The Effect of Green and Red Lentils in Half and Quarter-cup Serving Sizes on Acute Postprandial Blood Glucose and Plasma Insulin Response Compared to Multiple Starchy Controls in Healthy Adults.

by

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ABSTRACT

THE EFFECT OF GREEN AND RED LENTILS IN HALF AND QUARTER-CUP SERVING SIZES ON ACUTE POSTPRANDIAL BLOOD GLUCOSE AND PLASMA INSULIN RESPONSE COMPARED TO MULTIPLE STARCHY CONTROLS IN HEALTHY ADULTS.

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University of Guelph, 2018

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Alison M. Duncan
D. Dan Ramdath

Lentils can lower postprandial glycemic response but consumption is low and most studies have examined ½-1 cup serving sizes. This study examined the effects of lower serving sizes (½ and ¼ cup) of green and red lentils on postprandial glycemic response, compared to matched serving sizes of corn, macaroni, white potato, and white rice in two randomized crossover studies in 24 healthy adults. For both the half- and quarter-cup studies, reductions were found for glucose and insulin area under the curve, maximum concentration, and glucose and insulin at various time points for green and red lentils (which were not different from each other) compared to some of the starchy controls. These results show that green and red lentils can effectively reduce glycemic measures in half-cup and quarter-cup serving sizes compared to commonly consumed starchy foods, and provides evidence for consumption of lentils for type 2 diabetes prevention.
Acknowledgements

I am so grateful to have been able to conduct a human clinical trial in nutrition research and to have so much guidance and support from hardworking students and faculty in the Department of Human Health and Nutritional Sciences. I am not sure what I write here will be sufficient to say thank you, but I will be forever grateful to everyone who helped me get here!

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I would also like to thank Dr. Bettger for serving on my advisory committee, and for contributing to my education and career path plans throughout my undergraduate and graduate degree. I have been inspired by your ability to listen, guide and encourage and ultimately your passion for teaching, and this is something I hope to carry forward with me in my next chapter.

Thanks to Justine Tishinsky for giving me the opportunity to TA and for allowing me to grow in my role as a teacher. This experience has helped shape my career path, and you have been instrumental in that process, thank you!!

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encouragement, and support, and for teaching me how to coordinate a study. I would not have been able to do this without all of your help! Wes and Torie, I feel so privileged to have been able to work side by side with you as Masters students. Thank you for your hard work, your dedication, and for all the amazing memories that we made, and I look forward to watching your careers grow in the future! I am so fortunate to be able to say that I got to work on a study with two of my very best friends.

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I need to thank my amazing family for their love, support, and encouragement. Mom, Dad, Kelly and Joel, thank you for listening to me talk about science, for pushing me a little higher to reach my dreams, and for being my biggest cheerleaders. I am so happy to have you guys in my life, and I love you! Matt, you have been a huge part of this process. Thank you for encouraging me to dig a little deeper when I felt I couldn’t, thank you for celebrating the successes with me, regardless of how big or small they have been, and thank you for knowing exactly what to do or say to make me smile. Your patience, encouragement, and love has helped get me here.

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<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Repeated Measures Analysis of Variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CMAX</td>
<td>Maximum Concentration</td>
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<tr>
<td>CONSORT</td>
<td>Consolidated Standards of Reporting Trials</td>
</tr>
<tr>
<td>CPR</td>
<td>Cardiopulmonary Resuscitation</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>GL</td>
<td>Green Lentils</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Hemoglobin A1c, Glycated Hemoglobin</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HHNS</td>
<td>Human Health and Nutritional Sciences</td>
</tr>
<tr>
<td>HNRU</td>
<td>Human Nutraceutical Research Unit</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostasis Model Assessment of Insulin Resistance</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental Area Under the Curve</td>
</tr>
<tr>
<td>ID</td>
<td>Identification Number</td>
</tr>
<tr>
<td>Mac</td>
<td>Macaroni</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>NHPs</td>
<td>Natural Health Products</td>
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<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
</tr>
<tr>
<td>PHAC</td>
<td>Public Health Agency of Canada</td>
</tr>
<tr>
<td>REB</td>
<td>Research Ethics Board</td>
</tr>
<tr>
<td>RGR</td>
<td>Relative Glycemic Response</td>
</tr>
<tr>
<td>RL</td>
<td>Red Lentils</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 Diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>TLC Diet</td>
<td>Therapeutic Lifestyle Changes Diet</td>
</tr>
<tr>
<td>TMAX</td>
<td>Time to Maximum Concentration</td>
</tr>
<tr>
<td>WHMIS</td>
<td>Workplace Hazardous Materials Information System</td>
</tr>
<tr>
<td>WP</td>
<td>White Potato</td>
</tr>
<tr>
<td>WR</td>
<td>White Rice</td>
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Chapter I. BACKGROUND

