The Acute Effect of Soluble Dietary Fibre-Enriched Pudding Products on Glycemic and Insulinemic Response in Adults at Risk for Type 2 Diabetes

by

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ABSTRACT

The Acute Effect of Soluble Dietary Fibre-Enriched Pudding Products on Glycemic and Insulinemic Response in Adults at Risk for Type 2 Diabetes

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University of Guelph, 2016

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Type 2 Diabetes (T2D) is a chronic loss of the ability to regulate blood glucose, therefore dietary interventions capable of modulating glycemic response are rationalized. A randomized, double-blinded, crossover study was conducted to investigate effects of soluble dietary fibres (DF) (yellow mustard mucilage (YMM), fenugreek gum (FG), flaxseed mucilage (FM)) added to pudding on acute postprandial glycemic response in adults (n=15) at risk for T2D. Results showed significant time by treatment interactions for glucose and insulin, with the soluble DF puddings being lower than the control at some time points. Peak glucose and insulin were also significantly lower with all soluble DF puddings compared to control, although 2-hour incremental area under the curves did not differ. Therefore, YMM, FG and FM have the ability to alter glycemic response in individuals at risk for T2D, suggesting their potential as dietary strategies for individuals who could benefit from alterations in glycemic response.
ACKNOWLEDGEMENTS

I am so thankful for the opportunities I have had while working on my Master’s, while in the department of Human Health and Nutritional Sciences. Everyone in the department, and those involved with the Human Nutraceutical Research Unit (HNRU), have been so instrumental as mentors in guiding who I am as a researcher and a professional, and have really allowed me to grow. There are many people who have supported the research throughout the entire process, and I will be forever grateful to them.

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TABLE OF CONTENTS

ABSTRACT .................................................................................................................................................. ii

ACKNOWLEDGEMENTS ........................................................................................................................... iii

LIST OF TABLES .......................................................................................................................................... viii

LIST OF FIGURES ....................................................................................................................................... ix

LIST OF APPENDICES ............................................................................................................................... x

LIST OF ABBREVIATIONS ........................................................................................................................... xi

INTRODUCTION .......................................................................................................................................... 1
  1. Type 2 Diabetes ...................................................................................................................................... 1
      a. Prediabetes and Type 2 Diabetes: a Concern Worldwide and in Canada ..................................... 1
      b. Screening for Type 2 Diabetes .......................................................................................................... 2
      c. Type 2 Diabetes Disease Development and Symptoms ................................................................. 4
      d. Risk Factors for Type 2 Diabetes ...................................................................................................... 7
  2. Dietary Fibre ......................................................................................................................................... 10
      a. Dietary Fibre and its Physiochemical Properties ........................................................................... 10
      b. Dietary Fibre Canadian Recommendations .................................................................................... 11
  3. Type 2 Diabetes and Dietary Fibre ........................................................................................................ 12
      a. Type 2 Diabetes and Dietary Fibre – An Overview ...................................................................... 12
      b. Mucilage Gum Hydrocolloids – A Type of Soluble Fibre ............................................................. 14
      c. Yellow Mustard Seed and Yellow Mustard Mucilage ................................................................... 15
      d. The Role of Yellow Mustard Mucilage in Modulating Glycemic Response ............................... 16
      e. Fenugreek and Fenugreek Gum ....................................................................................................... 18
      f. The Role of Fenugreek Seeds and Fenugreek Gum in Modulating Glycemic Response .............. 18
         f.i. Fenugreek Seeds Acute Human Intervention Studies ................................................................. 19
         f.i.i. Fenugreek Gum Acute Human Intervention Studies ............................................................... 31
      g. Flaxseed and Flaxseed Mucilage ...................................................................................................... 33
      h. The Role of Flaxseed and Flaxseed Mucilage in Improving Glycemic Response ..................... 34
         h.i. Flaxseed Acute Human Intervention Studies ............................................................................... 35
         h.i.i. Flaxseed Mucilage Acute Human Intervention Studies .......................................................... 36

RESEARCH RATIONALE .......................................................................................................................... 40

PURPOSE, OBJECTIVES AND HYPOTHESIS .......................................................................................... 44
1. Research Purpose, Objectives and Hypotheses ................................................................. 44

METHODS.......................................................................................................................... 45

1. Study Design and Approvals ......................................................................................... 45
   a. Study Design ............................................................................................................... 45
   b. Study Approvals ........................................................................................................ 45

2. Participant Sample Size, Recruitment, Screening, and Orientation ............................. 46
   a. Participant Sample Size Determination ..................................................................... 46
   b. Participant Inclusion and Exclusion Criterion ......................................................... 47
   c. Participant Recruitment ............................................................................................ 49
   d. Study Screening Protocol ......................................................................................... 51
   e. Study Orientation ...................................................................................................... 52

3. Study Treatments ........................................................................................................... 52
   a. Pudding Ingredients and Preparation ...................................................................... 54
   b. Glucose Beverage Ingredients and Preparation ...................................................... 58
   c. Pudding Nutrient and Physical Composition .......................................................... 58

4. Treatment Randomization and Blinding ........................................................................ 62

5. Study Protocol and Study Visit Data Collection ........................................................... 63
   a. Study Protocol ............................................................................................................ 63
   b. Study Visit Data Collection ...................................................................................... 64
      b.i. Study Visit Anthropometric Measurements ....................................................... 65
      b.ii. Study Visit Blood Collection and Analysis ....................................................... 65
      b.iii. Pre-Study Visit Food Records ........................................................................ 68

6. Data and Statistical Analysis ........................................................................................ 69

RESULTS ............................................................................................................................ 70

1. Participant Flow ............................................................................................................. 70

2. Participant Characteristics and Baseline Information .................................................. 71
   a. Participant Characteristics ....................................................................................... 71
   b. Baseline Energy, Macronutrient, and Dietary Fibre Intakes ................................... 73
   c. Participants Anthropometric Characteristics During the Study ............................. 74
   d. Baseline Glycemic Response Biomarkers .............................................................. 75

3. Postprandial Glycemic Response Biomarkers ............................................................... 78
   a. Postprandial Blood Glucose ...................................................................................... 78
b. Postprandial Plasma Insulin .................................................................................................................. 81

DISCUSSION ............................................................................................................................................... 85

1. Participant Flow ...................................................................................................................................... 86

2. Participant Characteristics and Baseline Information ........................................................................... 87
   a. Participant Characteristics ................................................................................................................... 87
   b. Baseline Energy, Macronutrient, and Dietary Fibre Intakes .............................................................. 87
   c. Participants Anthropometric Characteristics During the Study ...................................................... 88
   d. Baseline Glycemic Response Biomarkers During the Study and Baseline HOMA-IR Values .......... 88

3. Postprandial Glycemic Response Biomarkers ....................................................................................... 89
   Postprandial Blood Glucose and Plasma Insulin Response ................................................................. 89
   a. Yellow Mustard Mucilage Postprandial Blood Glucose and Plasma Insulin Response ................. 90
   b. Fenugreek Gum Postprandial Blood Glucose Response and Plasma Insulin Response ............... 92
   c. Flaxseed Mucilage Postprandial Blood Glucose and Plasma Insulin Response ........................... 99
   d. Summary of Glycemic and Insulinemic Responses for Yellow Mustard Mucilage, Fenugreek Gum, and Flaxseed Mucilage ...........................................................(106

4. Study Strengths .................................................................................................................................... 107

5. Study Limitations .................................................................................................................................. 111

6. Future Research ...................................................................................................................................... 112

CONCLUSIONS ........................................................................................................................................... 114

REFERENCES .............................................................................................................................................. 116

APPENDICES ............................................................................................................................................ 123
**LIST OF TABLES**

| Table 1: | Canadian Diabetes Association 2013 Clinical Practice Guidelines for the Diagnosis of Prediabetes and Diabetes (9) | 3 |
| Table 2: | Study Inclusion and Exclusion Criterion with Rationale | 48 |
| Table 3: | Effective Recruitment Strategies for Enrolled Study Participants | 50 |
| Table 4: | Extraction Process for the Soluble Dietary Fibres (Yellow Mustard Mucilage, Fenugreek Gum, and Flaxseed Mucilage) from their Seed Products | 53 |
| Table 5: | Ingredient Composition for Pudding Treatments Standardized at a Volume of 500 mL | 57 |
| Table 6: | Nutritional Composition of the Pudding Treatments Standardized at a Volume of 500 mL | 61 |
| Table 7: | Treatment Codes and Corresponding Treatment Products | 63 |
| Table 8: | Summarization of Study Measures | 65 |
| Table 9: | Blood Sample Collection, Processing, and Storage Instructions | 67 |
| Table 10: | Participant Characteristics (n=15) | 73 |
| Table 11: | 3-Day Food Record Average Energy, Macronutrient, and Dietary Fibre Intakes Before Study Period (n=15) | 74 |
| Table 12: | Participant Characteristics During the First and Last Study Visit (Glucose Beverage Treatments), and During the Randomized Pudding Study Visits (n=15) | 75 |
| Table 13: | Fasting Blood Glucose and Fasting Plasma Insulin During the Study (n=15) | 76 |
| Table 14: | Fasting and Postprandial Blood Glucose and Plasma Insulin for the Glucose Beverage Treatment (n=15) | 77 |
| Table 15: | Postprandial Blood Glucose Response (n=15) | 80 |
| Table 16: | Postprandial Plasma Insulin Response | 84 |
LIST OF FIGURES

Figure 1: Study Design Summary ........................................................................................................... 45

Figure 2: Participant Flow Diagram ........................................................................................................ 71

Figure 3: Postprandial Glucose (a), and Insulin Response (b) for Glucose Beverage Treatment (n=15) .................................................................................................................................................. 77

Figure 4: Postprandial Blood Glucose Time Point Curves for Fenugreek Gum (a), Yellow Mustard Mucilage (b), Flaxseed Mucilage (c), and Control (d) Puddings (n=15) .................................................................................................................................................. 79

Figure 5: Postprandial Blood Glucose 2-Hour iAUC (n=15) .......................................................................... 80

Figure 6: Postprandial Blood Glucose Cmax (n=15) ...................................................................................... 81

Figure 7: Postprandial Plasma Insulin Time Point Curves for Fenugreek Gum (a), Yellow Mustard Mucilage (b), Flaxseed Mucilage (c), and Control (d) Puddings (n=15) .................................................................................................................................................. 83

Figure 8: Postprandial Plasma Insulin 2-Hour iAUC (n=15) .......................................................................... 84

Figure 9: Postprandial Plasma Insulin Cmax (n=15) ...................................................................................... 85
LIST OF APPENDICES

Appendix A: Acute Clinical Trials for the Glycemic Effect of Yellow Mustard Mucilage.... 123
Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds.................. 124
Appendix C: Acute Clinical Trials for the Glycemic Effect of Fenugreek Gum............. 131
Appendix D: Acute Clinical Trials for the Glycemic Effect of Flaxseeds....................... 133
Appendix E: Acute Clinical Trials for the Glycemic Effect of Flaxseed Mucilage........... 134
Appendix F: University of Guelph Research Ethics Board Approval.......................... 138
Appendix G: University of Guelph Environmental Health and Safety Biohazard Permit.... 139
Appendix H: The Canadian Diabetes Risk Questionnaire (CANRISK)......................... 140
Appendix I: Food Neophobia Questionnaire................................................................ 142
Appendix J: Three-Factor Eating Questionnaire..................................................... 144
Appendix K: Participant Recruitment Poster......................................................... 149
Appendix L: Screening-1 Questionnaire............................................................... 150
Appendix M: Screening-2 Consent Form............................................................... 152
Appendix N: Screening-2 Questionnaire............................................................... 156
Appendix O: Summary of Pudding Study Handbook............................................. 160
Appendix P: Study Consent Form............................................................................ 162
Appendix Q: Pudding and Glucose Beverage Ingredient Specifications.................... 168
Appendix R: Three-Day Food Record Instructions and Template.............................. 182
Appendix S: Blood Glucose and Plasma Insulin Compared Among Time Points Within Glucose Beverage Treatments............................................................... 188
Appendix T: Blood Glucose Compared Among Time Points Within Each Pudding Treatment........................................................................................................ 189
Appendix U: Plasma Insulin Compared Among Time Points Within Each Pudding Treatment........................................................................................................ 190
LIST OF ABBREVIATIONS

% = Percent
2hPG = 2-hour plasma glucose
AI = Adequate Intake
ANOVA = Repeated measures analysis of variance
AUC = Area under the curve
BMI = Body mass index
Blood glucose = Whole blood glucose (for this thesis’ data)
bpm = Beats per minute
BWb = Barley and white bread test meal (Ref 50)
BWFb-2.5 = Barley, wheat, and fenugreek bread test meal containing 2.5% fenugreek seed flour (Ref 50)
BWFb-5 = Barley, wheat, and fenugreek bread test meal containing 5% fenugreek seed flour (Ref 50)
BWFGb = Barley, wheat, fenugreek, and ginger bread test meal containing 1% fenugreek seed flour and 1% ginger flour (Ref 50)
CANRISK = Canadian Diabetes Risk Assessment Questionnaire
CDA = Canadian Diabetes Association
Cmax = Peak concentration
CVD = Cardiovascular disease
DF = Dietary fibre(s)
DNA = Deoxyribonucleic acid
DRI = Dietary Reference Intakes
EFSA = European Food Safety Authority
ELISA = Enzyme-linked immunosorbent assay
FBG = Fasting blood glucose (in appendices)
FDA = Food and Drug Administration
FG = Fenugreek gum
FG0 = Control group containing 0 g fenugreek seeds (Ref 47)
FG2.5 = Test group containing 2.5 g fenugreek seeds (Ref 47)
FG5 = Test group containing 5 g fenugreek seeds (Ref 47)
FM = Flaxseed mucilage
FPG = Fasting plasma glucose
GI = Glycemic Index
GL = Glycemic Load
GMPs = Good manufacturing practices
HbA1C = Glycated hemoglobin
HM = High mucilage (in appendices)
HMCS = High maltose corn syrup
HNRU = Human Nutraceutical Research Unit
HOMA-IR = Fasting insulin resistance
HPMC = Hydroxypropyl methylcellulose
iAUC = Incremental area under the curve
IAUC = Integrated area under the curve
IBD = Irritable bowel disorder
IF = Insoluble fibre (in appendices)
IFG = Impaired fasting glucose
II = Insulinemic index
IGT = Impaired glucose tolerance
ISO = International Organization Standardization
K₂EDTA = Potassium ethylene diamine tetraacetic acid
Kcal = Kilocalories (in appendices)
Kg = Kilogram
LM = Low mucilage (in appendices)
MTS = Modified tapioca starch
NHPs = Natural health products
NIDDM = Non-insulin dependent diabetes mellitus
NIH = National Institute of Health
NSP = Non-starch polysaccharides
OGTT = Oral glucose tolerance test
OMAFRA = Ontario Ministry of Agriculture and Food (in appendices)
PG = Random plasma glucose
RCF = Relative centrifugal force
REB = University of Guelph Human Research Ethics Board
rpm = Revolutions per minute
SAT = Subcutaneous adipose tissue
SD = Standard deviation
SE = Standard error
SF = Soluble fibre (in appendices)
SI = Small intestine
SIN = Social Insurance Number (in appendices)
SOP = Standard Operating Procedure
T1D = Type 1 diabetes
T2D = Type 2 diabetes
TCPS 2 = Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans
TF = Total fibre (in appendices)
TFEQ-CR = Three Factor Eating Questionnaire – Cognitive Restraint
Tmax = Time of peak concentration
UC = Ulcerative colitis
USA = United States of America
VAT = Visceral adipose tissue
WHC = Water-holding capacity
WWb = White bread control (Ref 50)
YM = Yellow mustard
YMB = Yellow mustard bran
YMM = Yellow mustard mucilage
INTRODUCTION

1. Type 2 Diabetes

   a. Prediabetes and Type 2 Diabetes: a Concern Worldwide and in Canada

   Diabetes is a chronic, non-communicable disease, where the human body loses the ability to effectively regulate blood glucose. There are several forms of the disease, however, the most common are Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D) (1-2). T1D is the form of diabetes where the β-cells of the pancreas, which are the cells of the body which produce and release insulin to the body for regulating blood glucose, fail to produce enough insulin due to cell damage or death (2). This in turn leads to improper blood glucose control. T1D typically develops in childhood or adolescence, however, it can also develop during adulthood (2). T2D is the form of diabetes in which the body cannot effectively use insulin produced, or cannot produce enough insulin, which in both cases leads to improper blood glucose control (2). T2D typically develops during adulthood, however it can also develop in childhood and adolescence (2). Prediabetes describes the condition when blood glucose levels are higher than normal, but have not reached a level high enough to classify the individual as having diabetes (1).

   As of 2012, diabetes was the fourth leading cause of mortality worldwide, responsible for 1.5 million fatalities (3). By 2014, the number of fatalities had increased to 4.9 million (4). Also, as of 2014, the number of individuals suffering from diabetes worldwide was 387 million, with the number of cases expected to increase to over 592 million by 2035 (3). It is estimated that one in four Canadians currently live with prediabetes or diabetes, and as of 2020, it is estimated that one in three individuals will be affected (6). It is also estimated that 50 % of the individuals with prediabetes will go on to develop T2D later in life, and, of the 2 million individuals diagnosed with diabetes in Canada, about 5 to 10 % have T1D, and 90 % have T2D.
(1-2). In 2015, the cost of health care for diabetes will be 14 billion Canadian dollars and this number is expected to rise to 16 billion Canadian dollars annually by 2020 (6).

Therefore, it is important to identify strategies to reduce the impact of diabetes for both individuals in the pre-stages of developing the disease, those suffering from the disease, and the Canadian health care system. As the rate of developing T2D from a prediabetes diagnosis is high, and the most prominent form of the disease is T2D, it is important to focus on disease detection, risk, and strategies aimed at improving disease prevention and severity (1-2, 6).

b. Screening for Type 2 Diabetes

Disease risk and development of T2D is multifactorial and complex for each individual, therefore, it is important to have proper screening techniques in place to allow for as early as possible identification of disease risk factors to minimize disease progression. It is currently estimated that greater than 2.8 % of adults have undiagnosed T2D, and this number can increase to greater than 10 % in some at risk populations (7). Therefore, the Canadian Diabetes Association (CDA) 2013 Clinical Practice Guidelines recommends physician screen for T2D every 3 years in adults over the age of 40 years, or in individuals who are deemed to be at a high risk for developing T2D, determined by the use of a validated risk scoring system, such as the Canadian Diabetes Risk Assessment (CANRISK) Questionnaire (7). The CANRISK questionnaire was developed by Health Canada to allow individuals to determine their risk for prediabetes or T2D (8). The questionnaire asks questions related to gender, age, height, body weight, waist circumference, physical activity, dietary lifestyle, blood pressure, blood sugar, child birth weight for females who have given birth, family history of diabetes, ethnicity, and education level (8). Each question assigns points for the specific answers provided, and, overall the questionnaire classifies individuals with a score of ≤20 at a low risk, a score of ≥21 but ≤32
at a moderate risk, and a score of ≥33 at a high risk, of developing prediabetes or T2D (8). It is also suggested that more frequent, and earlier in life, screenings be done for individuals at very high risk for developing the disease (7). The CDA recommends that screenings should take into account a variety of measurements including: fasting plasma glucose (FPG), glycated hemoglobin (HbA1C), random plasma glucose (PG) testing, and 2-hour plasma glucose (2hPG) after an oral glucose tolerance test (OGTT), using a beverage containing 75 g available carbohydrate (7). Screenings every 3 years for individuals over 40 years, or high risk individuals, should involve FPG and/or HbA1C measurements, however, in individuals that are at very high risk for developing T2D, it is recommended that FPG and/or HbA1C or 2hPG after an OGTT using 75 g available carbohydrate be measured (7). If individuals have a FPG and/or HbA1C value between the normal and prediabetes ranges, and have at least one risk factor for T2D, it is recommended that they also have a 2hPG test using a 75 g glucose OGTT (7).

Summarized in Table 1 are the CDA’s recognized values for FPG, HbA1C, random PG testing, and 2hPG after a 75 g OGTT, for adult individuals with prediabetes and T2D.

Table 1: Canadian Diabetes Association 2013 Clinical Practice Guidelines for the Diagnosis of Prediabetes and Diabetes (9).

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Normal</th>
<th>Prediabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (FPG) (mmol/L)</td>
<td>&lt;5.6</td>
<td>6.1-6.9</td>
<td>≥7.0</td>
</tr>
<tr>
<td>After no caloric intake for at least 8 hours</td>
<td></td>
<td>Impaired Fasting Glucose (IFG)</td>
<td></td>
</tr>
<tr>
<td>2-Hour Plasma Glucose (2hPG) after a 75 g Oral Glucose Tolerance Test (OGTT) (mmol/L)</td>
<td>-</td>
<td>7.8-11.0</td>
<td>≥11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impaired Glucose Tolerance (IGT)</td>
<td></td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>&lt;5.5</td>
<td>6.0-6.4</td>
<td>≥6.5</td>
</tr>
<tr>
<td>Random Plasma Glucose (PG) (mmol/L)</td>
<td>-</td>
<td>-</td>
<td>≥11.1</td>
</tr>
</tbody>
</table>
c. Type 2 Diabetes Disease Development and Symptoms

T2D development is highly multifactorial and complex, but is first stimulated by the inability to maintain a chronic nutrition surplus of macronutrients, primarily carbohydrates and fats (10). In normal nutrient homeostasis, during the fasted state, blood glucose is maintained through increased production and secretion of glucagon from pancreas α cells, and suppressed through increased production and secretion of insulin from pancreas β cells (10). The increased levels of glucagon cause increased endogenous glucose production by the liver, which results in a maintenance of blood glucose, which is transported to insulin-independent tissues, such as the brain (10).

In the fed state, blood glucose levels rise, and neuro-hormonal processes are activated, which causes an increase in insulin production and secretion from β cells, and a reduction of glucagon production and secretion from α cells (10). This results in decreased endogenous glucose production by glycogen breakdown in the liver, decreased adipose tissue lipolysis, and insulin-dependent tissues such as the heart, skeletal muscle, and adipose tissue, are stimulated to absorb glucose for use as energy, or for anabolic metabolism (10). Normally, β cells are able to adapt to the insulin needs of insulin-dependent tissues, and if tissues are less sensitive to insulin, the β cells respond by increasing production and secretion of insulin, or, if tissues are more sensitive to circulating insulin, there is a decrease in β cell insulin production and secretion (11).

Many complex mechanisms are involved in the dysregulation of proper nutrient homeostasis, which then leads to the development of T2D (10). Such possible mechanisms for glucose homeostasis dysregulation include dysfunctions in neuro-hormonal energy balance, and weight controls (10). Hormonal mechanisms that control hunger, satiety, appetite, physical activity, and body weight are all implicated in playing a role in the development of obesity when
imbalances occur, which leads to the progression of T2D (10). The chronic nutrition surplus (such as in overweight and obese states), leads to excesses of glucose and fatty acids in the blood and tissues (10-11). To avoid glucose toxicity, insulin-dependent tissues to become less responsive to circulating insulin, which leads to pancreas β cells producing and secreting more insulin to compensate in maintaining regulated blood glucose levels (10-11). Excess intake of fatty acids leads to an excess of adipose tissue stores (10-11). Blood glucose during fasting, and 2 hours after a meal, will slowly increase overtime, and stay elevated, due to the small changes in insulin-dependent tissue resistance to circulating insulin, and excess nutrients will be converted, and stored as adipose tissue (11). Although large changes in blood glucose are slow, pancreas β cells are susceptible to nutrient-induced damage, which overtime leads to a decrease in β cell function and insulin regulation, and progression into further β cell damage once there is pronounced hyperglycemia, and glucotoxic and glucolipotoxic mechanisms develop (11). In individuals susceptible to T2D, visceral adipose tissue (VAT) tissue becomes an increasing concern, as excess nutrients are stored as adipose tissue in visceral tissues, which causes adipose tissue hormonal dysregulation, inflammation, increased secretion of inflammatory cytokines, and increases in circulating non-esterified fatty acids (10-11). Chronic glucose toxicity, and lipotoxicity, causes irreversible damage overtime to both pancreas α and β cells, leading to impaired glucagon and insulin production and secretion, and to hepatic liver cells, as control of extrahepatic glucose production is impaired, and non-alcoholic fatty liver disease develops due to increases in VAT stores (10-11).

As T2D is a disease with progressive loss of blood glucose control, metabolic short term, and long term, complications arise as hyperglycemia progresses. Short term hyperglycemia, or hypoglycemia (from beginning treatments), can bring about increases in infection, slowed wound
healing, and diabetic ketoacidosis, due to increased production of ketones from adipose lipolysis, and due to insufficient uptake of glucose by insulin-dependent tissues for energy (12-13). As T2D develops, increases in loss of consciousness, confusion, and falls, can also occur (12). Long term complications of T2D, due to hyperglycemia, hypertension, and dyslipidemia, often result due to damage of both large (macrovascular) and small (microvascular) blood vessels, which goes on to disrupt and damage body tissue function (12). Body systems most affected by long term macrovascular and microvascular blood vessel damage from T2D include the cardiovascular system – resulting in increased risk of cardiovascular disease (CVD), the eyes – resulting in increased risk for diabetic retinopathy, cataracts, and glaucoma, the kidneys – with increased risk of nephropathy, kidney failure, and end-stage renal disease, the nervous system – with increased risk of nerve damage, causing increases in pain, tingling, and numbness, or delays in gastric emptying, the oral cavity – with increased risk of gingivitis and periodontitis, and the liver – leading to increased risk of non-alcoholic fatty liver disease (10, 12). As T2D affects many body systems, quality of life for individuals suffering from the disease is often impacted, as well as increased risk in mortality due to complications in body systems resulting from T2D organ damage (12).

Since T2D is a progressive loss of glycemic control, it is important to identify disease development as soon as possible to determine effective treatment strategies that can be implemented to reduce complications and the burden of the disease on individuals. Therefore, determining a person’s risk for developing T2D is important in understanding the chances of developing the disease, and being able to identify symptoms before disease detrimental disease progression occurs.
d. Risk Factors for Type 2 Diabetes

As T2D is the most common form of diabetes, it is important to understand the risk factors associated with its development to identify strategies for its prevention, and minimization of symptom development. There are many risk factors for the pathology of T2D, which include non-modifiable, and modifiable constituents. Non-modifiable risk factors include age, sex, ethnicity, family history, hereditability, history of gestational diabetes, genetics, and intrauterine environment (5, 10-11, 14-16). In general, T2D affects individuals as they increase in age (10). In developing countries, T2D affects individuals between the ages of 40 to 60 years most commonly, whereas in developed countries, T2D tends to develop in individuals after 60 years (10). However, although T2D was rarely seen in young individuals before the 1990s, the number of children and adolescence with T2D in some countries has surpassed those with T1D; the form of diabetes most commonly found in younger individuals (10). Another risk factor for developing diabetes is sex, in which males are at an increased risk of developing diabetes compared to females (5, 15). As of 2014, in Canada alone, 56 % of the population with diabetes were male, compared to only 44 % being female (5). Ethnicity is also an important risk factor for the predisposition of developing T2D (16). Certain ethnic minorities, including Indians, Native Americans, Africans, Hispanics, Latinos, and Asians, all have an increased prevalence for the development of T2D (16). High risk ethnic groups have a higher incidence rate for T2D in youths compared to low risk ethnic groups (15). Family history of diabetes is also related to a 2.4-fold increase in the risk of developing T2D (11). It is estimated that 15 to 25 % of first-degree relatives of individuals with T2D will go on to develop prediabetes or diabetes (11). Lifetime risk, by the age of 80 years, for T2D is estimated to be 38 % if one parent had the disease, or 60 % if both parents had T2D, by the age of 60 years (11). T2D heritability is
estimated to be greater than 50 %, with concordance rates for diabetes being between 38 to 58 % for monozygotic twins over the age of 60 years, which increases to 88 % if IGT occurs, and concordance rates between 17 to 20 % for dizygotic twins (10-11). Also, during fetal development, different in utero conditions can cause heritable alterations to gene expression which can affect how deoxyribonucleic acid (DNA) is methylated, and how DNA histones are modified (15). Fetal undernutrition, or over-nutrition, leading to very low, or very high, birth weights have also been attributed to increased risk of developing T2D (15). Furthermore, it is suggested that individuals whose mothers had gestational diabetes, or T1D, during pregnancy are at an increased risk of developing T2D at an earlier age, compared to individuals whose mothers were normoglycemic during pregnancy (10, 15). Gestational diabetes also increases a woman’s chance of later developing T2D by 7-fold compared to women who had a normoglycemic pregnancy (15). Finally, genetic advances in understanding T2D risk have linked over 65 gene loci to the disease, and over 50 loci to glycemic regulation, in genome-wide association studies (14).

Modifiable risk factors of T2D include environment, metabolic, and lifestyle factors (10, 15). Environmental factors such as synthetic organic pollutants, such as pesticides, and air pollution, have also been linked to the development of T2D due to their effects on endocrine cells (10, 15). Also, metabolic factors including previously identified glucose tolerance, abnormal lipid levels, including elevated levels of triglycerides and cholesterol, and low levels of high-density lipoprotein, hypertension, and inflammation, have all been implicated with an increased risk of the development of T2D (15). Finally, lifestyle factors play an integral part in the development of T2D (10, 15). Illnesses, such as sleeping disorders, and depression, leading to antidepressant medication use, and lifestyle factors, such as tobacco use, gut microbiota
changes (in early life, and due antibiotic use), and low socioeconomic status, have all been linked to an increased risk of T2D (7, 10, 15). Further, a Westernized lifestyle including a high-energy diet, along with low physical activity levels, has been strongly linked to both the rise in obesity, and T2D (10, 15). Obesity is a major predictor of T2D, leading to an increased lifetime risk of T2D in younger adults (25 to 40 years) due to a chronic nutrient surplus (10, 15). In relation to obesity, the distribution of adipose tissue is also a strong predictor of T2D risk (10, 15). VAT, as opposed to subcutaneous adipose tissue (SAT) stores, is an independent risk factor for insulin resistance and T2D, as expansion of adipose tissue into the visceral tissues such as the heart, skeletal muscle, liver, and pancreas cells, causes nutrient-induced damage to organs (10, 15).

Of the risk factors associated with T2D, it is important to understand which are modifiable, and which are non-modifiable. Dietary factors are modifiable risk factor towards improving obesity rates, reducing adipose tissue stores, and decreasing chronic overnutrition, therefore it is important to determine which aspects of nutrition are able to aid in the prevention of both obesity, and T2D development. If the progression of T2D is caught in prediabetes stages, effective management strategies such as lifestyle interventions to diet, and physical activity, can lower the risk of T2D development by 28 to 59 % (10). Also, in several other long term prevention studies, lifestyle changes, which included dietary changes such as changes to a low calorie, low fat, and high fibre diet, and at least 150 minutes of physical activity per week, aided in overall weight loss and a decreased risk of developing T2D by 58 % after 4 years (17). Particularly, increased consumption of foods rich in fibre, such as grain products using the whole grain, and low in refined dietary carbohydrates, have been linked to reduced risk and development of T2D (18-19). If effective management strategies can be employed, the
development, and burden, of T2D on individuals can be reduced, ultimately improving quality of life for individuals, and reducing stress on the Canadian healthcare system.

2. Dietary Fibre

   a. Dietary Fibre and its Physiochemical Properties

   Dietary fibre (DF) is an edible compound found in plant or algal material, which cannot be digested in the small intestine, but has the potential to be fermented by bacteria in the colon (20-21). The term DF encompasses several groups of carbohydrates, including non-starch polysaccharides (NSP), non-digestible carbohydrates, modified cellulosics, and synthesized carbohydrate polymers (20).

   The human physiological effects of DF depend upon the different physiochemical properties of each fibre type (20). The different physiochemical properties DF may possess are solubility in water, water-holding capacity (WHC), viscosity and gel formation, binding ability, bulking ability, and fermentability (20). DF can be classified into two broad groupings for water solubility properties, which are insoluble DF, which are fibres unable to solubilize in water, and soluble DF, which are fibres able to solubilize in water (21). Also, each fibre has a specific WHC, which is the amount of water retained by a set dry weight of DF in set conditions of temperature, soaking time, and duration and speed of centrifugation (20). WHC can also be altered by other factors such as gastrointestinal pH, fibre particle size, and the degree of food processing (22). In general, soluble DF have a higher degree of WHC compared to insoluble DF (22). DF, specifically soluble DF, are also able to provide viscosity (thickness), and gel-forming capacity, in solutions and products (20). In the gastrointestinal system, soluble DF can increase viscosity of the digested food contents to create gels, which provides more solidity to the digested food mass, lending to delayed gastric emptying from the stomach to the small intestines.
DF also has binding ability in the gastrointestinal system of bile acids excreted from the gallbladder into the SI (20). Soluble DF are the most capable of binding activity, as their gel matrix traps bile acids more readily, and therefore more bile acids are excreted in the feces (20). Some DF are also able to exert bulking ability of fecal material in the colon. Insoluble DF are the most capable of exerting fecal bulking ability in the colon, as they are minimally fermented by colonic bacteria, and therefore increase fecal material with particle formation and WHC (20-21). Finally, some DF are able to be fermented by microflora in the colon, however this process is highly variable between different fibre types (20-21). In general, soluble DF are more readily fermented in the colon compared to insoluble DF (20). Overall, all DF exert their physiological effects on the human body differently, depending on their physiochemical properties. Soluble DF are predominantly more capable of solubilizing in water, therefore typically have a higher WHC, which leads to a higher viscosity, and gel-forming capacity, a greater binding ability of nutrients and bile acids, and greater fermentability in the colon (20-22). Insoluble DF are less soluble in water, and therefore typically have a lower WHC compared to soluble DF, but are predominantly more capable of providing fecal bulking in the colon (20).

b. Dietary Fibre Canadian Recommendations

In Canada, DF is one of the 13 core nutrients that must be included on the Nutrition Facts Table on labelled food products (23), and has an Adequate Intake (AI) value as a part of the Dietary Reference Intakes (DRI) guidelines (24). AI values are “recommended average daily nutrient intake levels based on observed or experimentally determined approximations or estimates of nutrient intake,” and are values determined for healthy individuals with adequate nutritional intakes (24). The AI values for total fibre are based on consuming 14 g of fibre per
1000 kilocalories (24). Therefore, the AI for males 19 to 50 years is 38 g per day, and the AI for males 51 years or greater is 30 g per day (24). Also, the AI for women 19 to 50 years is 25 g per day, and the AI for women 51 years or greater is 21 g per day (24).

3. Type 2 Diabetes and Dietary Fibre
   a. Type 2 Diabetes and Dietary Fibre – An Overview

Lifestyle changes have been suggested to improve the risk, and delay the development, of T2D for adults, which is key to slowing disease prevalence. Specifically, there is much discussion about the role dietary fibre in the diet, and its effects on the incidence and severity of T2D in adults (18-19). Low fibre diets have been linked to an increased risk of T2D (19), whereas several meta-analyses and cohort studies have shown that increased consumption of DF promotes a reduction in T2D prevalence and development (18-19, 25-27). Some DF types have been shown to decrease the glycemic index (GI) of food products. The GI is a number given to a carbohydrate rich food product relating to the glycemic response, and the glycemic load (GL), which relates to the GI and a specific amount of carbohydrates in a given food product (25-26). Consumption of foods low on the GI, with a low GL have therefore been associated to a decreased risk of T2D, as high GI and GL foods have been associated with an increased risk of T2D (25-26).

