects of the amylose content of breakfast and lunch on postprandial variables in male volunteers. Am J Clin Nutr. 1993;58:180-6. (56) | • Crossover  
• Randomized  
• 4 treatments  
• 1-week washout periods  
• Blinding not described  
  
  *RS type & source: Starch was HAM (RS type not specified, deduced as RS$_2$)* | • n=22  
• Healthy males  
• Mean age 38.9 years  
• Non-obese | • High- and low-amylose starch breakfast and lunch  
• Breakfast was an apple compote-filled baguette (HAM starch was in bread and filling)  
• Lunch (second-meal) was a pizza (HAM starch was in pizza crust)  
• Meals matched for total carbohydrates  
• RS not quantified | • Fasting and postprandial glucose and insulin response to study treatments at breakfast and second meal | • Insulin AUC lower after high-amylose breakfasts  
• Glucose at 1 and 2 hours after second meal lower after the high-amylose lunches  
• Insulin and glucose AUC lower after high-amylose lunch (when combined with high-amylose breakfast) |

Abbreviations used: AUC=area under the curve; HAM=high-amylose maize.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospers J et al.</td>
<td>Crossover, Randomized, 4 treatments, No mention of washout periods, Blinding not described</td>
<td>n=16</td>
<td>4 types of cooked pasta consumed with tomato sauce, lean ground beef and cheese</td>
<td>Postprandial glucose and insulin response to test meals</td>
<td>When both high-amylose pastas were combined, they significantly reduced total and net glucose AUC over 1 and 3 hours compared to the regular (with and without regular cornstarch combined) pastas, and the total and net glucose AUC for the 1st hour compared to only the regular (with regular cornstarch) pasta. Considered individually, each high-amylose pasta reduced total glucose AUC compared to each regular pasta, and net glucose AUC when compared to only the regular pasta without cornstarch.</td>
</tr>
<tr>
<td>Amylose-to-amylopectin ratio in pasta affects postprandial glucose and insulin responses and satiety in males.</td>
<td>RS type &amp; source: Starch was HAM (RS type not specified, deduced as RS&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Healthy males, Mean age 44.75 years, Mean body weight 77.87 kg</td>
<td>Regular pasta (N) (flour was 100 durum flour)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RS form: Food (Pasta)</td>
<td></td>
<td>Cornstarch pasta (N+) (20% durum flour replaced by regular cornstarch)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High-amylose cornstarch pasta (H) (20% durum flour replaced with HAM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Very high-amylose cornstarch pasta (HH) (20% durum flour replaced with very high-amylose cornstarch)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treatments matched for total carbohydrates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; HAM=high-amylose maize.
Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
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<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granfeldt Y et al.</td>
<td>• Crossover</td>
<td>• n=9</td>
<td>• Arepas made from ordinary corn or high amylose corn flour</td>
<td>• Postprandial glucose and insulin responses to study treatments</td>
<td>• Both RS arepas reduced glucose and insulin iAUC</td>
</tr>
<tr>
<td>Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. J Nutr. 1995;125:459-65. (59)</td>
<td>• Randomization not described</td>
<td>• Healthy adults</td>
<td>• 1.8 g RS (control arepa)</td>
<td></td>
<td>• No difference between RS treatments</td>
</tr>
<tr>
<td>RS form: Food (Arepas)</td>
<td>• 3 treatments</td>
<td>• Mean age 34 years</td>
<td>• 15.9 g RS arepa (matched for total carbohydrate content)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS type &amp; source:</td>
<td>• 1 week washout periods</td>
<td>• Mean BMI 23 kg/m²</td>
<td>• 24.7 g RS arepa (matched for available carbohydrate content)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Blinding not described</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbreviations used:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI=body mass index; HAM=high-amylose maize; iAUC=incremental area under the curve.</td>
<td></td>
<td></td>
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</tbody>
</table>
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
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<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achour et al.</td>
<td>Crossover • Randomized • 2 treatments • 1-week washout period • Blinding not described</td>
<td>• N=8 • Healthy adults • Mean age 27 years • Mean BMI 22.4 kg/m²</td>
<td>• Porridge-like gel made with 20 g sucrose, water and either 50 g regular cornstarch or 50 g HAM cornstarch • Treatment consumed with a serving of cheese • Treatments consumed for breakfast and also dinner on same day • Treatments matched for total carbohydrates • RS not quantified</td>
<td>• Fasting and postprandial glucose and insulin response to test meal at breakfast (absorptive period) • Fasting (over 3-hr period) the morning following evening test meal consumption (postabsorptive period)</td>
<td>• HAM cornstarch meal reduced glucose and insulin AUC compared to regular starch meal (absorptive period)</td>
</tr>
<tr>
<td></td>
<td>RS form: Food (porridge-like gel with cheese)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**RS type & source:** Starch was heat-treated HAM (RS type not specified, deduced as RS₃)

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behall KM &amp; Hallfrisch J.</td>
<td>Crossover</td>
<td>n=25</td>
<td>Breads made with various proportions of high-amylose and standard cornstarch</td>
<td>Fasting and postprandial glucose, insulin, and glucagon response to bread tolerance test</td>
<td>70% amylose bread reduced 2-hour glucose AUC compared to 30-50% amylose breads</td>
</tr>
<tr>
<td>Plasma glucose and insulin reduction after consumption of breads varying in amylose content. Eur J Clin Nutr. 2002;56:913-20. (54)</td>
<td>Randomized</td>
<td>Healthy adults</td>
<td>30% high-amylose (2.0 g RS)</td>
<td>Glucose index</td>
<td>50-70% amylose breads reduced peak glucose compared to 30 and 40% amylose breads</td>
</tr>
<tr>
<td>RS type &amp; source: Starch was HAM (RS type not specified, deduced as RS₂)</td>
<td>6 treatment</td>
<td>Mean age ~41 years</td>
<td>40% high-amylose (3.8 g RS)</td>
<td>Insulin index</td>
<td>60% and 70% amylose bread reduced 2-hour insulin AUC compared to all other treatments</td>
</tr>
<tr>
<td></td>
<td>Washout periods not described</td>
<td>Mean BMI ~27.5 kg/m²</td>
<td>50% high-amylose (8.2 g RS)</td>
<td></td>
<td>70% amylose bread reduced insulin at 30 min compared to all other breads</td>
</tr>
<tr>
<td></td>
<td>Blinding not described</td>
<td></td>
<td>60% high-amylose (11.5 g RS)</td>
<td></td>
<td>60 and 70% amylose breads reduced insulin at 60 min compared to all other breads</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>70% high-amylose (13.4 g RS)</td>
<td></td>
<td>All breads increased glucagon at 60, 120 and 180 min compared to glucose beverage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control treatment was a glucose tolerance beverage</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Matched for total carbohydrate content</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reader et al.</td>
<td>Crossover</td>
<td>n=10</td>
<td>3 different snack bars:</td>
<td>Postprandial glucose and insulin response to test bar</td>
<td>RS bar reduced peak glucose (at 60 min) and glucose iAUC compared to other bars</td>
</tr>
<tr>
<td>Glycemic and</td>
<td>Randomized</td>
<td>Adults</td>
<td>RS bar – made with 6.75 g RS</td>
<td></td>
<td>RS bar reduced insulin at 90 minutes and insulin iAUC compared to the energy bar but not the Snickers Bar ®</td>
</tr>
<tr>
<td>insulinemic</td>
<td>3 treatments</td>
<td>Ages 43-74 years</td>
<td>Energy bar – no RS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>response of</td>
<td>3-10 day washout periods</td>
<td>Mean body weight 87 kg</td>
<td>Snickers Bar ® - no RS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>subjects with type</td>
<td>Double-blind</td>
<td>Diagnosed with T2D</td>
<td>Treatments similar in total carbohydrate and energy content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 diabetes after</td>
<td></td>
<td>HbA1c &lt;9.0%</td>
<td>Treatments varied greatly in macronutrient distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>consumption of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>three energy bars.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J Am Diet Assoc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2002;102:1139-42.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RS form: Food (snack bar)

RS type & source: RS from retrograded maltodextrin (RS type not specified, deduced as RS$_3$)

Abbreviations used: BMI=body mass index; HbA1c=glycated haemoglobin; iAUC=incremental area under the curve.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
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<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Glycemic Endpoints</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Yamada Y et al.</td>
<td>Crossover</td>
<td>n=20</td>
<td>Sliced bread baked with or without added RS₃</td>
<td>Postprandial glucose and insulin responses to study treatments</td>
<td>RS bread reduced glucose and insulin AUCs in borderline group only</td>
</tr>
<tr>
<td>Effect of bread containing resistant starch on postprandial blood glucose levels in humans. Biosci Biotechnol Biochem. 2005;69:559-66. (63)</td>
<td>Randomized</td>
<td>Normal glucose tolerance (n=8) and borderline glucose (n=12)</td>
<td>6 g RS</td>
<td>RS bread reduced glucose at 90 min in borderline group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 treatments</td>
<td>Mean age 20.5 years</td>
<td>0.9 g RS (control)</td>
<td>RS bread reduced change in glucose from baseline at 60 and 90 min in borderline group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-week washout period</td>
<td>Mean BMI not provided</td>
<td>Matched for total starch content</td>
<td>RS bread reduced change in insulin from baseline at 60 and 90 min in all participants, and at 60, 90 and 120 min in borderline group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Double blind</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RS type &amp; source:</strong> RS₃ from tapioca</td>
<td><strong>Food (bread)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; BMI=body mass index.
Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

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<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behall KM &amp; Scholfield DJ.</td>
<td>Crossover, Randomized, 4 treatments, Washout period not described, Blinding not described</td>
<td>n=24, 12 adults with hyper-insulinemia, 12 adults with normal insulinemia, Ages 25-57 years, Mean BMI 24.8 kg/m²</td>
<td>Corn chips or muffins made from high-amylose or standard corn starch, and high-amylose or standard cornmeal and starch, High- and low-amylose muffins, High- and low-amylose corn chips, Treatment portions provided 1 g available carbohydrate/kg body weight, Amount of RS unclear</td>
<td>Postprandial glucose, insulin and glucagon response to study treatments</td>
<td>High-amylose products reduced glucose AUC, High-amylose products reduced insulin AUC</td>
</tr>
</tbody>
</table>

*RS form: Food (corn muffins and corn chips)*

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| Behall KM et al. Consumption of both resistant starch and β-glucan improves postprandial plasma glucose and insulin in women. Diabetes Care. 2006;29:976-81. (62) | • Crossover  
• Randomized  
• 10 treatments  
• Washout period not described  
• Blinding not described | • n=20  
• Healthy women  
• Mean age 43 years  
• Normal weight control group n=10, overweight but without T2D group n=10 | • 9 muffins with varying amounts of RS and BG  
• Low: 0.9 g RS  
• Medium: 3.4 g RS  
• High: 6.5 g RS  
• Glucose beverage control  
• Treatments provided 1g total carbohydrate/kg body weight | • Postprandial glucose and insulin response to study treatments | • When both normal- and over-weight groups were combined (n=20):  
• Glucose AUCs reduced after high- or medium-RS/high-BG muffins (compared to glucose control, low-RS/low-BG muffin and low-RS/med-BG muffins)  
• Insulin AUCs reduced after high-RS/high-BG muffin compared to glucose control and all medium- and low-RS muffins) |

*RS form: Food (muffins)*

Abbreviations used: AUC=area under the curve; BG=beta-glucan; HAM=high-amylose maize.
## Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

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<tr>
<th>Reference</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behall KM at al. Barley β-glucan reduces glucose and insulin responses compared with resistant starch in men. Nutr Res. 2006;26:644-50. (69)</td>
<td>Crossover • Randomized • 10 treatments • Washout period not described • Blinding not described RS type &amp; source: RS$_2$ from HAM</td>
<td>• n=20 • Healthy men • Mean age • ~42 years • Normal weight control group n=10, overweight but without T2D group n=10</td>
<td>• 9 muffins with varying amounts of RS and BG • Low: 0 g RS • Medium: 6 g RS • High: 9 g RS • Glucose control beverage • Treatments matched for available carbohydrates</td>
<td>Postprandial glucose and insulin responses to study treatments</td>
<td>In high-BG muffin only, the medium- and high RS muffins reduced glucose AUC compared to medium-RS/medium-BG and low-RS/medium-BG muffins • High-, medium- and low-RS (all high-BG) muffins reduces insulin AUC compared to glucose control and all other muffins</td>
</tr>
</tbody>
</table>

**Abbreviations used:** AUC=area under the curve; BG=beta-glucan; HAM=high-amylose maize.
## Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Significant Effects of RS Treatment(s)</th>
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</thead>
</table>
| Li M et al.  
Postprandial glycaemic and insulinaemic responses to GM-resistant starch-enriched rice and the production of fermentation-related H\textsubscript{2} in healthier Chinese adults. Br J Nutr. 2010;103:1029-34. (60)  

\textit{RS form: Food (GM rice)}  
\textit{RS type & source: RS from high-amylose rice (RS type not specified, deduced as RS\textsubscript{2})}  

* Crossover  
* Randomized  
* 3 treatments  
* 1-week washout periods  
* Blinding not described  