Specifically, in epidemiological studies, the highest quartiles of whole grain and cereal fibre consumption, which are predominantly comprised of insoluble DF, have been linked to a decreased risk for T2D (28-29), however, in meta-analyses of clinical trials, both insoluble DF and soluble DF have shown improvements to HbA1C and FBG, with soluble DF potentially playing the greatest role in decreasing glycemic levels due to their increased viscosity characteristics, delayed gastric emptying ability, and fermentation ability (18, 27, 29-30).
Clinical trials of common fibre supplements, mostly comprised of soluble DF, have also shown improvements in glycemic and insulin responses in most cases studied (29-30).

Health claims related to the incorporation of DF into food products for decreasing the rate of diabetes, and improving glucose regulation, have started to emerge. For example, in Europe, the European Food Safety Authority (EFSA) has approved health claims for consumption of certain DF in decreasing the rise of blood glucose after a consumed meal (31). The approved DF types include arabinoxylan produced from wheat endosperm, β-glucans from oats and barley, hydroxypropyl methylcellulose (HPMC), pectins, and resistant starch, which are all soluble DF, or exhibit soluble DF physiochemical properties (31-32). In the United States of America (USA), the Food and Drug Administration (FDA) have allowed a Qualified Health Claim for psyllium husk in reducing the risk of T2D; however, they have concluded that there is still not enough scientific evidence for this claim (33). In Canada, there are currently no approved food health claims relating to the disease risk reduction, or function, of DF on improving diabetes risk or glycemic response (34).

However, in Canada, the CDA 2013 Clinical Practice Guidelines have identified the incorporation of DF into the diet for maintenance, and improvements, of T2D (35). The CDA recognizes that there is evidence to suggest that soluble DF improves postprandial blood glucose due to their role in delaying gastric emptying, and decreasing absorption of glucose in the SI (35). The CDA also mentions that high carbohydrate/high fibre diets decrease HbA1C, and the need for diabetic medications (35). However, the CDA also mentions that the disadvantages to high fibre diets may be gastrointestinal side effects (35). Another dietary strategy the CDA recommends relating to DF is the consumption of high carbohydrate/low GI diets (35). The data the CDA has presented suggests that a high carbohydrate/low GI diet decreases HbA1C,
hypoglycemia, and the need for diabetic medications (35). As the CDA acknowledges the beneficial effects DF has on diabetes, the recommendations for daily intake of DF for individuals with diabetes is higher than the DRI AI recommendations (35). Instead of consuming 14 g of total fibre per 1000 kilocalories per day, the CDA recommends consuming 15 to 25 g of DF per 1000 kilocalories per day, which equates to between 25 to 50 g of total fibre per day (35).

Overall, there is evidence to support the incorporation of DF into the diet as an effective lifestyle change towards reducing the prevalence, and development, of T2D. Both insoluble DF and soluble DF have been suggested to have a role in altering T2D physiological mechanisms; however, there has been more clinical evidence supporting the role of soluble DF in improving T2D disease mechanisms. Specific types of soluble DF have health claims in both Europe and the USA, and although there are no DF health claims relating to diabetes in Canada, the CDA has recognized that there is evidence to support soluble DF in regulating blood glucose. The CDA also recommends increasing DF consumption above the AI levels for individuals with diabetes to improve diabetic metabolic markers of the disease. Therefore, DF is an important dietary lifestyle modification to incorporate into the changes made in reducing the risk, and development, of T2D, and specifically, soluble DF may play an important role in controlling T2D.

b. Mucilage Gum Hydrocolloids – A Type of Soluble Fibre

Hydrocolloids are a type of soluble DF which are comprised of long chain polymers and polysaccharides, which all have the property of forming viscous dispersions and/or gels when in water, which is a property which may impact glycemic response (20, 32). For example, the DF types approved by EFSA in reducing glycemic response (i.e. arabinoxylan, β-glucans, HPMC, pectins, and resistant starch), are all hydrocolloid soluble DF, or exhibit hydrocolloid soluble DF
physiochemical properties (31-32). Hydrocolloids are hydrophilic, as they have a high affinity for binding water molecules due to their high number of hydroxyl groups, and they exhibit properties of colloids, as they produce dispersions when in water, which is an “intermediate between a true solution and a suspension” (32). Hydrocolloids are found in plant secretions, seeds, and seaweed extracts (20, 32).

Mucilage gums are a type of hydrocolloid, which are often derived from the outer layer of seeds, or plant soft stems (20, 32). Specific examples of mucilage gums include guar gum, locust bean gum, yellow mustard mucilage (YMM), psyllium gum, flaxseed mucilage (FM), and fenugreek gum (FG) (32). Mucilage gums tend to be very viscous in water and are able to form gels when in solutions (20, 32). Mucilage gums also have a high WHC, and are able to thicken, stabilize, and emulsify foods when added at low quantities (20, 32). Therefore, the use of mucilage gums in the food products is increasing in popularity due to their functional properties, and hydrocolloids are often used in food products such as soups, gravies, sauces, jams, and gelled deserts to create a desired viscosity, mouth feel, and texture (32).

c. Yellow Mustard Seed and Yellow Mustard Mucilage

Mustard seed is a Canadian crop which is produced in cool seasons, and has a relatively short growing time (36). The mustard seed crop is tolerant towards drought, heat, and frost, climate changes (36), making it a good fit for the Canadian climate. The Canadian agricultural industry is forecasted to harvest 129 kilo hectares of mustard seed crop in the 2015 to 2016 season, which is predicted to yield 0.99 tonnes of mustard seed per hectare of land, and produce between 685 to 715 Canadian dollars per tonne of mustard seed (37). The Canadian agricultural industry produces three types of mustard seed, being yellow, brown, and oriental, however, the
yellow mustard (YM) seeds, and its extracts, are the mildest for flavour, and are what is most commonly used for the condiment, and food industries (36).

YMM is a mucilage gum which can be extracted from both whole mustard seeds, and from yellow mustard bran (YMB), the outer husk of the seed (32, 38). The mucilage contains both neutral polysaccharides, such as glucose, and acidic polysaccharides, such as galacturonic and glucuronic acids, galactose, and rhamnose residues (32). YMM is able to form both viscous solutions and weak gels, but its ability to form solutions is highly dependent on the concentration of each polymer (32). YMM also has functional properties, such as a strong surface activity, and the ability to stabilize oil in water emulsions (39). Therefore, YMM is often used as a stabilizer, and bulking agent, for processed meats, and salad dressings (32, 39).

d. The Role of Yellow Mustard Mucilage in Modulating Glycemic Response

(Summarized in Appendix A)

YMM is a type of hydrocolloid soluble DF which may have an effect on improving the glycemic response in individuals, due to its high viscosity and weak gel-like properties, which may slow down absorption of glucose, delay gastric emptying of the stomach into the SI, and delay insulin secretion by pancreatic ß cells, like other soluble DF. Although YMM possesses similar attributes to other soluble DF which have been shown to positively influence glycemic response, the human clinical trial literature for YMM is lacking.

To date, only one study has looked at the role of YMM, specifically YMB, on the improvement of glycemic response (38). Lett et al. (2013) conducted an acute, randomized, crossover, intervention with potato and leek soup products, with or without (control), 5 g of YMB (containing approximately 1.5 g YMM), with equivalent amounts of carbohydrate (25 g/100 g of soup) (38). In this study, 10 healthy, young adults randomly consumed both the test
and control soups once, and provided a fasting blood sample, consumed the study visit soup within 15 minutes along with 200 mL of water, along with another 200 mL of water during the remaining 2 hours of the study visit, and provided blood samples over the next 2 hours, for the determination of blood glucose and glucose incremental area under the curve (iAUC). Overall, blood glucose concentrations were significantly different between the two soups, with the 15, 30 and 90 minute values, with more of a decrease in blood glucose with the YMB soup. The average time for blood glucose to peak, and peak blood glucose concentrations with the YMB soup compared to the control soup were decreased as well, however glucose iAUC did not significantly differ between the YMB and control soups.

Although the results from the one study using YMB did not show significant changes to glucose iAUC in healthy adults, the mean blood glucose concentrations at 15, 30, and 90 minutes were decreased, the time for glucose to peak was delayed, and the peak blood glucose concentrations were reduced, with the addition of 5 g of YMB in soup compared to a control soup matched for available carbohydrates (38). This was the first study in human participants to look at the incorporation of YMB into a food product for its role in improving glycemic response, which yielded some positive results to attenuation of glycemic response. However, the attenuation of glycemic response in this study may have also been attributed to the high protein, oil, and other DF components of the YMB, so although the YMB contained approximately 1.5 g of YMM, further clinical trials should be conducted to evaluate if the soluble DF portion of the YMB and YM seeds can attenuate glycemic response in the same manner when isolated. This study also did not look at insulin to make further conclusions about YMB role in attenuating the insulinemic response, therefore, further studies should incorporate glucose and insulin measurements to view the whole scope of the attenuation of glycemic response with YMM.
e. **Fenugreek and Fenugreek Gum**

Fenugreek is a legume with small-seeds, which is mainly grown in India, Ethiopia, Egypt, and Turkey, for its seeds, although it has slowly started to be grown in Canada as a forage crop (40). In 2009, a Saskatchewan based company, Emerald Seeds Products Limited, invested in Fenugreek crops in hopes of increasing the research conducted on the crop to generate health claims related to “defence against diabetes” (41). As of 2009, the fenugreek market in Canada was relatively small, with only 400 hectares of seed being grown (40). Although it has not been a key crop produced in Canada, it is used worldwide in a variety of food products and medicines, including spices, flavourings, and curries (40).

FG is a type of mucilage gum which is extracted most commonly from the endosperm of the fenugreek seed (42). FG is composed mainly of galactomannans, which are polysaccharides with a linear backbone of mannose sugars with galactose side chains (39, 42). It has a high content of galactose side chains, which makes FG very soluble in water, and able to form highly viscous solutions (42). FG also has a strong surface activity in solution, making it a strong emulsifier and stabilizer for oil in water emulsions (39). In the food industry, FG is less utilized for its thickening, stabilizing, and emulsifying properties, compared to other mucilage gums, however, it is an ideal candidate to be used in baking, bread making, gravies and soups, chocolate, and ice cream, for its functional properties (42-43).

f. **The Role of Fenugreek Seeds and Fenugreek Gum in Modulating Glycemic Response**

FG is a type of hydrocolloid soluble DF which may have an effect on improving the glycemic response in individuals, due to its high viscosity properties, which like some other soluble DF, may slow down absorption of glucose, delay gastric emptying from the stomach into
the SI, and delay insulin secretion by pancreatic β cells. Fenugreek seeds have been well studied in the literature for their role in altering the glycemic response in humans, however, the role of isolated FG has been less studied.

Fenugreek seeds have been studied in acute interventions, which have looked at fenugreek seeds incorporated into a glucose beverage during an OGTT, or have incorporated fenugreek seeds into a food matrix as the single bioactive, or in combination with other bioactives (44-50). All acute interventions looked at the immediate effects of fenugreek seeds on altering the postprandial glycemic response after the specific test OGTT or meal (44-50). FG has also been studied during acute interventions, which have looked at the addition of FG into a glucose beverage during an OGTT, or have incorporated FG into consumer beverage matrices (44, 51). Both acute interventions of FG looked at the immediate effects of FG on the postprandial glycemic response after the test OGTT or beverage (44, 51). Fenugreek has also been extensively studied in chronic interventions for its role on glycemic response, however, chronic interventions will not be covered in this literature review. The following is a summary of the literature focusing on the acute effects of fenugreek seeds on glycemic response both during an OGTT, and in food matrix forms (as a single bioactive, or with other bioactives) (44-50), and the acute effects of FG on glycemic response, both during an OGTT, and in food matrix forms (44, 51).

f.i. Fenugreek Seeds Acute Human Intervention Studies

(Summarized in Appendix B)

Fenugreek seeds have been acutely studied for their role in altering the glycemic response of human participants since the mid-1980s. Although the seeds have been studied for many decades, there is only a small subset of clinical trials that have focused on the acute role of
fenugreek seeds in attenuating the postprandial glycemic response (44-50). All acute fenugreek seed studies have focussed on directly adding the fenugreek seeds to a glucose beverage before conducting an OGTT, required participants to consume fenugreek seeds alongside, or slightly before, consuming a meal, or incorporated the fenugreek seeds into the food matrix before acutely testing the glycemic response to the test food (44-50). Incorporating the fenugreek seeds into a liquid or food matrix may make the seeds more palatable for consumption, instead of chewing the seeds alone, or in supplement form, as whole fenugreek seeds sometimes have a bitter taste (44), although food matrix may also cause variability in the results. Acute studies to date differ in the food matrix tested, and the dose and preparation of the fenugreek seeds used. Also, some acute studies presented which use fenugreek seeds, also use other bioactive ingredients which may have beneficial properties to altering the glycemic response when consumed (49-50). Therefore, those studies may have elicited changes to glycemic response due to the additive effect of the bioactive ingredients, which may or may not have been attributed to fenugreek seeds (49-50). This summary of the acute literature for fenugreek seeds will first discuss all studies involving only fenugreek seeds as the bioactive component within a glucose beverage during an OGTT, or within, or alongside, a food matrix during a postprandial meal test, and will then discuss studies involving fenugreek seeds as one of several bioactive ingredients (44-50).

The potential for acute fenugreek seed consumption to alter glycemic response was first studied by Sharma (1986), where several small trials were conducted using a randomized, crossover study design (44). Each small trial used a different formulation of fenugreek seed, combined with either 100 g of glucose in 250 mL water during an OGTT, or in combination with a meal. The portion of the trial which used fenugreek gum will be discussed in section f.i.i. of
this review. The first clinical trial looked at the incorporation of 25 g whole fenugreek seed powder (containing 12 g total fibre and 5 g FG) with 100 g glucose in 250 mL water, compared to the same beverage without fenugreek seeds (control), in 8 healthy adult participants. At each study visit, participants provided a fasting blood sample, consumed the study visit beverage, and had blood samples taken over the next 2.5 hours to measure postprandial blood glucose and insulin response, glucose integrated area under the curve (IAUC), and insulin IAUC. Overall, blood glucose response was significantly decreased at both 30 and 60 minutes, and glucose IAUC was also significantly decreased, after consuming the fenugreek seeds beverage, compared to the control beverage. Both insulin response at 30 and 60 minutes, and insulin IAUC, were also significantly decreased after consumption of the fenugreek seeds beverage compared to the control beverage. Therefore, in healthy adults, 25 g fenugreek seed powder in a glucose beverage significantly improved glycemic and insulinemic responses. The second portion of the trial followed the same randomized, crossover design, but looked at the incorporation of 25 g extracted fenugreek seed powder (defatted) (containing 12.92 g total fibre and 4.8 g FG) with 100 g glucose in 250 mL water, or the same beverage without fenugreek (control), with 6 healthy adult participants. At each study visit, participants provided a fasting blood sample, randomly consumed the study visit beverage, and had postprandial blood samples taken over the next 2.5 hours to measure postprandial blood glucose and insulin concentrations and IAUC. Overall, blood glucose at both 30 and 60 minutes and glucose IAUC were significantly decreased with the extracted fenugreek seeds beverage compared to the control beverage. Insulin response at 60 minutes, and insulin IAUC was also significantly decreased with the extracted fenugreek seeds beverage compared to the control beverage. Therefore, 25 g extracted fenugreek seeds in a glucose beverage significantly improved glycemic and insulinemic responses in healthy adults.
The third portion of the trial followed the same study design as the first and second trials, however, it incorporated 25 g of degummed fenugreek seed powder (FG removed) (total fibre content not specified) with 100 g glucose in 250 mL water, or the same beverage without fenugreek (control), with 6 healthy adult participants. For each study visit, participants provided a fasted blood sample, consumed the study visit beverage, and had postprandial blood samples taken over 2.5 hours to measure glucose and insulin response, glucose IAUC, and insulin IAUC. Overall, the glucose and insulin response, as well as the glucose and insulin IAUCs, were not significantly different between the 25 g degummed fenugreek seed powder and control beverages. This would suggest that perhaps the FG was required as a component of the fenugreek seed in order to illicit changes to the postprandial glycemic or insulinemic responses in healthy adults. The fourth portion of the trial was also a randomized, crossover intervention, which incorporated 25 g cooked fenugreek seeds (total fibre and FG content not specified) into a potato soup as a part of a larger meal (test meal), or had the same meal without the added cooked fenugreek seed soup (control meal), with 8 healthy adult participants. It was not mentioned if total or available carbohydrates for both meals were matched due to the addition of the potato soup, however, this was likely not feasible. Participants provided a fasted blood sample, consumed the study visit meal, and had blood samples taken over 2.5 hours to measure postprandial glucose and insulin response, glucose IAUC, and insulin IAUC. Overall, glucose IAUC was significantly reduced with the cooked fenugreek seeds test meal, compared to the control meal, however, there were no significant changes in insulin response or IAUC. It was also mentioned that the peak glucose concentration was decreased, and the time to reach peak glucose was delayed from 60 minutes to 90 minutes, with the cooked fenugreek seeds test meal, however, it was not indicated if this was significant. This trial may have had more significant
findings between the two meals if the carbohydrate challenge of each meal was matched. However, in healthy adults, a 25 g cooked fenugreek seed meal significantly improved glycemic response.

A study conducted by Madar et al. (1988) focussed on the effects of the incorporation of ground fenugreek seeds in water, with a meal, on glycemic response, in 21 individuals with non-insulin dependent diabetes mellitus (NIDDM) (45). In this crossover study, participants consumed either 15 g ground fenugreek seeds in water with a standardized meal, or the same meal without ground fenugreek seeds. At each study visit, participants provided a fasting blood sample, consumed the study visit meal, and had their blood sampled over the next 3 hours to determine blood glucose and insulin response. Overall, postprandial blood glucose values were significantly decreased with the test meal containing 15 g ground fenugreek seeds compared to the control meal, however, insulin values were not significantly different between either groups. Therefore, the incorporation of 15 g ground fenugreek seeds in water, with a meal, significantly improved glycemic response in adults with NIDDM, however, insulinemic response was not significantly improved.

Another study conducted by Neeraja and Rajyalakshmi (1996) looked at the role of processed fenugreek seeds within a traditional Pongal meal on glycemic response in 6 healthy, and 6 NIDDM, adult males (46). This crossover study incorporated 12.5 g of unprocessed dried fenugreek seeds [containing 5.98 g total fibre, and 2.35 g soluble DF (FG)], germinated then dried fenugreek seeds [containing 4.23 g total fibre, and 1.25 soluble DF (FG)], or boiled then dried fenugreek seeds [containing 3.75 g total fibre, and 0.38 g soluble DF (FG)], into Pongal breakfast dishes. The control meal was the same dish without the addition of any fenugreek seeds, and all meals were close but not matched in carbohydrate content (between 71 to 76 g). In
the morning of each study visit, all participants had a fasting blood sample taken, consumed the study visit breakfast, and had blood samples taken over the next 2 hours for analysis of blood glucose and glucose area under the curve (AUC). Overall, in the healthy participants, blood glucose values, and glucose AUC, was significantly decreased with the unprocessed and dried, and germinated and dried, fenugreek seed meals, compared to the control meal. Also, in the healthy participants, the time to reach peak glucose in both the unprocessed and dried, and germinated and dried, fenugreek seed meals was 90 minutes, compared to 60 minutes in the control meal, however, statistical significance was not reported. In the NIDDM participant group there was a similar trend with unprocessed and dried, and germinated and dried, fenugreek seed meals producing favourable glycemic results, however, the results were not significant, and there were many individual differences. Overall, a decreased glycemic response trend was noticed in the fenugreek seed compositions containing soluble DF, and therefore, FG. There were no significant results reported for the boiled and dried fenugreek seed test meal which contained only 0.38 g soluble DF. No significant results were reported in the NIDDM participant category, which may have been influenced by the medication therapy each participant used to manage T2D. Although each NIDDM participant was asked to refrain from medication therapy one day prior to each visit, this may not have been an adequate length of time for glycemic control to be dysregulated, and may have indicated why individual participant data greatly varied. Therefore, 12.5 g of unprocessed and dried, and germinated and dried, fenugreek seeds in a meal significantly improved glycemic response in healthy individuals, however, the same meal did not significantly attenuate glycemic response in individuals with NIDDM.

A study conducted by Bawadi et al. (2009) looked at the effects of fenugreek seeds in a drink solution during the postprandial state, in 166 adult individuals with T2D (47). In this
randomized, parallel-arm intervention, participants were randomly assigned into one of three groups, which were: FG0 (control group where participants consumed 0.8 g of dextrose in 25 mL warm water), FG2.5 (test group where participants consumed 2.5 g of fenugreek seeds in a dextrose solution), or FG5 (test group where participants consumed 5 g of fenugreek seeds in a dextrose solution). It was not mentioned if the control and test beverages were matched for carbohydrate content. After participants consumed their lunch, a blood sample was collected, and participants were then asked to consume the assigned beverage, and after 2 hours a final blood sample was taken, to compare pre- and post-test blood glucose. Overall, the difference between pre- and post-test blood glucose samples was significantly different compared to the control in only the FG5 group containing 5 g fenugreek seeds. Therefore, a decrease in glycemic response was noticed when consuming 5 g of fenugreek seeds in a dextrose solution while already in a postprandial state, for individuals with T2D, however, 2.5 g of fenugreek seeds did not significantly reduce postprandial glycemic response. The study could have been strengthened, however, if a standardized meal was provided to each participant as the pre-test meal, and if the dextrose solution provided was matched for carbohydrates between the three groups. It is also important to note that this was not a crossover intervention, therefore each individual could not act as their own control, which may have also strengthened the results.

The final acute study which used only fenugreek seeds as the test ingredient was conducted by Kumar et al. (2011), and looked at the glycemic response of fenugreek seeds consumed with rice, or whole wheat chapatti meals, in 25 adults with NIDDM (48). In this crossover, non-randomized intervention, participants participated in an OGTT with 50 g glucose in 250 mL water on the first study day, consumed 200 g of rice (50 g carbohydrate) on the second study day, consumed 103 g wheat chapatti (50 g carbohydrate) on the third day,
consumed 200 g rice with 12.5 g germinated fenugreek seed powder (containing 50 g carbohydrate, 6 g total fibre and 2.5 g FG) on the fourth day, and consumed 103 g wheat chapatti with 12.5 g germinated fenugreek seed powder (same composition as above) on the fifth day. On days 4 and 5, 15 of the 25 participants consumed the fenugreek seed powder in water 15 minutes before the meal, and the remaining 10 participants consumed the fenugreek seed powder in water during the meal. At each study visit, participants provided a fasting blood sample, consumed the study visit meal, and had blood samples taken over the next 2 hours for the determination of glucose AUC, and GI (test food glucose AUC/control glucose AUC multiplied by 100). Overall, all test meals had a significantly decreased glucose AUC compared to the OGTT beverage, however, it was not determined if the two meals containing germinated fenugreek seeds had more of a significant effect on decreasing blood glucose compared to the meals without fenugreek seeds. The two fenugreek meals also had a decreased GI compared to the rice and chapatti control meals, with the participants who consumed the fenugreek seeds 15 minutes before the test meal having a significantly greater GI decreases in both fenugreek meals, compared to the participants who consumed the fenugreek in water with each meal. Therefore, this suggests that germinated fenugreek seeds at 12.5 g have a role in reducing the glycemic response in individuals with NIDDM when compared to a glucose beverage, and can reduce the GI of a meal. This study (48) had greater decreases to glycemic responses in NIDDM participants compared to the study conducted by Neeraja and Rajyalakshmi (1996), which also used 12.5 g germinated fenugreek seeds in a meal, and found no significant effects on attenuating glycemic response in NIDDM individuals (46). It is important to note that the total fibre and FG content of the germinated fenugreek seeds used in this study were higher (6 g and 2.5 g respectively) (48) compared to the study by Neeraja and Rajyalakshmi (1996) (4.23 g and
1.25 g respectively), which may have explained the improvement to glycemic response in this study only (46). Therefore, it is important to note that processing may cause unfavourable alterations to the nutritional quality of the fenugreek seeds which could impact improvements to glycemic response endpoints.

There have also been 2 acute studies which have incorporated fenugreek seeds into food products along with other bioactive ingredients. The first study, by Pathak et al. (2000), incorporated fenugreek seeds, along with other bioactive ingredients such as foxtail millet, legumes, coconut oil, and amaranth, into traditional Indian snack foods, and measured the glycemic response to these products in 5 healthy female, and 5 NIDDM male, adults (49). In this randomized, crossover, intervention, participants consumed a 50 g carbohydrate glucose beverage during an OGTT (control), dhokla [250 g serving containing 50 g carbohydrate and 6.85 g crude fibre, incorporating millet, legumes, and fenugreek seeds (at 25 g)], uppuma [230 g serving containing 50 g carbohydrate and 8.03 g crude fibre, incorporating millet, legumes, fenugreek seeds (at 23 g), and coconut oil], and laddu [80 g serving containing 50 g carbohydrate and 4.86 g crude fibre, incorporating amaranth, millet, legumes, and fenugreek seeds (at 20g)]. During each visit, participants provided a fasted blood sample, then consumed the study visit food, with 200 mL water, and had blood samples taken over the next 2.5 hours to assess blood glucose, glucose AUC, and GI of each food product. Overall, blood glucose response, and glucose AUC, tended to be attenuated in both healthy and diabetic participants with all food products, however, significance was not reported. The GI of each food product were significantly different with both healthy and diabetic participants, however the comparison of the GI products between subject groups was not significant. Order of lowest to highest GI values were uppuma, followed by dhokla, and finally laddu. It is important to note that the highest
crude fibre content, and highest fenugreek seed content, was in uppuma, followed by dhokla, and then laddu, which may have been a contributing factor to why GI was the lowest in the higher fibre containing foods. Therefore, fenugreek seeds may have had a role in decreasing the GI, and glucose AUC, in each food product, in healthy and NIDDM individuals, however, these results cannot be attributed to fenugreek seeds alone as millet, legumes, coconut oil, and amaranth all contain DF.

Finally, a study conducted by Shakib and Gabrial (2010), focussed on the postprandial glycemic response of 20 healthy adults when consuming breads containing wheat, barley, ginger, and fenugreek seeds (50). In this randomized, crossover study, participants consumed different bread formulations which were: WWb (control containing 100 % refined wheat flour), BWb (50 % barley flour and 50 % refined wheat flour), BWFb-2.5 (97.5 % BWb recipe with 2.5 % fenugreek flour), BWFb-5 (95 % BWb recipe with 5 % fenugreek flour), and BWFGb (98 % BWb recipe with 1 % fenugreek flour and 1 % ginger flour). All recipes were matched for 50 g carbohydrate, therefore the serving for WWb was 100 g and all test recipes were a serving size of 119 g (BWFb-2.5 containing 2.98 g fenugreek flour, and BWFb-5 containing 5.95 g fenugreek flour). At each visit, participants provided a fasted blood sample, then consumed the study visit bread, and had blood samples taken over the next 2 hours for the analysis of glucose response, glucose AUC, and GI values. Overall, all breads containing barley had significantly decreased blood glucose responses at all postprandial time points compared to the WWb control, with the BWFGb having a significantly decreased glucose response at 30 minutes compared to the BWb bread, and the BWFb-5 bread having a significantly decreased blood glucose response at 60 minutes compared to the BWb bread. The glucose AUC values, and therefore the GI, were also significantly decreased compared to the WWb control for all barley bread products, with the
BWFGb and BWFb-5 breads being significantly decreased compared to the BWb bread. Barley contains a high concentration of soluble DF on its own (50), however, this study showed an additive effect of 5.95 g of fenugreek flour (in the test serving) in further decreasing blood glucose responses at 60 minutes, glucose AUC values, and GI, with the BWFb-5 bread. There was also an additive effect with the barley, fenugreek seed, and ginger flours, on further reducing glycemic response at 30 minutes, glucose AUC, and GI, with the BWFGb bread product, which likely could not be attributed to the fenugreek flour alone. Therefore, this study shows the potential of both 2.98 g and 5.95 g fenugreek flour having the ability to significantly attenuate postprandial glycemic responses in healthy adults when incorporated into barley and wheat breads, with further attenuation with 5.95 g fenugreek flour in combination with barley.

The literature examining the acute role of fenugreek seeds in attenuating glycemic response is conflicting, mainly with respect to the acceptable dosage range and processing effects on the fibre content of the seeds. In studies with healthy participants, in which fenugreek seeds were incorporated into either a glucose beverage or food matrix, compared to a similar control meal, doses of 12.5 g of unprocessed and dried, or germinated and dried fenugreek seeds, and 25 g of whole fenugreek seeds, extracted fenugreek seeds, or cooked fenugreek seeds, reduced glycemic and insulinemic response (44, 46). It is important to note that 12.5 g of boiled fenugreek seeds, and 25 g degummed fenugreek seeds, did not significantly alter glycemic or insulinemic response, which is perhaps due to their low FG content (44, 46). In studies with healthy participants, which incorporated both fenugreek seeds, and other bioactive ingredients, into food products, products with the highest level of crude fibre, and bread products with 2.98 g and 5.95 g of fenugreek seed flour, improved both the GI of the foods, and tended to, or significantly, decreased glycemic response (49-50). Therefore, in healthy adults, incorporation
of fenugreek seeds with high contents of DF, in doses of 2.98 g to 25 g, with or without other bioactives, has tended to improve glycemic response (44, 46, 49-50). In adults with T2D, or NIDDM, the results are also conflicting due to acceptable dosage range, and processing effects. In the study conducted by Bawadi et al., doses of 2.5 g of fenugreek seeds in a dextrose solution did not alter glucose response when consumed in a postprandial state after a lunch meal, however, 5 g of fenugreek seeds in a dextrose solution significantly decreased glycemic response in T2D participants (47). In the study conducted by Neeraja and Rajyalakshmi (1996), 12.5 g of unprocessed and dried, germinated and dried, or boiled and dried fenugreek seeds, in a meal, did not produce significant decreases to glycemic response in NIDDM participants, however, in the study conducted by Kumar et al. (2011), 12.5 g germinated fenugreek seeds with a meal, in NIDDM participants, decreased GI of the food products containing the seeds, which may have been explained by the higher DF and soluble DF content in the second study, perhaps due to processing differences (46, 48). The study conducted by Madar et al. (1988) also found significant effects on the reduction of glycemic response when consuming 15 g ground fenugreek seeds, however, insulinemic response was not significantly altered (45). Finally, in the study conducted by Pathak et al. (2000), in NIDDM males, fenugreek seeds, along with other bioactive ingredients, improved the GI, and seemed to attenuate glycemic response, in the food products with the highest levels of DF, however, significance was not reported (49). Although results are variable, fenugreek seed formulations with high soluble DF, and therefore, high levels of FG, seem to produce the most significant results in attenuating glycemic response in individuals with T2D, or NIDDM (45, 47, 48-49).
f.i.i. Fenugreek Gum Acute Human Intervention Studies

(Summarized in Appendix C)

FG has rarely been studied as an isolated food bioactive in acute human intervention studies. Although fenugreek seeds have been extensively researched, and the FG portion of the seeds, due to its high viscosity, may lead to improvements in glycemic response, few clinical trials have isolated the gum compound to be researched within food matrices alone. The two FG studies conducted have focussed on the addition of the gum isolate into either a glucose beverage as part of an OGTT, or have incorporated it into a consumer beverage product, and the literature for both studies will be discussed below (44, 51).

The first study using FG was a part of a larger subset of clinical trials published by Sharma (1986) (44). For this section of the clinical trial, 6 healthy adults participated in a randomized, crossover intervention, adding 5 g of gum isolate to 100 g glucose in 250 mL, or 100 g glucose in 250 mL water (control). At each visit, participants provided a fasted blood sample, consumed the study visit beverage, and had blood samples taken over the next 2.5 hours to determine glucose and insulin response, glucose IAUC, and insulin IAUC. Overall, 5 g of gum isolate significantly decreased glucose response at 30 and 60 minutes, and glucose IAUC, compared to the control beverage. Gum isolate also significantly reduced insulin response at 30 and 60 minutes, and insulin IAUC, compared to the control beverage. Therefore, 5 g FG consumed by healthy individuals significantly improved acute, postprandial glycemic response.

The second study to research FG was conducted by Mathern et al. (2009), which incorporated FG into consumer beverages, and monitored the postprandial glycemic response in 18 healthy, obese adults (51). In this randomized, crossover, single-blinded intervention, participants consumed a low-fibre breakfast along with Minute Maid Light beverages, which
contained either 0 g (control), 4 g (3.6 g FG), or 8 g (7.2 g FG), of isolated fenugreek extract. At each study visit, participants had a fasting blood sample taken, consumed the study visit beverage and breakfast, and had blood samples taken over the next 3 hours to calculate glucose and insulin AUC, and peak values for glucose and insulin. Overall, there were no significance reductions in glucose AUC, or peak glucose, with any treatment; however, insulin AUC and peak insulin was significantly increased with the 8 g fenugreek extract beverage compared to the control beverage. Peak insulin was significantly decreased for the 4 g FG extract beverage compared to the 8 g FG extract beverage, however, it was not significantly decreased than the control beverage. Therefore, this study did not show an improvement to glycemic response with either 3.6 g or 7.2 g FG in a beverage consumed before a breakfast meal, which is conflicting to the results reported by Sharma (1986), which showed an attenuation of glycemic response found with 5 g FG in a 100 g glucose in 250 mL water beverage (44, 51). The increase in insulinemic response also conflicts with Sharma (1986), which reported a significant reduction in insulin response with 5 g FG (44). Therefore, both 3.6 g and 7.2 g FG did not attenuate glycemic response, and 7.2 g FG increased insulinemic response, in healthy, obese adults (51).

Overall, the literature examining the acute role of FG in attenuating glycemic response is limited, and conflicting. In healthy participants, 5 g of FG within a 100 g glucose in 250 mL water beverage significantly attenuated both glycemic and insulinemic response, however, in healthy, obese adults, 3.6 g or 7.2 g FG did not significantly improve glycemic response, and 7.2 g FG increased insulinemic response (44, 51). Although both studies used isolated FG, perhaps extraction techniques differed, which may have contributed to the different responses in each study. Therefore, more research should be conducted to better understand the extraction techniques involved in isolating FG from the seed, in order to have the greatest bioactive
capacity. Also, more research should be conducted to determine the acute response that isolated FG has on attenuating glycemic response in a variety of different populations, as the role of FG on glycemic response has not been studied in adults with T2D which would benefit from decreases to glycemic response.

g. Flaxseed and Flaxseed Mucilage

Flax is an annual herb with blue flowers, which is native to the Mediterranean, and West Asia. However, since 1994, the world’s largest producer of flax has been Canada (52-53). For the 2015 to 2016 growing season, the Canadian agricultural industry is forecasted to reach a multi-year high in flaxseed production, with 649 kilo hectares of flaxseed being harvested, yielding 1.42 tonnes per hectare of land, with the average price per tonne being between 450 and 490 Canadian dollars (37). The flaxseeds are found in the five spherical compartments of the fruit capsule, where there are two seeds in each section (52). Flaxseeds have a nutty taste and, as they are high in α-linolenic acid, an omega 3 fatty acid, they are often used as an oilseed crop (52). However, flaxseeds are also high in dietary fibre, making them a valuable option for the incorporation into foods due to their high nutritional content (52).

FM is a type of mucilage gum that is secondary wall material for the outer layer of the seeds (32). It is composed of both neutral polysaccharides, such as xylose, arabinose, and galactose residues, and acidic polysaccharides, such as galactose, rhamnose, and galacturonic acid residues (32). FM has a high WHC, and is able to form highly viscous solutions and weak gels, mainly due to the neutral polysaccharide component of the structure (32, 54). Due to the high WHC, high viscosity, and weak gel, properties of FM, it can be used as a thickener, stabilizer, and a product to increase water holding for food products, however it is less commonly used compared to other gums in the food industry (32, 54).
h. The Role of Flaxseed and Flaxseed Mucilage in Improving Glycemic Response

FM is a type of hydrocolloid soluble DF which may have an effect on improving the glycemic response in individuals, due to its high viscosity properties, which like some other soluble DF, may slow down absorption of glucose, delay gastric emptying of the stomach into the small intestines, and delay insulin secretion by pancreatic β cells. Although soluble DF has been linked to a reduction in glycemic response, acute studies on the role of flaxseed, and FM, on attenuating glycemic response are limited.