* n=16  
* Healthy adults  
* Mean age 25 years  
* BMI 18-24 kg/m\textsuperscript{2}  

* 40 g rice (1 g RS) and water  
* 40 g rice (8 g RS) and water  
* Glucose control (40g glucose and water)  
* Treatments matched for total carbohydrate content  

* Postprandial glucose and insulin response to study treatments  

* Both rice treatments reduced peak glucose compared to glucose control, high RS rice reduced peak glucose compared to regular rice  
* High RS rice reduced glucose iAUC compared to regular rice and glucose control at 60, 90, 120 and 240 minutes  
* High RS rice reduced insulin iAUC at 45, 60, 90 and 120 min compared to regular rice  
* Insulin index was lower after high RS rice compared to control  

Abbreviations used: BMI=body mass index; iAUC=incremental area under the curve.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anderson GH et al.</strong>&lt;br&gt;Relation between estimates of cornstarch digestibility by the Englyst in vitro method and glycemic response, subjective appetite, and short-term food intake in young men. Am J Clin Nutr. 2010;91:932-9. (61) (Experiment 2 only)&lt;br&gt;&lt;br&gt;<strong>RS form:</strong>&lt;br&gt;<strong>Food (soup)</strong>&lt;br&gt;&lt;br&gt;<strong>RS type &amp; source:</strong> RS\textsubscript{2} from HAM</td>
<td>• Crossover&lt;br&gt;• Randomized&lt;br&gt;• 5 treatments&lt;br&gt;• 1-week washout periods&lt;br&gt;• Blinding not described</td>
<td>• n=16&lt;br&gt;• Healthy males&lt;br&gt;• Ages 20-30 years&lt;br&gt;• BMI 20-24.9 kg/m\textsuperscript{2}</td>
<td>• Various starches added to tomato soup&lt;br&gt;• 19 g RS (regular cornstarch)&lt;br&gt;• 23 g RS (HAM cornstarch)&lt;br&gt;• 27 g RS (whole-grain HAM cornstarch)&lt;br&gt;• 6 g RS in maltodextrin starch control&lt;br&gt;• Starch-containing soups matched for total carbohydrate content&lt;br&gt;• Starch-free control was plain tomato soup (not matched for total carbohydrates)</td>
<td>• Postprandial glucose response to study treatments</td>
<td>• The 19, 23 and 27 g RS treatments reduced glucose at 60 min compared to maltodextrin&lt;br&gt;• The 19 and 23 g RS treatments increased glucose at 2 hours, compared to the maltodextrin control&lt;br&gt;• All 3 high RS elicited glucose AUCs lower than maltodextrin control, and higher than the starch-free control&lt;br&gt;• Whole grain RS (27 g RS) reduced glucose AUC compared to regular starch (19g RS) treatment</td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
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<tr>
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<th>Study Design</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| Al-Tamimi EK et al. Consumption of cross-linked resistant starch (RS4XL) on glucose and insulin responses in humans. J Nutr Metab. 2010; ID 651063. (67) | • Crossover  
• Randomized  
• 3 treatments  
• 1-week washout periods  
• Semi-blind (2 snack bars were blinded but not beverage) | • n=13  
• Healthy adults  
• Mean age 27 years  
• Mean BMI 25 kg/m² | • Snack bars made with/without added RS  
• 0 g RS snack bar  
• 14 g RS in control bar  
• Control beverage was glucose solution  
• Matched for available carbohydrate content | • Postprandial glucose and insulin response to study treatments | • RS snack bar reduced peak glucose and glucose iAUC compared to control bar and beverage  
• RS snack bar reduced peak insulin and insulin iAUC compared to control bar and beverage |

Abbreviations used: iAUC=incremental area under the curve; BMI=body mass index.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hallstrom E et al.</td>
<td>Crossover Randomized 4 treatments Washout period not described Blinding not described</td>
<td>n=14 Healthy adults Ages 20-35 years Mean BMI 22.2 kg/m²</td>
<td>Pumpernickel bread baked with whole grain wheat flour or high amylose four with and without lactic acid (LA) treatment 4.8 g RS in whole grain bread 11.0 g RS in high-amylose bread with LA treatment 7.7 g RS in high-amylose bread without LA treatment 1.5 g RS in white bread control Matched for available starch content</td>
<td>Postprandial glucose and insulin responses study treatments Glycemic index</td>
<td>Both high amylose breads reduced 2-hour glucose iAUC compared to white bread control High amylose and whole grain breads elicited lower incremental peak glucose values compared to the white bread control High amylose breads increased insulin iAUC from 60-180 min compared to whole grain and white bread High amylose breads had lower glycemic index than whole grain and white bread</td>
</tr>
</tbody>
</table>

*RS form: Food (bread)*

**Abbreviations used:** BMI=body mass index; iAUC=incremental area under the curve.
### Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klosterbuer AS et al.</td>
<td>• Crossover</td>
<td>• n=20</td>
<td>• Breakfast meals (beverage, hot cereal and muffin)</td>
<td>• Postprandial glucose and insulin response to study treatments</td>
<td>• Both RS treatments reduced glucose AUC compared to control</td>
</tr>
<tr>
<td></td>
<td>• Randomized</td>
<td>• Healthy adults</td>
<td>• 25 g fibre from RS&lt;sub&gt;3&lt;/sub&gt; and 5 g pullulan</td>
<td></td>
<td>• The RS + pullulan treatment reduced insulin AUC compared to control</td>
</tr>
<tr>
<td></td>
<td>• 5 treatments</td>
<td>• Mean age 29 years</td>
<td>• 25 g soluble corn fibre</td>
<td></td>
<td>• Both RS treatments reduced insulin AUC compared to soluble corn fibre</td>
</tr>
<tr>
<td></td>
<td>• 3-week washout periods</td>
<td>• Mean BMI 23 kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>• 25 g soluble corn fibre and 5 g pullulan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Double-blind</td>
<td>25 g fibre from RS&lt;sub&gt;3&lt;/sub&gt;</td>
<td>• Control meal without added fibre</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>RS type &amp; source:</strong></td>
<td>25 g soluble corn fibre</td>
<td>• Matched for available carbohydrate content</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>**RS&lt;sub&gt;3&lt;/sub&gt; from heat-</td>
<td>5 g pullulan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>treated HAM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**RS form:** Food (muffin, cereal and beverage)

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize.
**Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued**

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| Ekstrom LMNK et al.  
On the possibility to affect course of glycaemia, insulinaemia, and perceived hunger/satiety to bread meals in healthy volunteers. Food Funct. 2013;4:522-9. (65) | • Crossover  
• Randomized  
• 5 treatments  
• 1-week washout periods  
• Blinding not described | • n=12  
• Healthy adults  
• Mean age 24.1 years  
• Mean BMI 23.3 kg/m² | • 5 breads with whole grain HAM corn starch guar gum  
• 6.0 g RS  
• 6.7g RS  
• 7.8g RS  
• 9.1 g RS  
• 1.1 g RS (white bread control)  
• Matched for available carbohydrate content | • Fasting and postprandial blood glucose and insulin response to study treatments | • Breads with >6.0 g RS reduced glucose incremental (i)Peak (difference from baseline to peak) compared to white and 6.0 g RS breads  
• Breads with >6.0g RS reduced insulin iPeak compared to white bread, and bread with >6.7 g RS reduced insulin iPeak compared to 6.0 g RS bread  
• The 7.8 g and 9.1 g RS breads had lower glycemic indexes than the control bread  
• The >6.0 g RS breads had lower glycemic profiles and insulin indexes than white bread |

*RS form:  
Food (bread)*

Abbreviations used: BMI=body mass index; HAM=high-amylose maize.
## Appendix C. Human Clinical Trials of the Glycemic Effects of Acute Consumption of RS-Enhanced Foods - continued

<table>
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<tr>
<th>Reference</th>
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<th>Glycemic Endpoints</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacNeil S et al.</td>
<td>Crossover</td>
<td>n=12</td>
<td>Bagels made with high-amylose cornstarch or standard wheat flour</td>
<td>Postprandial glucose and insulin response to study treatments and second meal</td>
<td>Treatment B reduced peak glucose, and glucose at 60, 90 and 120 min compared to other treatments</td>
</tr>
<tr>
<td></td>
<td>Randomized</td>
<td>Adults diagnosed with T2D</td>
<td>Various portions of each bagel to provide: A: 1.18 g RS (control bagel)</td>
<td></td>
<td>Treatment B reduced 3-hr glucose iAUC compared to treatments C and D</td>
</tr>
<tr>
<td></td>
<td>4 treatments</td>
<td>10 taking oral hypoglycemic agents, 2 using diet alone to manage condition</td>
<td>B: 20.76 g RS (matched for total carbohydrates of A)</td>
<td></td>
<td>Treatment B reduced insulin at 90 and 120 min compared to all other treatments</td>
</tr>
<tr>
<td></td>
<td>1-week washout periods</td>
<td>Mean age 60 years</td>
<td>C: 33.34 g RS (matched for available carbohydrates of A)</td>
<td></td>
<td>Treatment B reduced 3-hr insulin iAUC compared to treatments A and C</td>
</tr>
<tr>
<td></td>
<td>Blinding not described</td>
<td>Mean BMI 33 kg/m²</td>
<td>D: 21.2 g RS (matched for available carbohydrates of A and total fibre of B)</td>
<td></td>
<td>Treatment B reduced peak insulin compared to treatments A and D</td>
</tr>
<tr>
<td></td>
<td>RS type &amp; source: RS₂ from HAM</td>
<td></td>
<td></td>
<td></td>
<td>After 2nd meal challenge, Treatment C increased insulin iAUC compared to treatment A</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; HAM=high-amylose maize; iAUC=incremental area under the curve.
### Appendix D. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS Supplements

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<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robertson MD et al.</td>
<td>Crossover, Randomization not described, Two 1-day treatments, Washout period not described, Single-blind</td>
<td>n=10, Healthy adults, Mean age 47 years, Mean BMI 26.9 kg/m²</td>
<td>60 g RS added to jelly (divided into 4 doses in 24 hours), Starch-free jelly, Matched for available carbohydrate content</td>
<td>Fasting and postprandial glucose, insulin and C-peptide responses to standardized fibre-free test meal, Calculated postprandial insulin sensitivity (SI\text{ORAL}) using the minimal model (MINMOD) calculation from standardized fibre-free meal tolerance test (MTT) data, Calculated fasting insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B)</td>
<td>Reduced glucose during MTT, Reduced insulin during MTT, Increased postprandial insulin sensitivity (SI\text{ORAL}) during MTT, Increased ratio of C-peptide to insulin during MTT</td>
</tr>
</tbody>
</table>

RS form: Supplement (jelly)

Abbreviations used: BMI=body mass index; HAM=high-amylose maize; HOMA=homeostasis model assessment.
## Appendix D. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS Supplements - continued

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints and Calculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robertson MD et al.</td>
<td>Crossover</td>
<td>( n=10 ) Healthy adults</td>
<td>Pure starch supplement</td>
<td>Fasting and postprandial glucose and insulin response to standardized meal tolerance test (MTT)</td>
</tr>
<tr>
<td></td>
<td>Randomized</td>
<td>Mean age 49 years</td>
<td>0 g RS/day</td>
<td>Postprandial glucose, insulin and C-peptide response during euglycemic-hyperinsulinemic clamp (clamp)</td>
</tr>
<tr>
<td></td>
<td>Two 4-week interventions</td>
<td>Mean BMI 23.4 kg/m(^2)</td>
<td>30 g RS/day</td>
<td>Calculated postprandial insulin sensitivity (SI(_{ORAL})) using the minimal model (MINMOD) calculation with MTT data, and using the M/I calculation with clamp data</td>
</tr>
<tr>
<td></td>
<td>4-week washout period</td>
<td></td>
<td>Matched for available carbohydrate content</td>
<td>Calculated fasting insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-%B)</td>
</tr>
<tr>
<td></td>
<td>Single-blind</td>
<td></td>
<td></td>
<td>Reduced insulin AUC (MTT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RS treatment increased ratio of C-peptide to insulin (MTT)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased postprandial insulin sensitivity (MTT and clamp)</td>
</tr>
<tr>
<td></td>
<td>( RS ) form: ( RS_2 ) from HAM</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize; HOMA=homeostasis model assessment.
## Appendix D. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS Supplements - continued

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<th>Study Treatments</th>
<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| Johnston KL et al.  
**RS form:**  
Supplement | • Parallel-arm  
• Randomized  
• Two 12-week interventions  
• Single-blind  
  
  **RS type & source:**  
  RS2 from HAM | • n=20  
  • Insulin resistant (defined as fasting insulin >60 pmol/L) adults  
  • Mean age ~48 years  
  • Mean BMI ~31 kg/m² | • Pure starch supplement  
  • 0 g RS/day  
  • 40 g RS/day  
  • Matched for available carbohydrate content | • Fasting and postprandial glucose and insulin response to euglycemic-hyperinsulinemic clamp (clamp)  
• Calculated postprandial insulin sensitivity using M/I calculation with clamp data  
• Calculated fasting insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-%B) | • Increased postprandial insulin sensitivity (clamp) |
| Maki KC et al.  
**RS form:**  
Supplement | • Crossover  
• Randomized  
• Three 4-week interventions  
• 3-week washout periods  
• Double-blind  
  
  **RS type & source:**  
  RS2 from HAM | • n=33  
  • Healthy adults  
  • Mean age 49.5 years  
  • Mean BMI 30.6 kg/m²  
  • Waist circumference ≥102.0 cm (males) and ≥89.0 cm (females)  
  | • Pure starch supplement  
  • 0 g RS/day  
  • 15 g RS/day  
  • 30 g RS/ day  
  • Matched for available carbohydrate content in 15g RS treatment | • Fasting and postprandial glucose and insulin (insulin modified I.V. glucose tolerance test (IVGTT))  
• Calculated postprandial insulin sensitivity (SI_{IVGTT}) using the minimal model (MINMOD), glucose effectiveness (S_G), and acute insulin response (AIR_G) with IVGTT data  
• Calculated fasting insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-%B) | • In men only, both RS treatments increased postprandial insulin sensitivity (IVGTT) compared to control |

**Abbreviations used:** BMI=body mass index; HAM=high-amylose maize; HOMA=homeostasis model assessment.
### Appendix D. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS Supplements - continued

<table>
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<th>Reference</th>
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<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
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</thead>
<tbody>
<tr>
<td>Bodinham CL et al.</td>
<td>Crossover • Randomized • Two 4-week interventions • 4-week washout period • Single-blind</td>
<td>n=12 • Healthy adults • Mean age 37 years • Mean BMI 28.2 kg/m² • Insulin resistant (mean fasting insulin 96 pmol/L)</td>
<td>Pure starch supplement • 0 g RS/day • 40 g RS/day • Matched for available carbohydrate content</td>
<td>Fasting and postprandial glucose, insulin and C-peptide response to frequently sampled I.V. glucose tolerance test (FSIVGTT) • Calculated postprandial insulin sensitivity (SI&lt;sub&gt;IVGTT&lt;/sub&gt;) using the minimal model (MINMOD), glucose effectiveness (SG), first phase insulin response (AIRg) and disposition index with IVGTT data</td>
<td>Reduced fasting glucose • Increased insulin response (FSIVGTT) • Increased first phase insulin response (AIRg)</td>
</tr>
</tbody>
</table>

*RS form: Supplement

*RS type & source: RS<sub>2</sub> from HAM

Abbreviations used: BMI=body mass index; HAM=high-amylose maize.
### Appendix D. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS Supplements - continued

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<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
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</thead>
<tbody>
<tr>
<td>Robertson MD et al.</td>
<td>Crossover</td>
<td>n=15</td>
<td>Pure starch supplement</td>
<td>Fasting and postprandial glucose and insulin response to euglycemic-hyperinsulinemic clamp (clamp) and standardized meal tolerance test (MTT)</td>
<td>Reduced fasting glucose and insulin</td>
</tr>
<tr>
<td></td>
<td>Randomized</td>
<td>Insulin resistant adults</td>
<td>0 g RS/day</td>
<td>Calculated fasted insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B)</td>
<td>Reduced fasting insulin resistance (HOMA-IR)</td>
</tr>
<tr>
<td></td>
<td>Two 8-week interventions</td>
<td>Mean age 48.9 years</td>
<td>40 g RS/day</td>
<td>Calculated glucose clearance, glucose disposal (R_d) and endogenous glucose production (EGP) during clamp</td>
<td>Increased peripheral glucose uptake (clamp) during high-dose insulin infusion</td>
</tr>
<tr>
<td></td>
<td>8-week washout period</td>
<td>Mean BMI 34 kg/m²</td>
<td>Matched for available carbohydrate content</td>
<td></td>
<td>Increased glucose uptake (MTT)</td>
</tr>
<tr>
<td></td>
<td>Single-blind</td>
<td>Increased fasting insulin (60-156 pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>RS form:</em></td>
<td>Increased abdominal adiposity (Mean waist circumference = 160 cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**RS type & source:**
- *RS₂ from HAM*

Abbreviations used: BMI=body mass index; HAM=high-amylose maize; HOMA=homeostasis model assessment.
### Appendix D. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS Supplements - continued

<table>
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<tr>
<th>Reference</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
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</thead>
<tbody>
<tr>
<td>Bodinham CL et al.</td>
<td>Crossover</td>
<td>n=17</td>
<td>Pure starch supplement</td>
<td>Fasting and postprandial glucose and insulin response to euglycemic-hyperinsulinemic clamp</td>
<td>Reduced glucose 2hr AUC (MTT)</td>
</tr>
<tr>
<td>Efficacy of increased resistant starch consumption in human type 2 diabetes. Endocr Connect. 2014;3:75-84. (77)</td>
<td>Randomized</td>
<td>Adults with T2D (managed with oral hypoglycemic agents and lifestyle)</td>
<td>0 g RS/day</td>
<td>Fasting and postprandial glucose, insulin and C-peptide response to standardized meal tolerance test (MTT)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Two 12-week interventions</td>
<td>Mean age 55 years</td>
<td>40 g RS/day</td>
<td>HbA1c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12-week washout period</td>
<td>Mean BMI 30.6 kg/m²</td>
<td>Matched for available carbohydrate content</td>
<td>Calculated postprandial insulin sensitivity during MTT using the Matsuda index</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single-blind</td>
<td></td>
<td></td>
<td>Calculated fasting insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-%B)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RS form: Supplement</td>
<td></td>
<td></td>
<td>Calculated endogenous glucose production (EGP) and glucose disposal (Rd) during clamp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RS type &amp; source: RS₂ from HAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize; HOMA=homeostasis model assessment.
### Appendix E. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS-Enhanced Food

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
| Behall KM et al. Diets containing high amylose vs high amylopectin starches: effects on metabolic variables in human subjects. Am J Clin Nutr 1989;49:337-44. (78) | - Crossover  
- Randomized  
- Two 5-week interventions  
- No washout period  
- Blinding not described  
**RS form:** Food (pudding, muffins, crackers)  
**RS type & source:** Starch from HAM (RS type not specified, deduced as RS<sub>2</sub>) | - n=12  
- Healthy males  
- Mean age 34 years  
- Mean body weight 77.3 kg  
- RS intake was not quantified | - Diets in which 52% of energy from cornstarch, containing either 70% amylose or 70% amylopectin | - Fasting and postprandial glucose, insulin and glucagon response to oral glucose tolerance test (OGTT) after 4 weeks  
- Fasting and postprandial glucose, insulin and glucagon response to high- or low-amylose cracker after 5 weeks (starch tolerance test – STT)  
- Calculated glycemic index | - Glucose response to high-amylose STT was lower at 60 and 90 minutes, and higher at 180 minutes, compared to high-amylopectin STT  
- Insulin response to high-amylose STT was lower overall and at 30, 60 and 180 minutes than high-amylopectin STT  
- Glucagon response to high-amylose STT was lower at 30, 60 and 120 minutes compared to low-amylose STT |

Abbreviation used: HAM=high-amylose maize.
### Appendix E. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS-Enhanced Food - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Study Treatments</th>
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<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behall KM &amp; Howe JC. Effect of long-term consumption of amylose vs amylopectin starch on metabolic variables in human subjects. Am J Clin Nutr. 1995;61:334-40. (81)</td>
<td>• Crossover • Randomization not described • Two 14-week interventions • Washout period not described • Blinding not described</td>
<td>• n=24 • 10 adults with a normal insulin response to a glucose tolerance test (control group), and 14 adults with hyperinsulinemia (HI group) • Mean age 41 years • Mean body weight 83.7 kg</td>
<td>• Diets in which 51% of energy from carbohydrate, of which 55% of carbohydrate intake was either 70% amylose or 70% amylopectin starch • RS intake was not quantified</td>
<td>• Fasting and postprandial glucose and insulin response to study treatments during meal tolerance test (MTT) after 4, 8 and 13 weeks of each diet</td>
<td>• Insulin AUC (response to MTT) was reduced by high-amylose diet at weeks 8 and 13, within HI group and at weeks 4 and 13 within the control group, compared to amylopectin treatment</td>
</tr>
</tbody>
</table>

**RS form:** Food (bread, muffins, cookies, cereals, cheese puffs)

**RS type & source:** Starch from HAM (RS type not specified, deduced as RS\textsubscript{2})

Abbreviations used: AUC=area under the curve; HAM=high-amylose maize; HI=hyperinsulinemia.
### Appendix E. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS-Enhanced Food - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
</table>
Randomized  
Three 4-week interventions  
No washout period  
Blinding not described | n=23  
Overweight adults with either high plasma triglycerides or mild hypertension  
Mean age 51 years  
Mean BMI 29 kg/m² | High amylose diet (17-25 g RS/day)  
Low amylose diet  
High oat bran diet  
Acute treatment of high-amylose (RS) muffin (5.8 g RS) and low-amylose muffin (1.3 g RS) for meal tolerance test (MTT) | Fasting glucose and insulin after each treatment period  
Postprandial glucose and insulin response to high and low-amylose muffins (MTT) after the corresponding treatment period | RS diet reduced postprandial MTT glucose at 45 min (at C_MAX) compared to low-RS diet  
RS diet reduced postprandial MTT insulin at 75 min, and overall summed insulin response compared to low-RS diet |
Randomized  
3-week interventions  
Double-blind | n=25  
Healthy adult females  
Mean age 43 years  
Mean BMI 27.9 kg/m² (control group), 26.6 kg/m² (test group) | Mixture of freeze-dried food (dried whole grain, vegetables, mushrooms and seaweed)  
0 g RS/day  
24 g RS/day  
Matched for total starch content | Fasting glucose and insulin | Fasting glucose reduced within the RS treatment, but not compared to control |

Abbreviations used: BMI=body mass index; C_MAX = time of maximum concentration; HAM=high-amylose maize.
### Appendix E. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS-Enhanced Food - continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
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<th>Study Treatments</th>
<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weickert MO et al.</strong>&lt;br&gt;Impact of cereal fibre on glucose regulating factors. Diabetologia. 2005;48:2343-53. (79) (RS sub-study only)</td>
<td>• Crossover&lt;br&gt;• Randomized&lt;br&gt;• 4 1-day treatments&lt;br&gt;• 1-week washout period&lt;br&gt;• Single-blind</td>
<td>• n=9&lt;br&gt;• Adult females&lt;br&gt;• Mean age 23.6 years&lt;br&gt;• Mean BMI 21.3 km/m²</td>
<td>• 3 servings of bread divided throughout 1 day&lt;br&gt;• White bread (control)&lt;br&gt;• Wheat-fibre enriched bread&lt;br&gt;• Oat-fibre enriched bread&lt;br&gt;• RS-enriched bread (10.4 g RS per serving)</td>
<td>• Fasting and postprandial glucose, insulin and C-peptide response to 1 serving of bread (matched to provide 50 g carbohydrates) (acute sub-study)&lt;br&gt;• Fasting and postprandial glucose, insulin and C-peptide response to control bread on day after test bread consumption (3 servings)</td>
<td>• None</td>
</tr>
<tr>
<td><strong>Penn-Marshall M et al.</strong>&lt;br&gt;African Americans may have to consume more than 12 grams a day of resistant starch to lower their risk for type 2 diabetes. J Med Food. 2010;13:999-1004. (83)</td>
<td>• Crossover&lt;br&gt;• Randomized&lt;br&gt;• Two 6-week interventions&lt;br&gt;• 2-week washout period&lt;br&gt;• Double-blind</td>
<td>• n=15&lt;br&gt;• African American adults&lt;br&gt;• Mean age 36.6 years&lt;br&gt;• Mean BMI 37.7 kg/m²</td>
<td>• Sliced bread baked with and without added RS&lt;br&gt;• ~12g RS/day&lt;br&gt;• ~3 g RS/day&lt;br&gt;• Similar in soluble starch</td>
<td>• Fasting glucose and insulin&lt;br&gt;• HbA1c&lt;br&gt;• Calculated fasting insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B)</td>
<td>• None</td>
</tr>
</tbody>
</table>

Abbreviations used: BMI=body mass index; HAM=high-amylose maize; HOMA=homeostasis model assessment.
**Appendix E. Human Clinical Trials of the Glycemic Effects of Chronic Consumption of RS-Enhanced Food - continued**