Flaxseeds have been minimally studied in acute interventions on glycemic response. Only 2 studies have looked at flaxseeds, within a food matrix, on glycemic response (55-56). These acute interventions looked at the immediate effects of flaxseeds seeds within a food matrix on altering the postprandial glycemic response after the specific test meal (55-56). FM has also been minimally studied in acute interventions, which have looked at the consumption of FM in food matrices, and incorporated into a glucose solution during an OGTT (55-58). The acute interventions of FM looked at the immediate effects of FM on the postprandial glycemic response after the test food product (55-58). Flaxseeds have been more extensively studied in chronic interventions on glycemic response, and FM has also been minimally studied in chronic interventions, however, chronic interventions will not be covered in this literature review. The following is a summary of the literature focussing on the acute effects of flaxseeds on glycemic response in a food matrix (55-56), and the acute effects of FM on glycemic response, both in a food matrix form, and during an OGTT (55-58).
h.i. Flaxseed Acute Human Intervention Studies

(Summarized in Appendix D)

Flaxseed has been studied in 2 clinical trials, both incorporating flaxseed into a bread food matrix, to determine acute glycemic response (55-56). The first study to incorporate flaxseed into bread products to acutely measure glycemic response was conducted by Cunnane et al. (1993) (55). This paper also reports on an acute study with FM, which will be discussed in section h.i.i. In this randomized, crossover intervention, 6 healthy adults consumed breads made from flaxseed flour, and white flour (control), which were matched for 50 g carbohydrate, on different study visit days. The dose of flaxseed flour was not specified. At each study visit, fasted blood samples were collected, the bread was consumed, and postprandial blood samples were collected over 60 minutes for the determination of glucose iAUC. Overall, glucose iAUC was significantly decreased when the flaxseed bread was consumed compared to the white flour control bread. Therefore, in a study with healthy adults, consumption of a bread product containing flaxseed flour significantly improved glycemic response, however, it was unspecified how much flaxseed flour was actually consumed in the treatment product, making it difficult to draw accurate conclusions.

The second study to incorporate flaxseed within a bread food matrix was conducted by Kristensen et al. (2013) (56). FM was also studied in this trial, however, this aspect of the clinical trial will be discussed in section h.i.i. In this randomized, double-blinded, crossover intervention, 18 healthy, young adult males consumed two isocaloric meals on different study visit days; where each meal was comprised of two buns, cheese, butter, ham, and 400 mL of water, and were matched for carbohydrate content. The test meal contained 12.18 g whole flaxseed baked into the buns, whereas the control meal contained no added flaxseed. The control
meal contained 7 g DF, and the test meal, with flaxseed, contained 12 g DF. At each study visit, fasted blood samples were taken, and after consuming the study visit meal, postprandial blood samples were taken over 3 hours to determine glucose and insulin response. Overall, there was no significant decreases to either FBG or insulin response, compared to the control meal. Therefore, no significant changes to glycemic or insulinemic response were found in young, healthy males when consuming 12.18 g whole flaxseed baked into buns during a meal.

Overall, limited research is available on the acute role of flaxseed on glycemic response. The first study to incorporate flaxseed flour into bread products saw significant decreases to glycemic response in healthy adults, however, the dose of flaxseed was not indicated, which makes it hard to draw accurate conclusions (55), and the second study to incorporate 12.18 g flaxseed into a bun showed no significant differences to glycemic or insulinemic response in healthy adults (56). Therefore, more studies focussing on the acute role of flaxseeds, in both healthy populations, and individuals with T2D, should be conducted to understand the short term glycemic effects of the seeds.

h.i.i. Flaxseed Mucilage Acute Human Intervention Studies

(Summarized in Appendix E)

FM has been minimally studied as a food bioactive in acute human intervention trials. Although soluble DF has been linked to improving glycemic response, few clinical trials have isolated the FM compound to be researched without the remaining dietary components of flaxseed. Three clinical trials have incorporated FM into food matrices, and one trial has incorporated FM into a glucose solution during an OGTT (55-58). The literature for each clinical trial will be discussed below.
The first study focusing on the role of fibre found in flaxseed was conducted by Dahl et al. (2005) (57). In this crossover intervention, with 11 healthy adults, flax fibre comprised of both insoluble DF and soluble DF (FM) fractions were incorporated into loaves of bread. Participants consumed two different breads, in duplicate, matched for 50 g available carbohydrate, containing either 11 g of flax fibre [2.7 g soluble DF (FM)], or white flour (control). Blood samples were taken fasted, and after the consumption of the study visit bread, postprandial blood samples were taken over 2 hours for the determination of glucose response, and glucose iAUC. Overall, both glucose iAUC, and peak glucose concentration were significantly decreased after consuming the flax fibre bread compared to the control bread. Therefore, the consumption of 11 g flax fibre (2.7 g soluble DF) significantly improved acute glycemic response in healthy adults. However, the insoluble DF portion of the flaxseed was not removed, which may have aided in the decreased glucose response in the test bread.

The second study focusing on acute consumption of FM on glycemic response was conducted by Kristensen et al. (2013) (discussed previously) (56). In this section of the randomized, double-blinded, crossover intervention, 18 healthy, young adult males consumed three isocaloric meals on different study visit days; where each meal was comprised of two buns, cheese, butter, ham, and 400 mL of water, and were matched for carbohydrate content. The two test meals contained either 12.18 g FM baked into the buns (12 g DF) (low mucilage dose), or 17.27 g FM (17 g DF) (high mucilage dose), whereas the control meal contained no added flaxseed (7 g DF). Blood samples were taken fasted, and for 3 hours postprandially after the study visit meals, for the determination of blood glucose and insulin response. Overall, there were no significant changes to blood glucose, however, insulin response at 30 minutes was significantly decreased with the high mucilage meal compared to the control meal, and insulin
AUC was significantly decreased in both mucilage meals compared to the control meal. It is important to note that although 12.18 g and 17.27 g of FM were added to the low mucilage and high mucilage meals respectively, only 12 g and 17 g DF, respectively, were present in the meals. The control meal without any added FM also contained 7 g DF. Therefore, perhaps some FM was lost during the bun cooking and preparation. However, although there were FM losses, both 12.18 g and 17.27 g FM significantly improved insulinemic responses in healthy, young adults, even though no changes were present in glycemic response.

The final study incorporating FM into food matrices to determine acute effects on glycemic response was conducted by Au et al. (2014) (58). In this randomized, double-blinded, crossover intervention, 12 healthy adult males consumed 1.8 g FM in a glucose solution, 2.5 g FM in a dairy beverage, 2.5 g FM in a dairy pudding, two control glucose solutions (control reference), and a control dairy beverage and dairy pudding containing 0 g FM matched for viscosity. Other soluble DF types were investigated but will not be discussed in this review. The control dairy pudding contained 0.25 g κ-carrageenan (0.25 g fibre) to match viscosity. All treatments were 250 g, contained 50 g available carbohydrate, and were consumed with 125 mL water. At each study visit, participants provided a fasting blood sample, consumed the treatment in 5 minutes, and provided postprandial blood samples over the next 2 hours to determine glucose and insulin response, glucose and insulin iAUC, GI, and insulinemic index (II) (test food insulin AUC/control insulin AUC multiplied by 100). Overall, no significant differences were found between the reference control and the FM glucose solution for glucose or insulin endpoints. For the FM dairy treatments, glucose iAUC, GI, and peak glucose values, were significantly different compared to the control reference only, but there were no significant differences between treatments. Glucose time-to-peak was also significantly decreased with the
FM dairy pudding compared to the control reference only. Finally, most insulin endpoints were not significantly different between the control and FM dairy treatments, although insulin time-to-peak was significantly decreased with the FM dairy beverage compared to the control reference only. Overall, 1.8 g FM within a 50 g glucose solution did not improve glycemic or insulinemic response in healthy males, however, 2.5 g FM in a dairy beverage and dairy pudding significantly decreased glycemic response, and slightly decreased insulinemic response, compared to a 50 g glucose reference.

FM has also been incorporated into a standard OGTT glucose beverage. In a randomized, crossover intervention conducted by Cunnane et al. (1993) (discussed previously), 4 participants (unknown health status) consumed both 25 g FM in a 50 g glucose solution, and a 50 g glucose solution without added FM, on separate study days (55). At each study visit, participants provided a fasted blood sample, consumed the beverage, and had their blood taken over 2 hours for the determination of glucose iAUC. Overall, glucose iAUC was significantly reduced with the FM and glucose solution compared to the glucose control solution without added FM. Therefore, 25 g FM in a glucose solution significantly improved glycemic response, however, the health status of the participants who participated was not described, and the sample size was very low, so it is hard to draw accurate conclusions.

Overall, the literature on the acute glycemic response after consumption of FM is limited, and conflicting. Most literature on the acute effects of FM have only been done in healthy adults populations, and with one study, it is unknown what the health status of the participants were (55-58). With the consumption of 2.7 g FM in a bread matrix, glycemic response was significantly improved, however, this bread product also contained the insoluble DF portion of the flaxseed, which may have increased the beneficial effects to glycemic improvements (57).
Doses of 12.18 g and 17.27 g FM baked into a bun with a meal did not improve glucose response compared to a control bun, however, insulinemic response was significantly decreased with both FM buns, but a dose response effect was not present (56). Also, a dose of 1.8 g FM in a glucose beverage did not significantly alter glycemic and insulinemic responses compared to a reference glucose beverage, however, 2.5 g FM in a dairy beverage, and dairy pudding, significantly improved glycemic response compared to a reference glucose control only, but not a control pudding without added FM (58). Finally, a dose of 25 g FM in a glucose solution significantly improved glycemic response in 4 participants with unknown health status (55). Therefore, more clinical trials on the acute effects of incorporating FM on glycemic response are required for a definitive conclusion. The studies with FM in food matrices, and within glucose solutions, are conflicting, as well as doses being different between studies. Also, as results were so different, processing may have also been a contributing factor towards the variation in results. Clinical trials focusing on participants who are at risk, or have T2D, would also be beneficial, as this population would benefit from decreased glycemic responses.

**RESEARCH RATIONALE**

This research is rationalized in the context that T2D is a widely growing disease, with 1 in 4 individuals in Canada currently living with prediabetes or diabetes, which is expected to increase to 1 in 3 individuals by 2020 (6). Although there are many non-modifiable risk factors for the development of T2D, there are also many modifiable risk factors. If disease progression is caught in the prediabetes stages, lifestyle interventions to diet and physical activity, can decrease the risk of T2D development (10). If effective prevention and management strategies can be delivered, the rate at which prediabetes developing into T2D can be decreased, and the
burden of T2D on individuals can be reduced, ultimately improving quality of life for individuals, and a reduced stress on the Canadian healthcare system.

Particularly, increased consumption of foods rich in DF have been linked to reduced risk, and development, of T2D (18-19), however, Canadian adults may not be consuming enough DF (59). The CDA recognizes that soluble DF improves postprandial blood glucose, due to its role in delaying gastric emptying, and decreasing absorption of glucose in the SI (35). Therefore, the need for incorporating soluble DF into food products that the average consumer would eat in their daily lives is key to improving the overall consumption of DF towards the recommended amount and above, if an individual has T2D.

Although Canada currently has no approved health claims for DF in decreasing blood glucose or improving glycemic response, other countries, such as EFSA, have approved health claims for certain DF in decreasing blood glucose (31). The specific DF with approved health claims from EFSA include arabinoxylan, β-glucans, HPMC, pectins, and resistant starch, which are all hydrocolloid soluble DF, which are high in viscosity and exert gel-like properties, therefore, it is important to determine which other novel soluble DF may also improve glycemic response (31-32). As not all soluble DF have the same physiochemical properties, it is important to understand the mechanisms of each soluble DF, to determine whether it would improve glycemic response when added to food products. YM seeds, fenugreek seeds, and flaxseeds are all Canadian crops which contain soluble DF mucilage. However, the soluble DF component of the seeds has been minimally studied in the literature. If the soluble DF mucilage within each of these seed crops is able to illicit improvements in glycemic response, the Canadian agricultural economy could stand to benefit. Currently, there is very little research on the role YMM plays on glycemic response, however, the results on attenuation of acute glycemic response have so far
been positive in one study (38). Whole fenugreek seeds have been widely studied for their role on improving glycemic response, however, there have been few studies which have isolated FG to determine its isolated effects on attenuating glycemic response, and the two acute studies were conflicting (44, 51). Finally, whole flaxseeds have also been widely researched for their role in attenuating glycemic response. However, like the other two mucilages, FM has been less studied for its isolated role on improving acute glycemic responses. Acutely, FM has been able to attenuate glycemic responses in all studies (55-58), however, FM has only been researched in healthy populations, not prediabetic or T2D populations, which could stand to benefit most significantly from consuming additional fibre in their diets.

The ability of soluble DF to modulate glycemic response has partly been related to physicochemical properties, in particular, the ability to form viscous solutions and gel like structures in the gastrointestinal tract (60). Different soluble DF possess different physicochemical properties, depending on factors, including chemical composition, particle size, and molecular weight (60). This study is part of a broader project aimed at identifying the physicochemical properties and in vitro gastrointestinal behaviour and glycemic response of underutilized DF from agricultural by-products, i.e. YMM, FG and FM (Ontario Ministry of Agriculture, Food and Rural Affairs project #200603). In order to determine the appropriate level of each soluble DF to incorporate into the pudding products, experiments in simulated intestinal conditions were conducted to determine the DF concentration which would match the in vitro viscosity obtained by the specific amount of β-glucan in the EFSA health claim for β-glucan and glycemic response (61). This approach is rationalized in the context that viscosity induction of gut contents by soluble DF is understood to be a primary mechanism by which attenuations in glycemic response occur (60). Experiments were conducted on the basis of the EFSA health claim for β-glucan,
which states “consumption of ß-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal”, whereby the food must contain 4 g of ß-glucan, from oat or barley, per 30 g of available carbohydrate (61). This equates to 6.7 g of ß-glucan per 50 g of available carbohydrate, i.e. the amount of available carbohydrates routinely used in glycemic index testing (61-62). Therefore, each of the soluble DF concentrations for YMM, FG, and FM, were compared to oat gum containing 6.7 g of ß-glucan, and the soluble DF concentration of each fibre was chosen when each gum resulted in similar apparent viscosities (at 60 s\(^{-1}\)), using an ARES Rheometer (TA Instruments, Mississauga, Canada). In vitro viscosity determinations and rheological analyses were made by PhD student Nikolay Repin under the supervision of Professor Douglas Goff, in the Department of Food Science (60). Based on the initial testing, concentrations of YMM, FG, and FM to achieve equivalent in vitro apparent viscosities to 6.7 g of ß-glucan were calculated. This resulted in very low levels of soluble DF. Therefore, the concentration was increased by 3 times to 15.5, 5.9, and 11.4 g of YMM, FG, and FM, respectively. A secondary purpose of the broader project relates to comparing impacts of the source of available carbohydrates on glucose release and uptake. Therefore, all pudding products were formulated to contain 50 g of available carbohydrate, with 70 % of the carbohydrates coming from either high maltose corn syrup (HMCS), a simple carbohydrate, or modified tapioca starch (MTS), a complex starch carbohydrate, which would take longer to enzymatically digest, which was rationalized in the context that soluble DF would slow down starch breakdown (60). However, for the context of this research discussed, each pudding containing the same soluble DF were grouped into one, and carbohydrate source was not included as a key objective of study.
The rationale for utilizing a pudding matrix includes the fact that snack foods are popular products consumed by the Canadian population (63) and, as already thickened products, may represent a suitable vehicle for the addition of soluble DF. Interest in SF fortification of foods is high. Although FG has been used previously to make chocolates and ice cream (43) and FM was incorporated into dairy beverages and dairy puddings in a previous study (58), literature addressing the acute glycemic effects with YMM, FG and FM is scant. Therefore, this research examined the incorporation of YMM, FG, and FM, into a pudding product, as puddings are snack products which often have high viscosity already. As such textural changes should be minimally impactful.

Therefore, this research is rationalized in the context that isolated YMM, FG, and FM, have been minimally studied in the acute glycemic response literature, especially in at-risk, prediabetic, and T2D populations. This research aimed to expand the acute literature for the role each soluble DF plays on the attenuation of glycemic response in individuals who would be able to benefit from the incorporation of fortified fibre products into their diet.

**PURPOSE, OBJECTIVES AND HYPOTHESIS**

1. **Research Purpose, Objectives and Hypotheses**

   The purpose of this research was to investigate the postprandial glycemic and insulinemic responses following consumption of pudding treatments, matched for *in vitro* viscosity, containing novel soluble DF (YMM, FG, FM) in adults at risk for T2D. The specific objectives were to examine how each fibre-enriched pudding treatment, compared to a control low-fibre pudding treatment, were able to alter whole blood glucose (blood glucose) and plasma insulin responses over a 2 hour postprandial meal test. It was hypothesized that the soluble DF-containing pudding treatments would significantly lower postprandial glycemic and insulinemic
responses compared to the low-fibre control pudding treatments similarly, as all soluble DF treatments were matched for in vitro viscosity.

METHODS

1. Study Design and Approvals

   a. Study Design

   This randomized, double-blinded, crossover, controlled study was conducted at the Human Nutraceutical Research Unit (HNRU) of the University of Guelph. In total there were 10 study visits, with a minimum washout period between visits of 5 days. Study recruitment occurred from November 6th, 2014 to June 17th, 2015, and study visits commenced between December 2nd, 2014 to August 27th, 2015. Provided in Figure 1 is a summary of the study design of the clinical trial.

![Figure 1: Study Design Summary](image)

Figure 1: Study Design Summary

   b. Study Approvals

   The University of Guelph Human Research Ethics Board (REB) approved the study protocol (REB#14AP003) (see Appendix F) in accordance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2) (63). All study personnel
completed the TCPS 2 Course on Research Ethics before being involved with the research (64). The University of Guelph Biosafety Committee, in the Department of Environmental Health and Safety, approved the biohazard protocol (Number: H-254-11-16-07) (see Appendix G). Finally, the study was registered in the National Institute of Health (NIH) clinical trial registry (Identifier Number: NCT02289612; www.clinicaltrials.gov). In accordance with the REB and TCPS 2, all participants provided written informed consent to participate.

2. Participant Sample Size, Recruitment, Screening, and Orientation

a. Participant Sample Size Determination

Participant sample size was determined based on previous reports. Participant sample size was considered based on the International Organization Standardization (ISO) protocol ISO 26642:2010, titled “Food products — Determination of the glycaemic index (GI) and recommendation for food classification” which indicates that a minimum of 10 healthy participants must be used to determine adequate values (66). Brouns et al. (2005) (62) also recommends that, for a significant difference to be detected with a 80 % beta (power) level and a two-tailed alpha significance level of 5 %, 10 participants provides adequate results for GI testing with healthy participants (65). Using glucose response as the primary endpoint, a sample size calculation was completed with an online sample size calculator (http://hedwig.mgh.harvard.edu/), where for 10 participants, with a two-sided alpha significance level of 0.05 %, an 80 % beta (power) level, and a within patient standard deviation (SD) of 0.5, a difference between treatments of 0.706 mmol/L would be considered significant (67). Participants recruited for this study were required to be at risk for T2D, but not specifically screened on the basis of fasting glucose concentration. Since it was expected that variability in
fasting glucose could be higher for these study participants and to account for possible attrition, a total of 16 participants were recruited for the study.

b. Participant Inclusion and Exclusion Criterion

Participants were included in the study if they were at risk for Type 2 Diabetes (based on having a score of ≥21 on the CANRISK questionnaire (see Appendix H) (8), had a body mass index (BMI) of ≥25 and <40 kg/m² (classified as overweight to obese), and prior use of acetaminophen. Exclusionary criteria for the study included tobacco smokers, food or any life threatening allergies, gastrointestinal conditions or illnesses (including, but not limited to, lactose intolerance, Celiac disease, Crohn’s disease, Ulcerative Colitis (UC), or Irritable Bowel Disorder (IBD)), serious major medical conditions (i.e. renal or liver), pregnancy, trying to become pregnant or breastfeeding, a Food Neophobia Score between 30 to 54 (classified as food neophobic) (see Appendix I) (68), a Three Factor Eating Questionnaire – Cognitive Restraint (TFEQ-CR) Score of >16 (classified as a restrained eater) (see Appendix J) (69), typical alcohol consumption of >4 drinks per day, medications or natural health products (NHPs) used for diabetes (glycemic control), medications or NHPs contraindicated with acetaminophen, and recent or intended significant weight loss or gain (i.e. >4 kg in previous 3 months). Table 2 summarizes the study inclusion and exclusion criteria, along with rationale for each criteria.
<table>
<thead>
<tr>
<th>Participant Inclusion Criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males and Females</td>
<td>The study aimed to be inclusive of both sexes, as T2D can occur in all adult individuals.</td>
</tr>
<tr>
<td>18 to 70 years of age</td>
<td>The lower age limit was set so that the age of entry is a consenting adult and the upper age limit was set to include individuals who are not on any medications known to affect glucose metabolism and as age increases medication use often does as well. As age increases, the likelihood of disease is also higher, and we have chosen to study an at risk population which does not currently have T2D.</td>
</tr>
<tr>
<td>CANRISK Questionnaire score ≥21 (8)</td>
<td>This is a brief questionnaire designed by the Public Health Agency of Canada to assess risk of developing diabetes. A score of ≥21 identifies individuals with moderate to high risk of developing diabetes.</td>
</tr>
<tr>
<td>BMI ≥25 and &lt;40 kg/m²</td>
<td>A BMI score of ≥25 and &lt;40 kg/m² identifies individuals who are overweight or obese, which is a risk factor for developing diabetes.</td>
</tr>
<tr>
<td>Prior use of acetaminophen</td>
<td>The pudding products contained acetaminophen to determine gastric emptying. Therefore, individuals needed to have used acetaminophen previously to minimize risk of allergy.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participant Exclusion Criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco use</td>
<td>Tobacco use is known to increase risk of diabetes, however, to minimize variability, only non-smoking individuals were included in the study.</td>
</tr>
<tr>
<td>Food allergies or any life threatening allergies</td>
<td>To maximize safety of participants, any individuals with food allergies or any life-threatening allergies were excluded.</td>
</tr>
<tr>
<td>Gastrointestinal conditions or illnesses</td>
<td>As study endpoints related to gastric emptying, any gastrointestinal conditions or disease were excluded to minimize variability in gastric emptying due to disease.</td>
</tr>
<tr>
<td>Serious major medical condition</td>
<td>To minimize variability in participants, any individuals known to have a serious major medical condition were excluded.</td>
</tr>
<tr>
<td>Pregnancy or breastfeeding</td>
<td>To maintain safety of participants and their children, any women known to be pregnant or were breastfeeding were excluded.</td>
</tr>
</tbody>
</table>
### Participant Exclusion Criteria (Continued)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Rationale (Continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food Neophobia Scale score of 30 to 54 (68)</td>
<td>The Food Neophobia Scale was a questionnaire intended to exclude individuals who are unsure of consuming new products. As secondary satiety endpoints not included in this Thesis were measured, individuals with a score of 30 to 54 were excluded, as they were less likely to consume new products.</td>
</tr>
<tr>
<td>TFEQ-CR score &gt;16 (69)</td>
<td>The TFEQ-CR was a questionnaire intended to exclude individuals who were likely to restrict their eating habits. As secondary satiety endpoints not included in this Thesis were measured, individuals who were likely to restrict their eating habits were excluded.</td>
</tr>
<tr>
<td>Alcohol consumption &gt;4 drinks/sitting</td>
<td>As high alcohol consumption could increase the risk of other diseases, including liver disease, participants with high daily alcohol consumption were excluded.</td>
</tr>
<tr>
<td>Medication or natural health products (NHPs) used for diabetes (glycemic control)</td>
<td>The study was seeking to eliminate confounding influences medication and NHP use may play on altering glycemic end points, therefore, medications and NHPs used for diabetes were excluded.</td>
</tr>
<tr>
<td>Medication or NHPs contraindicated with acetaminophen</td>
<td>As each study treatment contained acetaminophen, for the safety of each participant, any medications or NHPs known to be contraindicated with acetaminophen were exclusionary criterion.</td>
</tr>
<tr>
<td>Recent or intended significant weight loss or gain (i.e. &gt;4 kg in previous 3 months)</td>
<td>As weight loss/gain can alter metabolism, participants who had lost significant amounts of weight before the trial were excluded as study endpoints may have been altered. Individuals planning to gain/lose weight were also excluded to reduce variability between study visits in the 10 weeks.</td>
</tr>
</tbody>
</table>

### c. Participant Recruitment

Adult (18-70 years old) males and females were recruited from Guelph, Ontario, and the surrounding areas. The REB approved posters (see Appendix K) were placed around the Guelph Community in eye-catching locations (i.e. grocery stores, on campus, shopping malls, community centres, and downtown Guelph), in order to achieve the greatest amount of attention for the target audience. Study personnel also attended a Retire in Style event held at a local...
community centre to advertise the study and hand out posters. Ads were also placed in the Guelph Tribune, Guelph Mercury, and Wellington Advertiser newspapers, and online with Kijiji (www.kijiji.ca), and a radio ad was announced on the local Guelph radio station (CJOY-Magic FM), to reach a greater amount of individuals in the community. Finally, word of mouth from individuals calling in, through social media, and also through past participants in the HNRU, also effectively recruited interested individuals. On each poster or advertisement, individuals were asked to email the study email (pudding@uoguelph.ca), or call the laboratory phone number (519-824-4120 ext. 58081).

Summarized in Table 3 are the recruitment strategies used that effectively recruited the enrolled participants in the study. The most effective recruitment strategies included local newspaper advertisements, participation in the Retire in Style Event held at a local community centre, word of mouth from previous participants in the HNRU, and posters distributed in the Guelph community.

Table 3: Effective Recruitment Strategies for Enrolled Study Participants

<table>
<thead>
<tr>
<th>Recruitment Strategy/Location</th>
<th>Number of Enrolled Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newspaper Article – Guelph Tribune</td>
<td>4</td>
</tr>
<tr>
<td>Newspaper Article – Guelph Mercury</td>
<td>3</td>
</tr>
<tr>
<td>Retire in Style Event</td>
<td>2</td>
</tr>
<tr>
<td>Previous participant in HNRU (flyer/email)</td>
<td>2</td>
</tr>
<tr>
<td>Recruitment poster in community</td>
<td>2</td>
</tr>
<tr>
<td>Recruitment poster on campus</td>
<td>1</td>
</tr>
<tr>
<td>Advertisement on Kijiji</td>
<td>1</td>
</tr>
</tbody>
</table>
d. Study Screening Protocol

Eligibility of each participant was assessed in a 2-step process according to the inclusion and exclusion criterion. Screening-1 was a brief over the phone questionnaire which addressed major inclusion and exclusionary criteria, including: age, BMI, allergies, pregnancy/breastfeeding, diagnosis of diabetes, medication and NHP use, and acetaminophen consumption (see Appendix L). If individuals were eligible after Screening-1, they were invited to visit the HNRU, following a 10 to 12 hour fast, to complete a Screening-2 visit, which included signing 2 copies of the Screening-2 consent form (see Appendix M), longer eligibility questionnaire (Screening-2 Questionnaire) addressing the remaining questions not covered by Screening-1 (see Appendix N), the CANRISK questionnaire (Appendix H) (8), the Food Neophobia Score questionnaire (Appendix I) (68), the TFEQ-CR questionnaire (Appendix J) (69), and body measurements were taken in a private area by a trained study personnel according to Standard Operating Procedures of the HNRU. Body measurements at Screening-2 included height to the nearest millimeter using a stadiometer (Model 217, SECA®, Hanover, USA), and fasted body weight to the nearest 0.5 kg on a digital scale (SVI-200F, Acculab®, Barrie, Canada), to determine BMI, waist circumference with a measuring tape held around the waist at the belly button according to the CANRISK questionnaire (Appendix H) (Model 201, SECA®, Hanover, USA), and blood pressure and heart rate, in duplicate (Model HEM-907XL, Omron®, Kyoto, Japan). All measurements followed the REB Standard Operating Procedure (SOP) Number 013 for Anthropometric Measurements (70), aside from waist circumference which followed the instructions provided in the CANRISK questionnaire (Appendix H) (8).

e. Study Orientation

If participants were eligible after Screening-2, they were invited to a Study Orientation session, where they were provided detailed information about the study, and were able to try one of the pudding products. During the Study Orientation, study personnel provided each potential participant with the Pudding Study Handbook (see Appendix O for Summary of Pudding Study Handbook), which thoroughly detailed information about the study and participant instructions. Study personnel thoroughly reviewed the handbook with the participant. After going over the study handbook, if they were still interested in participating in the study, they were provided a pudding sample, in order to assess if they could consume the pudding treatment in its entirety within 10 minutes during the study visits. If participants were still interested in participating after trying the pudding treatment, they were asked to sign 2 copies of the study consent form (Appendix P), were provided with a copy, and were asked to complete their 3-day food records before the first study visit, which will be discussed below. For all participants entering the study after the orientation, a study binder was created, which included all screening documents, consent forms, study flowsheets, laboratory documents, satiety documents, and food records.

3. Study Treatments

The study treatments were in the form of puddings that were developed under the direction of Professor Doug Goff in the Department of Food Science, and PhD student Nikolay Repin, at the University of Guelph. There were a total of 8 pudding treatments (2 control, 2 YMM, 2 FG, and 2 FM puddings) and 2 control 50 g glucose beverage treatments (Trutol®, model 401272P, Fisher Scientific Company, Waltham, USA).

The 2 puddings for each treatment were matched for all ingredients, except the source of available carbohydrates (MTS and HMCS). The varying source of available carbohydrate was
intended to support the aims of another project. The purpose of this thesis was to compare the
different soluble DF types in each pudding to the control puddings without added soluble DF.

Summarized in Table 4 below is the extraction process used to isolate the YMM, FG, and
FM soluble DF from their respective seed products (see Appendix Q for all product
specifications/ingredient packaging for each fibre type before extraction, pudding ingredient, and
glucose beverage product).

**Table 4: Extraction Process for the Soluble Dietary Fibres (Yellow Mustard Mucilage, Fenugreek Gum, and Flaxseed Mucilage) from their Seed Products**

<table>
<thead>
<tr>
<th>Product Specification Used for Each Soluble Fibre Type</th>
<th>Extraction Process to Obtain Soluble Fibre Component of Seeds</th>
</tr>
</thead>
</table>
| G.S. Dunn Limited, Dry Mustard Millers – Yellow Mustard Bran (Product 402, G.S. Dunn Limited, Hamilton, Canada) | - Yellow Mustard Bran soaked in cold tap water at a 1:20 ratio at room temperature for 15 hours.  
- Product was then filtered through a strainer and the liquid extract was centrifuged at 10000 g, at 10 °C, for 60 minutes. The bran layer on top was then discarded.  
- The remaining liquid was then freeze-dried and then soaked in a food grade ethanol solution (68% volume for volume) at a concentration of 10 mL ethanol solution for 1 g yellow mustard mucilage for 15 hours at room temperature.  
- The remaining extract was then recovered and allowed to air-dry for 72 hours at room temperature. |
| Emerald Seed Products Limited - CANAFEN® Gum (Fenugreek Gum) (Product FGEN401, Emerald Seeds Products Limited, Avonlea, Canada) | - CANAFEN® Gum was stirred with cold tap water at a ratio of 1:200 at room temperature for 15 hours.  
- Product was centrifuged at 10000 g, at 22 °C, for 60 min and the top layer of product was removed.  
- The remaining liquid was freeze-dried. |
a. Pudding Ingredients and Preparation

All pudding treatments were prepared in the HNRU Sensory Kitchen following good manufacturing practices (GMPs). To maintain blinding, all pudding treatments were prepared by a trained third party. Summarized in Table 5 below is the ingredient composition for each pudding product. Each of the pudding study treatments were produced in an amount of 500 mL each. Before pudding preparation, the extracted fibres were left to hydrate overnight in 500 mL of water at room temperature, with constant stirring at 400 revolutions per minute (rpm) using an electrical mixer (Model RW 20 digital, IKA® Works Inc., Wilmington, USA). After overnight hydration, to allow for complete solubilisation of the fibres, the YMM and FM solutions were heated to 80 °C for another 2 hours, and the FG solution was heated to 90 °C for another 3 hours, all with constant stirring at 1200 rpm with the same electrical mixer. The solution was then maintained at 80 °C and soy protein isolate (Product 2156, Now Foods, Bloomingdale, USA)
dissolved into the solution for 30 minutes with constant stirring at 1200 rpm, at the varying levels described in Table 5. At the same temperature of 80 °C, 9.80 g gelatine (Product Knox Kraft Foods, Northfield, USA) was then dissolved into the solution for 15 minutes with constant stirring at 1200 rpm. The solution was then allowed to cool at room temperature with constant stirring at 1200 rpm, until it reached 60 °C. After cooling, depending on the treatment, either 37.65g of MTS (Product TExTRA®PLUS (32596302), Batch BD 7509, Ingredion, Brampton, Canada), or 41.03 g of HMCS (Product 01550 High Maltose Corn Syrup, Batch 2203288124, Ingredion Canada Incorporated, Cardinal, Canada), both providing 35 g of dry matter, was dissolved in the mixture for 30 minutes with constant stirring at 1200 rpm. At the same temperature of 60 °C, 19.09 g of chocolate powder (NESQUIK 33% Less Sugar Powder, Nestlé, Halifax, Canada) was then added to the solution, and dissolved for 10 minutes with constant stirring at 1200 rpm. The chocolate powder and either the tapioca starch or high maltose corn syrup, depending on the treatment, resulted in 50 g of available carbohydrate in the final pudding products. After the addition of the chocolate powder, the solution was left at room temperature to cool down to 30 °C, and a mixture of acetaminophen (1500 mg) (Product Tylenol Extra Strength Caplets, DIN 00723908, McNeil Consumer Healthcare, Markham, Canada) and 20 mL of room temperature water previously boiled, was added to the solution to dissolve for 10 minutes with constant stirring at 1200 rpm. The finished pudding solution was then placed into a measuring cup and previously boiled, room temperature water was added to the measuring cup to obtain a volume of 500 mL. The pudding solution and added water were stirred together at room temperature for 5 minutes at 1200 rpm, to achieve homogeneity of the solution, and the contents of the measuring cup were poured into a white serving bowl. The bowl was then placed into the refrigerator, maintained at 4 °C, for a minimum of 24 hours (1 day), and a maximum of 72 hours
(3 days). It was determined that rheological characteristics of each of the pudding treatments at day 1 and day 3 were similar using an ARES Rheometer (TA Instruments, Mississauga, Canada) operating at 37 °C, and microbiological tests of total aerobic bacteria and enterobacteriaceae count of the puddings after 72 hours were safe for human consumption (analyzed by the laboratory of Dr. Keith Warriner of the Department of Food Science, University of Guelph, Guelph, Canada, on December 4th, 2014).
Table 5: Ingredient Composition for Pudding Treatments Standardized at a Volume of 500 mL

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>YMM-MTS</th>
<th>YMM-HMCS</th>
<th>FG-MTS</th>
<th>FG-HMCS</th>
<th>FM-MTS</th>
<th>FM-HMCS</th>
<th>Control-MTS</th>
<th>Control-HMCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Extracted Yellow Mustard Mucilage (g)</td>
<td>23.05</td>
<td>23.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2Extracted Fenugreek Gum (g)</td>
<td>0</td>
<td>0</td>
<td>7.38</td>
<td>7.38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3Extracted Flaxseed Mucilage (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17.80</td>
<td>17.80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4Soy Protein Isolate (g)</td>
<td>0</td>
<td>0</td>
<td>2.16</td>
<td>2.16</td>
<td>0.34</td>
<td>0.34</td>
<td>2.32</td>
<td>2.32</td>
</tr>
<tr>
<td>5Gelatine (g)</td>
<td>9.80</td>
<td>9.80</td>
<td>9.80</td>
<td>9.80</td>
<td>9.80</td>
<td>9.80</td>
<td>9.80</td>
<td>9.80</td>
</tr>
<tr>
<td>6Modified Tapioca Starch (g)</td>
<td>37.65</td>
<td>0</td>
<td>37.65</td>
<td>0</td>
<td>37.65</td>
<td>0</td>
<td>37.65</td>
<td>0</td>
</tr>
<tr>
<td>7High Maltose Corn Syrup (g)</td>
<td>0</td>
<td>41.03</td>
<td>0</td>
<td>41.03</td>
<td>0</td>
<td>41.03</td>
<td>0</td>
<td>41.03</td>
</tr>
<tr>
<td>8Chocolate Powder (g)</td>
<td>19.09</td>
<td>19.09</td>
<td>19.09</td>
<td>19.09</td>
<td>19.09</td>
<td>19.09</td>
<td>19.09</td>
<td>19.09</td>
</tr>
<tr>
<td>9Acetaminophen (mg)</td>
<td>1500.0</td>
<td>1500.0</td>
<td>1500.0</td>
<td>1500.0</td>
<td>1500.0</td>
<td>1500.0</td>
<td>1500.0</td>
<td>1500.0</td>
</tr>
</tbody>
</table>

Abbreviations used: YMM-MTS=Yellow mustard mucilage - modified tapioca starch pudding; YMM-HMCS=Yellow mustard mucilage - high maltose corn syrup pudding; FG-MTS=Fenugreek gum - modified tapioca starch pudding; FG-HMCS=Fenugreek gum - high maltose corn syrup pudding; FM-MTS=Flaxseed mucilage - modified tapioca starch pudding; FM-HMCS=Flaxseed mucilage - high maltose corn syrup pudding; Control-MTS=Control - modified tapioca starch pudding; Control-HMCS=Control - high maltose corn syrup pudding.

1Yellow Mustard Bran (Product 402, G.S. Dunn Limited, Hamilton, Canada).
2CANAFEN® Gum (Fenugreek Gum) (Product FGEN401, Emerald Seeds Products Limited, Avonlea, Canada).
3Flax Hull Lignans (Product Flax Hull Lignans, Natunola® Health Inc., Winchester, Canada).
4Soy protein isolate (Product 2156, Now Foods, Bloomingdale, USA).
5Gelatine (Product Gelatine, Knox Kraft Foods, Northfield, USA).
6MTS (Product TEXTRA®PLUS (32596302), Ingredion, Brampton, Canada).
7HMCS (Product 01550, Ingredion Canada Incorporated, Cardinal, Canada).
8Chocolate powder (NESQUIK 33% Less Sugar Powder, Nestlé, Halifax, Canada).
9Acetaminophen (Tylenol Extra Strength Caplets, DIN 00723908, McNeil Consumer Healthcare, Markham, Canada).
b. Glucose Beverage Ingredients and Preparation

To maintain a standardized volume of liquid between the pudding products and the glucose beverage study visits, a total volume of 500 mL was consumed on study visits with the glucose beverage as well. In order to standardize the volume, the 50 g glucose beverage (Trutol®, model 401272P, Fisher Scientific Company, Waltham, USA), which was a total of 296 mL, and 204 mL of water (Nestlé Pure Life, Nestlé, Halifax, Canada), which was poured into an identical glass for each participant and weighed on an electronic scale (Model S-8001, Denver Instrument, Bohemia, USA), was consumed at each glucose beverage study visit.

Acetaminophen (1500 mg) (Tylenol Extra Strength Caplets, DIN 00723908, McNeil Consumer Healthcare, Markham, Canada) was added to the glucose beverage solution in the morning of each study visit, and the bottle was capped and shaken for a minimum of 10 minutes until the 3 tablets were dissolved.

c. Pudding Nutrient and Physical Composition

Summarized in Table 6 is the nutrient composition for carbohydrate, protein, fat, and fibre, for each pudding product. Nutrient composition of each pudding product were matched according to calories, carbohydrate, protein, and fat concentration, and were all based on a serving size of 500 mL. As puddings were matched based on rheological testing for viscosity, the fibre amounts in each pudding product varied. Experiments in simulated intestinal conditions were conducted to determine the concentration of each DF which would match the in vitro viscosity obtained by the specific amount of β-glucan in the EFSA health claim for β-glucan and glycemic response (61). Therefore, each of the soluble DF concentrations for YMM, FG, and FM, were compared to oat gum containing 6.7 g of β-glucan, and the soluble DF concentration of each fibre was chosen when each gum resulted in similar apparent viscosities (at 60 s⁻¹), using
an ARES Rheometer (TA Instruments, Mississauga, Canada). In vitro viscosity determinations and rheological analyses (60) were made by PhD student Nikolay Repin under the supervision of Professor Douglas Goff, in the Department of Food Science. Based on the initial testing, concentrations of YMM, FG, and FM to achieve equivalent in vitro apparent viscosities to 6.7 g of β-glucan, resulted in very low levels of soluble DF, which were 3.8, 2.2, and 4.8 g of YMM, FG, and FM, respectively, per 500 mL of treatment. Therefore, the concentration was increased by 3 times to 15.5, 5.9, and 11.4 g of YMM, FG, and FM, respectively, per 500 mL of treatment.

Carbohydrate content was matched based on 50 g available carbohydrate, which was in the form of either tapioca starch or high maltose corn syrup, and the sugar content in the chocolate powder. As a secondary purpose of the broader research project related to comparing impacts of the source of available carbohydrates on glucose release and uptake, all pudding products were formulated to contain 50 g of available carbohydrate, with 70% of the carbohydrates coming from either HMCS, a simple carbohydrate, or MTS, a complex starch carbohydrate. The expectation was that the latter would take longer to enzymatically digest, and rationalized in the context that soluble DF would slow down starch breakdown (60).

Carbohydrate content of the extracted YMM, FG, and FM, were considered negligible, except for the SF component, which had no contribution to energy. Protein and ash analysis’ were carried out for each of the extracted YMM, FG, and FM, fibres by Nikolay Repin, using standardized American Association for Clinical Chemistry international methods 46-30.01 and 08-01.01 (71-72). Based on the protein and ash content of each SF, soy protein isolate was added to match each pudding for protein content. As the ethanol treatment during the extraction procedure removed any fat in the YMM and FM treatments, the level of fat in both extracted fibres were considered to be negligible. As per the product specifications for FG, fat content was
determined to be less than 1% on a dry weight basis, and negligible as well. SF concentrations of each extracted fibre were calculated by subtracting the protein and ash content from the weight of the freeze dried fibre mass. Protein, fat, carbohydrate, and fibre content of other ingredients were taken from the Nutrition Fact Panels from each label.
Table 6: Nutritional Composition of the Pudding Treatments Standardized at a Volume of 500 mL

<table>
<thead>
<tr>
<th></th>
<th>YMM-MTS</th>
<th>YMM-HMCS</th>
<th>FG-MTS</th>
<th>FG-HMCS</th>
<th>FM-MTS</th>
<th>FM-HMCS</th>
<th>Control-MTS</th>
<th>Control-HMCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>286</td>
<td>286</td>
<td>286</td>
<td>286</td>
<td>286</td>
<td>286</td>
<td>286</td>
<td>286</td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>66.9</td>
<td>66.9</td>
<td>57.2</td>
<td>57.2</td>
<td>62.8</td>
<td>62.8</td>
<td>51.4</td>
<td>51.4</td>
</tr>
<tr>
<td>Available Carbohydrates (g)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Total Dietary Fibre (g)</td>
<td>16.9</td>
<td>16.9</td>
<td>7.2</td>
<td>7.2</td>
<td>12.8</td>
<td>12.8</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Soluble Fibre from Extracted Fibres (g)</td>
<td>15.5</td>
<td>15.5</td>
<td>5.9</td>
<td>5.9</td>
<td>11.4</td>
<td>11.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Abbreviations used: YMM-MTS=Yellow mustard mucilage - modified tapioca starch pudding; YMM-HMCS=Yellow mustard mucilage - high maltose corn syrup pudding; FG-MTS=Fenugreek gum - modified tapioca starch pudding; FG-HMCS=Fenugreek gum - high maltose corn syrup pudding; FM-MTS=Flaxseed mucilage - modified tapioca starch pudding; FM-HMCS=Flaxseed mucilage - high maltose corn syrup pudding; Control-MTS=Control - modified tapioca starch pudding; Control-HMCS=Control - high maltose corn syrup pudding.
4. Treatment Randomization and Blinding

After the orientation session, participants were assigned a participant identification number, in numerical order from 1 to 16. Each participant identification number was randomized to the different pudding treatments by Nikolay Repin on June 18th, 2014, based on random orders being generated from Research Randomizer (www.randomizer.org), where 16 randomly generated orders for 8 treatments (numbered 1, 2, 3, 4, 5, 6, 7, 8) were requested (73). Study visits 1 and 10 were separately set, as participants consumed the 50 g glucose beverage and water control on both the first and last visit. Treatment codes were randomly given to each pudding and glucose beverage by Nikolay Repin on June 18th, 2014, to maintain double blinding in both participants and study personnel directly involved with the clinical trial. Table 7 shows the treatment codes and corresponding treatment products which were used during the study period. All participants and study team members were blinded for the duration of the data collection and analysis.
Table 7: Treatment Codes and Corresponding Treatment Products

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment Abbreviation</th>
<th>Treatment Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (Visit 1) – 50 g Glucose Beverage with 204 mL Water</td>
<td>Glucose (Visit 1)</td>
<td>305</td>
</tr>
<tr>
<td>Glucose (Visit 10) – 50 g Glucose Beverage with 204 mL Water</td>
<td>Glucose (Visit 10)</td>
<td>603</td>
</tr>
<tr>
<td>Yellow Mustard Mucilage – Modified Tapioca Starch</td>
<td>YMM-MTS</td>
<td>235</td>
</tr>
<tr>
<td>Yellow Mustard Mucilage – High Maltose Corn Syrup</td>
<td>YMM-HMCS</td>
<td>683</td>
</tr>
<tr>
<td>Fenugreek Gum – Modified Tapioca Starch</td>
<td>FG-MTS</td>
<td>890</td>
</tr>
<tr>
<td>Fenugreek Gum – High Maltose Corn Syrup</td>
<td>FG-HMCS</td>
<td>201</td>
</tr>
<tr>
<td>Flaxseed Mucilage – Modified Tapioca Starch</td>
<td>FM-MTS</td>
<td>460</td>
</tr>
<tr>
<td>Flaxseed Mucilage – High Maltose Corn Syrup</td>
<td>FM-HMCS</td>
<td>389</td>
</tr>
<tr>
<td>Control – Modified Tapioca Starch</td>
<td>Control-MTS</td>
<td>763</td>
</tr>
<tr>
<td>Control – High Maltose Corn Syrup</td>
<td>Control-HMCS</td>
<td>694</td>
</tr>
</tbody>
</table>

5. Study Protocol and Study Visit Data Collection

a. Study Protocol

Participants came to the HNRU after following a 10 to 12 hour fast, during which they were allowed to consume only water. Although water was encouraged, participants were asked to consume no more than 1 cup of water within the 1 hour prior to the morning study visit. Participants were also instructed to avoid alcohol, strenuous activity, and over-the-counter medication (especially acetaminophen) for 24 hours before each study visit. Participants were also asked to consume a similar dinner to the dinner before their first study visit on each subsequent occasion. Before the first study visit, participants were asked to record their dinner
meal in their Pudding Study Handbook, which they were asked to refer back to each week to maintain accuracy of the dinner meal consumed before each visit. Also, participants were reminded to maintain their usual lifestyle, dietary, and exercise habits at the end of every study visit. This also included maintaining their medication and NHP usage, and participants were asked to report any changes.

b. Study Visit Data Collection

Outcome measures, including anthropometric measurements, blood samples, and satiety measurements (not discussed in this Thesis), were taken at each study visit 1 through 10. Each participant was also asked to complete a 3-day food record before the first study visit. Table 8 shows all study visit measures that will be discussed in this thesis.
### Table 8: Summarization of Study Measures

<table>
<thead>
<tr>
<th>Anthropometric Study Measures</th>
<th>Materials Used</th>
<th>Timing of Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted Body Weight (kg)</td>
<td>Digital Scale (SVI-200F, Acculab®, Barrie, Canada)</td>
<td>Study Visits 1-10</td>
</tr>
<tr>
<td>Blood Pressure (Systolic/Diastolic) (mmHg)</td>
<td>Digital Blood Pressure Monitor (Model HEM-907XL, Omron®, Kyoto, Japan)</td>
<td>Study Visits 1-10</td>
</tr>
<tr>
<td>Heart Rate (beats per minute (bpm))</td>
<td>Digital Blood Pressure Monitor (Model HEM-907XL, Omron®, Kyoto, Japan)</td>
<td>Study Visits 1-10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Study Measures</th>
<th>Materials Used</th>
<th>Timing of Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (mmol/L)</td>
<td>HemoCue® 201 Glucose Reader (HemoCue®, Ängelholm, Sweden) HemoCue® Microcuvettes (Model HEM-110705, HemoCue®, Ängelholm, Sweden)</td>
<td>Study Visits 1-10, at 0, 15, 30, 60, 90, and 120 minutes</td>
</tr>
<tr>
<td>Plasma Insulin (µIU/ml)</td>
<td>Enzyme-Linked Immunosorbent Assay (ELISA) (Model 80-INSHU-E10.1, ALPCO, Salem, USA)</td>
<td>Study Visits 1-10, at 0, 15, 30, 60, 90, and 120 minutes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food Record Study Measures</th>
<th>Materials Used</th>
<th>Timing of Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Day Food Records</td>
<td>ESHA The Food Processor SQL (Version 10.13.1, Salem, USA)</td>
<td>3 Days Prior to Study Visit 1</td>
</tr>
</tbody>
</table>

#### b.i. Study Visit Anthropometric Measurements

Fasted body weight, blood pressure, and heart rate were measured at every study visit the same way as during the Screening-2 visit, as per the REB SOP number 013 (70). All anthropometric measurements were taken by a trained study coordinator in a private area.

#### b.ii. Study Visit Blood Collection and Analysis

Blood was collected by finger prick before (fasted) and after (at 15, 30, 60, 90, and 120 minutes) consumption of the study treatment based on the University of Guelph REB SOP number 011 (74). Before every finger prick blood sample, participants warmed their hands in a
heating pad for a minimum of 5 minutes (Model 731AO-CN, Sunbeam®, Brampton, Ontario).

A tourniquet was then placed tightly on the wrist about 1.5 minutes before each blood sample, and the participant was asked to lower their hand to maximize blood pooling in the fingers. Participants were also asked to open and close their hands lightly about 45 seconds before each blood sample to improve blood pooling. Qualified study personnel put on gloves, and an alcohol wipe (Model SC06450, Safecross First Aid Ltd, Toronto, Canada) was used about 30 seconds before each blood sample to clean the finger and was allowed to air dry. The hand was massaged gently before finger pricking, and pressure was placed on the desired finger during finger pricking, on the side of the finger, with the blade perpendicular to the lines of the finger print, with a BD Microtainer® Contact-Activated Lancet (1.5 mm width x 2.0 mm depth) (Model 366594, BD®, Franklin Lakes, USA). Light pressure was applied to the hand and finger after pricking to create blood drops which were collected into a 500 µL BD Microtainer® MAP Microtube for Automated Process 1.0 mg potassium ethylene diamine tetraacetic acid (K₂EDTA) additive tube (Model 363706, BD®, Franklin Lakes, USA) until the desired 500 µL of blood was collected. After the desired amount of blood was collected, the tourniquet was removed, and non-woven gauze (Model A2103-CH, AMD – RITMED Inc., Lachine, Canada), was applied to the finger prick to allow for adequate blood clotting for a band aid (Model 15210EMD, Derma Sciences Inc., Houston, USA) to be applied.

After each blood collection, the microtainer tubes were handed to another study team member to deliver to the laboratory. The microtainer tubes were slowly inverted 8 times, as per manufacturing instructions, and 5 µL whole blood samples in duplicate were immediately pipetted onto a hydrophobic surface (Parafilm M™) (Model PM-996, Pechiney Plastic Packaging, Menasha, USA) to be drawn up into two HemoCue® microcuvettes.
(Model HEM-110705, HemoCue®, Ängelholm, Sweden) for glucose analysis with the
HemoCue® 201 glucose reader (HemoCue®, Ängelholm, Sweden). After glucose analysis, the
tube was closed and immediately centrifuged at 2000 relative centrifugal force (RCF) (g) and
21°C for 3 minutes (Model Allegra™ X-22R Centrifuge, Beckman Coulter®, Mississauga,
Canada) and blood plasma was then pipetted into 1.2 mL Corning Cryovials (Model C430658,
Corning Inc., Corning, USA) stored at -80°C until analysis. Summarized in Table 9 are the
blood sample collection, processing, and storage instructions for blood glucose analysis, and
plasma storage.

Table 9: Blood Sample Collection, Processing, and Storage Instructions

<table>
<thead>
<tr>
<th>Study Measure</th>
<th>Time Point</th>
<th>Type of Blood Collection Tube</th>
<th>Blood Sample Collection, Processing, and Storage Instructions</th>
</tr>
</thead>
</table>
| Blood Glucose | 0, 15, 30, 60, 90, 120 minutes | 500 µL K₂EDTA microtainer microtube¹ | - Collect blood into microtube and gently invert 8 times.  
  - Using a pipette, transfer two 5 µL drops of whole blood onto a hydrophobic surface² and immediately close microtube.  
  - Immediately draw up the two drops of blood with two microcuvettes³, and immediately place into the glucose⁴ reader for glucose analysis. |
| Plasma Insulin | 0, 15, 30, 60, 90, 120 minutes | 500 µL K₂EDTA microtainer microtube¹ | - Immediately after glucose analysis, store microtube in refrigerator until centrifuging, or centrifuge⁵ immediately for 3 minutes at 2000 g, at 21 °C.  
  - Aliquot plasma (minimum 50 µL) into cryovials⁶ and store at -80 °C until analysis. |

¹500 µL BD Microtainer® MAP Microtube for Automated Process 1.0 mg K₂EDTA additive tube (Model 363706, BD®, Franklin Lakes, USA).  
²Parafilm M (Model PM-996, Pechiney Plastic Packaging, Menasha, USA).  
³HemoCue® microcuvettes (Model HEM-110705, HemoCue®, Ängelholm, Sweden).  
⁴HemoCue® 201 glucose reader (HemoCue®, Ängelholm, Sweden).  
⁵Centrifuge (Model Allegra™ X-22R Centrifuge, Beckman Coulter®, Mississauga, Canada).  
⁶1.2 mL Corning Cryovials (Model C430658, Corning Inc., Corning, USA).
Plasma insulin was analyzed in duplicate using an enzyme-linked immunosorbent assay (ELISA) (Model 80-INSHU-E10.1, Lot 03974, ALPCO, Salem, USA) according to the manufacturers’ protocols. The limit of sensitivity for this assay was 0.399 µIU/ml insulin with normal ranges between 5 to 25 µIU/ml insulin. In total, 24 plates were utilized, which had an inter-assay variability for the controls of 10.73%, and an intra-assay variability of all duplicated samples of 7.53%. Finally, fasting insulin resistance (HOMA-IR) was calculated using fasting blood glucose and plasma insulin data and the homeostasis model assessment (version 2.2.3, Headington, UK) (75).

b.iii. Pre-Study Visit Food Records

Participants were asked to complete an initial 3-day food record between their Study Orientation session and their first study visit day. The 3-day food record was completed for two weekdays and 1 weekend day, and participants were given detailed instructions (both written and image examples) to provide as accurate as possible records (see Appendix R). Participants were also asked to try to plan to record their food intake on days that would typically mimic their usual diet. Participants were provided with blank food records in their Pudding Study Handbook which allowed them to write the time of the food consumption, the type of food consumed, the amount, and any food descriptions or preparation methods. 3-day food records were used to provide an overview of a typical diet for each participant. All food record data was entered individually for each day, and as an average of all three days, into ESHA The Food Processor SQL (Version 10.13.1, Salem, USA).
6. Data and Statistical Analysis

Statistical analysis was performed using the Statistical Analysis Software (Version 9.4, Cary, USA) with significance considered when $P \leq 0.05$. All data was examined for normal distribution using the univariate procedure, with examination of stem leaf diagrams and box plots. It was determined that insulin data were not normally distributed and were therefore natural log transformed before statistical analysis.

Since the statistical analysis revealed no significant differences in glucose or insulin between the two types of available carbohydrate (HMCS and MTS) for each pudding treatment (YMM, FG, FM, control), the HMCS and MTS puddings for each treatment type were combined. In addition, since the purpose of this thesis was to compare the puddings with different soluble DF (YMM, FG, FM, control), the two glucose beverage treatments were analyzed in combination to characterize the participants, and were excluded from treatment comparisons.

Participant anthropometric data (body weight, blood pressure, heart rate) were compared among the treatments using repeated measures analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons.

Fasting blood glucose, and natural log-transformed fasting plasma insulin were compared between pudding treatments using ANOVA followed by the Tukey’s test for multiple comparisons.

Postprandial blood glucose and plasma insulin were compared among pudding treatments using ANOVA, controlling for treatment and time to test for a treatment by time interaction. Since there was a significant treatment by time interaction for both glucose and insulin, pudding
treatments were compared at each time point separately using ANOVA followed by Tukey’s test for multiple comparisons.

Postprandial blood glucose and plasma insulin were analyzed for 2-hour iAUC, peak concentration (Cmax), and time of peak concentration (Tmax), using GraphPad Prism (Version 6.07, La Jolla, USA). Blood glucose and plasma insulin 2-hour iAUC and Cmax were then compared among pudding treatments using ANOVA followed by Tukey’s test. Blood glucose and plasma insulin Tmax were compared among grouped pudding treatments using Chi square analysis.

RESULTS

1. Participant Flow

Participant flow through Screening-1, Screening-2, Study Orientation, randomization, and study analysis is summarized in Figure 2. In total, 249 people were screened for eligibility at Screening-1 of which 41 were eligible to proceed to Screening-2. Of the eligible Screening-2 participants, 23 individuals were not eligible to continue, leaving 18 people who progressed to the Study Orientation. At that point, 2 individuals did not progress to randomization, as one person did not want to consume the pudding products and another lost interest. In total, 16 participants were enrolled into the study and randomized to the 8 pudding treatments, with the glucose beverage visits scheduled for the first and last study visit days to ensure sufficient spread. One person was subsequently removed from the study, as they were consistently not complying with pre-study visit protocols. Therefore, data and statistical analysis was performed on data from the 15 participants who completed all 10 study visits.
2. Participant Characteristics and Baseline Information

   a. Participant Characteristics

   Participant characteristics are summarized in Table 10. Overall, 2/3 of the participant sample was male (n=10), and 1/3 were female (n=5), which may have been due to males being at greater risk for developing diabetes based on the CANRISK questionnaire. Participants, on average, were 55.1 years old, with a height of 1.7 m, body weight of 87.6 kg, and BMI of 29.5 kg/m². Their waist circumference, which was measured for the CANRISK questionnaire, was on average, 103.7 cm. On average, participants were considered hypertensive, with systolic and
diastolic blood pressures of 148.4 and 90.9 mmHg, respectively (76). Prescription medication and NHP use was minimal, with an average of 1.5 prescription medications, and 3 NHPs reported. All participants were at risk for T2D, with an average CANRISK score of 27.7, indicating a moderate risk of developing T2D, based on the score range of 21 to 32 in the CANRISK scoring system (8). In general, 12 of the 15 participants fell in the moderate risk of developing T2D on the CANRISK questionnaire, with their scores between 21 and 32. Three participants were at a high risk of developing T2D on the CANRISK questionnaire, with a CANRISK score greater than 33 (8). Finally, on average, participants had a HOMA-IR value of 1.2, as a measure of insulin resistance, which was within normal ranges for HOMA-IR as it was under the cut off value of 2.6 (77).
Table 10: Participant Characteristics (n=15)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)(^1)</td>
<td>10 male / 5 female</td>
</tr>
<tr>
<td>Age (years)(^1)</td>
<td>55.1 ± 12.0</td>
</tr>
<tr>
<td>Height (m)(^1)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Body Weight (kg)(^1)</td>
<td>87.6 ± 17.4</td>
</tr>
<tr>
<td>BMI (kg/m(^2))(^1)</td>
<td>29.5 ± 3.4</td>
</tr>
<tr>
<td>Waist Circumference (cm)(^1)</td>
<td>103.7 ± 13.3</td>
</tr>
<tr>
<td>Blood Pressure (Systolic) (mmHg)(^1)</td>
<td>148.4 ± 14.8</td>
</tr>
<tr>
<td>Blood Pressure (Diastolic) (mmHg)(^1)</td>
<td>90.9 ± 12.4</td>
</tr>
<tr>
<td>Prescription Medication Use (n)(^1)</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>Natural Health Product Use (n)(^1)</td>
<td>3.0 ± 2.3</td>
</tr>
<tr>
<td>CANRISK Score(^1)</td>
<td>27.7 ± 5.9</td>
</tr>
<tr>
<td>HOMA-IR(^2)</td>
<td>1.2 ± 0.8</td>
</tr>
</tbody>
</table>

Data are means ± SD, except for Sex which is n.
\(^1\)Data from Screening-2 Visit.
\(^2\)Data from First Study Visit (Glucose Beverage).
Abbreviations used: BMI=Body mass index; CANRISK=Canadian diabetes risk assessment questionnaire.

b. Baseline Energy, Macronutrient, and Dietary Fibre Intakes

Table 11 shows the average 3-day food record data for energy, macronutrient, and dietary fibre intakes at baseline. Overall, average intakes were 2293.0 kcal, 90.8 g of protein, 92.9 g of fat, 31.7 g of saturated fat, 1.6 g of trans fat, 264.3 g of carbohydrate, and 21.6 g of DF.
Table 11: 3-Day Food Record Average Energy, Macronutrient, and Dietary Fibre Intakes Before Study Period (n=15)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>3-Day Food Record Averages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2293.0 ± 220.0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>90.8 ± 9.3</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>92.9 ± 24.0</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>31.7 ± 8.2</td>
</tr>
<tr>
<td>Trans Fat (g)</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>264.3 ± 28.4</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>21.6 ± 2.5</td>
</tr>
</tbody>
</table>

Data are means ± standard error (SE).

c. Participants Anthropometric Characteristics During the Study

Summarized in Table 12 are the participants’ body weight, systolic blood pressure, diastolic blood pressure, and heart rate during the study. Body weight, systolic blood pressure, diastolic blood pressure, and heart rate did not significantly change during the study.
Table 12: Participant Characteristics During the First and Last Study Visit (Glucose Beverage Treatments), and During the Randomized Pudding Study Visits (n=15)¹

<table>
<thead>
<tr>
<th>Study Treatment²</th>
<th>Body Weight (kg)</th>
<th>Systolic Blood Pressure (mmHg)</th>
<th>Diastolic Blood Pressure (mmHg)</th>
<th>Heart Rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>87.9 ± 4.6</td>
<td>135.1 ± 5.2</td>
<td>87.4 ± 3.0</td>
<td>74.5 ± 5.6</td>
</tr>
<tr>
<td>YMM</td>
<td>88.6 ± 4.7</td>
<td>140.0 ± 5.6</td>
<td>87.3 ± 2.8</td>
<td>70.8 ± 2.9</td>
</tr>
<tr>
<td>FG</td>
<td>88.5 ± 4.7</td>
<td>138.6 ± 5.5</td>
<td>88.3 ± 2.9</td>
<td>71.0 ± 2.7</td>
</tr>
<tr>
<td>FM</td>
<td>88.2 ± 4.6</td>
<td>136.4 ± 5.2</td>
<td>86.8 ± 2.8</td>
<td>71.9 ± 2.4</td>
</tr>
<tr>
<td>Control</td>
<td>88.3 ± 4.7</td>
<td>138.0 ± 5.1</td>
<td>87.0 ± 3.2</td>
<td>72.3 ± 3.1</td>
</tr>
</tbody>
</table>

¹Data are means ± SE.

²Glucose beverage treatments, and both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.

Abbreviations used: bpm=Beats per minute; YMM=Yellow mustard mucilage treatments; FG=Fenugreek gum treatments; FM=Flaxseed mucilage treatments.

d. Baseline Glycemic Response Biomarkers

Participant fasting blood glucose and plasma insulin concentrations did not significantly differ among the glucose or pudding treatments, as summarized in Table 13. The Canadian Diabetes Association 2013 Clinical Practice Guidelines for the Diagnosis of Prediabetes and Diabetes classifies a normal fasting blood glucose as <5.6 mmol/L. Therefore, with an average fasting blood glucose value of 5.5 mmol/L, the study participants would be classified as having normal fasting blood glucose, although they were at a moderate risk of developing T2D according to the CANRISK questionnaire (9).
Table 13: Fasting Blood Glucose and Fasting Plasma Insulin During the Study (n=15)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fasting Blood Glucose&lt;sup&gt;2&lt;/sup&gt; (mmol/L)</th>
<th>Fasting Plasma Insulin&lt;sup&gt;3&lt;/sup&gt; (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5.6 ± 0.1</td>
<td>41.4 (30.6, 55.8)</td>
</tr>
<tr>
<td>YMM</td>
<td>5.5 ± 0.1</td>
<td>43.2 (31.8, 58.2)</td>
</tr>
<tr>
<td>FG</td>
<td>5.5 ± 0.1</td>
<td>42.0 (30.6, 58.2)</td>
</tr>
<tr>
<td>FM</td>
<td>5.5 ± 0.1</td>
<td>40.2 (28.2, 56.4)</td>
</tr>
<tr>
<td>Control</td>
<td>5.4 ± 0.1</td>
<td>38.4 (27.6, 52.8)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Glucose beverage treatments, and both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.  
<sup>2</sup>Data are means ± SE.  
<sup>3</sup>Data are geometric means (95% confidence intervals).  
Abbreviations used: YMM=Yellow mustard mucilage treatments; FG=Fenugreek gum treatments; FM=Flaxseed mucilage treatments.

Data for the glucose beverage treatments is summarized in Table 14, Figure 3 and Appendix S. On average, both blood glucose and plasma insulin generally increased from baseline and peaked at either 30 or 60 minutes, then returned to be not significantly different from baseline at 120 minutes. On average, the Cmax was 11.02 mmol/L for blood glucose and 340.8 pmol/L for plasma insulin. Tmax primarily occurred at 30 (63.3 % of participants) and 60 minutes (34.0 % of participants) for blood glucose, and at 30 (56.7 % of participants) and 60 minutes (28.0 % of participants) for plasma insulin. For blood glucose, Tmax occurred at 15 minutes for only 0.7 % of participants and at 90 minutes for 2.0 % of participants. For plasma insulin, Tmax occurred at 15 minutes for only 6.0 % of participants, at 90 minutes for only 8.7 % of participants, and at 120 minutes for only 0.7 % of participants. Of note is that these treatments closely mimicked a standard OGTT, except 50 g glucose was used, as opposed to the standard 75 g glucose in an OGTT.
Table 14: Fasting and Postprandial Blood Glucose and Plasma Insulin for the Glucose Beverage Treatment (n=15)¹

<table>
<thead>
<tr>
<th>Time Point (Minutes)</th>
<th>Blood Glucose (mmol/L)²</th>
<th>Plasma Insulin (pmol/L)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.58 ± 0.12</td>
<td>41.4 (30.6, 55.8)</td>
</tr>
<tr>
<td>15</td>
<td>7.96 ± 0.21</td>
<td>219.6 (166.2, 290.4)</td>
</tr>
<tr>
<td>30</td>
<td>10.29 ± 0.31</td>
<td>340.8 (258.0, 450.6)</td>
</tr>
<tr>
<td>60</td>
<td>9.93 ± 0.52</td>
<td>258.0 (190.8, 306.0)</td>
</tr>
<tr>
<td>90</td>
<td>8.43 ± 0.49</td>
<td>176.4 (114.6, 270.6)</td>
</tr>
<tr>
<td>120</td>
<td>6.52 ± 0.39</td>
<td>77.4 (49.2, 121.8)</td>
</tr>
</tbody>
</table>

¹Glucose beverage treatments were averaged since their results did not significantly differ.
²Data are means ± SE.
³Data are geometric means (95% confidence intervals).

Figure 3: Postprandial Blood Glucose (a), and Plasma Insulin Response (b) for Glucose Beverage Treatment (n=15)
Glucose beverage treatments were averaged since their results did not significantly differ.
Data are means ± SE for blood glucose (a).
Data are geometric means (95% confidence intervals) for plasma insulin (b).
3. Postprandial Glycemic Response Biomarkers

a. Postprandial Blood Glucose

The postprandial blood glucose time curves for each pudding treatment are summarized in Appendix T (comparison of time points within each treatment) and in Figure 4 (comparison of fibre treatments to control at each time point). For all study treatments, blood glucose generally increased from baseline and peaked at either 30 or 60 minutes, then returned to be not significantly different from baseline at 120 minutes (Appendix T). There was a statistically significant time by treatment interaction for blood glucose which justified a treatment comparison within each time point separately (Figure 4). At 30 minutes, blood glucose was significantly lower with consumption of all soluble DF treatments (YMM, FG, FM) when compared to the control treatment (P<0.005). At 90 and 120 minutes, blood glucose was significantly higher for the YMM and FM (but not FG) treatments when compared to the control treatment (P<0.05). At 15 and 60 minutes, blood glucose did not significantly differ among any of the treatments.

Postprandial blood glucose 2-hour iAUC is summarized in Table 15 and Figure 5, and blood glucose Cmax is summarized in Table 16, and Figure 6. Blood glucose 2-hour iAUC did not significantly differ among any of the treatments. However, blood glucose Cmax was significantly lower for all soluble DF treatments (YMM, FG, FM) compared to the control treatment (P<0.05). Blood glucose Tmax was not significantly different among any treatments (P=0.12). Blood glucose Tmax primarily occurred at 30 (64.2 % of participants) and 60 minutes (33.3 % of participants). Tmax occurred at 15 minutes for only 0.8 % of participants and at 90 minutes for 1.7 % of participants.
Figure 4: Postprandial Blood Glucose Time Point Curves for Fenugreek Gum (a), Yellow Mustard Mucilage (b), Flaxseed Mucilage (c), and Control (d) Puddings (n=15)
Data are means ± SE.
Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.
Data with a superscript *(P<0.005) or ** (P<0.05) indicate a significant difference at that time point compared to the control pudding treatment.
Table 15: Postprandial Blood Glucose Response (n=15)\textsuperscript{1,2,3}

<table>
<thead>
<tr>
<th>Postprandial Blood Glucose Biomarkers</th>
<th>YMM</th>
<th>FG</th>
<th>FM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose 2-Hour iAUC (mmol/L x 120 minutes)</td>
<td>195.1 ± 17.3</td>
<td>187.7 ± 17.9</td>
<td>193.0 ± 19.0</td>
<td>202.9 ± 20.8</td>
</tr>
<tr>
<td>Blood Glucose Cmax (mmol/L)</td>
<td>8.4 ± 0.3*</td>
<td>8.8 ± 0.2*</td>
<td>8.4 ± 0.3*</td>
<td>9.5 ± 0.3</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are means ± SE.
\textsuperscript{2}Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.
\textsuperscript{3}Data with a superscript * (P<0.05) indicate a significant difference compared to the control pudding treatment.