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Glycemic Endpoints and Calculations</th>
<th>Significant Effects of RS Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwak JH et al.</td>
<td>• Parallel-arm</td>
<td>• n=85</td>
<td>• Individual packets of 210 g of cooked refined rice with or without added RS</td>
<td>• Fasting and postprandial glucose and insulin response to standardized meal tolerance test (MTT)</td>
<td>• RS rice decreased glucose at 60 and 120 min and glucose AUC of MTT compared to control rice (adjusted for baseline)</td>
</tr>
<tr>
<td></td>
<td>• Randomized</td>
<td>• Adults</td>
<td>• 0 g RS/day</td>
<td>• Calculated fasting insulin resistance (HOMA-IR)</td>
<td>• RS rice increased postprandial insulin sensitivity as measured by Gutt index (adjusted for baseline)</td>
</tr>
<tr>
<td></td>
<td>• 4-week interventions</td>
<td>• Mean age ~51 years</td>
<td>• 6.51 g RS/day</td>
<td>• Calculated postprandial insulin sensitivity (Gutt index)</td>
<td>• RS rice reduced fasting insulin, insulin AUC and insulin resistance (HOMA-IR) compared to control rice, though, unadjusted for baseline</td>
</tr>
<tr>
<td></td>
<td>• 2-week run in period (regular diet plus cooked refined rice)</td>
<td>• Mean BMI ~25 km/m²</td>
<td></td>
<td>• HbA1c</td>
<td></td>
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<tr>
<td></td>
<td>• Double-blind</td>
<td>• Newly diagnosed with T2D or diagnosis of pre-diabetes</td>
<td></td>
<td>• RS rice decreased glucose at 60 and 120 min and glucose AUC of MTT compared to control rice (adjusted for baseline)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fasting plasma glucose ≥ 100 mg/dL</td>
<td></td>
<td>• RS rice increased postprandial insulin sensitivity as measured by Gutt index (adjusted for baseline)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 2-hour oral glucose tolerance test ≥140 mg/dL</td>
<td></td>
<td>• RS rice reduced fasting insulin, insulin AUC and insulin resistance (HOMA-IR) compared to control rice, though, unadjusted for baseline</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• HbA1c ≥5.7%</td>
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</tr>
</tbody>
</table>

*RS form: Food (rice mix)*

**Abbreviations used:** AUC=area under the curve; BMI=body mass index; HAM=high-amylose maize; HbA1c=glycated hemoglobin; HOMA=homeostasis model assessment.
Appendix F. University of Guelph Research Ethics Board Approval

RESEARCH ETHICS BOARDS
Certification of Ethical Acceptability of Research Involving Human Participants

APPROVAL PERIOD: July 5, 2013
EXPIRY DATE: July 5, 2015
REB: NPES
REB NUMBER: 13MY041
TYPE OF REVIEW: Full Board
PRINCIPAL INVESTIGATOR: Duncan, Alison (amduncan@uoguelph.ca)
DEPARTMENT: Human Health & Nutritional Sciences
SPONSOR(S): OMAFRA - Ontario Food Processing Research Program
TITLE OF PROJECT: The Better Bagel Study: The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human participants in the above-named research project and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement, 2nd Edition.

The REB requires that researchers:

- Adhere to the protocol as last reviewed and approved by the REB.
- Receive approval from the REB for any modifications before they can be implemented.
- Report any change in the source of funding.
- Report unexpected events or incidental findings to the REB as soon as possible with an indication of how these events affect, in the view of the Principal Investigator, the safety of the participants, and the continuation of the protocol.
- Are responsible for ascertaining and complying with all applicable legal and regulatory requirements with respect to consent and the protection of privacy of participants in the jurisdiction of the research project.

The Principal Investigator must:

- Ensure that the ethical guidelines and approvals of facilities or institutions involved in the research are obtained and filed with the REB prior to the initiation of any research protocols.
- Submit a Status Report to the REB upon completion of the project. If the research is a multi-year project, a status report must be submitted annually prior to the expiry date. Failure to submit an annual status report will lead to your study being suspended and potentially terminated.

The approval for this protocol terminates on the EXPIRY DATE, or the term of your appointment or employment at the University of Guelph whichever comes first.

Signature: Date: July 8th, 2014

A. Papdopoulos
Chair, Research Ethic Board-NPES
Appendix G. University of Guelph Environmental Health and Safety Biohazard Permit

UNIVERSITY
of GUELPH

BIOSAFETY COMMITTEE

BIOHAZARD PERMIT

PRINCIPAL INVESTIGATOR: ALISON DUNCAN

DEPARTMENT: HUMAN HEALTH AND NUTRITIONAL SCIENCES

TITLE OF PROJECT:
The Better Bagel Study: The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer.

NUMBER: H-254-10-15-06

LOCATION: Bldg. 88, Rm. 143; Bldg. 70, Rms. 305, 370; Bldg. 140, 3204A

APPROVED FOR THE PERIOD: 2013 June 19 TO 2015 June 30

The members of the University of Guelph Biosafety Committee have examined the protocol which describes the use of biohazardous materials in the above-named project and it considers the procedures, as described by the applicant, to conform to the University’s requirements for work with biohazardous materials. All persons working with biohazardous materials under this permit shall adhere to the administrative procedures and rules as set forth by the Biosafety Policy, Biosafety Manual, and any directives supplemental to the application.

Approved: ________________________ Approved: ________________________
Chair, Biosafety Committee University Biosafety Officer

Date: June 19, 2013 Date: 2013 06 19
Appendix H. CANRISK Questionnaire

THE CANADIAN DIABETES RISK QUESTIONNAIRE

CANRISK

Are you at risk?

The following questions will help you to find out if you are at higher risk of having pre-diabetes or type 2 diabetes. Pre-diabetes is a condition where a person's blood sugar levels are higher than normal, but not high enough to be diagnosed as diabetes. You can have pre-diabetes or undiagnosed type 2 diabetes without having any obvious warning signs or symptoms.

Knowing your risk can help you make healthy choices now that will reduce your risk or even prevent you from developing diabetes.

Please answer the questions as honestly and completely as you can. If you wish, a friend or family member can help you to complete this form. The answers to these questions are completely confidential. Answer all questions. Enter your scores for each question in the box on the right-hand side and then add them up to calculate your total risk score.

This questionnaire is intended for adults aged 40 to 74 years.

→ AS YOU GET OLDER, YOUR RISK OF DEVELOPING DIABETES GOES UP.

1. Select your age group:
   - 40-44 years: 0 points
   - 45-54 years: 7 points
   - 55-64 years: 13 points
   - 65-74 years: 15 points

2. Are you male or female?
   - Male: 6 points
   - Female: 9 points

→ BODY SHAPE AND SIZE CAN AFFECT YOUR RISK OF DIABETES.

3. How tall are you and how much do you weigh?

   On the left-hand side of the BMI chart below, circle your height, then on the bottom of the chart circle your weight.

   Find the square on the chart where your height crosses with your weight, and note which shaded area you fall into.

   For example, if you were 5 feet 2 inches (157.5 cm) and 163 pounds (74 kg) you would fall in the LIGHT GREY area.

   Select your BMI group from the following choices:
   - White (BMI less than 25): 0 points
   - Light grey (BMI 25 to 29): 4 points
   - Dark grey (BMI 30 to 39): 9 points
   - Black (BMI 35 and over): 14 points

4. Using a tape measure, place it around your waist at the level of your belly button. Measure after breathing out (do not hold your breath) and write your results on the line below.

   Then check the box that contains your measurement. (Note: this is not the same as the "waist size" on your pants).

   **MEN — Waist circumference:** ______ inches OR ______ cm
   - Less than 44 cm or 37 inches: 0 points
   - Between 44.102 cm or 37.40 inches: 4 points
   - Over 102 cm or 40 inches: 6 points

   **WOMEN — Waist circumference:** ______ inches OR ______ cm
   - Less than 35 cm or 31.5 inches: 0 points
   - Between 35.08 cm or 31.5-35 inches: 4 points
   - Over 35 cm or 35 inches: 6 points

<table>
<thead>
<tr>
<th>Height (in)</th>
<th>Weight (lbs)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>4’5”</td>
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<td>165</td>
<td>73</td>
</tr>
<tr>
<td>6’6”</td>
<td>170</td>
<td>75</td>
</tr>
</tbody>
</table>

175
→ YOUR LEVEL OF PHYSICAL ACTIVITY AND WHAT YOU EAT CAN AFFECT YOUR RISK OF DEVELOPING DIABETES.

5. Do you usually do some physical activity such as brisk walking for at least 30 minutes each day?
   ○ Yes [ ] 1 point
   ○ No [ ] 0 points

6. How often do you eat vegetables or fruits?
   ○ Every day [ ] 2 points
   ○ Not every day [ ] 0 points

→ HIGH BLOOD PRESSURE, HIGH BLOOD SUGAR, AND PREGNANCY-RELATED FACTORS ARE ASSOCIATED WITH DIABETES.

7. Have you ever been told by a doctor or nurse that you have high blood pressure OR have you ever taken high blood pressure pills?
   ○ Yes [ ] 4 points
   ○ No or don’t know [ ] 0 points

8. Have you ever been found to have high blood sugar either from a blood test, during an illness, or during pregnancy?
   ○ Yes [ ] 14 points
   ○ No or don’t know [ ] 0 points

9. Have you ever given birth to a large baby weighing 9 pounds (4.1 kg) or more?
   ○ Yes [ ] 1 point
   ○ No, don’t know, or not applicable [ ] 0 points

→ SOME TYPES OF DIABETES RUN IN FAMILIES.

10. Have any of your blood relatives ever been diagnosed with diabetes?
    Check ALL that apply.
    ○ Mother [ ] 2 points
    ○ Father [ ] 2 points
    ○ Brothers/Sisters [ ] 2 points
    ○ Children [ ] 2 points
    ○ Other [ ] 0 points
    ○ Not/Don’t know [ ] 0 points

    Your combined score cannot be more than 8 points.
    2 points for each category, do not count multiple children or siblings twice.

11. Please check off which of the following ethnic groups your biological (blood) parents belong to:
    ○ White (Caucasian) [ ] 0 points
    ○ Aboriginal [ ] 3 points
    ○ Black (African Caribbean) [ ] 5 points
    ○ East Asian (Chinese, Vietnamese, Filipino, Korean, etc.) [ ] 10 points
    ○ South Asian (East Indian, Pakistani, Sri Lankan, etc.) [ ] 11 points
    ○ Other non-white (Latin American, Arab, West Asian) [ ] 3 points

    Choose only one score, the highest.
    Do not add mother plus father scores together. (Your score cannot be more than 11 points for this section).

→ OTHER FACTORS ARE ALSO RELATED TO DEVELOPING DIABETES.

12. What is the highest level of education that you have completed?
    ○ Some high school or less [ ] 5 points
    ○ High school diploma [ ] 1 point
    ○ Some college or university [ ] 0 points
    ○ University or college degree [ ] 0 points

    Add up your points from questions 1 to 12

These risk scores are in no way a substitute for actual clinical diagnosis.
If you have any concerns, please consider discussing your results with a health care practitioner (e.g., family doctor, nurse practitioner, pharmacist).

Lower than 21 → Low risk
Your risk of having pre-diabetes or type 2 diabetes is fairly low, though it always pays to maintain a healthy lifestyle.

21-32 → Moderate risk
Based on your identified risk factors, your risk of having pre-diabetes or type 2 diabetes is moderate. You may wish to consult with a health care practitioner about your risk of developing diabetes.

33 and over → High risk
Based on your identified risk factors, your risk of having pre-diabetes or type 2 diabetes is high. You may wish to consult with a health care practitioner to discuss getting your blood sugar tested.

Diabetes is a serious chronic disease and uncontrolled diabetes can lead to heart disease, kidney disease and other conditions.
While you can’t change some factors such as age, gender, family history, and ethnocultural background, other risk factors for diabetes may respond to lifestyle changes. These include weight, physical activity, diet, and smoking.
If your BMI is 25 or higher, lowering your weight may help you reduce your risk of developing type 2 diabetes. Even a small change in body weight or physical activity can reduce your risk. Embrace a healthy balanced diet which emphasizes vegetables, fruit, and whole grains.
Consult Canada’s Food Guide for helpful suggestions. If you are not active, begin slowly and increase your activity gradually. Check with your doctor before beginning any exercise program.
If you smoke, it’s never too late to quit. Every step you take to improve your health counts!
Thank you for completing the Canadian Diabetes Risk Questionnaire.

Public Health Agency of Canada, 2011
Appendix I. Participant Recruitment Poster

Better Bagel Study

Adults 40+ years old are needed for a nutrition study on the effect of consuming a bagel high in resistant starch on risk of diabetes and colon cancer.

This study includes two 57-day treatment periods which will each involve:

- Consume 1 bagel each day for 57 days
- Attend one 3-hr study visit on days 1 and 57 for an oral glucose tolerance test (consume a glucose drink and have blood samples over 3 hours)
- Attend one 3-hr study visit for a food intake satiety test
- Attend 20-minute study visits on days 15, 29 and 43
- Provide a fecal sample at the beginning and end
- Complete periodic study questionnaires and food records

*Financial Compensation Provided*

This study is being conducted by the Department of Human Health and Nutritional Sciences and has received clearance from the University of Guelph Research Ethics Board (REB#13MY041)

To find out more about the study and your eligibility as a participant please contact:

519-824-4120 x58081 or bagel@uoguelph.ca
Screening-1 Questionnaire

The effect of resistant starch bagels on mitigation of risk for T2D and colorectal cancer

Participant Screening ID: ________________ Phone # or Email: See master list

1. How did you hear about the study? _______________________________

2. How old are you? ________

3. Do you currently smoke? YES  NO

4. What is your height? _______m  weight? ______kg  (BMI = ______)

5. Do you have diabetes? YES  NO

6. Do you have any other diseases, conditions or health issues? YES  NO

   If YES, what are they? ____________________________________________
   ______________________________________________________________

7. Are you on any prescription medications or NHPs? YES  NO

   If YES, what are they?