Abbreviations used: YMM=Yellow mustard mucilage treatments; FG=Fenugreek gum treatments; FM=Flaxseed mucilage treatments; iAUC=Incremental area under the curve; Cmax=Maximum concentration.

Figure 5: Postprandial Blood Glucose 2-Hour iAUC (n=15)

Data are means ± SE.
Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.
Abbreviations used: iAUC=Incremental area under the curve.
Figure 6: Postprandial Blood Glucose Cmax (n=15)
Data are means ± SE.
Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ. Data with a superscript * (P<0.05) indicate a significant difference compared to the control pudding treatment.
Abbreviations used: Cmax=Maximum concentration.

b. Postprandial Plasma Insulin

The postprandial plasma insulin time curves for each pudding treatment are summarized in Appendix U (comparison of time points within each treatment) and Figure 7 (comparison of fibre treatments to control at each time point). In general for all the study treatments, plasma insulin increased after 0 minutes, peaked at either 30 or 60 minutes, returned towards baseline, yet with the exception of the control treatment, remained significantly higher than baseline at 120 minutes. There was a statistically significant time by treatment interaction which justified a treatment comparison within each time point separately (Figure 7). At 15 minutes, plasma insulin was significantly lower with the FG and FM (but not YMM) treatments compared to the control treatment (P<0.05). Also, at 30 minutes, plasma insulin was significantly lower with each of the soluble DF treatments (YMM, FG, FM) compared to the control treatment (P<0.05). Finally, at 120 minutes, the plasma insulin for the FM (but not YMM or FG) treatment was
significantly higher than the control treatment (P<0.05). At 60 and 90 minutes, plasma insulin did not significantly differ among any of the treatments.

Postprandial plasma insulin 2-hour iAUC is summarized in Table 16 and Figure 8, and plasma insulin Cmax is displayed in Table 17 and Figure 9. Postprandial plasma insulin 2-hour iAUC was not significantly different among any of the pudding treatments. However, plasma insulin Cmax was significantly decreased for all soluble DF treatments (YMM, FG, FM) compared to the control treatment (P<0.05). Plasma insulin Tmax was not significantly different among any treatments (P=0.74). Plasma insulin Tmax primarily occurred at 30 (56.7 % of participants) and 60 minutes (30.0 % of participants). Plasma insulin Tmax occurred at 15 minutes for only 6.7% of participants, 90 minutes for only 5.8 % of participants, and 120 minutes for only 0.8 % of participants.
Figure 7: Postprandial Plasma Insulin Time Point Curves for Fenugreek Gum (a), Yellow Mustard Mucilage (b), Flaxseed Mucilage (c), and Control (d) Puddings (n=15)

Data are geometric means (95% confidence interval).
Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ. Data with a superscript * (P<0.05) indicate a significant difference at that time point compared to the control pudding treatment.
Table 16: Postprandial Plasma Insulin Response (n=15)\textsuperscript{1,2,3}

<table>
<thead>
<tr>
<th>Postprandial Plasma Insulin Biomarkers</th>
<th>YMM</th>
<th>FG</th>
<th>FM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin 2-Hour iAUC (pmol/L x 120 minutes)</td>
<td>15549.0 (11765.4, 20550.0)</td>
<td>14211.0 (10986.0, 18382.8)</td>
<td>15241.2 (11287.8, 20579.4)</td>
<td>21628.2 (16365.0, 28584.0)</td>
</tr>
<tr>
<td>Insulin Cmax (pmol/L)</td>
<td>290.4 (224.4, 375.6)*</td>
<td>287.4 (232.2, 356.4)*</td>
<td>293.4 (226.8, 379.8)*</td>
<td>483.6 (373.8, 625.8)</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are geometric means (95 % confidence intervals).
\textsuperscript{2}Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.
\textsuperscript{3}Data with a superscript * (P<0.05) indicate a significant difference compared to the control pudding treatment.

Abbreviations used: YMM=Yellow mustard mucilage treatments; FG=Fenugreek gum treatments; FM=Flaxseed mucilage treatments; iAUC=Incremental area under the curve; Cmax=Maximum concentration.

Figure 8: Postprandial Plasma Insulin 2-Hour iAUC (n=15)

Data are geometric means (95 % confidence intervals).
Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.
Abbreviations used: iAUC=Incremental area under the curve.
DISCUSSION

The purpose of this research was to determine the postprandial glycemic and insulinemic responses in adults at risk for T2D following consumption of pudding treatments containing 3 different soluble DF, including YMM, FG, and FM, compared to control pudding treatments without added soluble DF. This study utilized a randomized, double-blinded, crossover design, where a 2-step screening process was used to determine participants’ level of T2D risk with a variety of measurements included in the CANRISK questionnaire (Appendix H) (8) to include participants at risk for T2D. Participants consumed the 10 treatments, with the 50 g glucose beverage treatments being consumed on the first and last study visit, and the 8 pudding treatments being consumed randomly across study visits 2 through 9 and a minimum wash-out period of 5 days between each visit. This study adds to the limited literature regarding the acute metabolic effects of YMM, FG, and FM. As these soluble DF are sourced from Canadian crops, the agricultural industry could stand to benefit if they are able to attenuate glycemic and
insulinemic responses. Further, this is the first clinical trial to study any of these soluble DF in individuals at risk for T2D. As T2D involves a progressive loss of glycemic regulation, it is important to identify strategies which could aid in preventing the development of the disease. Individuals determined to be at risk for T2D were chosen to participate as they may have been in the beginning stages of impaired glycemic regulation.

1. Participant Flow

It was determined that a sample size with appropriate power would be 10 participants, and therefore, 16 participants were recruited to account for participant attrition. As only 1 participant was excluded during the duration of the trial, the participant attrition rate was 6% for this study. In general, most clinical trials which have acutely studied YMM, FG, and FM, either as isolated soluble DF, or within the whole seed, have reported low attrition rates (100% completion). However, 2 studies (57-58) which looked at isolated FM soluble DF reported attrition rates of 8%, due to pre-study protocols not being followed (57) or due to impaired fasting glucose in a healthy participant population (58). Although the present study was a 10 week commitment, participant attrition was relatively low. This may have been due to a thorough explanation of the time commitment and study details during the orientation session before the study began and also because of a good report with the participants. Also, participants were emailed at least 24 hours before each study visit to remind them of the pre-study visit protocols.
2. Participant Characteristics and Baseline Information

a. Participant Characteristics

The methods of recruitment in the 2-step screening process were aimed at identifying individuals with T2D disease risk factors. The Screening-1 Questionnaire (Appendix K) identified key inclusion criteria for entry into the study, however, Screening-2 was a more thorough screening process, which included the CANRISK questionnaire (Appendix H) (8) to identify risk. Overall, participants had an average score of 27.7 on the CANRISK questionnaire, classifying them as having a moderate risk of developing T2D (8). Participants were middle-aged, with an average age of 55.1 years; and were classified as overweight, with a weight of 87.6 kg and a BMI of 29.5 kg/m²; which increases an individuals’ risk of developing T2D (10, 15). The CANRISK questionnaire (Appendix H) (8) gives more points to males and this may have contributed to the male/female distribution of 66.7/33.3 %. As males in the Canadian population are at a higher risk of developing diabetes (5), the gender balance in this study reflects the distribution risk in Canadian adults.

b. Baseline Energy, Macronutrient, and Dietary Fibre Intakes

Participants completed a 3-day food record between the orientation session and their first study visit, to obtain information about each participant’s regular diet. On average, DF intake for participants was 21.6 g per day. This equates to approximately 9.4 g per 1000 kcal of the participants diet, given that on average participants consumed 2293 kcal per day. The DRI AI levels for DF suggest an average intake of 14 g of DF per 1000 kcal (24), therefore, participants DF intake was lower than the recommended intakes for Canadians.
c. Participants Anthropometric Characteristics During the Study

Over the course of the 10 study visits, participants’ anthropometric data including body weight, systolic and diastolic blood pressure, and heart rate were collected. Among all of the 10 treatments, and over time, all anthropometric measures did not significantly differ. This provides strength to the study’s results, as it reduces the confounding effect that anthropometric measures may play on glycemic response end points, and validates that the effect of the treatments were more likely due to the effect of each soluble DF, and not due to anthropometric biomarker changes over the 10 treatments, or over time.

d. Baseline Glycemic Response Biomarkers During the Study and Baseline HOMA-IR Values

Participants in this study were at a moderate risk of developing T2D with an average CANRISK score of 27.7. However, participants’ average fasting blood glucose for all treatments (5.5 mmol/L) was lower than the prediabetes cut off value of 5.6 mmol/L (9), indicating that participants did not have prediabetes. Also, on average, participants’ fasting plasma insulin for all treatments was 41.04 pmol/L. This is in the normal insulin value range between 30 to 150 pmol/L (78). Finally, on average, participants’ HOMA-IR value based on their first glucose beverage study visit was 1.2, which indicated that participants were not insulin resistant, as the value was within the normal HOMA-IR cut off value of 2.6 (77). This verifies that participants did not have T2D, but were an at risk group (9, 77-78). The fact that participant fasting blood glucose and plasma insulin concentrations did not differ across visits, also provides strength to the study’s results, as it reduces potential confounding effect different fasting blood glucose or fasting plasma insulin values may play on overall postprandial glycemic response end points.
3. Postprandial Glycemic Response Biomarkers

Postprandial Blood Glucose and Plasma Insulin Response

Postprandial blood glucose and plasma insulin endpoint measurements included a comparison of time points within treatments, a time by treatment comparison for each time point separately between treatments, postprandial blood glucose and plasma insulin 2-hour iAUC, Cmax, and Tmax.

Time points within each treatment were compared, and it was determined that baseline and 120 minute blood glucose values were not significantly different within any of the treatments (YMM, FG, FM, or control). Therefore, all treatments had their blood glucose values return towards baseline after 120 minutes, and although the soluble DF treatments (YMM, FG, FM) all had slightly higher values at 120 minutes compared to baseline, they were not significantly different from the respective baseline values. However, all soluble DF treatments (YMM, FG, FM) had 120 minute plasma insulin concentrations significantly higher than their respective baseline values. The control treatment had slightly, but not significantly, higher 120 minute plasma insulin concentrations compared to baseline. This means that, although blood glucose concentrations for all treatments (YMM, FG, FM, control) were close to baseline after 120 minutes, plasma insulin for all soluble DF treatments (YMM, FG, FM) remained elevated at 120 minutes. Therefore, although plasma insulin was slower to clear from the blood stream after the soluble DF (YMM, FG, FM) treatment challenges, blood glucose values were not significantly higher than baseline, which suggested that the elevation in plasma insulin at 120 minutes for all soluble DF treatments still allowed adequate clearance of blood glucose into the tissues.

Postprandial glucose and insulin response for the 2-hour iAUC, Cmax, and Tmax biomarkers did not significantly differ among the soluble DF treatments (YMM, FG, FM),
however, both blood glucose and plasma insulin Cmax for all soluble DF treatments were significantly different than the control treatment. This was not unexpected since, although the soluble DF concentrations differed in terms of weight, the puddings were matched for simulated intestinal digestion viscosity and digesta viscosity which had been shown to be a major factor in glycemic response (60). The finding of similar glycemic and insulinemic response in this study supports that hypothesis. Each of the soluble DF treatments will be discussed separately, in comparison to the control treatment and the existing literature.

a. **Yellow Mustard Mucilage Postprandial Blood Glucose and Plasma Insulin Response**

Postprandial blood glucose and plasma insulin response at 30 minutes was significantly decreased for the YMM treatment compared to the control treatment. Also, at 90 and 120 minutes, blood glucose concentrations for the YMM treatment were significantly higher compared to the control treatment, although postprandial blood glucose and plasma insulin 2-hour iAUC, and Tmax were not significantly different between the YMM and control treatments. It was important to note that Cmax for blood glucose and plasma insulin for the YMM treatment were both significantly decreased compared to the control treatment values. Overall, these results pointed to the modulation and attenuation of postprandial blood glucose and plasma insulin response with the soluble DF investigated.

To date, there has only been one other acute study, by Lett et al. (2013), which has looked at the attenuation of glycemic response by YMM (38), and there have currently been no acute studies looking at insulinemic response for YMM. In the study with 10 healthy males by Lett et al. (2013), 5 g YMB, containing approximately 1.5 g YMM, in potato and leek soup, had similar glycemic responses to the current study. Significant time by treatment interactions were
observed with decreased glucose responses with the YMB soup compared to the control soup. This is similar to the significantly decreased blood glucose response at 30 minutes with the YMM treatment compared to the control treatment in the current study. The previous study also found that YMB soup significantly increased glucose response in comparison to the control soup at some time points, which is similar to the current study. Similar to the present study, Lett et al. (2013) had no significant differences in postprandial 2-hour glucose iAUC with and without YMB. Lower values of blood glucose Cmax were observed in both studies in the presence of yellow mustard gum, although lower values of blood glucose Tmax with the DF were only observed in the previous study.

Therefore, this study adds to the minimal existing literature showing the potential for YMM to attenuate the glycemic response when added to a food matrix, and was the first study to look at insulinemic response with the consumption of YMM. This was also the first study to investigate isolated YMM from the YM seeds or YMB, and it found similar glycemic attenuation to a soup product containing 5 g YMB, containing approximately 1.5 g YMM (38). Although this study had higher levels of soluble DF (15.5 g) than the previous study containing 1.5 g YMM, it did not contain any other nutritional components of the seed which may have also had an impact on glycemia (i.e. high protein and fat content) (38). This was also the first study to look at individuals at risk for T2D, which found similar improvements to glycemic response compared to healthy male individuals in the previous study (38). While 2-hour blood glucose and plasma insulin iAUC were not significantly improved, Cmax was significantly decreased for both blood glucose and plasma insulin, and both glycemic and insulinemic response curves were modulated compared to the control treatment, which does suggest some improvements to the
attenuation and modulation of glycemic and insulinemic response and requires further investigations.

b. Fenugreek Gum Postprandial Blood Glucose Response and Plasma Insulin Response

Postprandial plasma insulin response at 15 minutes and blood glucose and plasma insulin response at 30 minutes were significantly decreased for the FG treatment compared to the control treatment, although, postprandial blood glucose and plasma insulin 2-hour iAUC, and Tmax were not significantly different between the treatments. However, Cmax for blood glucose and plasma insulin for the FG treatment were significantly decreased compared to the control treatment values. Overall, this research suggests that some biomarkers of blood glucose and plasma insulin response can be modulated and attenuated with FG.

To date, there have been minimal acute studies which have looked at the impact of fenugreek seeds or isolated FG on glycemic response (44-51). Also, only 2 studies have looked at the role of fenugreek seeds or FG on time by treatment insulinemic response (44-45). In the studies with OGTT glucose beverages containing 25 g whole fenugreek seeds (5 g FG), 25 g defatted fenugreek seeds (4.8 g FG) and 5 g FG, in healthy participants both glucose and insulin response at certain time points were significantly decreased compared to a control beverage (44); meals containing 15 g ground fenugreek seed powder (FG concentration not reported) in participants with NIDDM significantly decreased blood glucose compared to a control meal (45); meals containing 12.5 g unprocessed dried fenugreek seeds (2.35 g FG) and 12.5 germinated then dried fenugreek seeds (1.25 g FG) significantly decreased blood glucose in healthy participants compared to a control meal (46); dextrose solutions containing 5 g of fenugreek seeds (FG concentration not reported) in participants with T2D significantly decreased glucose
response compared to a control beverage (47); and barley bread products containing 2.98 g and 5.95 g fenugreek seed flour (FG concentrations not reported), and 1.19 g fenugreek seed flour (FG concentration not reported) with 1.19 g ginger flour, significantly decreased blood glucose at all time points compared to white bread, and barley bread with 5.95 g fenugreek flour and the 1.19 g fenugreek seed flour with 1.19 g ginger flour bread further significantly decreased blood glucose at some time points compared to the barley bread in healthy participants (50). However, in studies with OGTT beverages containing 25 g of degummed fenugreek seeds (0 g FG), and a meal with 25 g cooked fenugreek seeds (FG concentration not reported) in healthy participants did not significantly decrease glucose or insulin response at any time point compared to a control beverage or meal (44); a meal containing 15 g fenugreek seed powder (FG concentration not reported) in participants with NIDDM did not significantly alter insulin response at any time point (45); meals containing 12.5 g unprocessed dried fenugreek seeds (2.35 g FG) and 12.5 germinated then dried fenugreek seeds (1.25 g FG) did not significantly alter glycemic response in participants with NIDDM, and 12.5 g boiled and dried fenugreek seeds (0.38 g FG) did not alter glycemic response in either healthy or NIDDM participants (46); and dextrose solutions containing 2.5 g of fenugreek seeds (FG concentration not reported), did not significantly change glucose response in participants with T2D (47). Therefore, in studies with whole fenugreek seeds, those with the highest concentration of FG, ranging from 1.25 to 5 g, or concentrations of whole fenugreek seeds, ranging from 2.98 to 25 g without FG extracted, were able to significantly decrease blood glucose response in those that measured time versus treatment compared to control treatments (44-47). This is similar to the current study, which found that blood glucose was significantly decreased at 30 minutes with the FG treatment containing 5.9 g FG compared to the control, which supports the hypothesis that FG can attenuate glycemic
response. Also, the results found by Sharma (1986) with glucose beverages containing between 4.8 to 5 g FG (44) significantly decreased insulin response, which is similar to the 5.9 g FG treatment in this study, which significantly decreased plasma insulin at 15 and 30 minutes. This adds to the existing literature to support FG decreasing insulinemic response and supports the hypothesis that FG can attenuate insulinemic response.

In the current literature with fenugreek seeds and FG, in studies which looked at postprandial blood glucose AUC, iAUC, or IAUC, most found a significant decrease in blood glucose AUC, iAUC, or IAUC with the fenugreek seed or FG treatment, compared to the control treatment (44, 46, 48, 50). In the studies focusing on blood glucose AUC, iAUC, or IAUC, 25 g whole fenugreek seed powder (5 g FG) in healthy participants (44), 25 g defatted fenugreek seed powder (4.8 g FG) in healthy participants (44), 25 g cooked fenugreek seeds (FG concentration not reported) in healthy participants (44), 12.5 g unprocessed dried fenugreek seeds (2.35 g FG) and 12.5 g germinated then dried fenugreek seeds (1.25 FG) in healthy participants (46), 12.5 g germinated fenugreek seed powder (2.5 g FG) in NIDDM participants (48), barley bread with 2.98 and 5.95 g fenugreek flour, and barley bread with 1.19 g fenugreek flour and 1.19 g ginger flour, in healthy participants (50), and 5 g FG in healthy participants (44), significantly decreased blood glucose AUC, iAUC, or IAUC measurements compared to the control treatment. In contrast, in the current study, 5.9 g FG treatment did not significantly decrease blood glucose 2-hour iAUC compared to the control treatment. However, the current study is similar to some fenugreek seed and FG literature, which has shown no significant effects to glucose AUC or IAUC with fenugreek seed and FG treatments (44, 46, 51), or where significance was not reported in the results (49). In those studies, 25 g degummed fenugreek seeds (0g FG) in healthy participants (44), 12.5 g unprocessed dried fenugreek seeds (2.35 g FG), 12.5 g germinated then
dried fenugreek seeds (1.25 FG), and 12.5 g boiled then dried fenugreek seeds (0.38 g FG) in NIDDM participants (46), 12.5 g boiled then dried fenugreek seeds (0.38 g FG) in healthy participants (46), and 3.6 and 7.2 g FG in healthy, obese participants (51), did not significantly decrease blood glucose AUC or IAUC compared to the control treatments, and in one study with fenugreek seeds concentrations at 20, 23, and 25 g (FG concentrations not reported) in foods with multiple bioactives, significance of blood glucose AUC was not reported in healthy and NIDDM participants (49). Therefore, the non-significantly different results in the current study for 5.9 g FG treatments on blood glucose 2-hour iAUC are similar to some fenugreek seed and FG literature which ranged in FG content and did not have significant glucose AUC or IAUC results (44, 46, 49, 51).

Importantly, only 2 studies have looked at the role of fenugreek seeds or FG on insulin AUC, or IAUC and the results from those studies were contrasting (44, 51). For insulin AUC and IAUC, glucose beverages containing 25 g whole fenugreek seed powder (5 g FG), 25 g defatted fenugreek seed powder (4.8 g FG), and 5 g FG, in healthy participants, significantly decreased insulin IAUC compared to a control beverage; however, 25 g degummed fenugreek seeds (0 g FG) in a glucose beverage, and 25 g cooked fenugreek seeds (FG concentration not reported) in a meal, in healthy participants, did not significantly change insulin IAUC compared to the controls (44). Also, 3.6 g FG in a beverage with breakfast did not significantly decrease insulin AUC compared to the control beverage and meal, and 7.2 g FG increased insulin AUC compared to the control and 3.6 g FG beverage and meal, in 18 healthy, obese participants (51). The current study found no significant change to plasma insulin 2-hour iAUC with a 5.9 g FG treatment compared to the control treatment, which is similar to the non-significantly different results with the consumption of 25 g degummed fenugreek seeds (0 g FG), 25 g cooked
fenugreek seeds (FG concentration not reported), and 3.6 g FG (44, 51). Overall, the role of FG on insulin AUC, IAUC, and iAUC remains equivocal. However, this study supports the current literature that indicates that insulin iAUC is not significantly changed by FG.

Overall, results for blood glucose and insulin AUC, iAUC, and IAUC are conflicting. Some studies with FG concentrations between 1.25 to 5 g have shown significant decreases in glucose AUC, iAUC, and IAUC, when compared to a control treatment in healthy and NIDDM participants (44, 46, 48, 50). However, other reports, including the current study, have found no significant decreases in blood glucose response with FG concentrations between 0 to 7.2 g in healthy, healthy and obese, at risk for T2D, and NIDDM participants (44, 46, 49, 51). Also, in studies looking at insulin AUC or IAUC, consumption of 4.8 to 5 g FG significantly decreased insulin IAUC compared to a control treatment in healthy participants, however, in other studies, including the current study, products with unknown FG concentrations, and concentrations between 3.6 g to 5.9 g FG did not significantly decrease insulin AUC, iAUC, or IAUC, and concentrations of 7.2 g FG significantly increased AUC compared to a control and 3.6 g FG beverage and meal (44, 51).

Finally, the available literature for fenugreek seeds and isolated FG is limited in discussing results for Cmax and Tmax for both blood glucose and insulin. The current study is the first to report results for insulin Tmax. In studies with meals containing 25 cooked fenugreek seeds (FG concentration not reported) in healthy participants, blood glucose Cmax was significantly decreased, and blood glucose Tmax was prolonged from 60 to 90 minutes, in healthy participants (44); meals with 12.5 g unprocessed dried fenugreek seeds (2.35 g FG) and 12.5 g germinated then dried fenugreek seeds (1.25 g FG) significantly prolonged blood glucose Tmax from 60 minutes to 90 minutes in both healthy and NIDDM participants (46); meals with
12.5 g germinated fenugreek seed powder (2.5 g FG) decreased blood glucose Cmax, and blood glucose Tmax was prolonged from 60 to 90 minutes, in NIDDM participants (48), however, statistical significance for Cmax and Tmax results in all studies discussed was not reported. Finally, meals containing 3.6 and 7.2 g FG in healthy, obese participants did not significantly improve blood glucose Cmax, and 3.6 g FG did not significantly change insulin Cmax compared to the control; however, 7.2 g FG significantly increased insulin Cmax compared to the control and 3.6 g FG beverage and meal (51). In the current study, both blood glucose and plasma insulin Cmax were significantly decreased with the 5.9 g FG treatment when compared to the control treatment, which is similar to the results with treatments containing 25 g cooked fenugreek seeds (FG concentration not reported) and 12.5 g germinated fenugreek seed powder (2.5 g FG) which showed decreases to blood glucose Cmax but did not indicate significance (44, 48), but conflicts with the results found by Mathern et al. (2009) which did not find that 3.6 or 7.2 g FG significantly improved blood glucose Cmax (51). The current study also did not find significant differences in blood glucose Tmax with the 5.9 g FG treatment compared to the control treatment. This conflicts with the current literature showing that blood glucose Tmax was prolonged from 60 to 90 minutes in all 3 studies, however, significance was not reported (44, 46, 48). Also, the present study conflicts with the Cmax literature for insulin, as the 5.9 g FG treatment in this study significantly decreased insulin Cmax compared to the control treatment, however 3.6 g FG did not change insulin Cmax, and 7.2 g FG significantly increased insulin Cmax. Therefore, this study is the first to report significant decrease to insulin Cmax with a 5.9 g FG treatment compared to a control. This is also the first study to report on the role of FG on insulin Tmax, where 5.9 g FG treatment did not significantly improve insulin Tmax.
compared to the control. Therefore, the evidence for FG in decreasing blood glucose and insulin 
Cmax, and altering blood glucose Tmax is conflicting in the literature.

Overall, this study adds to the minimal existing literature on the attenuation of glycemic and insulinemic response with FG. The available literature is conflicting in regards to time by treatment interactions for glucose and insulin response, glucose and insulin iAUC, glucose and insulin Cmax, and glucose Tmax endpoints. This current study adds to the existing literature which supports a significant time by treatment interaction at 30 minutes for decreasing blood glucose and significant decreases at 15 and 30 minutes to plasma insulin compared to the control treatment, non-significant changes to blood glucose and plasma insulin 2-hour iAUC, significant decreases to blood glucose and plasma insulin Cmax compared to the control treatment, and non-significant differences to blood glucose and plasma insulin Tmax. Also, this was the first study to report on insulin Tmax, and found no significant differences compared to the control. Although minimal changes to glycemic and insulinemic response were seen with FG in this current study, blood glucose was significantly lower at 30 minutes, and plasma insulin was significantly lower at 15 and 30 minutes with the FG treatment, and both blood glucose and plasma insulin Cmax were decreased, which lends to FG being able to overall modulate both glycemic and insulinemic responses, independent of being able to attenuate responses. Therefore, FG seemed to be able to steadily and slowly control the uptake of blood glucose, ultimately reducing blood glucose Cmax, and after the peak concentration was achieved, blood glucose slowly returned towards baseline, which may have been attributed to the steady concentration of plasma insulin response. Plasma insulin Cmax was significantly reduced, and the blood glucose response appeared to be steadier in comparison to the control treatment, with overall blood glucose Cmax also being reduced, which suggests that less plasma insulin was
required with FG to steadily regulate the uptake of blood glucose into the tissues. The decrease in plasma insulin Cmax was hypothesized, as it was also hypothesized that blood glucose Cmax would be attenuated with FG treatments, and therefore, the results are in agreement with the hypotheses. These attenuations are beneficial to populations requiring regulation to glycemic and insulinemic responses, including individuals at risk, or who currently have T2D. Also, this could ultimately benefit the agricultural and manufacturing industries in developing products to modulate glycemic and insulinemic response, as although overall both glycemic and insulinemic response did not decrease, both peak glucose and insulin were able to be attenuated, and the response curves were steadier compared to the control treatment.

c. Flaxseed Mucilage Postprandial Blood Glucose and Plasma Insulin Response

Postprandial blood glucose concentration at 30 minutes, and plasma insulin concentration at 15 and 30 minutes were significantly decreased for the FM treatment compared to the control treatment, however, at 90 and 120 minutes for blood glucose and 120 minutes for plasma insulin, concentrations for the FM treatment were significantly higher compared to the control treatment. Both postprandial blood glucose and plasma insulin 2-hour iAUC and Tmax were not significantly different between the FM and control treatment. The Cmax for blood glucose and plasma insulin for the FM treatment was significantly decreased compared to the control treatment. Therefore, this research suggests that some biomarkers of blood glucose and plasma insulin response can be modulated and attenuated with the consumption of FM.

To date, there have been minimal acute studies which have looked at the attenuation of glycemic and insulinemic response with flaxseeds or isolated FM (55-58). In the 2 studies using whole flaxseeds (55-56), only one study looked at a time by treatment comparison for both glucose and insulin response, with a meal containing 12.18 g whole flaxseed (FM concentration
not reported) versus a control meal, in healthy participants, and found no significant differences at any time point for blood glucose or insulin over 3 hours (56). In the 4 studies with isolated FM, only one study, which included meals with 12.18 and 17.27 g of FM, looked at a time by treatment comparison for both glucose and insulin (56). They found that the FM meals compared to the low-fibre control meal were not significantly different at any of the time points for blood glucose over 3 hours, although the 17.27 g FM meal significantly decreased insulin response at some time points compared to the control meal (56). The previous study (56) contrasts the results found in this present study, as there was a significant decrease to blood glucose at 30 minutes, and significant increases to blood glucose at 90 and 120 minutes with the 11.4 g FM treatment compared to the control treatment. However, the present study with the 11.4 g FM treatment significantly decreased plasma insulin response at 15 and 30 minutes compared to the control treatment, which is similar to the results found in the previous study with the 17.27 g FM meal (56), and although this study had less FM than the 12.18 g FM meal in the previous study, it still showed decreases to plasma insulin. Therefore, the current study is the first to demonstrate that 11.4 g FM was able to decrease blood glucose concentration at 30 minutes, and increased concentrations at 90 and 120 minutes, compared to a control treatment. Also, this was the first study to report increases to plasma insulin concentration at 120 minutes compared to the control treatment, and agreed with the previous literature on decreasing insulin response earlier on in the curve (56). The glycemic and insulimemic responses in the current study with 11.4 g FM were similar, in that both curves had significantly lower responses earlier in the curve, and had higher concentrations of both blood glucose and plasma insulin later in the curve, compared to the control treatment. Although both blood glucose and plasma insulin concentrations were significantly higher later on in the curve compared to the control, overall, the glycemic and
insulinemic responses were modulated with the FM treatment compared to the control. Therefore, these results add to the minimal existing literature available for both glycemic and insulinemic response to flaxseed and FM which showed modulating effects at different time points compared to the control treatments.

The existing literature for flaxseed and isolated FM has examined blood glucose iAUC (55-58), and only 2 previous studies have looked at the role of flaxseed and FM on insulin AUC and iAUC (56, 58). In the previous literature, flaxseed bread (flaxseed and FM concentration not reported) and bread containing 11 g flax fibre (2.7 g FM) consumed by healthy participants significantly decreased blood glucose iAUC compared to a control bread (55, 57); a glucose beverage 25 g FM in a glucose beverage consumed by participants with unknown health status significantly decreased glucose iAUC compared to a white bread or glucose beverage control (55); and dairy beverages and puddings containing 2.5 g FM were able to decrease blood glucose iAUC compared to OGTT glucose beverages in healthy participants (58). For insulin AUC, both 12.18 g FM and 17.27 g FM meals significantly decreased insulin AUC compared to the control meal in healthy participants (56). In the current study, 11.4 g FM did not significantly decrease either blood glucose or plasma insulin 2-hour iAUC compared to the control treatment, which conflicts with the current literature which has shown the ability of concentrations of FM at 2.5 to 25 g to decrease blood glucose iAUC (55, 57, 58), and concentrations of 12.18 and 17.27 g FM to decrease insulin AUC (56). However, some previous studies showed similar results to the current study for both blood glucose and insulin AUC and iAUC (56, 58). It was shown that meals with 12.18 g whole flaxseed (FM concentration not reported), 12.18 g FM, and 17.27 g FM, in healthy participants did not significantly decrease blood glucose iAUC compared to a control meal, and the 12.18 g whole flaxseed meal did not significantly alter insulin AUC.
compared to the control (56); and in healthy participants, dairy beverages and puddings
containing 2.5 g FM were not able to significantly decrease blood glucose iAUC compared to the
control puddings, and were not able to significantly alter insulin iAUC compared to any control
treatments (puddings or OGTT beverages) (58). Therefore, the current study which contained
11.4 g FM is similar to previous literature, where concentrations of FM between 2.5 to 17.27 g
FM did not significantly improve blood glucose iAUC, and a meal containing 12.18 g whole
flaxseeds, and dairy beverages and puddings containing 2.5 g FM did not significantly alter
insulin AUC or iAUC (56, 58). Overall, the results for FM on attenuating blood glucose and
insulin iAUC is conflicting. The current study indicates that FM does not significantly decrease
blood glucose or insulin iAUC. In the previous studies which showed improvements to both
glucose and insulin iAUC or AUC, FM concentrations were not reported (55), were higher than
what was used in this study (55, 56), contained other aspects of the flaxseed (i.e. insoluble DF)
which could have improved glucose iAUC independently of FM (57), or the FM treatments were
only significantly different for glucose iAUC compared to the glucose beverage, and not the
control dairy or pudding treatments (58). Although some of the previous literature does support
the attenuation of blood glucose and insulin iAUC, the concentrations used were not comparable
to the current study, and studies which used whole flaxseed, or had lower concentrations of FM
did not find significant differences to either blood glucose or insulin AUC or iAUC (56, 58).
Therefore, perhaps higher concentrations of FM are required for the attenuation of glycemic and
insulinemic iAUC.

Finally, the available literature for flaxseed and isolated FM is limited in discussing
results for Cmax and Tmax. Only 2 studies with FM have reported results for blood glucose
Cmax (57-58), and one study has reported results of blood glucose Tmax (58). The literature on
FM and insulin response is even scarcer, as only 1 study has reported on insulin Cmax and insulin Tmax results for FM treatments (58). In the previous literature, bread containing 11 g flax fiber (2.7 g FM) in healthy participants was able to decrease blood glucose Cmax compared to the control bread (57); and with dairy beverages and puddings containing 2.5 g FM in healthy participants, blood glucose Cmax was significantly decreased compared to an OGTT glucose beverage, but not compared to control dairy beverages and puddings (58). Insulin Cmax was also not significantly changed by 2.5 g FM addition to a dairy beverage and pudding compared to the control treatments (58). In the present study, both blood glucose and plasma insulin Cmax were significantly decreased with the 11.4 g FM treatment compared to the control treatment, which is comparable to the glucose Cmax results with bread and dairy beverages and puddings containing 2.5 to 2.7 g FM (57-58). The current study did not observe the same effects on plasma insulin Cmax as compared to the study with dairy beverage and puddings containing 2.7 g FM, as in this study insulin Cmax was significantly decreased by the 11.4 g FM treatment compared to the control treatment, which suggested that a higher dose of FM was required to alter peak insulin. Blood glucose and insulin Tmax has only been reported in one study, which found that blood glucose Tmax was significantly decreased with the 2.5 g dairy pudding compared to the glucose control, however, all other treatments were not significantly different between each other (58). Also, insulin Tmax was significantly decreased only by the 2.5 g FM dairy beverage compared to the glucose control, but not the dairy control beverages, and the 2.5 g FM dairy pudding did not significantly change insulin Tmax (58). This is conflicting to the current study where 11.4 g FM in puddings did not significantly change either blood glucose or insulin Tmax compared to the control treatments, however, it is similar to the results for the 2.5 g FM dairy beverage which did not significantly change blood glucose Tmax, and the 2.5 g FM
dairy pudding which did not significantly change insulin Tmax (58). It is important to note however, that both the dairy beverage and pudding were not significantly different compared to the control beverage and pudding, which is similar to the results found in this study with the FM treatment compared to the control. Therefore, the blood glucose Cmax and Tmax results in the present study are comparable with most of the existing literature with small doses of FM between 2.5 to 2.7 g, which tended to decrease blood glucose Cmax, but 2.5 g FM dairy beverages did not alter Tmax (57-58). It is important to note however, that 2.5 g FM dairy puddings were able to significantly decrease blood glucose Tmax compared to the glucose beverage, but not the control puddings, which is similar to the results found in this study which compared 11.4 g FM treatments to control puddings, so perhaps having FM in a food matrix compared to a beverage limits the FM capabilities of altering Tmax. However, this was the first study to report significant decreases to insulin Cmax with FM treatments, which suggests that perhaps a higher dose of soluble DF was required to decrease insulin Cmax. This study also agrees with the existing literature that insulin Tmax was not significantly changed by FM in pudding treatments, which again supports that perhaps FM in a food matrix is limited in altering Tmax, although attenuations to both Cmax have been displayed.

Overall, this study adds to the minimal existing literature on the attenuation of glycemic and insulinemic response with FM. This was the first study to report significant blood glucose treatment versus time comparisons for a FM treatment compared to a low-fibre control treatment. The previous literature is currently conflicting in regards to attenuation of glucose iAUC, and this current study adds to the existing literature which reported non-significantly different changes to glucose iAUC compared to a control treatment. In general, the previous literature supports that FM is significantly able decreases to Cmax compared to control treatments, and
this study is in agreement with the previous literature. Finally, only one study had previously looked at blood glucose Tmax, and reported that 2.5 g FM in dairy pudding was significantly able to decrease blood glucose Tmax compared to a glucose beverage, however, it was not significantly different compared to the control pudding, which is different than the current study results, and the 2.5 g FM dairy beverage did not significantly change blood glucose Tmax compared to any other treatments, which is in agreement with the current study (58). This suggests that FM is able to change blood glucose Cmax independent of it being able to alter blood glucose Tmax. It is important to note that in the previous study, although the 2.5 g FM dairy pudding and beverage treatments were matched for in vitro viscosity, their results for blood glucose Tmax were conflicting and although both products contained the same concentration of 2.5 g FM, their glycemic attenuation properties were different (58). This conflicts with the current study, which found that soluble DF treatments (YMM, FG, FM) matched for in vitro viscosity had similar glycemic attenuation properties for all biomarker endpoints (2-hour iAUC, Cmax, Tmax). In regards to insulinemic response, this study adds to the available literature which is conflicting in regards to treatment by time interactions for insulin response, insulin iAUC, Cmax, and Tmax. This current study adds to the existing literature which supports significant treatment by time interactions for decreasing plasma insulin, and is the first study to report increases to insulin response compared to the control treatment. This study also adds to the literature supporting no significant changes to plasma insulin 2-hour iAUC. This is the first study to report significant decreases to insulin Cmax with the 11.4 g FM treatment compared to the control treatment, and adds to the literature supporting no significant differences to insulin Tmax with FM, although a 2.5 g dairy beverage was able to decrease insulin Tmax compared to a glucose beverage, but not to the control dairy beverage (58), which suggests that FM in food
matrices may be unable to alter insulin $T_{\text{max}}$. Overall, both blood glucose and plasma insulin was altered at different time points with the FM treatment, and both blood glucose and plasma insulin $C_{\text{max}}$ were decreased, which suggests that FM can play a beneficial role in altering both glycemic and insulinemic responses, and rationalizes more research being conducted on FM to understand if there is a consistent trend in food products containing FM having the ability to improve glycemic response for adults at risk, and who currently have, T2D.

d. Summary of Glycemic and Insulinemic Responses for Yellow Mustard Mucilage, Fenugreek Gum, and Flaxseed Mucilage

Overall, the soluble DF treatments (YMM, FG, FM) were not significantly different from each other, however, they were significantly different from the control pudding treatment. As each of the soluble DF treatments (YMM, FG, FM) were matched for *in vitro* viscosity before consumption, it was expected that each soluble DF would behave similarly in regards to the attenuation of glycemic and insulinemic response since, although the soluble DF concentrations differed, all soluble DF puddings were hypothesized to have similar intestinal digestion viscosity.

In regards to the blood glucose and plasma insulin biomarkers analyzed, each soluble DF treatment (YMM, FG, FM) behaved similarly. Comparisons of baseline blood glucose to 120 minute blood glucose concentrations within treatments found no significant differences between any of the treatments (YMM, FG, FM, control). However, plasma insulin at baseline compared to 120 minutes was significantly higher with all the soluble DF treatments (YMM, FG, FM) but was not significantly different with the control treatments. Also, although all soluble DF treatments (YMM, FG, FM) were not able to significantly decrease either blood glucose or plasma insulin 2-hour iAUC or $T_{\text{max}}$, all soluble DF treatments (YMM, FG, FM) were able to
significantly alter both blood glucose and plasma insulin responses at different time points, and both blood glucose and plasma insulin Cmax concentrations. Overall, all soluble DF treatments (YMM, FG, FM) were not successfully able to attenuate all glycemic or insulinemic response endpoints measured which were the initially hypothesized to decrease, however, blood glucose and plasma insulin response was modulated with all soluble DF treatments (YMM, FG, FM) and both blood glucose and plasma insulin Cmax was attenuated. Therefore, the modulation and attenuation of glycemic and insulinemic response found in this study shows the rationalization of researching each soluble DF further, in hopes of incorporating each soluble DF into food products for individuals looking to alter glycemic and insulinemic responses.

4. Study Strengths

The current study had many strengths, which aids in supporting the validity of the results. These include strengths in terms of study design, treatment design, participant recruitment, screening and follow up, and data collection and analysis. This study followed a randomized, double-blinded, crossover intervention, which had a washout period of a minimum of 5 days between treatments, and chose a minimum participant sample size of 10 to have an 80% power level. The 2 glucose beverage visits were on the first and last study days, while the 8 pudding treatments were randomized throughout study visits 2 to 9. As participants consumed each of the pudding treatments at random, this ensured that changes to glycemic response were not based on additive effects of the pudding treatments at any point, and also reduced bias from either the participant or the researcher for any of the pudding treatments. The glucose beverage visits were chosen to be on the first and last study visit as a determination of participant baseline and endpoint glycemic and insulinemic response similar to an OGTT. Also, all pudding treatments were assigned a treatment code and were produced by a third party who did not work with the
participants, which eliminated both researcher and participant bias, as participants and researchers did not know which pudding treatment participants were consuming at any study visit. A crossover study design was chosen so that participants served as their own control for each of the treatments, which improved inter-individual variation between each participant for the 10 treatments. Also, a washout period of a minimum of 5 days was chosen to allow participants to return to pre-study visit values before each study visit, so that glycemic response was not altered by any treatment beforehand. Also, choosing a minimum 5 day washout allowed participants to complete the study to its entirety within 10 weeks, which was not too long of a timeframe for participants, as attrition rate was not affected due to time commitments. Participants were also asked before each study visit to consume a similar dinner each night before the study visit at a similar time. This allowed participants to come to the study visit with similar feelings of satiety and glycemic response. Finally, a participant sample size of 10 was deemed necessary to have a statistical power of 80%. Choosing 16 participants to allow for attrition was appropriate, as 15 participants finished the study to completion, which was well above the desired sample size. This allowed the study to have appropriate statistical power, and also allowed for the ability to tease out blood glucose and plasma insulin differences more effectively with a larger sample size.

The pudding treatments were created with the snacking industry in mind, as the consumer snacking market is popular with the Canadian population (63). The puddings were chocolate flavour, as chocolate is a typical flavour of pudding products, and was chosen in hopes of improving palatability for participants. A strength of this study was that all pudding treatments were matched for the same macronutrient composition, including available carbohydrates, protein, and fat. Also, although the concentration of each soluble DF (YMM, FG, FM) were
different between the treatment types, they were all matched on viscosity properties, which validated the reason for different soluble DF concentrations across treatment types, and based on the results, concluded that, although each soluble DF was utilized at a different concentration, their similar *in vitro* viscosity was associated with similar attenuations of blood glucose and plasma insulin. Therefore, the pudding products were chosen based on palatability of the products for participants, and were matched for macronutrient intake, which aided in minimizing glycemic response changes due to differences in nutritional composition of the products, and focussed on changes due to the soluble DF (YMM, FG, FM).

Another strength of the study was in participant recruitment and screening, as well as participant follow up throughout the clinical trial to reduce participant attrition, as well as strength of screening protocols. Participants were screened in a 2-step process, which improved identifying individuals who were at risk for T2D, and allowed for identifying thorough participant details before the study began. The screening process also included an orientation session, which allowed the researchers to thoroughly provide each participant with study details before the study began, and to try the pudding product to allow participants to understand what they were going to be consuming for 8 study visits. The thorough 2-step screening process and study orientation improved participant understanding of the study, which allowed for minimal participant attrition. Finally, during the duration of the clinical trial, every effort was taken to maintain a good rapport with each participant, in order for them to feel welcome and informed throughout the study. Participants were contacted every week 24 hours before their study visit to confirm their appointment, and to provide reminders about the pre-study visit protocols. Participants were also followed up with if they had any questions, and were encouraged to contact the researchers if they needed any information. This was important in minimizing
participant attrition, as most of the participants expressed enjoyment in coming to the study visits each week. Finally, the screening process incorporated the validated CANRISK questionnaire (8) (Appendix H) which allowed for accurate assessment of T2D risk based on multiple risk factors for T2D. The CANRISK questionnaire (Appendix H) (8) incorporated key risk factors for determining diabetes risk, which represent a large demographic of the adult North American population. Although key factors such as gender, and more advanced age were considered in the questionnaire, other risk factors such as BMI, waist circumference, physical activity, dietary lifestyle, blood pressure, blood glucose, family history, and ethnicity were also considered which allowed for younger populations also at risk to be considered. Using a validated T2D risk assessment tool for screening allowed for a greater ability to identify an appropriate at risk participant population from a wide variety of risk factors and a wide subset of the population, which are all implicated in the etiology of T2D.

Finally, the thoroughness of the data collection and analysis provided strength to the completeness of the study results. The research was completed by a small team, which were all trained by the head study coordinator and all data collection and analysis was completed by the team. All study protocols for data collection and participant interactions followed protocols as determined by each individual piece of machine equipment, and the HNRU SOP protocols. All finger prick blood samples were taken by a small team of M.Sc. students, which all practiced the technique thoroughly to minimize chances of hemolysis. Also, all blood glucose analysis was completed in duplicate with the same 2 HemoCue® 201 glucose readers (HemoCue®, Ängelholm, Sweden), after ensuring standards were within range each morning before the study visits began. Also, all insulin ELISA assays were completed by the same individual to minimize inter-individual variation in assay technique, and followed all assay protocols provided by the
company. Where possible, samples for each participant were analyzed in separate batches with as few assay plates as possible to minimize variability. Finally, all data was entered by the study team, and a separate team member checked every entry for completeness and accuracy.

5. Study Limitations

There were some study limitations with the treatment design and with the participant characteristics. First, the pudding serving size of 500 mL was a large portion, and was a lot higher than a typical Canadian pudding serving of 80 to 140 mL (79), and second, the puddings contained 1500 mg of acetaminophen to measure gastric emptying, which was very bitter. Although effort was made to ensure palatability of the puddings, the large portion and bitterness of the acetaminophen caused some participants to express difficulty in consuming the puddings to their entirety. Finally, although the soluble DF concentrations for all soluble DF (YMM, FG, FM) puddings was determined based on in vitro viscosity testing based on the EFSA health claim for β-glucans (61), there were no control pudding products used in the current study which contained β-glucan as a reference. Pudding products containing 6.7 g of β-glucan as per the EFSA health claim, and puddings containing 3 times the concentration for the EFSA health claim, could have been utilized to understand if there would have been similar attenuations to glycemic and insulinemic regulation.

Secondly, a limitation of the study was that the ethnicity of the all 15 participants was Caucasian. This is not representative of the ethnic diversity in the Canadian population, and in the increased risk of T2D associated with certain ethnic ancestries including Indian, Native American, African, Hispanic, Latino, and Asian (16). Although the CANRISK questionnaire assigns increased points to certain ethnicities (8), most participants who inquired about the study and were enrolled from the Guelph community were primarily Caucasian.
6. Future Research

This research strengthens the literature showing that YMM, FG, and FM have potential to modulate postprandial glycemic response in persons at risk for T2D. Future research directions could include looking at individuals which would benefit from attenuation of glycemic response, using insulinemic response as a biomarker more frequently, standardizing optimal doses of the different soluble DF types, and standardizing for commercially relevant serving sizes across studies.

This was the first study in each of the 3 soluble DF types to look at the glycemic and insulinemic response for participants who were at risk for T2D. Most studies for all 3 soluble DF types have looked at healthy populations (38, 44, 46, 49-51, 55-58), and few studies have looked at the acute role of these soluble DF types in participants with NIDDM, or T2D (45-48). As attenuation of glycemic response is important for individuals at risk for T2D, or who currently have prediabetes or diabetes, which could stand to benefit from improvements in blood glucose regulation, it is important to look at these populations to determine if YMM, FG, and FM could stand to alter glycemic response.

Also, the previous literature currently available for the role each of the 3 soluble DF types play on the attenuation of insulinemic response is minimal, as most studies have only investigated glycemic response. Therefore, this is one of the first studies to report results for each of the 3 soluble DF on acute insulinemic responses. As insulin homeostasis is key to maintaining blood glucose levels (11), it is important to also identify if the 3 soluble DF are able to play an acute role in attenuating insulin response.

Although there is minimal literature currently for each of the 3 soluble DF, the doses of each of the soluble DF for each study also very greatly between studies in the current literature,
which makes it difficult to draw accurate conclusions. Also, similar doses in the literature have not been able to reproduce similar attenuation effects to glycemic response, and the evidence for a dose dependent response is conflicting. Therefore, being able to establish a standardized method of soluble DF extraction for each type, and trying to find the optimal dose of soluble DF is important in understanding if each of the 3 soluble DF are able to improve glycemic and insulinemic response. The optimal dose of soluble DF for each treatment would also need to be understood in terms of customer acceptance and palatability. Therefore, understanding optimal doses in improving glycemic and insulinemic response while maintaining palatability of the food products is important.

Finally, going forward, standardizing treatment portions across studies would also aid in understanding the role each of the 3 soluble DF play on attenuating glycemic and insulinemic response. Currently, products such as soups (38), glucose beverages (44, 55), seeds or gum/mucilage baked into a meal (44, 46, 48, 58), in water with a meal (45), dextrose solutions (47), snacks (49), breads (50, 55, 57), juices (51), dairy beverages (58), and dairy puddings (58), have all been used to look at the role each of the 3 soluble DF types play in attenuating glycemic and insulinemic response but vary in their nutrient composition and available carbohydrates. The differences in food matrices may have aided in the variability of the data presented between each of the studies currently present in the literature, however, standardizing the commercially relevant portions of the food products across studies, and their amounts of available carbohydrates would be beneficial in understanding the role each of the 3 soluble DF play on attenuating glycemic response, as although the food matrix would change between studies, comparisons could be made if the same amounts of available carbohydrates were utilized, and similar commercially relevant portion sizes of the meals were achieved.
CONCLUSIONS

This research generated evidence related to the postprandial glycemic and insulinemic responses with acute consumption of pudding treatments containing novel soluble DF (YMM, FG, FM) in adults at risk for T2D using a randomized, double-blinded, crossover intervention. The puddings were matched for in vitro viscosity, recognizing the role that viscosity plays in the glycemic response of a meal. Overall, the results show that the addition of YMM, FG and FM to a convenience snack food was effective at improving some acute glycemic and insulinemic markers of T2D risk.

It was hypothesized that the soluble DF pudding treatments would lower postprandial glycemic and insulinemic responses compared to the low-fibre control pudding treatment. This was investigated by comparing glucose and insulin 2-hour iAUC, Cmax, and Tmax. The hypothesis that the soluble DF pudding treatments (YMM, FG, FM) would significantly lower postprandial blood glucose and plasma insulin 2-hour iAUC and Tmax relative to the control was rejected. However, the hypothesis that the soluble DF pudding treatments (YMM, FG, FM) would lower blood glucose and plasma insulin Cmax compared to the control pudding treatment was accepted. The Cmax results suggest modulations of glycemic and insulinemic response with the inclusion of soluble DF (YMM, FG, FM) and are important because of the potential to decrease the risk of developing T2D or aid individuals in managing T2D. The finding is also important because it supports that soluble DF-containing products matched for in vitro viscosity will similarly modulate glycemic response. Further research is required for each of the soluble DF (YMM, FG, FM), in particular to understand the response in different individuals and to determine what the optimal dose of each of the different soluble DF types (YMM, FG, FM). In summary, this research supports the role soluble DF (YMM, FG, FM) has on modulating acute
glycemic and insulinemic responses, confirming that soluble DF, and YMM, FG and FM, in particular, are dietary strategies to minimize the risk of developing T2D.
REFERENCES


50. Shakib MCR, Gabrial SGN. Post-prandial responses to different bread products based on wheat, barley and fenugreek or ginger or both in healthy volunteers and their effect on the glycemic index of such products. Journal of American Science. 2010;6:89-96.


61. The European Commission. Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID


## APPENDICES

### Appendix A: Acute Clinical Trials for the Glycemic Effect of Yellow Mustard Mucilage

<table>
<thead>
<tr>
<th>Reference</th>
<th>YMM Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with YMM</th>
</tr>
</thead>
</table>
| Lett AM et al.  
Yellow mustard bran attenuates glycaemic response of a semi-solid food in young healthy men.  
Int J Food Sci Nutr. 2013;64:140-146. (Ref 38) | - YMB with 30% SF (YMM) content                                                 | - Potato and leek soup with 5 g YMB (containing ~1.5 g YMM) (test product)  
- Potato and leek soup without YMB (control product)  
- Both matched for total available carbohydrate (25 g carbohydrate/100 g soup) | - n=10  
- Males  
- Healthy  
- 18-30 years (mean age 21.1 years)  
- BMI <30 kg/m² (mean BMI 23.3 kg/m²)  
- Moderately active  
- Non-smoker  
- FBG <6.1 mmol/L | - Randomized  
- Crossover  
- Blinding not described  
- 2 treatments (test and control)  
- 1 day washout | - Fasting and postprandial glucose response after each study treatment | - Significantly reduced peak blood glucose with YMB test product  
- Significantly delayed average time for glucose curve to peak with test product  
- Significantly different mean blood glucose at 15, 30, and 90 minutes favouring YMB test product |

Abbreviations used: YMM=Yellow mustard mucilage; YMB=Yellow mustard bran; SF=Soluble fibre; BMI=Body mass index; FBG=Fasting blood glucose
Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharma RD.</td>
<td>A: Whole fenugreek seed powder</td>
<td>A: - 25 g whole fenugreek seed powder (12 g TF and 5 g FG) and 100 g glucose in 250 mL water (test) - 100 g glucose in 250 mL water (control)</td>
<td>A: - n=8 - Gender not mentioned - Healthy - Age range not specified (mean age 35 years) - BMI range not specified - % ideal body weight 102</td>
<td>Studies (A-D): - Randomized - Crossover - Blinding not described - 2 treatments (test and control) - 1 week washout - 3 days prior to each visit participants consumed diets containing 250 g carbohydrate per day</td>
<td>Studies (A-D): - Fasting and postprandial glucose and insulin response after each study treatment</td>
<td>A: - Significant decrease in blood glucose response at 30 and 60 minutes in test compared to control - Significant reduction in glucose IAUC in test compared to control - Significant decrease in insulin response at 30 and 60 minutes in test compared to control - Significant reduction in insulin IAUC in test compared to control</td>
</tr>
<tr>
<td></td>
<td>B: Extracted fenugreek powder (defatted)</td>
<td>B: - 25 g extracted fenugreek seed powder (defatted) (12.92 g TF and 4.8 g FG) and 100 g glucose in 250 mL water (test) - 100 g glucose in 250 mL water (control)</td>
<td>B: - n=6 - 1 female and 5 males - Healthy - Age range not specified (mean age 34 years) - BMI range not specified - % ideal body weight 104</td>
<td></td>
<td>B: - Significant decrease in blood glucose response at 30 and 60 minutes in test compared to control - Significant reduction in glucose IAUC in test compared to control - Significant decrease in insulin response at 60 minutes in test compared to control - Significant reduction in insulin IAUC in test compared to control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C: Degummed fenugreek seeds</td>
<td>C: - 25 g degummed fenugreek seeds (total fibre content not specified) and 100 g glucose in 250 mL water (test) - 100 g glucose in 250 mL water (control)</td>
<td>C: - n=6 - Gender not mentioned - Healthy - Age range not specified (mean age 32 years) - BMI range not specified - % ideal body weight 110</td>
<td></td>
<td>C: - No significant difference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D: Cooked fenugreek seeds</td>
<td>D: - 25 g cooked fenugreek seeds (TF and FG content not specified) in potato soup (weight not specified) within a meal containing 200 g rice, 200 g potato, 25 g onion, and 50 g vegetable oil (test) - Same meal without soup (1350 kilo calories) (control) - Not mentioned if matched for carbohydrate content</td>
<td>D: - n=8 - Gender not mentioned - Healthy - Age range not specified (mean age 31 years) - BMI range not specified - % ideal body weight 102</td>
<td></td>
<td>D: - Significant reduction in glucose IAUC in test compared to control</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used: TF=Total Fibre; FG=Fenugreek gum; BMI=Body mass index; IAUC=Integrated AUC
Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
</table>
| Madar Z et al. | Ground fenugreek seeds | - 15 g ground fenugreek seeds in water with a meal containing 500 kcal (55% carbohydrate, 15% protein, and 30% fat) (test) | - n=21  
- Gender not mentioned  
- Classified with NIDDM  
- Age range not specified (mean age 58 years)  
- BMI range not specified  
- Weight range not specified (mean weight 74 kg)  
- Medication interventions not excluded (4 participants not on glucose lowering medication) | - Randomization not described  
- Crossover  
- Blinding not described  
- 2 treatments (test and control meal)  
- 4-7 day washout | - Fasting and postprandial glucose and insulin response after each study treatment | - Postprandial glucose levels significantly decreased with test meal compared to control |

Abbreviations used: Kcal=Kilocalories; %=percent; NIDDM=Non-insulin dependent diabetes mellitus; BMI=Body mass index; Kg=kilogram
## Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neeraja A and Rajyalakshmi P.</td>
<td>Whole dried fenugreek seed powder - Germinated and dried fenugreek seeds - Boiled and dried fenugreek seeds</td>
<td>Traditional Pongal breakfast meal containing 2 green chilies, 20 g onion, 0.14 g mustard, 0.3 g cumin seeds, 5 g oil, 0.12 g turmeric, 60 g rice and 30 g greengram dhal in 200 mL water, and salt for taste - Test products contained 12.5 g of either whole, germinated, or boiled, and dried fenugreek seeds within meal - Control contained no fenugreek seeds - Meals similar in nutrient composition, containing: 405-445 kilo calories, 15-18 g protein, 7-7.9 g fat, and 71-76 g carbohydrate - 12.5 g whole dried fenugreek seeds contained 5.98 g total fibre and 2.35 g SF (FG) - 12.5 g germinated and dried fenugreek seeds contained 4.23 g total fibre and 1.25 g SF (FG) - 12.5 g boiled and dried fenugreek seeds contained 3.75 g total fibre and 0.375 g SF (FG)</td>
<td>Healthy Participants - n=6 - Males - Healthy - 27-31 years (mean age not provided) - 60-80 kg - BMI range not specified - No signs and symptoms of deficiency - No drug therapies NIDDM Participants - n=6 - Males - Classified with NIDDM - 42-57 years (mean age not provided) - 51-76 kg - BMI range not specified</td>
<td>- Randomization not described - Crossover - Blinding not described - 4 treatments (3 test and 1 control) - 7 day washout - NIDDM participants refrained from medication one day prior to each study visit</td>
<td>- Fasting and postprandial glucose response after each study treatment</td>
<td>Healthy Participants - Significant decrease in blood glucose response with whole dried, and germinated, fenugreek seeds compared to control - Significant reduction in glucose AUC with whole dried, and germinated, fenugreek seeds compared to control NIDDM Participants - Not significant</td>
</tr>
</tbody>
</table>

Abbreviations used: SF=Soluble fibre; FG=Fenugreek gum; BMI=Body mass index; NIDDM=Non-insulin dependent diabetes mellitus; AUC=Area under the curve
Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
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<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bawadi HA et al. The postprandial hypoglycemic activity of fenugreek seed and seeds’ extract in Type 2 Diabetics: A pilot study. Phcog Mag. 2009;4:134-138. (Ref 47)</td>
<td>- Whole fenugreek seeds</td>
<td>- FG0 (control) – 0.8 g of dextrose in 25 mL warm water  - FG2.5 (test) – 2.5 g fenugreek seeds in a dextrose solution at a ratio of 1:5 (weight:volume)  - FG5 (test) – 5 g fenugreek seeds in a dextrose solution at a ratio of 1:5 (weight:volume)  - Not mentioned if matched for carbohydrate content</td>
<td>- n=166  - 91 females and 75 males  - Classified with T2D  - Age range not mentioned (mean age for each group 58, 59.7, and 53.3 years)  - BMI range not mentioned – not an inclusion or exclusion criteria  - Weight range not specified</td>
<td>- Randomized - Parallel-arm - Blinding not described - 3 treatments (control and 2 test) - Treatments consumed in non-fasted, postprandial state</td>
<td>- Difference between pre- and post-test blood glucose values</td>
<td>- Difference between pre- and post-test blood glucose values statistically lowered in FG5 group only, compared to FG0 group</td>
</tr>
</tbody>
</table>

Abbreviations used: FG0=Control group containing 0 g fenugreek seeds; FG2.5=Test group containing 2.5 g fenugreek seeds; FG5=Test group containing 5 g fenugreek seeds; BMI=Body mass index; T2D=Type 2 Diabetes
## Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kumar S et al.</strong>&lt;br&gt;Comparative study of fenugreek seeds on glycemic index in high and medium dietary fiber containing diets in NIDDM patients. NJIRM. 2011;2:29-37. (Ref 48)</td>
<td>- Germinated fenugreek seed powder</td>
<td>- OGTT with 50 g glucose in 250 mL water (control)&lt;br&gt;- 200 g of rice (control)&lt;br&gt;- 103 g wheat chapatti (control)&lt;br&gt;- 200 g rice with 12.5 g germinated fenugreek seed powder (test)&lt;br&gt;- 103 g wheat chapatti with 12.5 g germinated fenugreek seed powder (test)&lt;br&gt;- 12.5 g germinated fenugreek seed powder contained 6 g total fibre, and 2.5 g FG&lt;br&gt;- 15 participants consumed fenugreek seeds in water 15 minutes before meal&lt;br&gt;- 10 participants consumed fenugreek seeds in water during the meal&lt;br&gt;- Treatments matched for 50 g carbohydrate</td>
<td>- n=25&lt;br&gt;- 12 females and 13 males&lt;br&gt;- Classified with NIDDM&lt;br&gt;- 40-60 years (mean age 50 years)&lt;br&gt;- BMI range not mentioned&lt;br&gt;- Weight range not mentioned&lt;br&gt;- Consumed oral hypoglycemic medication</td>
<td>- Not randomized&lt;br&gt;- Crossover&lt;br&gt;- Blinding not described&lt;br&gt;- 5 treatments (3 control and 2 test)&lt;br&gt;- No washout period between study visits</td>
<td>- Fasting and postprandial glucose response after each study treatment&lt;br&gt;- GI of food products (test food glucose AUC/control glucose AUC * 100)</td>
<td>- Glucose AUC for all control and test meals significantly lower than OGTT&lt;br&gt;- GI significantly lower for test meals in group consuming 12.5 g germinated fenugreek seeds 15 minutes before meal compared to group consuming seeds with meal</td>
</tr>
</tbody>
</table>

Abbreviations used: NIDDM=Non-insulin dependent diabetes mellitus; OGTT=Oral glucose tolerance test; FG=Fenugreek gum; GI=Glycemic index; AUC=Area under the curve
Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
</table>
- Dhokla - 250 g serving incorporating millet, legumes, and 25 g fenugreek seeds (6.85 g crude fibre) (test)  
- Uppuma - 230 g serving incorporating millet, legumes, 23 g fenugreek seeds, and coconut oil (8.03 g crude fibre) (test)  
- Laddu - 80 g serving incorporating amaranth, millet, legumes, and 20 g fenugreek seeds (4.86 g crude fibre) (test)  
- Treatments matched for 50 g carbohydrate | Healthy Participants  
- n=5  
- Females  
- Healthy  
- 22-25 years (mean age not provided)  
- BMI 22.5-28.5 kg/m² (mean BMI not provided)  
- No drug therapies  
- Non-smoker  
NIDDM Participants  
- n=5  
- Males  
- Classified with NIDDM  
- 57-70 years (mean age not provided)  
- BMI 22.5-28.5 kg/m² (mean BMI not provided)  
- No drug therapies  
- Non-smoker | - Randomized  
- Crossover  
- Blinding not described  
- 4 treatments (1 control and 3 test)  
- Washout period not described | - Fasting and postprandial glucose response after each study treatment  
- GI of food products (test food glucose AUC/control glucose AUC * 100)  
- GI of each food product significantly different in both healthy and NIDDM participants  
- GI from highest to lowest was dhokla, laddu, and uppuma |

Abbreviations used: OGTT=Oral glucose tolerance test; BMI=Body mass index; NIDDM=Non-insulin dependent diabetes mellitus; GI=Glycemic index; AUC=Area under the curve
### Appendix B: Acute Clinical Trials for the Glycemic Effect of Fenugreek Seeds - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Seed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Seeds</th>
</tr>
</thead>
</table>
| Shakib MCR and Gabriel SGN.             | Fenugreek Seed Flour      | WWb – 100% refined wheat flour (control)  
- BWb – 50% barley flour and 50% refined wheat flour (test)  
- BWFb-5 – 95% BWb recipe and 5% fenugreek seed flour (5.95 g fenugreek seed flour in treatment serving) (test)  
- BWFb-2.5 – 97.5% BWb recipe and 2.5% fenugreek seed flour (2.98 g fenugreek seed flour in treatment serving) (test)  
- BWFGb – 98% BWb recipe, 1% fenugreek seed flour, and 1% ginger flour (test)  
- All treatments matched for 50 g carbohydrate  
- Water allowed to be consumed during study visit | n=20  
- 8 females and 12 males  
- Healthy  
- Age range not specified (mean age 40 years)  
- BMI range not specified (mean BMI 21.5 kg/m²)  
- Non-Smoking  
- No drug therapies | Randomized  
- Crossover  
- Blinding not described  
- 5 treatments (1 control and 4 test)  
- Washout period not described | Fasting and postprandial glucose response after each study treatment  
- GI of food products (test food glucose AUC/control glucose AUC * 100) | All test breads significantly decreased blood glucose at all postprandial time points compared to WWb control  
- BWFGb significantly decreased blood glucose further at 30 minutes compared to BWb  
- BWFb-5 significantly decreased blood glucose further at 60 minutes compared to BWb  
- All test breads significantly decreased glucose AUC compared to WWb control  
- All test breads had significantly lower GI compared to WWb control  
- Glucose AUC and GI for BWFb-5 and BWFGb were significantly lower than BWb values |

Abbreviations used:  
WWb=White bread control; BWb=Barley and white bread test meal; BWFb-5=Barley, wheat, and fenugreek bread test meal containing 5% fenugreek seed flour; BWFb-2.5=Barley, wheat, and fenugreek bread test meal containing 2.5% fenugreek seed flour; BWFGb=Barley, wheat, fenugreek, and ginger bread test meal containing 1% fenugreek seed flour and 1% ginger flour; BMI=Body mass index; GI=Glycemic index; AUC=Area under the curve
### Appendix C: Acute Clinical Trials for the Glycemic Effect of Fenugreek Gum

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Gum Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Gum</th>
</tr>
</thead>
</table>
| Sharma RD. | Isolated FG from fenugreek seeds | - 5 g isolated FG and 100 g glucose in 250 mL water (test)  
- 100 g glucose in 250 mL water (control) | n=6  
- Gender not mentioned  
- Healthy  
- Age range not specified (mean age 25 years)  
- BMI range not specified  
- % ideal body weight 102 | Randomized  
- Crossover  
- Blinding not described  
- 2 treatments (test and control)  
- 1 week washout period  
- 3 days prior to each visit participants consumed diets containing 250 g carbohydrate per day | Fasting and postprandial glucose and insulin response after each study treatment | - Significant decrease in blood glucose response at 30 and 60 minutes in test compared to control  
- Significant reduction in glucose IAUC in test compared to control  
- Significant decrease in insulin response at 30 and 60 minutes in test compared to control  
- Significant reduction in insulin IAUC in test compared to control |

Abbreviations used: FG=Fenugreek gum; BMI=Body mass index; IAUC=Integrated area under the curve; AUC=Area under the curve
### Appendix C: Acute Clinical Trials for the Glycemic Effect of Fenugreek Gum - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Fenugreek Gum Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Fenugreek Gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mathern JR et al. Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. Phytother Res. 2009;23:1 543-1548. (Ref 51)</td>
<td>- Purified fenugreek fibre</td>
<td>- 0 g fenugreek fibre in 8 ounces Minute Maid Light with 4 ounces crushed ice (control)</td>
<td>- n=18</td>
<td>- Randomized</td>
<td>- Fasting and postprandial glucose and insulin response after each study treatment</td>
<td>- Insulin AUC significantly increased after 8 g fenugreek fibre meal test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 4 g fenugreek fibre (3.6 g FG) in 8 ounces Minute Maid Light with 4 ounces crushed ice (test)</td>
<td>- 10 females and 8 males</td>
<td>- Crossover</td>
<td></td>
<td>- Peak insulin significantly greater in 8 g fenugreek fibre test meal compared to control meal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 8 g fenugreek fibre (7.2 g FG) in 8 ounces Minute Maid Light with 4 ounces crushed ice (test)</td>
<td>- Healthy</td>
<td>- Single-blinded</td>
<td></td>
<td>- Peak insulin significantly lower in 4 g fenugreek fibre test meal compared to 8 g fenugreek fibre test meal, but not compared to control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- All beverages consumed with same low-fibre test meal</td>
<td>- Obese</td>
<td>- 3 treatments (control and 2 test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- All meals matched in nutrients except fibre</td>
<td>- 18-65 years (mean age 32 years)</td>
<td>- 48 hour washout period</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- BMI ≥ 30 kg/m^2 (mean BMI 36 kg/m^2)</td>
<td>- Participants avoided high-fibre diets the day before each visit</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Non-smoker</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations used: FG=Fenugreek gum; BMI=Body mass index; AUC=Area under the curve
Appendix D: Acute Clinical Trials for the Glycemic Effect of Flaxseeds

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flaxseed Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Flaxseeds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cunnane SC et al.</strong> High α-linolenic acid flaxseed (Linum usitatissimum): some nutritional properties in humans. Br J Nutr. 1993;69:443-453. (Ref 55) (Only discussing flaxseed flour and control groups)**</td>
<td>- Flaxseed flour</td>
<td>- Bread made from flaxseed flour (amount of flaxseed flour not provided) – nutrition composition not provided - Bread made from white flour – nutrition composition not provided (control) - Both breads matched for 50 g carbohydrate</td>
<td>- n=6 - 1 female and 5 male - Healthy - Age range not specified (mean age 30 years) - BMI range and mean not specified</td>
<td>- Randomized - Crossover - Blinding not specified - 2 treatments (1 test and 1 control) - Washout period between study visits not described</td>
<td>- Fasting and postprandial glucose response after consuming treatment breads matched for 50 g carbohydrate</td>
<td>- Significantly decreased glucose iAUC with flaxseed bread compared to white bread</td>
</tr>
<tr>
<td><strong>Kristensen M et al.</strong> Flaxseed dietary fibers suppress postprandial lipemia and appetite sensation in young men. Nutr Metab Cardiovasc Dis. 2013;23:136-43. (Ref 56) (Only discussing control and whole flaxseed glycemic results)**</td>
<td>- Whole flaxseed</td>
<td>- Control and test meal consisted of two buns with cheese, butter, and ham, and 400mL of water - 12.18 g whole flaxseeds baked into the two buns in test meal (whole meal contained 12 g DF) - Control meal contained no flaxseed (whole control meal contained 7 g DF) - Test meals contained ~40% of their daily energy requirements - Test and control meal isocaloric and matched for 147 g carbohydrate, 49 g fat, and 44 g protein</td>
<td>- n=18 - Males - Healthy - 18-40 years (mean age 27.2 years) - BMI range 22-30 kg/m² (mean BMI 25.4 kg/m²)</td>
<td>- Randomized - Crossover - Double-blinded - 2 treatments (1 control and 1 test) - Washout period between the treatments not described</td>
<td>- Fasting and postprandial glucose and insulin response after each study meal</td>
<td>- No significant difference</td>
</tr>
</tbody>
</table>