<table>
<thead>
<tr>
<th>Medication or NHP</th>
<th>Purpose</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

8. Do you have any anaphylactic food allergies? YES  NO

If individual meets these eligibility requirements and are still interested, explain that the screening process continues with a more detailed eligibility questionnaire, some body measurements, and a blood screening visit. They will receive a cookbook ($30 value) after completion of the screening process.

Screening-2 Visit: Date: ________________  Time: ________________
CONSENT TO PARTICIPATE IN RESEARCH

The Better Bagel Study
The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer

Body Measurement and Questionnaire Screening-2 Study Visit

INTRODUCTION
You are being asked to participate in screening for a research study directed by Professor Alison Duncan of the Department of Human Health and Nutritional Sciences (HHNS) at the University of Guelph. The results of this research will contribute to the thesis of University of Guelph M.Sc. student Sarah Dainty, and to the research activities of HHNS M.Sc. coursework and B.Sc. students at the University of Guelph. This research is funded by the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) and the study bagels are provided by Canada Bread (Maple Leaf Foods).

RESEARCHER CONTACT INFORMATION
If you have any questions or concerns, please don’t hesitate to contact:

Alison Duncan, Ph.D., R.D.
University of Guelph Study Director
Professor, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x53416 or email: amduncan@uoguelph.ca

Sarah Dainty, B.Sc.
University of Guelph Study Coordinator
M.Sc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or email: sdainty@uoguelph.ca

PURPOSE AND DESCRIPTION OF RESEARCH
Resistant starch, a dietary fibre, is a potential dietary strategy to reduce the risk of type 2 diabetes and colorectal cancer. The purpose of this research is to determine if eating bagels made with resistant starch every day for 8 weeks can reduce risk factors for these diseases, as well as increase feeling of fullness (known as satiety). Research to
date has shown that daily consumption of resistant starch can reduce the postprandial
glucose and insulin response compared to other foods, which leads to improved insulin
sensitivity in a variety of populations. However, this research varies in the amount of
resistant starch that people have consumed, the duration it was consumed for, and the
form that was consumed (food versus supplement form). In order to realize the
potential of this food ingredient when baked into a healthy bread product, resistant
starch bagels have been developed for this trial.

This study is a double-blinded study which means that neither the researchers nor the
participants know which treatment any participant is consuming. This study is also a
randomized crossover design, which means the participants will be randomly assigned
to one of two types of bagel treatments for the first eight weeks, then consume neither
study treatments for four weeks, and finally, consume the other type of treatment bagels
for the remaining 8 weeks. At specific time points throughout the study, participants’
blood samples will be analyzed for markers of type 2 diabetes risk, including fasting and
postprandial glucose and insulin levels, cholesterol and triglycerides. Additionally,
participants’ fecal samples will be analyzed for markers of colon cancer risk. A total of
25 participants will be included in this study.

Before the research study is started, participants must be fully screened to ensure they
meet the eligibility criteria. Part of this includes taking body measurements, which is the
focus of this consent form and is described below.

**STUDY SCREENING PROCEDURES**

If you choose to volunteer to participate in the screening process for this study, you
would be asked to do the following:

- Come to the Human Nutraceutical Research Unit (HNRU), located in room 144 of
  the Food Science Building, 88 McGilvray St., University of Guelph; phone 519-
  824-4120 x53925.

- Have your height, body weight, waist- and hip- circumference measured. This will
  be done by a trained study coordinator in a private area.

- Complete a study eligibility questionnaire that will gather information about your
  medical history, dietary habits and lifestyle habits.

- Complete a questionnaire that evaluates diabetes risk called the CANRISK
  questionnaire. This is a 2-page questionnaire adapted from Health Canada that
  gathers information on your lifestyle, medical and family history, height, body
  weight and waist circumference to determine your risk of diabetes.

- Complete a questionnaire that evaluates your eating habits.
POTENTIAL RISKS AND DISCOMFORTS
There are minimal risks associated with participation in this screening. The following summarizes the potential risks and how we will act to minimize potential discomfort associated with this study.

- All body measurements will be completed by a trained study coordinator in a private area.
- All information about you, your health, medical and family history that is obtained for screening purposes will be collected privately and kept confidential.
- Every effort to ensure your comfort and safety will be made during the course of this screening. In the unlikely event of a study-related injury, study staff from the University of Guelph will engage appropriate emergency response services to assist in your care.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY
If you participate in this screening step, you will have benefit of gaining experience participating in research. The overall research project will generate knowledge that may contribute to dietary recommendations for individuals who are at risk for developing type 2 diabetes and/or colon cancer. This research may lead to the use of resistant starch in bread-based food products for the reduction of diabetes and/or colon cancer risk.

PAYMENT FOR PARTICIPATION
You will not receive any compensation from participating in this screening visit. If you proceed to the next phase of the screening process (screening-3), you will receive a cookbook (approximate value $30).

COSTS FOR PARTICIPATION
There is no direct cost for participating in this study. You will only be responsible for covering any costs related to ensuring you are able to attend your scheduled study visits (i.e. gas money, parking fees, public transportation fees, child care fees, etc.).

CONFIDENTIALITY
Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. All participants will be assigned a number, and a study code will be used. Your name will never be used in communicating any aspect of the study. Records will be kept on a password-protected computer and/or in a locked file cabinet in a locked office. In following these guidelines, participants’ confidentiality will be maintained to the best of our ability. Results from the study may be published but will be presented as group data. All data will be kept for 25 years, in accordance with the guidelines set by Health Canada.

If requested, direct access to your research records for this study will be granted to study monitors, auditors, the University of Guelph Research Ethics Board, and regulatory authorities for the verification of study procedures and/or data. Your confidentiality as a study participant will not be violated during this process, to the extent permitted by applicable laws and regulations. By signing this written informed consent form you are agreeing to authorize such access.
PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you don’t want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise that warrant doing so. The researchers may withdraw you if participation is no longer in your best interest, or if you fail to follow the directions of the study. If you decide to participate, you agree to cooperate fully with study procedures. We will tell you about new information that may affect your health, welfare, or willingness to stay in this study. You will be given a copy of this consent form to keep.

RIGHTS OF RESEARCH PARTICIPANTS
You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. This study has been reviewed and received ethics clearance through the University of Guelph Research Ethics Board. If you have questions regarding your rights as a research participant, contact:

Director, Research Ethics Telephone: (519) 824-4120, ext. 56606
University of Guelph E-mail: sauld@uoguelph.ca
437 University Centre Fax: (519) 821-5236
Guelph, ON N1G 2W1

SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE
I have read the information provided for the study “The Better Bagel Study: The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer- Body Measurement and Questionnaire Screening-2 Study Visit” as described herein. My questions have been answered to my satisfaction, and I agree to participate. I have been given a copy of this form.

<table>
<thead>
<tr>
<th>NAME OF PARTICIPANT</th>
<th>SIGNATURE OF PARTICIPANT</th>
<th>DATE</th>
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<table>
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<tr>
<th>NAME OF WITNESS</th>
<th>SIGNATURE OF WITNESS</th>
<th>DATE</th>
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Screening-2 Questionnaire

The effect of resistant starch bagels on mitigation of risk for T2D and colorectal cancer

The purpose of this questionnaire is to gather more information about you as a participant in this study. Please feel free to not answer any questions you are uncomfortable with answering. All information provided in this questionnaire will be kept strictly confidential.

CONTACT INFORMATION

Participant Screening ID: ___________ Date________________
Address: See master list
Phone: Work: See master list Home: See master list Email: See master list
Best way to communicate: ______________ Date of Birth: __________ Age: ______

DIET-RELATED QUESTIONS:

1. Are you allergic or sensitive to gluten? YES NO
2. Do you have any other food allergies or sensitivities? YES NO
   If YES, please describe: __________________________________________
3. Are you on a special diet? YES NO
   Details: ______________________________________________________
4. Do you consume caffeine (coffee, tea, pop)? YES NO
   If so, how much per day? __________________
5. Do you consume alcohol? YES NO
   If so, how many drinks per week? _____
   (1 drink = 12oz beer, 5oz wine, 1.5oz hard liquor)
6. Completion of brief 24-hour dietary recall. When you have completed the questionnaire, the study coordinator will ask you what you consumed in the last 24 hours to get a rough idea of your diet.
HEALTH AND LIFESTYLE-RELATED QUESTIONS

If male, skip to question 10.

7. Are you postmenopausal?  
   If YES, are you taking hormone replacement therapy?  
   If YES, are you able to stay on a consistent dose regimen for the study?

8. Do you currently smoke?  
   If NO, have you ever smoked?  
   If YES how long ago did you stop smoking? ________________

9. Do you use recreational drugs?  

10. Do you have, or have had, any of the following health conditions:

<table>
<thead>
<tr>
<th>Health Condition</th>
<th>Currently Have</th>
<th>Have Had in Past</th>
<th>Have Never Had</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Cholesterol</td>
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<tr>
<td>Heart Disease</td>
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<tr>
<td>Cancer</td>
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<td>Type 1 Diabetes</td>
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<td>Type 2 Diabetes</td>
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<tr>
<td>Pre-Diabetes</td>
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<td></td>
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<tr>
<td>High Blood Pressure</td>
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<tr>
<td>Impaired Liver Function</td>
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<tr>
<td>Impaired Kidney Function</td>
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<tr>
<td>Celiac Disease</td>
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<tr>
<td>Crohn’s Disease</td>
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<tr>
<td>Ulcerative Colitis</td>
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<tr>
<td>Constipation</td>
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<tr>
<td>Irritable Bowel Syndrome</td>
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<tr>
<td>Arthritis</td>
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<tr>
<td>Depression</td>
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</table>

11. Are there any other health conditions you have or have had?  
   If YES, what are they? __________________________________________
12. Are you currently taking any prescription medications?  
   YES  NO  
   If YES, please complete the following table:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Purpose</th>
<th>Duration of Use</th>
<th>Notes</th>
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13. Do you feel it would be possible for your medications to remain stable in terms of type and dose for the entire study?  
   YES  NO

14. Have you taken antibiotics in the last 6 months?  
   YES  NO  
   If YES, when did you stop using them? ________________________

15. Have you travelled internationally in the last 6 months?  
   YES  NO  
   If YES, did you experience traveler’s diarrhea?  
   YES  NO

16. Have you experienced food poisoning or severe diarrhea in the past 6 months?  
   YES  NO

17. Have you had any surgeries or medical events in the last 6 months?  
   YES  NO  
   If YES, what are they? ________________________________________

18. Do you use any over-the-counter medications, including pain relievers?  
   YES  NO  
   If YES, what are they? ________________________________________
   ____________________________________________________________
19. Do you take vitamin, mineral or herbal supplements (natural health products)?

If YES, please complete the following table:

<table>
<thead>
<tr>
<th>Natural Health Product</th>
<th>Purpose</th>
<th>Duration of Use</th>
<th>Notes</th>
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20. Depending if the natural health product is known to affect study endpoints, would you be willing to discontinue it for the duration of the study? YES NO

21. Do you have any allergies (drug, food, environmental)? YES NO

   If YES, what are they ________________________________

22. Do you exercise? YES

   NO

   Can you describe your exercise? ________________________________

   How often and how intense? ________________________________

23. Has your body weight changed

   In the past 3 months? YES NO If YES, by how much? __________

   In the past year? YES NO If YES, by how much? __________

   If necessary, please explain: ________________________________

24. It will be very important to maintain your body weight throughout this study. Will you be OK with this? YES NO
STUDY LOGISTIC QUESTIONS

25. Do you have any issues with having your blood taken?  YES  NO

26. Is there a particular weekday that you absolutely could NOT have a study visit?
   MONDAY  TUESDAY  WEDNESDAY  THURSDAY  FRIDAY

27. Is there a particular weekday that you prefer to have study visits?
   MONDAY  TUESDAY  WEDNESDAY  THURSDAY  FRIDAY

28. Are you currently involved in any other research study?  YES  NO

29. Have you ever been involved in a research study before?  YES  NO

   If YES, please expand briefly

30. Why do you want to be in this study?

   __________________________________________________________
   __________________________________________________________

Thank you for completing this questionnaire. The study coordinator will now review the questionnaire with you and answer any questions you may have. Thank you again for your time and cooperation.
Appendix M. Screening-3 Consent Form

(Printed on University of Guelph, College of Biological Sciences letterhead)

CONSENT TO PARTICIPATE IN RESEARCH

The Better Bagel Study:
The effect of resistant starch bagels on risk factors of
type 2 diabetes and colorectal cancer

Blood Sample and Body Measurement Screening-3 Study Visit

INTRODUCTION
You are being asked to participate in screening for a research study directed by
Professor Alison Duncan of the Department of Human Health and Nutritional Sciences
(HHNS) at the University of Guelph. The results of this research will contribute to the
thesis of University of Guelph M.Sc. student Sarah Dainty, and to the research activities
of HHNS M.Sc. coursework and B.Sc. students at the University of Guelph. This
research is funded by the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA)
and the study bagels are provided by Canada Bread (Maple Leaf Foods).