Abbreviations used: DF=Dietary fibre; BMI=Body mass index
### Appendix E: Acute Clinical Trials for the Glycemic Effect of Flaxseed Mucilage

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flaxseed Mucilage Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Flaxseed Mucilage</th>
</tr>
</thead>
</table>
| Dahl WJ et al.     | BakeOmega flax fibre   | - Bread product containing 11 g flax fiber [2.7 g SF (FM)] per 125 g of bread (test)  
- 105 g of control bread containing no added flax fibre  
- Both breads matched for 50 g available carbohydrate  
- Also contained IF flax fibre components | - n=11  
- Females and males (breakdown not provided)  
- Healthy  
- 20-26 years (mean age 22.8 years)  
- BMI range 19.6-29.5 kg/m² (mean BMI 22.6 kg/m²)  
- Normal fasting glucose (range not specified) | - Randomization not specified  
- Crossover  
- Blinding not specified  
- 2 treatments (1 control and 1 test bread given 2 times each)  
- Washout period between the treatments not described | - Fasting and postprandial glucose response after each treatment | - Significantly decreased glucose iAUC with flax fibre bread compared to control bread  
- Significantly decreased peak blood glucose with flax fibre bread compared to control bread |

Abbreviations used: FM=Flaxseed mucilage; SF=Soluble fibre; IF=Insoluble fibre; BMI=Body mass index; iAUC=Incremental area under the curve
### Appendix E: Acute Clinical Trials for the Glycemic Effect of Flaxseed Mucilage - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flaxseed Mucilage Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Flaxseed Mucilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kristensen M et al. Flaxseed dietary fibers suppress postprandial lipemia and appetite sensation in young men. Nutr Metab Cardiovasc Dis. 2013;23:136-43. (Ref 56) (Only discussing control and FM glycemic results)</td>
<td>- Extracted FM</td>
<td>- Control and test meals consisted of two buns with cheese, butter, and ham, and 400mL of water - 12.18 g FM baked into the two buns in test meal (whole meal contained 12 g DF) (LM) - 17.27 g FM baked into the two buns in test meal (whole meal contained 17 g DF) (HM) - Control meal contained no flaxseed (whole control meal contained 7 g DF) (control) - Test meals contained ~40% of their daily energy requirements - Test and control meal isocaloric and matched for 147 g carbohydrate, 49 g fat, and 44 g protein</td>
<td>- n=18 - Males - Healthy - 18-40 years (mean age 27.2 years) - BMI range 22-30 kg/m² (mean BMI 25.4 kg/m²)</td>
<td>- Randomized - Crossover - Double-blinded - 3 treatments (1 control and 2 test) - Washout period between the treatments not described</td>
<td>- Fasting and postprandial glucose and insulin response after each study meal</td>
<td>- Significantly decreased insulin response at 30 minutes with HM meal compared to control meal - Significantly decreased insulin AUC for LM and HM meals compared to control (not significantly different between FM doses)</td>
</tr>
</tbody>
</table>

Abbreviations used: FM=Flaxseed mucilage; DF=Dietary fibre; LM=Low mucilage; HM=High mucilage; BMI=Body mass index; AUC=Area under the curve
### Appendix E: Acute Clinical Trials for the Glycemic Effect of Flaxseed Mucilage - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flaxseed Mucilage Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Flaxseed Mucilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au MMC et al. Effects of soy-soluble fiber and flaxseed gum on the glycemic and insulinemic responses to glucose solutions and dairy products in healthy adult males. J Am Coll Nutr. 2013;32:98-110. (Ref 58) (Only discussing control and FM glycemic results)</td>
<td>- Extracted FM</td>
<td>- 1.8 g FM in a glucose solution (250 g volume) (test)  - 2.5 g FM in a dairy beverage (250 g volume) (test)  - 2.5 g FM in a dairy pudding (250 g volume) (test)  - Control glucose solution (consumed twice) (control reference) (250 g volume)  - Dairy beverage, and dairy pudding containing 0 g FM (250 g volumes)  - Control dairy pudding contained 0.25 g κ-carrageenan (0.25 g fibre) for thickness  - All treatments matched for 50 g available carbohydrate  - 125 mL water consumed with all treatments</td>
<td>- n=12  - Males  - Healthy  - 19-40 years (mean age 25.3 years)  - BMI range 18.5-26.0 kg/m² (mean BMI 22.7 kg/m²)  - Non-smokers  - Normal fasting blood glucose &lt; 5.6 mmol/L</td>
<td>- Randomized  - Crossover  - Double-blinded  - 7 treatments (3 test and 4 control)  - Washout period of 7 days between study visits</td>
<td>- Fasting and postprandial glucose and insulin response after each study meal  - GI of food products (test food glucose iAUC/control glucose iAUC * 100)  - II of food products (test food insulin iAUC/control insulin iAUC * 100)</td>
<td>- Significantly decreased glucose iAUC, GI, and peak glucose values for the FM dairy treatments compared to the reference  - Glucose time-to-peak significantly decreased in FM dairy pudding compared to reference  - Insulin time-to-peak significantly decreased in FM dairy beverage compared to reference</td>
</tr>
</tbody>
</table>

Abbreviations used: FM=Flaxseed mucilage; BMI=Body mass index; GI=Glycemic index; iAUC=Incremental area under the curve; II=Insulinemic index
Appendix E: Acute Clinical Trials for the Glycemic Effect of Flaxseed Mucilage - Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flaxseed Mucilage Form</th>
<th>Study Treatments</th>
<th>Participant Characteristics</th>
<th>Study Design</th>
<th>Glycemic Endpoints</th>
<th>Significant Glycemic Results with Flaxseed Mucilage</th>
</tr>
</thead>
</table>
| Cunnane SC et al.  
High α-linolenic acid flaxseed (Linum usitatissimum): some nutritional properties in humans. Br J Nutr. 1993;69:443-453. (Ref 55) (Only discussing FM and control groups) | - Extracted FM | - 25 g FM in 50 g glucose solution (test)  
- 50 g glucose solution without FM (control) | - n=4  
- Gender not specified  
- Health status not specified  
- Age range and mean not specified  
- BMI range and mean not specified | - Randomized  
- Crossover  
- Blinding not specified  
- 2 treatments (1 test and 1 control)  
- Washout period between study visits not specified | - Fasting and postprandial glucose response after consuming treatments matched for 50 g carbohydrate | - Significantly decreased glucose iAUC with FM glucose solution compared to glucose solution control |

Abbreviations used: FM=Flaxseed mucilage; BMI=Body mass index; iAUC=Incremental area under the curve
Appendix F: University of Guelph Research Ethics Board Approval

UNIVERSITY
OF GUELPH
RESEARCH ETHICS BOARDS
Certification of Ethical Acceptability of Research
Involving Human Participants

APPROVAL PERIOD: June 16, 2014
EXPIRY DATE: June 16, 2016
REB: NPES
REB NUMBER: 14AP003
TYPE OF REVIEW: Full Board
PRINCIPAL INVESTIGATOR: Duncan, Alison (amduncan@uoguelph.ca)
DEPARTMENT: Human Health & Nutritional Sciences
SPONSOR(S): OMAFRA
TITLE OF PROJECT: The effect of fibre-enriched pudding products on glycemic and satiety response in adults at risk for type 2 diabetes

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: November 10, 2015

I. Kuczynski
Chair, Research Ethic Board-General
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 Sweet Drink Study: The effect of fibre-enriched beverages on glycemic and satiety response in adults at risk for type 2 diabetes

NUMBER: H-254-11-16-07

LOCATION: Bldg 88, Room 143; Bldg 70, Room 316

APPROVED FOR THE PERIOD: 2014 July 08 TO 2016 July 31

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: [Signature]  Approved: [Signature]
Chair, Biosafety Committee University Biosafety Officer

Date: July 9, 2014 Date: JULY 9, 2014
Appendix H: The Canadian Diabetes Risk Questionnaire (CANRISK)

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 may 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 score 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: 0 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 (or 1.575m) and 163 pounds (or 74kg) 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 34): 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 at 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 34 inches OR 86 cm: 0 points
   - Between 34-38 inches OR 86-96 cm: 4 points
   - Over 38 inches OR 96 cm: 6 points

   **WOMEN** - Waist circumference: ________ inches OR ________ cm
   - Less than 31 inches OR 78 cm: 0 points
   - Between 31-35 inches OR 78-88 cm: 4 points
   - Over 35 inches OR 88 cm: 6 points
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: 0 points  
   - No: 1 point

6. How often do you eat vegetables or fruits?  
   - Every day: 0 points  
   - Not every day: 2 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 a 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?  
    - Mother: 2 points  
    - Father: 2 points  
    - Brothers/Sisters: 2 points  
    - Children: 2 points  
    - Other relatives: 0 points  
    - None: 0 points

Add your score. Your combined score cannot be more than 6 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) parent(s) belong to.  
    - Mother:  
      - White (Canadian): 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 visible (Latin American, Arab, West Asian): 3 points
    - Father:  
      - White (Canadian): 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 visible (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

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.
Appendix I: Food Neophobia Questionnaire

Study Name: The Pudding Study

Participant Screening Number: 

REB Number: 14AP003
Researcher Initials: 
Date: 

Food Neophobia Questionnaire

Please complete the following standardized questionnaire by circling the response that is MOST appropriate to you. There are no right or wrong answers.

1. I am constantly sampling new and different foods
   A  B  C  D  E  F  G
   Agree Agree Agree Neither Agree Disagree Disagree Disagree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely

2. I do not trust new foods
   A  B  C  D  E  F  G
   Disagree Disagree Disagree Neither Agree Agree Agree Agree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely

3. If I don’t know what a food is, I won’t try it
   A  B  C  D  E  F  G
   Disagree Disagree Disagree Neither Agree Agree Agree Agree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely

4. I like foods from different countries
   A  B  C  D  E  F  G
   Agree Agree Agree Agree Neither Agree Disagree Disagree Disagree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely

5. Ethnic food looks too weird to eat
   A  B  C  D  E  F  G
   Disagree Disagree Disagree Neither Agree Agree Agree Agree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely

6. At dinner parties, I will try a new food
   A  B  C  D  E  F  G
   Agree Agree Agree Agree Neither Agree Disagree Disagree Disagree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely

7. I am afraid to eat things I have never had before
   A  B  C  D  E  F  G
   Disagree Disagree Disagree Neither Agree Agree Agree Agree
   Extremely Moderately Slightly or Disagree Slightly Moderately Extremely
8. I am very particular about the foods I eat

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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</thead>
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<tr>
<td></td>
<td>Disagree</td>
<td>Disagree</td>
<td>Disagree</td>
<td>Neither Agree or Disagree</td>
<td>Agree</td>
<td>Agree</td>
<td>Agree</td>
</tr>
<tr>
<td></td>
<td>Extremely</td>
<td>Moderately</td>
<td>Slightly</td>
<td>or Disagree</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Extremely</td>
</tr>
</tbody>
</table>

9. I will eat almost anything

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agree</td>
<td>Agree</td>
<td>Agree</td>
<td>Neither Agree or Disagree</td>
<td>Disagree</td>
<td>Disagree</td>
<td>Disagree</td>
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<tr>
<td></td>
<td>Extremely</td>
<td>Moderately</td>
<td>Slightly</td>
<td>or Disagree</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Extremely</td>
</tr>
</tbody>
</table>

10. I like to try new ethnic restaurants

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Agree</td>
<td>Agree</td>
<td>Agree</td>
<td>Neither Agree or Disagree</td>
<td>Disagree</td>
<td>Disagree</td>
<td>Disagree</td>
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<tr>
<td></td>
<td>Extremely</td>
<td>Moderately</td>
<td>Slightly</td>
<td>or Disagree</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Extremely</td>
</tr>
</tbody>
</table>
Appendix J: Three-Factor Eating Questionnaire

Study Name: The Pudding Study
Participant Screening Number: __________
REB Number: 14AP003
Researcher Initials: __________
Date: ________________

Three Factor Eating Questionnaire

Please complete the following standardized questionnaire by circling the response that is MOST appropriate to you. There are no right or wrong answers.

PART ONE
Please mark (X) True or False according to how you feel each of the following statements apply to you.

<table>
<thead>
<tr>
<th>#</th>
<th>Question</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I usually eat too much at social occasions, like parties and picnics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I am usually so hungry that I eat more than three times a day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>When I have eaten my quota of calories, I am usually good about not eating any more</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Dieting is hard for me because I just get so hungry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I deliberately take small helpings as a means of controlling my weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sometimes things just taste so good that I keep on eating even when I am no longer hungry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>When I feel anxious, I find myself eating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Life is too short to worry about dieting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Since my weight goes up and down, I have gone on reducing diets more than once</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>I often feel so hungry that I just have to eat something</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>When I am with someone who is overeating, I usually overeat too</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>I have a pretty good idea of the number of calories in common foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Sometimes when I start eating, I just can’t seem to stop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>It is not difficult for me to leave something on my plate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Question</td>
<td>True</td>
<td>False</td>
</tr>
<tr>
<td>----</td>
<td>--------------------------------------------------------------------------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>17</td>
<td>At certain times of the day, I get hungry because I have gotten used to eating then</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Being with someone who is eating often makes me feel hungry enough to eat also</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>When I feel blue, I often overeat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>I enjoy eating too much to spoil it by counting calories or watching my weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>When I see a real delicacy, I often get so hungry that I have to eat right away</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>I often stop eating when I am not really full as a conscious means of limiting the amount that I eat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>I get so hungry that my stomach often feels like a bottomless pit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>My weight has hardly changed at all in the last ten years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>I am always hungry so it is hard for me to stop eating before I finish the food on my plate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>When I feel lonely, I console myself by eating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>I consciously hold back at meals in order not to gain weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>I sometimes get very hungry late in the evening or at night</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>I eat anything I want, any time I want</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Without even thinking about it, I take a long time to eat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>I count calories as a conscious means of controlling my weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>I do not eat some foods because they make me fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>I am always hungry enough to eat at any time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>I pay a great deal of attention to changes in my figure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### PART TWO

| 37. How often are you dieting in a conscious effort to control your weight?  
**Please mark (X) your best answer** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rarely</td>
</tr>
<tr>
<td>Sometimes</td>
</tr>
<tr>
<td>Usually</td>
</tr>
<tr>
<td>Always</td>
</tr>
</tbody>
</table>

| 38. Would a weight fluctuation of 5 pounds affect the way you live your life?  
**Please mark (X) your best answer** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
</tr>
<tr>
<td>Slightly</td>
</tr>
<tr>
<td>Moderately</td>
</tr>
<tr>
<td>Very much</td>
</tr>
</tbody>
</table>

| 39. How often do you feel hungry?  
**Please mark (X) your best answer** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Only at mealtimes</td>
</tr>
<tr>
<td>Sometimes between meals</td>
</tr>
<tr>
<td>Often between meals</td>
</tr>
<tr>
<td>Almost always</td>
</tr>
</tbody>
</table>

| 40. Do your feelings of guilt about overeating help you to control your food intake?  
**Please mark (X) your best answer** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
</tr>
<tr>
<td>Rarely</td>
</tr>
<tr>
<td>Often</td>
</tr>
<tr>
<td>Always</td>
</tr>
</tbody>
</table>

| 41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?  
**Please mark (X) your best answer** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy</td>
</tr>
<tr>
<td>Slightly difficult</td>
</tr>
<tr>
<td>Moderately difficult</td>
</tr>
<tr>
<td>Very difficult</td>
</tr>
</tbody>
</table>

| 42. How conscious are you of what you are eating?  
**Please mark (X) your best answer** |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
</tr>
<tr>
<td>Slightly</td>
</tr>
<tr>
<td>Moderately</td>
</tr>
<tr>
<td>Extremely</td>
</tr>
</tbody>
</table>
43. How frequently do you avoid “stocking up” on tempting foods?

<table>
<thead>
<tr>
<th>Please mark (X) your best answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almost never</td>
</tr>
<tr>
<td>Seldom</td>
</tr>
<tr>
<td>Usually</td>
</tr>
<tr>
<td>Almost always</td>
</tr>
</tbody>
</table>

44. How likely are you to shop for low calorie foods?

<table>
<thead>
<tr>
<th>Please mark (X) your best answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlikely</td>
</tr>
<tr>
<td>Slightly likely</td>
</tr>
<tr>
<td>Moderately likely</td>
</tr>
<tr>
<td>Very likely</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Please mark (X) your best answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
</tr>
<tr>
<td>Rarely</td>
</tr>
<tr>
<td>Often</td>
</tr>
<tr>
<td>Always</td>
</tr>
</tbody>
</table>

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

<table>
<thead>
<tr>
<th>Please mark (X) your best answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlikely</td>
</tr>
<tr>
<td>Slightly likely</td>
</tr>
<tr>
<td>Moderately likely</td>
</tr>
<tr>
<td>Very likely</td>
</tr>
</tbody>
</table>

47. How frequently do you skip dessert because you are no longer hungry?

<table>
<thead>
<tr>
<th>Please mark (X) your best answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almost never</td>
</tr>
<tr>
<td>Seldom</td>
</tr>
<tr>
<td>Usually</td>
</tr>
<tr>
<td>Almost always</td>
</tr>
</tbody>
</table>

48. How likely are you to consciously eat less than you want?

<table>
<thead>
<tr>
<th>Please mark (X) your best answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlikely</td>
</tr>
<tr>
<td>Slightly likely</td>
</tr>
<tr>
<td>Moderately likely</td>
</tr>
<tr>
<td>Very likely</td>
</tr>
</tbody>
</table>
49. Do you go on eating binges though you are not hungry?  
*Please mark (X) your best answer*

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td></td>
</tr>
<tr>
<td>Rarely</td>
<td></td>
</tr>
<tr>
<td>Often</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never “giving in”), what number would you give yourself?  
*Please mark (X) your best answer*

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0. Eat whenever you want, whenever you want it</td>
<td></td>
</tr>
<tr>
<td>1. Usually eat whenever you want, whenever you want it</td>
<td></td>
</tr>
<tr>
<td>2. Often eat whatever you want, whenever you want it</td>
<td></td>
</tr>
<tr>
<td>3. Often limit intake, but often “give in”</td>
<td></td>
</tr>
<tr>
<td>4. Usually limit food intake, rarely give “in”</td>
<td></td>
</tr>
<tr>
<td>5. Constantly limiting food intake, never “giving in”</td>
<td></td>
</tr>
</tbody>
</table>

51. To what extent does this statement describe your eating behaviour? “I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.”  
*Please mark (X) your best answer*

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not like me</td>
<td></td>
</tr>
<tr>
<td>Little like me</td>
<td></td>
</tr>
<tr>
<td>Pretty good description of me</td>
<td></td>
</tr>
<tr>
<td>Describes me perfectly</td>
<td></td>
</tr>
</tbody>
</table>
Appendix K: Participant Recruitment Poster

Pudding Study

Adult males and females 18-70 yrs old are needed for a nutrition study on the effects of consuming a fibre-enriched pudding product on type 2 diabetes risk.

This study will involve:

• Ten study visits which will each involve:
  A 3-hr morning study visit each week for 10 weeks where a pudding product or glucose drink will be consumed and blood samples will be taken over two hours

*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 Human Research Ethics Board (REB#14AP003)

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

519-824-4120 x58081 or pudding@uoguelph.ca
Appendix L: Screening-1 Questionnaire

Study Name: The Pudding Study
REB Number: 14AP003

Participant Screening Number: ___________
Researcher Initials: ___________
Date: ___________

The effect of fibre-enriched pudding products on glycemic and satiety responses in adults at risk for type 2 diabetes

Name of study coordinator: _______________ Date: _______________ Time: _______________

Name of caller: _______________ Gender: _______________

Phone #: _______________ Email: _______________ Best way to get in touch: _______________

1. How did you hear about the study? _______________

2. How old are you? _______________

3. Are you male or female? _______________

4. How tall are you? _______________

5. How much do you weigh? _______________ BMI _______________

6. Do you smoke? YES NO

7. Are you currently pregnant or breastfeeding or planning to become pregnant? YES NO

8. Have you ever been diagnosed with diabetes? YES NO

9. Are you currently taking any 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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<td></td>
<td></td>
</tr>
</tbody>
</table>

10. Do you have any medical conditions? YES NO
   a. If YES, please describe _______________

11. Do you routinely consume acetaminophen containing products? (i.e. Tylenol) YES NO
   a. If YES, how often? _______________

12. Do you have any food allergies? YES NO
   a. If YES, please describe _______________

CANRISK Scores:
- 0 points for <44 years
- 7 points for 45-54 years
- 13 points for 55-64 years
- 15 points for 65-70 years

- 0 points for FEMALE
- 8 points for MALE

- 0 points for BMI <25 kg/m²
- 4 points for BMI 25-29 kg/m²
- 9 points for BMI 30-34 kg/m²
- 14 points for BMI >35 kg/m²
13. Are you vegetarian or vegan?  
   a. If YES, please describe  
   b. If YES, are you comfortable with consuming pork gelatin?  
      (as this is an ingredient in the Kraft Knox Gelatin to thicken the puddings)  
      YES  NO

14. Do you have any allergies?  
   a. If YES, please describe  

15. Do you consume alcohol?  
   YES  NO  
   If so, how many drinks per week? _____  
   If so, how many typically in one setting? _____  
   (1 drink = 12oz beer, 5oz wine, 1.5oz hard liquor)  

16. Do you usually do some physical activity such as brisk walking for at least 30 minutes each day?  
   YES  NO  

17. How often do you eat vegetables or fruits? Would you say:  
   a. Everyday  
   b. Not everyday  

18. 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?  
   a. Yes  
   b. No or Don’t Know  

19. Have you ever been found to have a high blood sugar either from a blood test, during an illness, or during pregnancy?  
   a. Yes  
   b. No or Don’t Know  

20. Have any of your blood relatives ever been diagnosed with diabetes?  
   YES  NO  
   a. If YES, please indicate which family member  

21. Please indicate which ethnic groups your biological (blood) parents belong to:  
   a. Mother  
   b. Father  

CANRISK Score:
0 points for WHITE/CAUCASIAN  
3 points for ABORIGINAL/OTHER NON-WHITE (LATIN AMERICAN, ARAB, WEST ASIAN)  
3 points for BLACK (AFRO-CARIBBEAN)  
10 points for EAST ASIAN (CHINESE, VIETNAMESE, FILIPINO, KOREAN, etc.)  
11 points for SOUTH ASIAN (EAST INDIAN, PAKISTANI, SRI LANKAN, etc.)
Appendix M: Screening-2 Consent Form

(Will be printed on University of Guelph Letterhead)

CONSENT TO PARTICIPATE IN RESEARCH

The Pudding Study
The effects of fibre-enriched pudding products on glycemic and satiety responses in adults at risk for type 2 diabetes.

Body Measurements and Questionnaire Screening-2 Study Visit

INTRODUCTION
You are being asked to participate in screening for a research study directed by Professors Alison Duncan and Amanda Wright of the Department of Human Health and Nutritional Sciences (HHNS), and Professor Doug Goff at the University of Guelph. The results of this research will contribute to the theses of University of Guelph M.Sc. student Brittney Kay and Ph.D. student Nikolay Repin and to the research activities of HHNS M.Sc. and B.Sc. students at the University of Guelph. This research is funded by the Ontario Ministry of Agriculture and Food (OMAFRA).

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

Brittney Kay, 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: brittney@uoguelph.ca

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

Amanda Wright, Ph.D.
University of Guelph Study Director
Associate Professor, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x54697 or email: ajwright@uoguelph.ca

PURPOSE AND DESCRIPTION OF RESEARCH
Adequate dietary fibre intake is a potential dietary strategy to reduce the risk of and to help manage type 2 diabetes. Research to date has shown that consumption of a variety of fibre types can effectively lower the postprandial glucose and insulin response, leading to improved insulin sensitivity in several populations. The purpose of this research is to determine if eating pudding products made with a variety of less
common fibre sources (fenugreek gum, soluble flaxseed gum, and yellow mustard gum) will reduce the absorption of glucose in the blood stream (known as lowering glycemic response), as well as increase feelings of fullness (known as satiety). The study aims to assess which fibre types are most effective at improving glycemic response and satiety and to support the potential development of dietary fibre fortified products.

This study is double-blinded which means that neither the researchers nor the participants know which treatment a participant is consuming at any time. This study is also a randomized design, which means that participants will be randomly assigned an order in which to consume each of the 8 pudding products. At each study visit, participants' blood samples will be analyzed for markers of type 2 diabetes, including fasting and postprandial glucose and insulin levels. Paracetamol (acetaminophen) absorption as an indicator of gastric emptying related to satiety and glucose absorption will also be studied. A total of 15 participants between the ages of 18 to 70 years of age, who are at risk for type 2 diabetes, 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, and answering three screening questionnaires, which is the focus of this consent form and is described below.

**STUDY SCREENING PROCEDURES**
The screening process for this study will take approximately 30 minutes. If you choose to volunteer to participate, 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-circumference, and blood pressure, measured 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 the CANRISK questionnaire which is a standard tool used to assess type 2 diabetes risk. This is a 2-page questionnaire adapted from Health Canada that gathers information on your lifestyle, medical and family history, height, and body measurements to determine your risk of diabetes.
- Complete two questionnaires that assess 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.
• Every effort to ensure your comfort and safety will be made during the course of this screening.
• All body measurements will be completed by a trained study coordinator in a private area and according to set standard operating procedures.
• All information obtained about you, your health, medical and family history will be collected privately and kept confidential.
• 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 take part 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, or have already developed, type 2 diabetes. This research may lead to the use of the fibre types listed above in pudding-based food products aimed at reducing the risk of diabetes.

PAYMENT FOR PARTICIPATION
You will not receive any compensation from participating in this screening visit.

COSTS FOR PARTICIPATION
There is no direct cost for participating in this study screening visit. You will only be responsible for covering any costs related to ensuring you are able to attend your scheduled study visit (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
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 Pudding Study: The effect of fibre-enriched pudding products on glycemic and satiety response in adults at risk for type 2 diabetes - 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>
</tr>
</thead>
</table>

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

**Study Name:** The Pudding Study  
**REB Number:** 14AP003

**Participant Screening Number:** ______  
**Researcher Initials:** ______  
**Date:** __________________

---

**The effect of fibre-enriched pudding products on glycemic and satiety response in adults at risk for type two diabetes**

*The purpose of this questionnaire is to gather information about you to assess your potential eligibility to be 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: Researcher will record on master list

Phone: Work: See master list  
Home: See master list  
Email: See master list

Best way to communicate: ______________  
Date of Birth: See master list  
Age: ________

---

**BODY WEIGHT AND HEIGHT MEASUREMENTS:**

1. The study coordinator will measure your body weight, height and blood pressure.

Body Weight: ___________  
Height: ___________  
BMI: ___________

Blood pressure reading 1: ________  
Blood pressure reading 2: ___________

Waist circumference: ____________

---

**DIET-RELATED QUESTIONS:**

1. Do you have any food allergies or sensitivities?  
   YES  
   NO
   If YES, please describe: __________________________

2. Do you suffer from any gastrointestinal illnesses such as lactose intolerance, irritable bowel syndrome or celiac disease?  
   YES  
   NO
   a. If YES, please expand__________________________

3. Are you on a special diet?  
   YES  
   NO
   Details: __________________________________________}_
4. Do you consume caffeine (coffee, tea, pop, energy drinks, supplements) YES NO
   If so, how much per day? ________________

5. Do you consume alcohol? YES NO
   If so, how many drinks per week? _____
   If so, how many drinks per serving? _____
   (1 drink = 12oz beer, 5oz wine, 1.5oz hard liquor)

6. Do you consume breakfast on a regular basis? YES NO

7. 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**

8. How would you describe your general health?
   POOR GOOD VERY GOOD EXCELLENT

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

10. Are you currently pregnant or breast feeding or trying to become pregnant?
    YES NO

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

    | Health Condition                  | Currently Have | Have Had in Past |
    |-----------------------------------|----------------|------------------|
    | High Cholesterol                  |                |                  |
    | Heart Disease                     |                |                  |
    | Cancer                            |                |                  |
    | Type 1 Diabetes                   |                |                  |
    | Type 2 Diabetes                   |                |                  |
    | Pre-Diabetes                      |                |                  |
    | High Blood Pressure               |                |                  |
    | Impaired Liver Function           |                |                  |
    | Impaired Kidney Function          |                |                  |
    | Celiac Disease                    |                |                  |
    | Crohn’s Disease                   |                |                  |
    | Ulcerative Colitis                |                |                  |
    | Constipation                      |                |                  |
    | Irritable Bowel Syndrome          |                |                  |
    | Arthritis                         |                |                  |
    | Depression and/or Anxiety         |                |                  |
12. Are there any other health conditions you have or have had?  
   If YES, what are they? ____________________________

13. Are you currently taking any prescription medications?  
   If YES, please complete the following table:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Purpose</th>
<th>Duration of Use</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

14. 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

15. Have you taken antibiotics in the last 3 months?  
   If YES, when did you stop using them? ________________

16. Have you had any surgeries or medical events in the last 6 months?  
   If YES, what are they? ____________________________

17. Do you use any over-the-counter medications, including pain relievers (i.e. Tylenol)?  
   If YES, what are they, and how often? ____________________________

18. Do you routinely use acetaminophen containing products (i.e. Tylenol)?  
   YES  NO

19. Depending if the over-the-counter medications contain acetaminophen, would you be willing to discontinue its use for 24 hours prior to each study visit?  
   YES  NO

20. 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

21. If a natural health product you are taking is known to affect study endpoints, would you be willing to discontinue it for the duration of the study?  
   YES  NO

22. Do you have any allergies (drug, food, environmental)?  
   If YES, what are they ____________________________
23. Do you have any allergies or sensitivities to acetaminophen containing products (i.e. Tylenol)?  
   YES  NO

24. Do you exercise?  
   YES  NO
   Can you describe your exercise? ____________________________________________ 
   How often and how intense? ____________________________________________

25. Are you currently on shift work?  
   YES  NO

26. 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: ____________________________________________________________________

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

STUDY LOGISTIC QUESTIONS

28. Do you have any issues with having your blood taken by finger prick?  YES  NO

29. This study requires 10 morning visits to the University of Guelph of approximately 3 hours each. Visits will start between 8:30 and 9:00 a.m. and take place once a week (2.5 months total). Can your schedule accommodate these visits?  
   YES  NO

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

31. Are there particular weekdays you prefer to have study visits?  
   MONDAY  TUESDAY  WEDNESDAY  THURSDAY  FRIDAY

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

33. Have you ever been involved in a research study before?  YES  NO
   If YES, please expand briefly ____________________________________________

34. 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 O: Summary of Pudding Study Handbook

### Table 1: Summary of Pudding Study Handbook

<table>
<thead>
<tr>
<th>Section of Study Handbook</th>
<th>Description of Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Welcome</td>
<td>- A brief welcome to the study providing a brief overview of the research, a description of the handbook, and a thank you for their participation.</td>
</tr>
<tr>
<td></td>
<td>- Contact information for the principle investigators, and the lead Masters student.</td>
</tr>
<tr>
<td>Contact Information</td>
<td>- Location information for the HNRU.</td>
</tr>
<tr>
<td></td>
<td>- Contact information for the entire research team.</td>
</tr>
<tr>
<td>Research Summary</td>
<td>- An overview of the study design, protocol, and study products.</td>
</tr>
<tr>
<td></td>
<td>- An overview of the study endpoints.</td>
</tr>
<tr>
<td></td>
<td>- An overview of how the research benefits the scientific literature and food industry, and how their participation contributed to the research.</td>
</tr>
<tr>
<td>Study Calendar and Activities</td>
<td>- An illustration of the study timeline.</td>
</tr>
<tr>
<td></td>
<td>- A brief description of the study visit schedule, including the date of each study visit, and an overview of what occurs at each study visit.</td>
</tr>
<tr>
<td></td>
<td>- A detailed summary for each study visit day, including reminders for the entire duration of the study period, and for each study visit.</td>
</tr>
<tr>
<td>Pudding Study Treatments and Background</td>
<td>- A description of the ingredients in each pudding product, and where they were prepared.</td>
</tr>
<tr>
<td>Diet/Lifestyle</td>
<td>- Nutritional composition of each pudding product, including calories and fibre content of each pudding.</td>
</tr>
<tr>
<td></td>
<td>- Notes about how the pudding products will be consumed at each study visit.</td>
</tr>
<tr>
<td></td>
<td>- Detailed notes about maintaining a similar diet and lifestyle throughout the entire study period, including reminders about changes to medication and NHP use, and maintaining body weight.</td>
</tr>
<tr>
<td>Section of Study Handbook – Continued</td>
<td>Description of Contents – Continued</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Study Measurements</td>
<td>- A summary of all study endpoints, and body measurements and samples being collected over the study period.</td>
</tr>
<tr>
<td></td>
<td>- Detailed examples of the satiety questionnaires at each study visit.</td>
</tr>
<tr>
<td>Food Records</td>
<td>- Detailed instructions on completing 3-day food records, and weighed food records.</td>
</tr>
<tr>
<td></td>
<td>- All blank food records provided to be completed and handed in.</td>
</tr>
</tbody>
</table>
Appendix P: Study Consent Form

(Will be printed on University of Guelph Letterhead)

CONSENT TO PARTICIPATE IN RESEARCH

The Pudding Study


Study Informed Consent Form

INTRODUCTION
You are being asked to participate in a research study directed by Professors Alison Duncan and Amanda Wright of the Department of Human Health and Nutritional Sciences (HHNS), and Professor Doug Goff at the University of Guelph. The results of this research will contribute to the theses of University of Guelph M.Sc. student Brittney Kay and Ph.D. student Nikolay Repin and to the research activities of HHNS M.Sc. and B.Sc. students at the University of Guelph. This research is funded by the Ontario Ministry of Agriculture and Food (OMAFRA).

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

Brittney Kay, 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: brittney@uoguelph.ca

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

Amanda Wright, Ph.D.
University of Guelph Study Director
Associate Professor, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x54697 or email: ajwright@uoguelph.ca

PURPOSE AND DESCRIPTION OF RESEARCH
Adequate dietary fibre intake is a potential dietary strategy to reduce the risk of and to help manage type 2 diabetes. Research to date has shown that consumption of a variety of fibre types can effectively lower the postprandial glucose and insulin response, leading to improved insulin sensitivity in several populations. The purpose of this research is to determine if eating pudding products made with a variety of less common fibre sources (fenugreek gum, soluble flaxseed gum, and yellow mustard gum)
will reduce the absorption of glucose in the blood stream (known as lowering glycemic response), as well as increase feelings of fullness (known as satiety). The study aims to assess which fibre types are most effective at improving glycemic response and satiety and to support the potential development of dietary fibre fortified products.

This study is double-blinded which means that neither the researchers nor the participants know which treatment a participant is consuming at any time. This study is also a randomized design, which means that participants will be randomly assigned an order in which to consume each of the 8 pudding products. At each study visit, participants’ blood samples will be analyzed for markers of type 2 diabetes, including fasting and postprandial glucose and insulin levels. Paracetamol (acetaminophen) absorption as an indicator of gastric emptying related to satiety and glucose absorption will also be studied. Paracetamol is absorbed in the small intestine into the blood stream, similar to glucose, as they are both minimally absorbed by the stomach. Gastric emptying rate is the speed in which food leaves the stomach and enters the small intestine, and it is measured because the rate as which gastric emptying occurs effects how quickly glucose is absorbed into the blood from the small intestines. A total of 15 participants between the ages of 18 to 70 years of age, who are at risk for type 2 diabetes, will be included in this study.