RESEARCHER CONTACT INFORMATION
If you have any questions or concerns, please don’t hesitate to contact:

Alison Duncan, Ph.D., R.D.
University of Guelph Study Director
Professor, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x53416 or email: amduncan@uoguelph.ca

Sarah Dainty, B.Sc.
University of Guelph Study Coordinator
M.Sc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or email: sda@uoguelph.ca

PURPOSE AND DESCRIPTION OF RESEARCH
Resistant starch, a dietary fibre, is a potential dietary strategy to reduce the risk of type
2 diabetes and colorectal cancer. The purpose of this research is to determine if eating
bagels made with resistant starch every day for 8 weeks can reduce risk factors for
these diseases, as well as increase feeling of fullness (known as satiety). Research to
date has shown that daily consumption of resistant starch can reduce the postprandial
glucose and insulin response compared to other foods, which leads to improved insulin
sensitivity in a variety of populations. However, this research varies in the amount of
resistant starch that people have consumed, the duration it was consumed for, and the
form that was consumed (food versus supplement form). In order to realize the potential of this food ingredient when baked into a healthy bread product, resistant starch bagels have been developed for this trial.

This study is a double-blinded study which means that neither the researchers nor the participants know which treatment any participant is consuming. This study is also a randomized crossover design, which means the participants will be randomly assigned to one of two types of bagel treatments for the first eight weeks, then consume neither study treatments for four weeks, and finally, consume the other type of treatment bagels for the remaining 8 weeks. At specific time points throughout the study, participants’ blood samples will be analyzed for markers of type 2 diabetes risk, including fasting and postprandial glucose and insulin levels, cholesterol and triglycerides. Additionally, participants’ fecal samples will be analyzed for markers of colon cancer risk. A total of 25 participants will be included in this study.

Before the research study is started, participants must be fully screened to ensure they meet the eligibility criteria. Part of this includes a blood sample, body measurements and 3-day food record, as described below.

**STUDY SCREENING PROCEDURES**

If you choose to volunteer to participate in the screening process for this study, you would be asked to do the following:

- Come to the Human Nutraceutical Research Unit (HNRU), located in room 144 of the Food Science Building, 88 McGilvray St., University of Guelph; phone 519-824-4120 x53925 after having fasted for 12 hours (nothing to eat or drink except water), and after having avoided strenuous exercise, alcohol and over-the-counter medications for 24 hours prior.

- Have your body weight measured. This will be done by a trained study coordinator in a private area.

- Have your blood pressure and heart rate measured. This will be done by a trained study coordinator in a private area.

- Provide a blood sample. A qualified and trained medical technician will draw a blood sample from your forearm using a needle. The total amount of blood will be 5 mL which is very small in comparison to the amount to the 450 mL that is collected when blood is donated to Canadian Blood Services. There is a chance that the process of the blood sample could cause you some slight discomfort as the needle is inserted and, as with any venipuncture, there may be some minimal bruising afterwards. These risks and potential discomforts from the blood draws will be managed by having a qualified and experienced medical technician taking your blood. In addition, consuming plenty of water the night before and morning of the visit can facilitate blood sampling. Also, applying compression the blood draw site will help to minimize bruising.
• Enjoy a light snack.

• **Three-Day Food Record:** You will be provided with instructions and blank forms to record everything you eat and drink for three days, on typical days for you. This only needs to be completed if you are fully eligible and decide to participate in the study. Therefore this does not need to be started until after your participation in the study is decided upon.

**STUDY SAMPLE LABORATORY ANALYSIS**
Blood samples will be processed at the HNRU and sent to an independent medical laboratory (LifeLabs® Medical Laboratory Services) for same-day analysis of markers of type 2 diabetes risk (glucose) and liver enzymes AST and ALT (markers to ensure healthy liver function) and kidney function (creatinine).

**POTENTIAL RISKS AND DISCOMFORTS**
There are minimal risks associated with participation in this screening. The following summarized the potential risks:

- A qualified and trained medical technician, employed by the University of Guelph, will draw a blood sample from your forearm using a needle. There is a chance that this process could cause you some slight discomfort as the needle is inserted and, as with any blood sample procedure, there may be some minimal bruising afterwards. These risks and potential discomforts from the blood samples will be managed by having a qualified and experienced medical technician, employed by the University of Guelph, taking your blood. In addition, consuming plenty of water the night before and the morning of can facilitate blood sampling. Also, applying compression to the blood draw site immediately after will minimize bruising.

- Every effort to ensure your comfort and safety will be made during the course of this screening. In the unlikely event of a study-related injury, study staff from the University of Guelph will engage appropriate emergency response services to assist in your care.

**POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY**
If you participate in this screening step, you will have benefit of gaining experience participating in research. The overall research project will generate knowledge that may contribute to dietary recommendations for individuals who are at risk for developing type 2 diabetes and/or colon cancer. This research may lead to the use of resistant starch in bread-based food products for the reduction of diabetes and/or colon cancer risk.

**PAYMENT FOR PARTICIPATION**
You will receive compensation in the form of a cookbook (approximate value of $30).
COSTS FOR PARTICIPATION
There is no direct cost for participating in this study. You will only be responsible for covering any costs related to ensuring you are able to attend your scheduled study visits (i.e. gas money, parking fees, public transportation fees, child care fees, etc.).

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PARTICIPATION AND WITHDRAWAL
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Director, Research Ethics
University of Guelph
437 University Centre
Guelph, ON  N1G 2W1

Telephone: (519) 824-4120, ext. 56606
E-mail: sauld@uoguelph.ca
Fax: (519) 821-5236
SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE
I have read the information provided for the study “The Better Bagel Study: The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer – Blood Sample and Body Measurement Screening-3 Study Visit” as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

<table>
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<tr>
<th>NAME OF PARTICIPANT</th>
<th>SIGNATURE OF PARTICIPANT</th>
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<td>V.b. Foods and Natural Health Products to Avoid During the Study</td>
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<td>IX. Study Diary</td>
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</table>
CONSENT TO PARTICIPATE IN RESEARCH
The Better Bagel Study:
The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer

INTRODUCTION
You are being asked to participate in a research study directed by Professor Alison Duncan of the Department of Human Health and Nutritional Sciences at the University of Guelph. The results of this research will contribute to the thesis of University of Guelph M.Sc. student Sarah Dainty and to the research activities of HHNS M.Sc. coursework and B.Sc. students at the University of Guelph. This research is funded by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the study bagels are provided by Canada Bread of Maple Leaf Foods.

RESEARCHER CONTACT INFORMATION
If you have any questions or concerns, please don't hesitate to contact:

Alison Duncan, Ph.D., R.D.
University of Guelph Study Director
Professor, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x53416 or email: amduncan@uoguelph.ca

Sarah Dainty, B.Sc.
University of Guelph Study Coordinator
M.Sc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or email: sdainty@uoguelph.ca

PURPOSE AND DESCRIPTION OF RESEARCH
Resistant starch, a dietary fibre, is a potential dietary strategy to reduce the risk of type 2 diabetes and colorectal cancer. The purpose of this research is to determine if consuming bagels made from a corn flour high in resistant starch every day for 8 weeks can reduce risk factors for these diseases, as well as increase feeling of fullness (known as satiety). Research to date has shown that daily consumption of resistant starch can reduce the postprandial glucose and insulin response compared to other foods, which leads to improved insulin sensitivity in a variety of populations. However, this research
varies in the amount of resistant starch that people have consumed, the duration it was consumed for, and the form that was consumed (food versus supplement form). In order to realize the potential of this food ingredient when baked into a healthy bread product, resistant starch bagels have been developed for this research.

If you decide to participate in this study, you will be asked to consume two types of bagels for an 8-week period each, separated by a 4-week break. The bagels will contain either contain approximately 30g of resistant starch per day (treatment) or no added RS (control). This study is double-blinded, which means that neither the researchers nor yourself will know which type of bagel you are consuming during each treatment period. This study is also a randomized crossover study, which means that the order in which you are consuming the two types of bagels has been randomly assigned, and that over the course of the study, you will consume both types of study bagels. Most of the data we will collect during this study will come from the analysis of your blood samples. At specific time points during both treatment periods, blood samples will be collected and analyzed for markers of type 2 diabetes risk, including fasting and postprandial glucose and insulin levels, cholesterol and triglycerides. Additionally, body measurements will be taken throughout the study, and fecal samples will be analyzed for markers that relate to colon cancer risk. A total of 25 participants will be included in this study.

Overall, this research has potential to contribute to dietary recommendations for people wanting to reduce their risk of type 2 diabetes and colorectal cancer, as well as provide insight into resistant starch’s satiety producing effect.

**STUDY PROCEDURES**

If you are eligible and if you choose to volunteer to participate in this study, you would be asked to do the following things for the duration of each 8-week treatment period. Note that the treatment periods are separated by a 4-week break:

- **Consume 1 study bagels / day, which will be provided to you in frozen, 2-week supplies.** These bagels are produced by Canada Bread, Maple Leaf Foods. They are made with the primary ingredients of either standard flour or high resistant starch corn flour.

- **Record in a daily study diary how and when you consumed your study bagels, health issues, over-the-counter medication use, changes in your medications, and anything you feel would be relevant to record.**

- **Maintain your usual dietary and lifestyle habits.**

Each 8-week treatment period will involve a total of 6 study visits that will occur at the Human Nutraceutical Research Unit (HNRU), located in room 144 of the Food Science, Guelph Food Technology Centre Building, 88 McGilvray St. at the University of Guelph; phone 519-824-4120 x53925. The following describes exactly what will happen at each of these study visits:
Study Day 1 Visit (Baseline):
You will be asked to come to the HNRU after a 12-hour overnight fast (except water which is encouraged) and having avoided alcohol, strenuous exercise and over-the-counter medications for 24 hours prior. Upon arrival at the HNRU, you will:

- Return your fecal sample which will be collected within the 4 days before your study day 1 visit. You will receive comprehensive instructions on how to do this.
- Return a pre-study 3-day food record (2 weekdays and 1 weekend day) that you will have completed before your study day 1 visit.
- Have your blood pressure and body weight measured. This will be done by a trained study coordinator in a private area.
- Provide a fasting blood sample (19 mL). This will be completed by a qualified and trained medical technician.
- Complete an oral glucose tolerance test (OGTT). This involves drinking 75 g of glucose (sugar) in an orange flavoured solution (300 mL) over 5-10 minutes, and further blood samples (11 mL each) will be taken at 30, 60, 90, 120 and 180 min after starting the glucose drink from a catheter that will be inserted into your arm. The total amount of blood will about 75 mL which is very small in comparison to the amount to the 450 mL that is collected when blood is donated to Canadian Blood Services.
- Have a snack.
- Take with you your first 2 weeks of study bagels.

Study Day 2 or 3 or 4 (depending on scheduling) Visit (Satiety Study Visit):
- In advance of this visit, you will have been asked to keep a record of your dietary intake the day before this study visit. Please bring it to this study visit.
- You will be asked if you are fasted (no food since last night, no more than 500mL of water since 8:30pm last night), have avoided unusual physical activity for 24 hours.
- Complete a satiety questionnaire.
- Consume a breakfast (it will be provided and includes the study bagel)
- Stay at the HNRU for the following 3 hours and complete a satiety questionnaire at period time points. During this time you may work individually, read, listen to music, chat with others, etc.
- Consume an unlimited pizza lunch until you are comfortably full. The pizza will be Delisio 4-cheese thin crust.
- Receive instructions about how to complete a weighed 24-hour food record for the remainder of the day.

Study Day 15 Visit:
- Arrive having avoided food and beverage (except water) that morning.
- Return your empty study bagel packaging.
- Return your completed weighed food record from your Satiety Study Day.
- Have your fasted body weight measured. This will be done by a trained study coordinator in a private area.
• Complete a sensory questionnaire about the previous 2 weeks of your study bagel consumption.
• Have a snack.
• Take with you your next 2-week supply of study bagels.

Study Day 29 Visit:
• In advance of this Day 29 study visit, you will have completed a 3-day food record (2 weekdays and 1 weekend day), please return it at this study visit.
• Arrive having avoided food and beverage (except water) that morning.
• Return your empty study bagel packaging.
• Have your fasted body weight measured. This will be done by a trained study coordinator in a private area.
• Complete a sensory questionnaire about the previous 2 weeks of your study bagel consumption.
• Have a snack.
• Take with you your next 2-week supply of study bagels.

Study Day 43 Visit:
• Arrive having avoided food and beverage (except water) that morning.
• Return your empty study bagel packaging.
• Have your fasted body weight measured. This will be done by a trained study coordinator in a private area.
• Complete a sensory questionnaire about the previous 2 weeks of your study bagel consumption.
• Have a snack.
• Take with you your next 2-week supply of study bagels.

Study Day 57 Visit:
You will be asked to come to the HNRU after a 12-hour overnight fast (except water which is encouraged) and having avoided alcohol, strenuous exercise and over-the-counter medications for 24 hours prior. Upon arrival at the HNRU, you will:
• Return your fecal sample which will be collected within the 4 days before your study day 57 visit. You will receive comprehensive instructions on how to do this.
• Have your blood pressure and body weight measured. This will be done by a trained study coordinator in a private area.
• Provide a fasting blood sample (19 mL). This will be completed by a qualified and trained medical technician.
• Complete an oral glucose tolerance test (OGTT). This involves drinking 75 g of glucose (sugar) in an orange flavoured solution (300 mL) over 5-10 minutes, and further blood samples (11 mL each) will be taken at 30, 60, 90, 120 and 180 min after starting the glucose drink from a catheter that will be inserted into your arm. The total amount of blood will about 75 mL which is very small in comparison to the amount to the 450 mL that is collected when blood is donated to Canadian Blood Services.
• Complete a questionnaire about the previous 2 weeks of your study bagel consumption.
• Have a snack.
• For treatment period 2, complete the study exit questionnaire and paper work for your study compensation. This will then be submitted to allow you to receive compensation within 4-6 weeks.

Washout Period
This is the 4-week period in between the two treatment periods. During this time, please maintain your usual diet and lifestyle and if possible not to start any new natural health products. If you are prescribed any new medications during this time, please inform the researchers.

STUDY SAMPLE LABORATORY ANALYSIS
Blood samples will be processed at the HNRU and sent to LifeLabs, Guelph, ON for analysis of lipids and HbA1c. Analysis of glucose and insulin will occur at the University of Guelph. All plasma samples will be labeled with participant number (not name) for confidentiality. After the blood samples have been analyzed for the study measurements, they will be destroyed.

Fecal samples will be delivered the laboratory of Professor Emma Allen-Vercoe in the Department of Molecular and Cellular Biology at the University of Guelph. Following analysis for types and microbes, they will be destroyed.

Dr. Allen-Vercoe’s laboratory is seeking some fecal samples to culture to recover live bacterial isolates that will be characterized and curated and used in future research, but they will be labeled with participant number (not name) for confidentiality, as above. Please indicate here if you consent for your fecal sample to be retained for this analysis:

YES: _____  NO:_________  Participant Initials: _______

STUDY RESULTS AND PUBLICATION
Results from this study may be published, but will always be presented as group data and with no ability to link data back to an individual (i.e. data will always remain confidential). Your decision to be a participant in this study is voluntary and you are free to withdraw yourself, your samples and/or your data from the study at any time. Following completion of the study analyses, a summary of your individual results will be mailed to you.

POTENTIAL RISKS AND DISCOMFORTS
There are minimal risks associated with participation in this study. The following summarizes the potential risks:

• At four study visits (Study Days 1 and 57 for each treatment period), a qualified and trained technician will insert a catheter into your arm for blood samples during the oral glucose tolerance test. There is a chance that this process could
cause you some slight discomfort as the needle is inserted and, as with any blood sample procedure, there may be some minimal bruising afterwards. These risks and potential discomforts from the blood samples will be managed by having a qualified and experienced medical technician taking your blood. In addition, consuming plenty of water the night before and the morning of can facilitate blood sampling. Also, applying compression to the blood draw site immediately after the catheter is removed will minimize bruising.

- The potential side effects of the sugar drink consumed for the oral glucose tolerance test may include headache, dizziness, nausea, bloating, flatulence, or diarrhea. Mild headache, dizziness and/or nausea may occur during the test in about 1 in 10 people, but the incidence of more severe symptoms is uncommon.

- Every effort to ensure your comfort and safety will be made during the course of this study. In the unlikely event of a study-related injury, study staff will engage appropriate emergency response services to assist in your care.

**POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY**

If you participate in this research, you will have the benefit of gaining experience participating in a research study. You will receive a written summary of your individual study data. In relation to larger benefits to this research, the knowledge gained from this study may contribute to dietary recommendations for individuals who are at risk for developing type 2 diabetes and colorectal cancer. This research may lead to the use of resistant starch in bread-based food products for the reduction of diabetes and/or colon cancer risk.

**PAYMENT FOR PARTICIPATION**

You will be compensated for your time and effort for this study in the amount of $400 upon completion of the study. If you withdraw from the study before its completion, your compensation will be pro-rated accordingly.

**COSTS FOR PARTICIPATION**

There is no direct cost for participating in this study. You will only be responsible for covering any costs related to ensuring you are able to attend your scheduled study visits (i.e. gas money, public transportation fees, child care fees, etc.). It is our intention that, through the financial compensation that is provide for your time and effort participating in this study, it partially reimburses you for some of these costs you may incur.

**CONFIDENTIALITY**

Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. All participants will be assigned a number, and a study code will be used. Your name will never be used in communicating results of the study. Records will be kept on a password-protected computer and/or in a locked file cabinet in a locked office. In following these guidelines, participants’ confidentiality will
be maintained to the best of our ability. Results from the study may be published but will be presented as group data. All data will be kept for 25 years, in accordance with the guidelines set by Health Canada.

Microbial isolates (bacterial cultures) obtained from your fecal sample may be used for further experimentation, but these isolates will be labeled with your participant number only, to ensure confidentiality.

If requested, direct access to your research records for this study will be granted to study monitors, auditors, the University of Guelph Research Ethics Board, and regulatory authorities for the verification of study procedures and/or data. Your confidentiality as a study participant will not be violated during this process, to the extent permitted by applicable laws and regulations. By signing this written informed consent form you are agreeing to authorize such access.

PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you don’t want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise that warrant doing so. The researchers may withdraw you if participation is no longer in your best interest, or if you fail to follow the directions of the study. If you decide to participate, you agree to cooperate fully with study procedures. We will tell you about new information that may affect your health, welfare, or willingness to stay in this study. You will be given a copy of this consent form to keep.

RIGHTS OF RESEARCH PARTICIPANTS
You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. This study has been reviewed and received ethics clearance through the University of Guelph Research Ethics Board. If you have questions regarding your rights as a research participant, contact:

Director, Research Ethics  Telephone: (519) 824-4120, ext. 56606
University of Guelph  E-mail: sauld@uoguelph.ca
437 University Centre  Fax: (519) 821-5236
Guelph, ON   N1G 2W1
SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE
I have read the information provided for the study “The Better Bagel Study: The effect of resistant starch bagels on risk factors of type 2 diabetes and colorectal cancer” as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

<table>
<thead>
<tr>
<th>NAME OF PARTICIPANT</th>
<th>SIGNATURE OF PARTICIPANT</th>
<th>DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NAME OF WITNESS</th>
<th>SIGNATURE OF WITNESS</th>
<th>DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix P, Participant E-mail Reminder Scripts (Treatment Period 1 - Study Day 1 visit only)
*******************************************************************************
TREATMENT PERIOD 1 - Study Day 1 Appointment Reminder

Welcome to the BBS: Study Day 1 Appointment Reminder (to send after Orientation and before Study Day 1)

Hi [Participant Name],

Welcome to the Better Bagel Study! Your first study visit (Study Day 1) has been scheduled for [Date] at [Time] at the Human Nutraceutical Research Unit (HNRU) in room 144 of the Food Science Building at the University of Guelph (phone 519-824-4120 x53925).

This study visit will last approximately 3.5 hours and will involve:
- Fasted blood sample and oral glucose tolerance test (after which a snack will be provided)
- Body weight measurement
- Handing in your pre-study 3-day food record (2 weekdays and 1 weekend day)
- Handing in your pre-study day 1 fecal sample collected within 4 days of this study visit (if not already done)
- Picking up your 2-week supply of bagels.

During this study visit there may be some waiting time between blood samples, so please feel free to bring a book, magazine, laptop, or DVD to watch on our TV.

As a reminder, remember to:
- Avoid food and non-water beverages for 12 hours prior (however drinking water is OK and is encouraged for easier blood sampling)
- Avoid over-the-counter medication for 24 hours prior
- Avoid any unusual physical activity the day prior
- Avoid alcohol the evening prior
- Wear clothing that will permit access to your upper arm for blood sampling

Finally, as with all study visits, please remember to bring your BBS Study Handbook with you.

Thanks and please let us know if you have any questions or concerns, we look forward to seeing you!

[Researcher name] and the Better Bagel Study team
*******************************************************************************
Appendix Q. Maple Leaf Foods/Canada Bread Communication Log

Production of the bagels for the clinical trial covered an extensive time period due to multiple challenges and delays in bagel formulation, production and analysis. Table 1 summarizes the individuals involved in this process, and Table 2 details the chronology of events that occurred between August 22, 2002 and January 18, 2014 when the bagels were finally ready for the clinical trial.

Table 1. Individuals involved in the study bagel development

<table>
<thead>
<tr>
<th>Name and Position</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Industry Professionals</strong></td>
</tr>
<tr>
<td>John Webb, Ph.D. – <strong>Director of Emerging Science, Maple Leaf Foods</strong></td>
</tr>
<tr>
<td>Stephen Donaldson – <strong>Senior Director of Product Development, Canada Bread (CB)</strong> (no longer with company)</td>
</tr>
<tr>
<td>Kevin Hashimoto – <strong>Director of Product Development, Canada Bread</strong></td>
</tr>
<tr>
<td>Bruce McKeown – <strong>Product Development Manager, Canada Bread</strong></td>
</tr>
<tr>
<td>Christine Pelkman, Ph.D. – <strong>Senior Nutrition Scientist &amp; Clinical Nutrition Manager, Ingredion, Inc.</strong></td>
</tr>
<tr>
<td><strong>University of Guelph Faculty (part of research team)</strong></td>
</tr>
<tr>
<td>Michael J. Emes, Ph.D. – Professor, MCB &amp; Dean, College of Biological Science</td>
</tr>
<tr>
<td>Alison M Duncan, Ph.D., R.D. – Professor, HHNS</td>
</tr>
<tr>
<td>Ian Tetlow, Ph.D. – Associate Professor, MCB</td>
</tr>
<tr>
<td>Emma Allen-Vercoe, Ph.D. – Associate Professor, MCB</td>
</tr>
<tr>
<td><strong>Students</strong></td>
</tr>
<tr>
<td>Sarah Dainty, B.Sc. – <strong>M.Sc. Candidate, HHNS</strong></td>
</tr>
<tr>
<td>Laura Montgomery, B.Sc. – <strong>M.Sc. Candidate, HHNS</strong></td>
</tr>
<tr>
<td>Ian Brown, B.Sc. – <strong>M.Sc. Candidate, MCB</strong></td>
</tr>
<tr>
<td>Sarah Massey, B.Sc. – <strong>M.Sc. Candidate, MCB</strong></td>
</tr>
</tbody>
</table>