STUDY PROCEDURES
If you decide to participate in this study, each visit will take place 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 in more detail what will happen at each of these study visits:

If you decide to participate in this study, prior to each visit it is critical that you fast for 10-12 hours prior and that you arrive for the visit fasted. Before starting each fast, you will need to consume a standard dinner meal which will be created based on your food diary. You will have some choice in the meal options. After consumption of the standard dinner meal, you should drink water, but should not consume any other drinks or any foods to ensure you are fasted for the study visit. In the morning, please consume only one glass of water one hour prior to your study visit. Please do not consume over-the-counter paracetamol (acetaminophen) containing products for 24 hours before each visit, and we also ask that you avoid drinking alcohol, and participating in strenuous exercise for 24 hours before each visit. During each visit, you will have your blood pressure, heart rate, and body weight measured. This will be done by a trained study coordinator in a private area.

If you decide to participate in this study, at each study visit the study coordinator will measure your body weight, height, blood pressure and heart rate in a private area. At fasting, one finger prick blood samples will be taken by a trained technician, the trained study coordinator, or by self-pricking. You will be then be asked to consume either a standard sugary glucose beverage (control) and 204 mL of water, or one of 8 pudding products (containing acetaminophen) during the study visit within 10 minutes and one
finger prick blood sample will be taken at the 15, 30, 60, 90, and 120 min intervals, following the start of ingestion of the food. In total, there will be 6 finger prick blood samples per study session. At the end of each finger prick, a bandage or dressing will be applied. Five minutes before each blood sample, you will be asked to complete a satiety questionnaire. Satiety is defined as the process that inhibits further intake of foods and beverages after an eating session. Different foods have varying levels of satiation. During the 2 hours you will remain seated with minimal activity. At a point close to when you consumed the study pudding, you will be asked to complete a questionnaire that asks about your liking of the study pudding. There will be magazines and movies available to watch, but you are also invited to bring work to do on a computer or books to read. After the two hours, you will be provided with an unlimited pizza lunch meal, which you will consume until you are comfortably full. The pizza will be Delisio 4-cheese thin crust, and you will be able to consume water during the meal as well.

The data we will collect during this trial will come from the analysis of your blood samples, and from the satiety questionnaires. All blood samples will be collected and analyzed for markers of type 2 diabetes risk, including fasting and postprandial glucose and insulin levels, and gastric emptying, including paracetamol absorption.

On the final visit you will be asked to complete a brief study exit questionnaire and paperwork for your study compensation, which will include providing your Social Insurance Number (SIN) so that the University can reimburse you for your participation in the study. The paperwork will then be submitted to the University financial department for processing and you should receive compensation within 4-6 weeks.

**STUDY SAMPLE LABORATORY ANALYSIS**
Blood samples will be processed at the HNRU for analysis of glucose, insulin, and paracetamol, and will be stored until publishing of the results. All stored plasma samples for analysis will be labeled with participant number (i.e. not identifiable by participant name) for confidentiality.

**STUDY RESULTS AND PUBLICATION**
Results from this study may be published and presented at scientific conferences. However, results 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:

- Please note that the pudding products contain pork gelatin, which could be a food restriction for certain specialty diets.
• At each of the 10 study visits a trained technician, or trained study coordinator, will take finger prick blood samples. There is a chance that this process could cause you some slight discomfort. These risks and potential discomforts from the blood draws will be managed by consuming plenty of water the night before and the morning of (up to one glass one hour prior to the study visit) can facilitate blood sampling and 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.

• The potential side effects for consuming the pudding products may be bloating, flatulence or diarrhea from the fibre present in the products. As each product will contain no more than 15g of fibre, side effects from fibre intake should be minimal as this amount does not exceed the daily adequate intake levels for adults. Also, as the pudding products will contain paracetamol (acetaminophen) at 1500mg, please consult a doctor if wheezing, rash, itching, increased sweating, nausea, vomiting, stomach pain, and loss of appetite occurs, however, the amount of paracetamol in the products is well below the maximum daily intake for a given day (4000mg). The dose in the pudding treatments (1500mg) is the equivalent to consuming three extra strength Tylenol tablets (500mg each). To prevent over-consumption, please refrain from consuming acetaminophen containing products for the rest of the day (24 hours) following each study visit.

• 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
There will be no clinical benefit to participating in this research, however, if you participate in this research, you will have the benefit of gaining experience participating in a research study and you will receive a written summary of your individual study data. Although you will receive a written summary of your individual study information, the results were not obtained from a licensed medical laboratory and thus should not be used for diagnostic purposes. If you have concerns about the results, you should seek the advice of a physician. More generally, the knowledge gained from this study may contribute to dietary recommendations for individuals who are at risk for developing type 2 diabetes. This research may lead to the use of a variety of fibre sources in puddings and other food products for the reduction of risk for type 2 diabetes and the improvement of satiety.
PAYMENT FOR PARTICIPATION
You will be financially compensated for your time and effort for this study in the amount of $60 upon completion of each study visit (for a total of $600 – 10 visits x $60 per visit). As such, you will be required to provide your Social Insurance Number for compensation purposes. If you withdraw from the study before its completion, your compensation will be pro-rated accordingly. For example, if you complete 4 study visits, you will receive $240.

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 the 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, participant 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
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 Pudding Study: The effect of fibre-enriched pudding products on glycemic and satiety response in adults at risk for type 2 diabetes” 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>
<tr>
<td>NAME OF WITNESS</td>
<td>SIGNATURE OF WITNESS</td>
<td>DATE</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Appendix Q: Pudding and Glucose Beverage Ingredient Specifications

YELLOW MUSTARD BRAN # 402

DESCRIPTION: The hulls of #1CW Yellow Mustard Seed from which most of the flour has been removed, with none of fixed oil removed.


GENERAL REQUIREMENTS: Material supplied under this specification will comply with all aspects of the Canadian Food and Drug Act and Regulations and U.S. Food & Drug Act and Regulations. Product will be stored by the manufacturer or agent under conditions that will ensure that product is received in optimum condition.

PACKAGING: 20 kg or 25kg net multi wall paper bags

LABELING: Shall conform to all Canadian & U.S. Regulations. Product name, Code and Lot number shall be clearly marked on each container.

1. Physical Specifications
   Colour: Light brownish yellow
   Flavour: Clean characteristic mustard bran flavour, free from off flavours.
   Odour: Mild without mustiness.

2. Analytical Specifications

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.0 % maximum</td>
</tr>
<tr>
<td>Volatile Oil</td>
<td>Trace</td>
</tr>
<tr>
<td>Protein (NX6.25)</td>
<td>15.0 % minimum</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>45-60 %</td>
</tr>
<tr>
<td>Fixed Oil</td>
<td>12.0 % minimum</td>
</tr>
<tr>
<td>Ash</td>
<td>5.0 % maximum</td>
</tr>
<tr>
<td>Ash Insoluble in HCl</td>
<td>3.0 % maximum</td>
</tr>
<tr>
<td>Standard Plate Count</td>
<td>100,000 /g maximum</td>
</tr>
<tr>
<td>Yeast &amp; Mold</td>
<td>500 /g maximum</td>
</tr>
<tr>
<td>Coliform</td>
<td>100 /g maximum</td>
</tr>
<tr>
<td>E. Coli</td>
<td>Negative</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Negative /25g</td>
</tr>
<tr>
<td>Sulfites</td>
<td>None added or used during manufacturing</td>
</tr>
</tbody>
</table>

3. Shall be free of hard lumps, impurities and foreign matter, off flavours and aromas.

4. Shelf life of minimum 24 months in a clean, dry, infestation free warehouse below 75% relative humidity. Product is light sensitive if package left open and should be resealed after opening to ensure product integrity.

Issue date: 05/11

UNCONTROLLED COPY

Figure 1: Yellow Mustard Bran (Product 402, G.S. Dunn Limited, Hamilton, Canada)
**NUTRITIONAL INFORMATION**

**ON G.S. DUNN PRODUCT # 402**

**YELLOW MUSTARD BRAN**

<table>
<thead>
<tr>
<th>Component</th>
<th>g/100 g</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOISTURE</td>
<td>&lt; 9</td>
<td></td>
</tr>
<tr>
<td>CRUDE FIBRE</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>FOOD ENERGY (KCALORIES)</td>
<td>415</td>
<td>CALCULATED</td>
</tr>
<tr>
<td>PROTEIN (N x 6.25)</td>
<td>15</td>
<td>BY DIFFERENCE</td>
</tr>
<tr>
<td>TOTAL CARBOHYDRATE</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>ASH</td>
<td>&lt; 5</td>
<td>BHUYAN et. al. (1991)</td>
</tr>
<tr>
<td>FAT</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>SATURATED</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>POLYUNSATURATED</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>MONOUNSATURATED</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>CHOLESTEROL</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TRANS FATTY ACIDS</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>FREE SUGARS</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>DIETARY FIBER</td>
<td>49.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>mg/100 g</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODIUM</td>
<td>15</td>
<td>AOAC 2.129</td>
</tr>
<tr>
<td>POTASSIUM</td>
<td>N/D</td>
<td>3.014</td>
</tr>
<tr>
<td>CALCIUM</td>
<td>200</td>
<td>18.040</td>
</tr>
<tr>
<td>IRON</td>
<td>6</td>
<td>24.016</td>
</tr>
<tr>
<td>PHOSPHORUS</td>
<td>N/D</td>
<td>(1984)</td>
</tr>
<tr>
<td>MAGNESIUM</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>ZINC</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>COPPER</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>MANGANESE</td>
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<td></td>
</tr>
<tr>
<td>VITAMIN A (IU)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>THIAMINE</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>RIBOFLAVIN</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>NIACIN</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

*NO ARTIFICIAL ANTIOXIDANT, PRESERVATIVES, TARTRAZINE, LACTOSE*

*PLEASE NOTE THAT MUSTARD IS A NATURAL PRODUCT, THEREFORE ALL RESULTS ARE PROXIMATE DUE TO A TYPICAL RANGE OF CROP.*

04/08

**Figure 1 (Continued): Yellow Mustard Bran (Product 402, G.S. Dunn Limited, Hamilton, Canada)**
## R803 Product Specifications

**Product Name** CANAFEN® Gum  
**Product Code** FGEN401

### A. Identity

<table>
<thead>
<tr>
<th>Common Names</th>
<th>Fenugreek Gum, Fenugreek Fiber, Fenugreek Galactomannan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant source</td>
<td>Fenugreek, <em>Trigonella foenum-graecum</em></td>
</tr>
<tr>
<td>Plant Part</td>
<td>Endosperm Gum</td>
</tr>
<tr>
<td>Color</td>
<td>Off White</td>
</tr>
<tr>
<td>Flavor</td>
<td>Bland</td>
</tr>
<tr>
<td>Manufacture</td>
<td>Water-based separation of endosperm from fenugreek seeds</td>
</tr>
</tbody>
</table>

### B. Regulatory Information

<table>
<thead>
<tr>
<th>Origin</th>
<th>Product of Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS Tariff Classification No.</td>
<td>1302.19</td>
</tr>
<tr>
<td>GRAS Status</td>
<td>Self-Affirmed: Only applicable to product manufactured by Emerald Seed Products</td>
</tr>
<tr>
<td>GMO Status</td>
<td>Produced from Non GMO Seed</td>
</tr>
<tr>
<td>Sterilization</td>
<td>Available if Required (Non-Irradiation Techniques Used)</td>
</tr>
<tr>
<td>Kosher Status</td>
<td>Certified Orthodox Union</td>
</tr>
<tr>
<td>Halal Status</td>
<td>Certificate No: EME.5212.110004.CA</td>
</tr>
<tr>
<td>Health Canada</td>
<td>Natural Health Products Directorate Site Licence No. 300395</td>
</tr>
</tbody>
</table>

### C. Proximate Analysis

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>&lt; 15 %</td>
</tr>
<tr>
<td>Total Dietary Fiber</td>
<td>&gt; 85 % dwb</td>
</tr>
<tr>
<td>Soluble Dietary Fiber</td>
<td>&gt; 75 % dwb</td>
</tr>
<tr>
<td>Insoluble Dietary Fiber</td>
<td>&lt; 15 % dwb</td>
</tr>
<tr>
<td>Protein</td>
<td>&lt; 5.0 % dwb</td>
</tr>
<tr>
<td>Ash</td>
<td>&lt; 3.0 % dwb</td>
</tr>
<tr>
<td>Fat</td>
<td>&lt; 1.0 % dwb</td>
</tr>
</tbody>
</table>

### D. Microbial Profile

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Aerobic Count</td>
<td>&lt; 3000</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Yeasts</td>
<td>&lt; 450</td>
</tr>
<tr>
<td>Molds</td>
<td>&lt; 450</td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Fecal Coliforms</td>
<td>&lt; 100</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Not Detected</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Not Detected</td>
</tr>
<tr>
<td><em>Staphylococcus aureas</em></td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

---

Figure 2: CANAFEN® Gum (Fenugreek Gum) (Product FGEN401, Emerald Seeds Products Limited, Avonlea, Canada)
R803 Product Specifications

E. Inorganic Contaminants

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>&lt; 0.5 µg/g</td>
</tr>
<tr>
<td>Cadmium</td>
<td>&lt; 0.2 µg/g</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt; 0.5 µg/g</td>
</tr>
<tr>
<td>Mercury</td>
<td>&lt; 0.1 µg/g</td>
</tr>
<tr>
<td>Chromium</td>
<td>&lt; 0.5 µg/g</td>
</tr>
</tbody>
</table>

F. Gluten Free Status

Gluten containing seeds: < 20 ppm

G. Physical Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size</td>
<td>99.0% &lt; 40 Mesh (420 microns)</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>0.8 to 0.9 kg/L</td>
</tr>
</tbody>
</table>

H. Shelf-Life

From Date of Manufacture: 4 Years Under Appropriate Storage Conditions

I. Packaging

Food Grade Plastic Lined Fiber Drums:
- Net Weight per Package: Available as 20 kg or 25 kg (44 lbs or 55 lbs)
- Package Size: 38 cm x 38 cm x 30.5 cm (15" x 15" x 12")

Food Grade Plastic Lined Mesh Totes:
- Net Weight per Package: 1000 kg (2200 lbs)
- Package Size: 90 cm x 90 cm x 105 cm (35" x 35" x 65")

Food Grade Paper Bags:
- Net Weight per Package: Available as 20 kg or 25 kg (44 lbs or 55 lbs)
- Package Size: 13 cm x 43 cm x 91 cm (5" x 17" x 65")

J. Label Information

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>343 Cal/100 g</td>
</tr>
<tr>
<td>Trans Fat</td>
<td>0 g/100 g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0 mg/100 g</td>
</tr>
<tr>
<td>Sugars</td>
<td>0.6 g/100 g</td>
</tr>
<tr>
<td>Calcium</td>
<td>115 mg/100 g</td>
</tr>
</tbody>
</table>

The above specifications are provided for general information purposes only.

www.emeraldseedproducts.com

101 Wood Mountain Trail East • Box 149, Avonlea, SK Canada S0H 0C0 • T: 306-868-2030 • F: 306-868-2032

Figure 2 (Continued): CANAFEN® Gum (Fenugreek Gum) (Product FGEN401, Emerald Seed Products Limited, Avonlea, Canada)
Natunola® Flax Hull Lignans

Product Description:
Natunola® Flax Hull Lignans is a concentrated ingredient, containing predominantly the outer shells or hulls of the flaxseed which provides a quality source of fiber and the plant lignan Secoisolariciresinol diglycoside (SDG). Flaxseed lignans are in high demand because of the growing amount of research that has found that SDG and other lignans found within the hull of the flaxseed may protect against hormone related cancers, such as breast and prostate cancer.

This product is: kosher certified, non-GMO, gluten free, and safe for most individuals with nut allergies.

SDG levels within the flaxseed vary depending on growing conditions including: weather, soil nutrients, length of growing season, etc. Batch calculation results, stated below, provide an approximate value of SDG levels within the Natunola® Flax Hull Lignans product.

Flaxseed Benefits:
Flaxseed is a rich source of the essential fatty acid alpha-linolenic acid (ALA) which is found within the seed’s inner meat, also known as the kernel. Flaxseed is also a rich source of fiber and lignans which are found within the seed’s outer shell, also known as the hull. Overall, flaxseed use is well known for its positive health benefits and has been linked to: decreased risk of cardiovascular disease, decreased risk of developing hormone associated cancers, improved immune function, and protection against type II diabetes.

Product Application
Natunola® Flax Hull Lignans can be used in a number of applications and is a novel ingredient in prepared foods such as:

Bars (snack, energy, etc)  Snack Foods  Cereals  Muffins  Crackers

Product Specifications
Appearance: Brown flakes with some yellow flakes
Texture: Course
Oil Content: Average 20%
Protein Content: Average 15%
Moisture Content: Maximum 8%
Usage Level: 5 – 100% (wt/wt)
SDG Level: 180 – 300 mg / 10 g
Baking Stability: Up to 350°C for up to 2 hours with minimal nutritional loss
Shelf Life: 12 Months
Pack Size: 15 kg bag (40 bags per pallet)
Storage: Recommended storage temperature below 20°C To extend shelf life keep in a cool place away from sunlight.

Typical Nutritional Profile
Nutritional Analysis (g/100g)*
Energy (Cal/100g): 483
Energy (kJ/100g): 2023
Fat (including Omega-3): 22.0
Omega-3: 12.8
Omega-6: 2.9
Carbohydrate (including fiber): 55.4
Protein: 15.7
Ash: 2.0
Moisture: 4.3

*Batch analysis only – variances may occur within crops which may cause deviations from these values.

Natunola is a registered trademark of Natunola Health Inc.
Specifications are based on information available at the time of printing. This information is provided in good faith and is subject to the following conditions: 1. Natunola makes no warranty of any kind concerning any product, formulation or procedure or health claim or other matter contained in the information including, without limitation, any warranty that the sale or use of any product, formulation or procedure will not infringe any patent or other third party rights. 2. The user of the information will not provide it to third parties and will indemnify and hold Natunola harmless from any liability arising out of the recipient’s use of the information. (April, 2013)

Natunola Health Inc.
861 St. Lawrence Street, Winchester, ON Canada K0C 2K0
Tel: (613) 774-9908  * Fax: (613) 774-2228  * flax@natunola.com  * www.natunola.com

Figure 3: Flax Hull Lignans (Product Flax Hull Lignans, Natunola® Health Inc., Winchester, Canada)
ETHYL ALCOHOL (95% VOL. MIN.)

SPECIFICATIONS

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density, kg/L @ 20°C</td>
<td>0.8103 (max.)</td>
</tr>
<tr>
<td>Colour, APHA</td>
<td>2.5 (max.)</td>
</tr>
<tr>
<td>Acids, g/100 ml</td>
<td>As acetic acid</td>
</tr>
<tr>
<td>Esters, g/100 ml</td>
<td>As ethyl acetate</td>
</tr>
<tr>
<td>Aldehydes, g/100 ml</td>
<td>As acetaldehyde</td>
</tr>
<tr>
<td>Higher alcohols, g/100 ml</td>
<td>As isobutyl alcohol</td>
</tr>
<tr>
<td>Nonvolatile matter, g/100 ml</td>
<td>0.0020 (max.)</td>
</tr>
</tbody>
</table>

Ethyl alcohol (95% vol. min.) conforms with all U.S. Pharmacopoeia (USP), British Pharmacopoeia (BP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP) and Food Chemicals Codex (F.C.C.) Standards, with the exception of alcohol strength, specific gravity, and density in the case of BP, EP and JP.

The specified specific gravity and density values in the above references correspond to an alcohol strength of 96 to 96.6% v/v in the case of BP, of 95.1 to 96.9% v/v in the case of EP and of 95.1 to 95.6% in the case of JP, therefore 95% v/v alcohol will not conform to the above, by virtue of it’s lower minimum alcohol strength.

Effective: July 25, 2000  Redated June 4, 12 with no change
Replaces: March 8, 1999
Ref: 4.10-35  Rev: 05

Chatham, Ontario (519) 436-1130  Brampton, Ontario (905) 790-7500  Tiverton, Ontario (519) 368-7723  Boucherville, Quebec (450) 655-7504

Figure 4: Ethanol Solution (Product Ethyl Alcohol (95 % volume minimum), Commercial Alcohols, Brampton, Canada)
May 9, 2013

Subject: Food/ Beverage Use Declaration for Ethyl Alcohol 95% US Proof USP, Ethyl Alcohol 95.5% US Proof USP/EP, and Ethyl Alcohol Anhydrous / Dehydrated Alcohol

To whom it may concern:

This is to confirm that Ethyl Alcohol 95% USP, Ethyl Alcohol 100 US Proof, USP, Ethyl Alcohol 95.5% USP/EP, Ethyl Alcohol 191 US Proof USP/EP, Ethyl Alcohol Anhydrous, (Dehydrated Ethyl Alcohol) sold by Commercial Alcohols, meet Food Chemicals Codex criteria for Ethyl Alcohol and as such are suitable for use in food and beverage applications.

Yours truly,

[Signature]

Ron Shamash
Alcohol QA, Technical Services and Regulatory Affairs Manager

Document redated from Aug. 2010 to May 2013 with otherwise no change.

Figure 4 (Continued): Ethanol Solution (Product Ethyl Alcohol (95 % volume minimum), Commercial Alcohols, Brampton, Canada)
NOW® Non-GMO Soy Protein is a good vegetable source of high quality complete protein that is very low in fat and carbohydrates, and contains an excellent amino acid profile. Soy products, including Soy Protein, are high in phytoestrogens, which may positively support healthy natural estrogen levels in women. Soy Protein also provides beneficial proteins such as Genistein and Diadzein, which have been shown to support good health through various biochemical processes.* Make sure you’re getting the basic building blocks of good health with high quality Non-GMO Soy Protein Isolate from NOW.

**Suggested Usage**

As a dietary supplement, mix a 1/3 cup of soy protein isolate powder daily into at least 8 oz. of water, milk, or juice. Add fruit and ice, if desired, and blend.

- Soy Products FAQs

**Nutrition Info**

<table>
<thead>
<tr>
<th>Nutrition Info</th>
<th>Amount Per Serving</th>
<th>% Daily Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving Size 1/3 Cup (24 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Servings Per Container 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Calories from Fat</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total Fat</td>
<td>0.5 g</td>
<td>1%*</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>&lt;0.5 g</td>
<td>&lt;1%*</td>
</tr>
<tr>
<td>Protein</td>
<td>20 g</td>
<td>40%*</td>
</tr>
<tr>
<td>Calcium</td>
<td>170 mg</td>
<td>17%</td>
</tr>
<tr>
<td>Iron</td>
<td>3 mg</td>
<td>17%</td>
</tr>
<tr>
<td>Magnesium</td>
<td>20 mg</td>
<td>5%</td>
</tr>
<tr>
<td>Sodium</td>
<td>200 mg</td>
<td>8%</td>
</tr>
<tr>
<td>Potassium</td>
<td>80 mg</td>
<td>2%</td>
</tr>
</tbody>
</table>

* Percent Daily Values are based on a 2,000 calorie diet.
† Daily Value not established.

**Details**

**Ingredient:** Non-GMO Soy Protein Isolate. Vegetarian/Vegan Product.

**Contains no:** sugar, salt, starch, yeast, wheat, gluten, com, milk, egg, shellfish or preservatives.

Notice: Use this product as a food supplement only. Not intended as a meal replacement.

This product is sold by weight not volume.

NOW® Non-GMO Soy Protein Isolate is a good vegetable source of high quality complete protein that is very low in fat and carbohydrates.

Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease. One serving of NOW® Non-GMO Soy Protein Isolate Powder provides 20 grams of soy protein.

This product contains an average of 42 mg of Isoflavones per serving which have been shown to aid in maintaining good health.*

Store in a cool, dry place. Please Recycle.

*These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

This information current as of 12/15/14 5:50 PM.

**Figure 5:** Soy Protein Isolate (Product 2156, Now Foods, Bloomingdale, USA)
**Figure 6:** Gelatine (Product Gelatine, Knox Kraft Foods, Northfield, USA)

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
<th>Valeur nutritive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per 1/4 pouch (1.8 g)</td>
<td>pour 1/4 sachet (1.8 g)</td>
</tr>
<tr>
<td>Amount</td>
<td>% Daily Value</td>
</tr>
<tr>
<td>Calories / Calories</td>
<td>5</td>
</tr>
<tr>
<td>Fat / Lipides</td>
<td>0 g</td>
</tr>
<tr>
<td>Carbohydrate / Glucides</td>
<td>0 g</td>
</tr>
<tr>
<td>Protein / Protéines</td>
<td>1 g</td>
</tr>
</tbody>
</table>

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

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

Ingredients: Gelatine.

Directions:
One envelope of Knox Gelatine will set 2 cups (500 mL) of liquid or 1 1/2 cups (375 mL) solids.

Ingrédients: Gélatine.

Mode d’emploi:
Une enveloppe de gélatine Knox permet de faire prendre en gelée 2 tasses (500 mL) de liquide ou 1 1/2 tasse (375 mL) de solide.

Store in a cool, dry place.
Garder dans un endroit frais et sec.
Figure 7: Modified Tapioca Starch (Product TEXTRA®PLUS (32596302), Ingredion, Brampton, Canada)
Figure 8: High Maltose Corn Syrup (Product 01550, Ingredion Canada Incorporated, Cardinal, Canada)
Figure 9: Chocolate powder (NESQUIK 33% Less Sugar Powder, Nestlé, Halifax, Canada)

### INGREDIENTS

Sugar, cocoa, soya lecithin, salt, sodium ascorbate, ferric orthophosphate, tricalcium phosphate, mucoalbumin, vitamin A palmitate, flavour and artificial flavour. May contain milk, soya and wheat.

### NUTRITION INFORMATION

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
<th>% DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>1g</td>
<td>1 %</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>1g</td>
<td>3 %</td>
</tr>
<tr>
<td>Trans Fat</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0mg</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>25mg</td>
<td>1 %</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>12g</td>
<td>4 %</td>
</tr>
<tr>
<td>Fibre</td>
<td>1g</td>
<td>3 %</td>
</tr>
<tr>
<td>Sugars</td>
<td>11g</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1g</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>20 %</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>20 %</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>10 %</td>
<td></td>
</tr>
</tbody>
</table>
Figure 10: Acetaminophen (Tylenol Extra Strength Caplets, DIN 00723908, McNeil Consumer Healthcare, Markham, Canada)
Figure 11: Glucose Beverage (Trutol®, model 401272P, Fisher Scientific Company, Waltham, USA)
Appendix R: Three-Day Food Record Instructions and Template

**Food Records**

Completing Accurate Food Records

As part of this research, you will be completing 24-hour detailed food records for three days (two weekdays and one weekend day) at the beginning of this study. Your accuracy and honesty is crucial to this study! When filling out these forms, please remember the following:

- **Start a new food record sheet for each day**
- **Record the following:**

1. The **time** you eat.
2. The **type** of food (e.g. 1% milk, chicken noodle soup, cream cheese: light? fat-free? **BRANDS** are very helpful!).
3. How the food is **prepared** (e.g. fried in 2 tablespoons of canola oil, steamed, baked).
4. The **amount** consumed: use household measures (cups, fluid ounces, table/teaspoons) or weight if you know it (grams, ounces); read labels to help you determine serving sizes.
5. Include all **condiments** and amounts (e.g. 2 tsp sugar added to coffee).
6. Include amounts of all **liquids** (including water!).
7. If you know the **RECIPE** of the dish you ate, please include/attach the recipe to the food record and record approximately the serving size eaten (e.g. if a chili serves 5 people, and you had a serving, then 1/5 of the dish was consumed).

Here is an example of the amount of detail that we are requesting:

12:30 pm: cheese burger
- 3 ounces of lean ground beef
- 2 Tbsp. ketchup; 1 slice of tomato
- 1 slice skim milk processed cheese (Kraft)
- 1 white sesame seed bun
MORE HELPFUL TIPS

• Whenever possible, list the BRAND NAME. If you have the label, please turn it in with your food record (e.g. candy wrapper, cereal box label etc.).

• Don’t forget things like: coffee/tea, condiments, sauces, candy, water, oil type... we want to know EVERYTHING.

• Which brings us to our next point: DETAILS, DETAILS, DETAILS!!!! Please be as specific and detailed as possible. Draw pictures if you find it hard to describe an amount (e.g. thickness of meat). Include anything that might be helpful.

• Carry your food record sheets with you EVERYWHERE you go and record foods as you consume them (This is much easier than trying to recall all the little details at the end of the day).

• Lastly, frequently refer back to these instructions and please do not hesitate to call 519-824-4120 ext. 58081, or email us if you have ANY questions. We will check our email and voicemail every night to answer your questions promptly.

Some Quick and Easy Portion Reference Guides

• A deck of cards is the same size as a 3-oz serving of meat; a golf ball is about the size of 1-oz of meat.
• Four dice equals 1-oz of cheese.
• Think about a quarter ($0.25). One quarter-size of dry noodles provides about 2 servings of cooked spaghetti noodles.
• Two handfuls of chips or pretzels will provide about 1 oz.
• One ounce of nuts is about the size of your thumb.
• One teaspoon of margarine is about the size of the tip of your thumb.
• Two tablespoons of peanut butter is about the size of a ping pong ball.
## Tips on Estimating your Serving Sizes!

<table>
<thead>
<tr>
<th>Serving Size</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ounces of meat</td>
<td>The size and thickness of a deck of cards</td>
</tr>
<tr>
<td>1 cup of cold cereal</td>
<td>About equal to the size of your fist</td>
</tr>
<tr>
<td>½ c. cooked cereal, rice, pasta</td>
<td>About equal to a small computer mouse</td>
</tr>
<tr>
<td>1 medium fruit</td>
<td>About equal to a baseball</td>
</tr>
<tr>
<td>1 ounce of cheese</td>
<td>About the size of four stacked dice or two cheese slices</td>
</tr>
<tr>
<td>½ cup of ice cream</td>
<td>About the size of a tennis ball</td>
</tr>
<tr>
<td>1 cup of vegetables or mashed potatoes</td>
<td>About the size of your fist. A baked potato is also about the size of your fist.</td>
</tr>
<tr>
<td>1 teaspoon of butter or peanut butter</td>
<td>About equal to the size of your thumb</td>
</tr>
</tbody>
</table>
# Sample Food Record Sheet

**Participant ID:** 352  
**Date of Food Record:** Monday June 3, 2013

<table>
<thead>
<tr>
<th>Time</th>
<th>Food Eaten</th>
<th>Amount</th>
<th>Food Description and/or Preparation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>10am</td>
<td>Danone yogurt</td>
<td>1 portion (100g)</td>
<td>Vanilla, 2.9% fat</td>
</tr>
<tr>
<td>10am</td>
<td>Banana (1 medium)</td>
<td>1</td>
<td>Untoasted</td>
</tr>
<tr>
<td>10am</td>
<td>Rye bread (Dempster's)</td>
<td>1 slice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peanut butter (Kraft Regular, smooth)</td>
<td>2 tbsp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Welch’s grape jelly</td>
<td>1 tbsp.</td>
<td></td>
</tr>
<tr>
<td>10am</td>
<td>Green tea</td>
<td>1 cup</td>
<td>Hot water, tea bag. No sugar added.</td>
</tr>
<tr>
<td>12pm</td>
<td>Water</td>
<td>1.5 cups</td>
<td>Tall glass</td>
</tr>
<tr>
<td>12:30pm</td>
<td>Lindt chocolates</td>
<td>2</td>
<td>Individually wrapped holiday truffles</td>
</tr>
<tr>
<td>1pm</td>
<td>Danone yogurt</td>
<td>1 portion (100g)</td>
<td>Strawberry, 2.9% fat</td>
</tr>
<tr>
<td>1:30pm</td>
<td>Cooked white basmati rice</td>
<td>1.5 cups</td>
<td>Salt added.</td>
</tr>
<tr>
<td></td>
<td>Chicken stir-fry (see recipe)</td>
<td>1 cup</td>
<td>2 boneless, skinless chicken breasts (fat removed), 1 green pepper, 1 head broccoli, 1 large carrot, 1 cup white button mushrooms (chopped), 4 tbsp. sunflower oil, ½ tbsp. Cool Runnings seasoning salt, ½ c VH Pad Thai stir-fry sauce.</td>
</tr>
<tr>
<td>Time</td>
<td>Meal Type</td>
<td>Details</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>1:30pm</td>
<td>Water</td>
<td>1.5 cups</td>
<td></td>
</tr>
<tr>
<td>5pm</td>
<td>Tea (hot water + Tetley tea bag)</td>
<td>1 mug (1 cup)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk (2%)</td>
<td>2 tbsp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
<td>1 tsp</td>
<td></td>
</tr>
<tr>
<td>6:45pm</td>
<td>Cooked white basmati rice</td>
<td>½ cup</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chicken stir-fry (see recipe)</td>
<td>Salt added.</td>
<td></td>
</tr>
<tr>
<td>7pm</td>
<td>Water</td>
<td>1.5 cups</td>
<td></td>
</tr>
<tr>
<td>7:30pm</td>
<td>Grilled cheese sandwich</td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(used sandwich grill, not fried)</td>
<td>Dempster’s soft sliced white bread</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 slices</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black Diamond processed cheese (thin-slice)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 tbsp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heinz Ketchup</td>
<td></td>
</tr>
<tr>
<td>7:45pm</td>
<td>Water</td>
<td>1.5 cups</td>
<td></td>
</tr>
<tr>
<td>9:15pm</td>
<td>Grapefruit, pink</td>
<td>½ fruit</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cut in half, sprinkled with ½ tsp sugar</td>
<td></td>
</tr>
</tbody>
</table>
# Food Record Sheet

Participant ID: _____________________  Date of Food Record: _____________________

<table>
<thead>
<tr>
<th>Time</th>
<th>Food Eaten</th>
<th>Amount</th>
<th>Food Description and/or Preparation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
Appendix S: Blood Glucose and Plasma Insulin Compared Among Time Points Within Glucose Beverage Treatments

Figure 1: Postprandial Blood Glucose (a), and Plasma Insulin Response (b) for Glucose Beverage Treatments (n=15)
Data are means ± SE for blood glucose (a).
Data are geometric means (95% confidence intervals) for plasma insulin (b).
Data with different subscripts are significantly different (P<0.05).
Both available carbohydrate types (MTS and HMCS) were averaged since their results did not significantly differ.
Appendix T: Blood Glucose Compared Among Time Points Within Each Pudding Treatment

![Graphs showing blood glucose levels over time for different pudding treatments](image)

Figure 1: Postprandial Blood Glucose With Time Points Compared Within Each Treatment for Fenugreek Gum (a), Yellow Mustard Mucilage (b), Flaxseed Mucilage (c), and Control (d) Puddings (n=15)

Data are means ± SE.

Data with different subscripts within a treatment are significantly different (P<0.05).

Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.
Appendix U: Plasma Insulin Compared Among Time Points Within Each Pudding Treatment

Figure 1: Postprandial Plasma Insulin With Time Points Compared Within Each Treatment for Fenugreek Gum (a), Yellow Mustard Mucilage (b), Flaxseed Mucilage (c), and Control (d) Puddings (n=15)
Data are geometric means (95% confidence interval).
Data with different subscripts within a treatment are significantly different (P<0.05).
Both available carbohydrate types (MTS and HMCS) for each pudding treatment (YMM, FG, FM, control) were averaged since their results did not significantly differ.