Abbreviations used: CB = Canada Bread; HHNS = Department of Human Health and Nutritional Sciences, University of Guelph; I = Ingredion; MCB = Department of Molecular and Cellular Biology, University of Guelph.
<table>
<thead>
<tr>
<th>Date</th>
<th>Type of Communication / Event</th>
<th>People Involved</th>
<th>Purpose</th>
</tr>
</thead>
</table>
| August 22, 2012    | Overall Team Meeting          | -JW-CB -SD-CB         | -Students presented “high-level overview” of their parts of the research to date  
<p>|                    |                               | -MJE -AMD             | -SD and AMD presented plan for human study phase of this research         |
|                    |                               | -EAV -IJT             |                                                                         |
|                    |                               | -IB -SM               |                                                                         |
|                    |                               | -SD                   |                                                                         |
| November 28, 2012  | University of Guelph Researcher Team Meeting | -MJE -AMD        | -Discussed human study design, inclusion and exclusion criteria       |
|                    |                               | -EAV -IB              |                                                                         |
|                    |                               | -SD -LM               |                                                                         |
| November 28, 2012 to January 3, 2013 | Emails                  | -AMD -JW-CB           | -Requests for meeting and to advance study bagel formulation          |
|                    |                               | -KH-CB                |                                                                         |
| February 7, 2013   | Meeting at Maple Leaf Foods ThinkFOOD! Centre | -JW-CB -SD             | -Discussed study bagel formulation with CB team                              |
|                    |                               | -KH-CB -AMD           | -Will use 1.5 bagels of previous recipes to achieve 30g dose              |
|                    |                               | -LM                   | -Talked about timeline, told it may take until mid to late summer to schedule bagel production |
| February 15 to May 14, 2013 | Emails                  | -JW-CB -AMD           | -Requests to schedule bagel development and production date           |
|                    |                               | -KH-CB                |                                                                         |
| May 23-28, 2013    | Emails                        | -BM-CB -AMD          | -Request from BM-CB to meet with AMD, SD and LM about bagel formulation requirements |
|                    |                               |                       | -Meeting booked for June 6, 2013                                       |
| June 6, 2013       | Meeting at UG                 | -BM-CB -AMD          | -Discussed bagel formulation and nutritional requirements, ingredients required, and next steps |
|                    |                               | -SD -LM               |                                                                         |
| June 13 to August 19, 2013 | Emails                  | -BM-CB -AMD          | -Emails to communicate and finalize ingredients to be used (Amioca, gluten, flavour modulator issues cleared up) |
|                    |                               | -JW-CB -SD            | -BM-CB ordered required ingredients, scheduled flavour modulator lab tests for August 21, 2013 – SD to attend |</p>
<table>
<thead>
<tr>
<th>Date</th>
<th>Activity</th>
<th>Participants</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 22, 2013</td>
<td>Lab test at Canada Bread Test kitchen (Maple Leaf Foods ThinkFOOD! Centre)</td>
<td>BM-CB, SD-Brandon Guild (CB Co-op student)</td>
<td>-Tested 5 different flavour modulators in RS bagel prototypes (to test bitterness masking ability) -Smoothenol product determined best option</td>
</tr>
<tr>
<td>August 23 to September 5, 2013</td>
<td>Emails</td>
<td>BM-CB, SD-Rivemedee plant manager and staff</td>
<td>-BM-CB ordered ingredients, waiting for regulatory approval at Rivemedee plant -Scheduling meeting at Rivemedee plant to discuss production (set for September 17, 2013)</td>
</tr>
<tr>
<td>September 17, 2013</td>
<td>Meeting at CB frozen bakery, Rivemedee plant</td>
<td>BM-CB, SD-Rivemedee plant manager and staff</td>
<td>-Discussed number of bagels required, how to schedule around ongoing production -Scheduled production for October 5, 2013</td>
</tr>
<tr>
<td>October 3-4, 2013</td>
<td>Emails</td>
<td>BM-CB, SD-Rivemedee plant staff</td>
<td>-Production logistics planned, final details arranged, recipes finalized</td>
</tr>
<tr>
<td>October 5, 2013</td>
<td>Bagel Production Day</td>
<td>BM-CB, SD-AMD, SD-SD</td>
<td>-Approximately 3000 RS and 3000 control bagels produced, each from single batch -Most being stored at Rivemedee, some brought to UG to be sent for nutrition composition analysis</td>
</tr>
<tr>
<td>October 7-21, 2013</td>
<td>Nutrition analysis of Bagels</td>
<td>Maxxam Analytics</td>
<td>-RS Results much lower than expected -Macronutrient distribution results acceptable</td>
</tr>
<tr>
<td>October 26 to November 21, 2013</td>
<td>Emails</td>
<td>BM-CB, BD-JB-CB</td>
<td>-Looking for explanation of difference between actual and expected RS values -Communication with Maxxam representatives, Stacey Dundas (previous researcher), Ingredion representatives, Sanaa Ragae, Ph.D. in department of Food Science, UG -Ingredion to re-test bagel samples – suspect problem is in the method of analysis -Ingredion will use in-house method (modified Englyst) and Maxxam’s method (AOAC 2002.02)</td>
</tr>
<tr>
<td>Date</td>
<td>Activity Description</td>
<td>Responsible Parties</td>
<td>Notes</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>---------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>November 25 – December 11, 2013</td>
<td>Bagel and Hi-maize-260 Analysis of RS – Ingredion, NJ</td>
<td>-CP-I -SD -AMD -SD</td>
<td>-Re-testing RS content of the bagels with in house method and AOAC method -In-house method is consistent with expected results</td>
</tr>
<tr>
<td>December 13, 2013</td>
<td>Phone call &amp; email</td>
<td>-CP-I -SD -AMD -SD</td>
<td>-Discussion of results, determined in-house (modified Englyst) is most appropriate for foods with RS -RS analysis results allow treatment to be reduced to 1 bagel/day (estimated 22-25g RS/day depending on final bagel weight)</td>
</tr>
<tr>
<td>December 13, 2013</td>
<td>Emails</td>
<td>-AMD -BM-CB -RD -SD</td>
<td>-Results explained, request to schedule bagel production -Production date set for January 12, 2014</td>
</tr>
<tr>
<td>January 12, 2014</td>
<td>Emails</td>
<td>-BM-CB -AMD -SD</td>
<td>-Production rescheduled January 18, 2014 due to weather</td>
</tr>
<tr>
<td>January 18, 2014</td>
<td>Final Bagel Production Day</td>
<td>-BM-CB -AMD -SD -RD</td>
<td>-Approximately 3000 RS bagels and 3000 Control bagels produced -Portion of boxes delivered to UG for frozen storage, rest to remain frozen at CB until needed</td>
</tr>
<tr>
<td>January 30, 2014</td>
<td>Bagels shipped to Ingredion for RS analysis</td>
<td>-CP-I -SD -AMD -SD</td>
<td>-Bagel samples shipped to Ingredion (NJ, USA) for analysis by Nutrition R&amp;D and Medallion Labs -Results found to be acceptable -Bagels ready for clinical trial</td>
</tr>
</tbody>
</table>

Abbreviations used: AOAC = Association of Analytical Communities; CB = Canada Bread; RS = Resistant starch; UG = University of Guelph.
Appendix R. Example Flowsheet (Study Day 1 visit only)

<table>
<thead>
<tr>
<th>University of Guelph</th>
<th>Participant ID: _____ TPeriod: I II Treatment: A B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better Bagel Study</td>
<td>Study Day: Scr-1 Scr-2 Scr-3 Orient 1 Satiety 15 29 43 57</td>
</tr>
<tr>
<td>Study Flowsheet</td>
<td>Date: _______________ Researcher: __________________</td>
</tr>
</tbody>
</table>

Study Day 1

1. Greet the potential participant and thank them for coming. Ask them if they have any questions or concerns, any health issues to report or any changes in their medications.

2. Collect fecal sample and complete table if not already done.

<table>
<thead>
<tr>
<th>Participant ID: _____</th>
<th>TP I or II</th>
<th>Study Day (-4, -3, -2, -1, 1)</th>
<th>Date</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Fecal Sample Collected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Participant ID: _____</th>
<th>Day 1 Study Visit</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Complete fasting blood samples and OGTT according to tables on next page.

- Ask participant the following questions:
  - Have they fasted for last 12 hours? YES NO
  - Have they avoided alcohol since last evening? YES NO
  - Have they avoided any unusual physical activity yesterday? YES NO
  - Have they avoided over-the-counter medications for last 24 hours? YES NO
### OGTT Blood Samples Day 1

<table>
<thead>
<tr>
<th>Blood Sample</th>
<th>Target Time</th>
<th>Actual Time</th>
<th>Green Heparin tube</th>
<th>Gold SST tube</th>
<th>Lavender EDTA tube</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>n/a</td>
<td>4 mL</td>
<td>2 x 5 mL</td>
<td>4 mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Consume Trutol Solution (within 5 minutes) Start Time: __________  End Time: __________

<table>
<thead>
<tr>
<th>Time</th>
<th>Amount</th>
<th>Processing Instructions</th>
<th>Notes</th>
</tr>
</thead>
</table>
| 15 min| 4 mL   | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH |       |
| 30 min| 4 mL   | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH |       |
| 60 min| 4 mL   | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH |       |
| 90 min| 4 mL   | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH |       |
| 120 min| 4 mL | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH |       |
| 180 min| 4 mL | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH |       |

**Blood Processing Instructions**

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Amount</th>
<th>Processing Instructions</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Gold SST (insulin) and Gold SST (lipids) | 5 mL       | -mix by gentle inversion 5 times  
- sit tube at room temp for 30 min to clot  
- centrifuge for 15 min, 3000 rpm, 4-8°C  
- from the 4 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of insulin  
- from the 5 mL SST aliquot serum into two 1 mL cryovials and store frozen for analysis of lipids by GGH | * Blood should be collected in to vacutainers in the following order: Gold tubes first, then Green tubes, then lavender tubes |
| Lavender EDTA   | 4 mL       | -mix thoroughly by gentle inversion 8 times  
- place tube in plastic bag with LifeLabs requisition form for analysis of HbA1C  
- store in refrigerator |                                                      |
| Green Heparin   | 4 mL       | -mix thoroughly by gentle inversion 8 times  
- store in fridge 30 minutes then centrifuge for 15 min, 3000 rpm, 4-8 °C  
- aliquot plasma into two 1 mL cryovials  
- store frozen for analysis for glucose | * Spin each green top tube with the gold top from the same timepoint  |
5. During OGTT, review completed pre-study 3-day food record.

6. Provide snack.

7. Provide 2-week supply of bagels and complete table.

**Study Day 1 Bagel Dispense Summary:**

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Date</th>
<th>Study Day</th>
<th>Number Bagels Returned A</th>
<th>Number Bagels Provided A</th>
<th>Notes</th>
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<tbody>
<tr>
<td></td>
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<td>1</td>
<td>N/A</td>
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</table>

8. Remind participant about their upcoming Satiety study visit and the following instructions:
   - Maintain their habitual lifestyle, dietary and exercise habits.
   - Avoid unusual physical activity and alcohol the day before.
   - Consume a usual dinner and record foods consumed the day before.
   - Avoid food (water is OK) after 8:00pm the night before.
   - Avoid food and all beverages (including water) the morning of.

9. Remind the participant to:
   - Maintain their habitual lifestyle, dietary and exercise habits.
   - Consume their study bagels every day and retain the empty bags to return at their Study Day 15 visit.
   - Consume a ‘typical’ dinner the night before their satiety study visit and record the food and beverages consumed in the ‘Pre-Satiety Study Food Record’ and return to the satiety study
   - Complete their daily study diary every day and return to their Study Day 15 visit.
# The Better Bagel Study Diary

**Treatment Period I**  
Study Days 1-7  
Participant ID: __________

_Please return this at your Study Day 15 visit_

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Date</th>
<th>Study Bagel Consumption Details (Time and Preparation details i.e. Toasted? Toppings?)</th>
<th>Notes (sickness, change in medication routine, extra medications, change in exercise, anything else you think is relevant)</th>
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</thead>
<tbody>
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</table>
Appendix T. Sensory Questionnaire (Day 57)

Study Name: Better Bagel Study
REB Number: REB#13MY041

The Better Bagel Study
Bagel Sensory Questionnaire
Study Day 57

The purpose of this questionnaire is to evaluate qualities related to your liking of the study bagels you consumed during the 2 weeks since your last study visit. Please answer each question to the best of your ability by placing a checkmark (✓) under the response that best corresponds to your feelings.

1. Please describe how you consumed your bagels during the last 2 weeks.

_________________________________________________________________
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________

2. **OVERALL**, how much did you like or dislike your study bagels during the last 2 weeks?

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<tr>
<td></td>
<td>Dislike Extremely</td>
<td>Dislike Very Much</td>
<td>Dislike Moderately</td>
<td>Dislike Slightly</td>
<td>Neither Like or Dislike</td>
<td>Like Slightly</td>
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3. How much did you like or dislike the **APPEARANCE** of your study bagels during the last 2 weeks?

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4. How much did you like or dislike the **AROMA** of your study bagels before consuming them during the last 2 weeks?

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5. How much did you like or dislike the **FLAVOUR** of your study bagels during the last 2 weeks?

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6. How much did you like or dislike the **TASTE** of your study bagels during the last 2 weeks?

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7. How much did you like or dislike the **TEXTURE** of your study bagels during the last 2 weeks?

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8. Have you noticed any of the following adverse symptoms associated with consuming your study bagels during the last 2 weeks? (check all that apply)
   - Abdominal bloating, swelling
   - Flatulence (gas)
   - Constipation
   - Diarrhea
   - Stomach pain
   - Nausea
   - Flatulence (gas)
   - Constipation
   - Diarrhea
   - Stomach pain
   - Nausea
   - Belching
   - Vomiting
   - Choking/difficulty swallowing
   - None
   - Other: __________________

9. Please indicate which of the following statements best reflects your feelings on consuming these bagels if they were available after the study. Please circle only one response.

   I would eat this food every opportunity I had
   I would eat this food very often
   I would frequently eat this
   I like this and would eat it now and then
   I would eat this if available but would not go out of my way
   I do not like it but would eat it on an occasion
   I would hardly ever eat this
   I would eat this only if there were no other food choices
   I would eat this only if I were forced to
10. Please feel welcome to use this space to provide any other feedback you may have about what you liked about your study bagels during the last 2 weeks.

______________________________________________________________________

______________________________________________________________________

______________________________________________________________________

______________________________________________________________________

11. Please feel welcome to use this space to provide any other feedback you may have about what you did not like about your study bagels during the last 2 weeks.

______________________________________________________________________

______________________________________________________________________

______________________________________________________________________

______________________________________________________________________
12. Before answering this question (which is below), please review this background information in regards to a function claim. A function claim is a statement found on food packages that claims specific beneficial effects that the consumption of a food or a constituent of a food has on normal functions of the body. A function claim can only appear on a food package if it is approved by Health Canada. Currently, Health Canada is working to advance function claims related to satiety. An example of a function claim related to satiety could be:
“A serving of a resistant starch bagel is more filling than a serving of a non-resistant starch bagel for up to 2 hours”

“A serving a resistant starch bagel gives feelings of fullness longer than a serving of a non-resistant starch bagel”

“A serving of a resistant starch bagel is more filling for longer than a serving non-resistant starch bagel”

“A serving of a resistant starch bagel containing resistant starch helps reduce the desire to eat for up to 4 hours”

**QUESTION:** If a function claim that relates resistant starch to satiety appeared on the study bagels, how likely is it that you would choose these bagels based on the functional claim? Please circle only one response.

- [ ] I would eat this food every opportunity I had
- [ ] I would eat this food very often
- [ ] I would frequently eat this
- [ ] I like this and would eat it now and then
- [ ] I would eat this if available but would not go out of my way
- [ ] I do not like it but would eat it on an occasion
- [ ] I would hardly ever eat this
- [ ] I would eat this only if there were no other food choices
- [ ] I would eat this only if I were forced to

Thank you for completing this sensory questionnaire.