A. Type 2 Diabetes

A.i. Type 2 Diabetes: Prevalence and Diagnosis

Type 2 diabetes (T2D) is a metabolic disease that is rapidly growing worldwide. As of 2015, 415 million people were diagnosed with diabetes, globally, and it is estimated that an additional 193 million people have undiagnosed diabetes (Chatterjee et al. 2015). The global incidence of diabetes is expected to increase to 642 million people by 2040 (International Diabetes Federation 2015), with 90-95% of cases being diagnosed as T2D (Chatterjee et al. 2015; International Diabetes Federation 2015; Guariguata et al. 2013). The majority of T2D cases occur in low to middle income countries, but the highest prevalence rates are found in North America, and Eastern Europe where 12.6%, and 14.3% of the population have been diagnosed with T2D (Guariguata et al. 2013). As the sixth leading disability in 2015, diabetes places a socioeconomic burden on the individual and the healthcare system, as it is estimated to cost $825 billion (USD) globally (Chatterjee et al. 2015).

Diabetes is characterized by elevated levels of circulating blood glucose. Type 1 diabetes (T1D) is an autoimmune disorder in which the β cells of the pancreas are destroyed by the host’s immune system (Cigolle et al. 2009). The immune-mediated destruction of pancreatic β cells and loss of β cell function results in the lack of insulin production, which prevents tissues from glucose uptake, and ultimately leads to hyperglycemia, a state of elevated blood glucose (Cigolle et al. 2009; Bock et al. 2006; Insel et al. 2015). Individuals diagnosed with T1D typically require insulin injections for appropriate glycemic control (Cigolle et al. 2009). T2D is characterized by increased hyperglycemia, from impaired insulin secretion due to β cell dysfunction, and impaired insulin action from insulin resistance in target tissues (Chatterjee et al. 2015; Public Health
Agency of Canada 2011a; Ozougwu et al. 2013). Management of blood glucose focuses on maintaining blood glucose concentrations within normal ranges (fasting blood glucose: 4.0 -7.0 mmol/L; 2 hour-postprandial blood glucose: 5.0-10.0 mmol/L) (Cheng 2013). T2D onset can be delayed, or even prevented with lifestyle modification including regular physical activity, maintenance of a healthy body weight, smoking cessation and dietary modification (Public Health Agency of Canada 2011a; Cheng 2013).

A.ii. Type 2 Diabetes: Risk Factors

Patients with T2D have an increased risk of all-cause mortality, an increased risk for macrovascular and microvascular issues such as myocardial infarction, stroke, nephropathy, retinopathy, and reduced blood flow to nervous tissues, compared to those without diabetes (Tancredi et al. 2015; Emerging Risk Factors Collaboration 2010; Caspersen et al. 2012; Public Health Agency of Canada 2011b). Therefore, it is critical to determine and understand the risk factors for T2D to help prevent its onset and development, and its associated complications, and improve quality of life. T2D development is multifactorial and includes non-modifiable and modifiable risk factors such as genetic, environmental and lifestyle risk factors (Ozougwu et al. 2013; Chan et al. 2009; Colaguiri 2010).

A variety of non-modifiable risks have been identified in the literature. Age is a clear non-modifiable risk factor that increases T2D risk, as shown in 2008-2009 Canadian T2D prevalence of 2.6%, 16.6% and 25% in Canadians 35-39 years old, 60-64 years old and 70 years and older, respectively (Public Health Agency of Canada 2011a). Ethnicity is another non-modifiable risk factor for T2D. People of European descent are less likely to develop diabetes compared to African, Asian and Hispanic American descents (Public Health Agency of Canada 2011a). Finally, genetics also play a role in the development of T2D. One study demonstrated
that T2D prevalence was 37% in dizygotic twins, but was 65% in monozygotic twins (Beck-Nielsen et al. 2003). Furthermore, the risk of developing T2D is higher when one parent has T2D, and even higher if both parents have T2D (Vimaleswaran and Loos 2010), demonstrating a heritable component of T2D. As of 2016, more than 80 susceptibility loci have been identified for T2D through genome wide association studies (Imamura et al. 2016).

Modifiable risk factors have also been identified in the development and progression of T2D. Smoking status, and increased visceral adiposity from being overweight or obese is associated with an increased risk for T2D. As body weight increases (leading to overweight and obesity), there is an increase in insulin resistance and an associated increase in hyperglycemia (Paulweber et al. 2010; Burr et al. 2012). As of 2017, 60% of patients with T2D were classified as obese (body mass index (BMI) ≥30 kg/m²) (Chatterjee et al. 2015). Various studies have demonstrated an increased risk of T2D prevalence, incidence and a poorer metabolic profile with increased sedentary time and reduced physical activity (Healy et al. 2008; Healy et al. 2011; Cooper et al. 2014). Diet is also a clear contributing factor to the development of T2D. In developed nations, an obesogenic environment has emerged with increased availability of poor quality energy-dense foods with larger portion sizes, and increased total energy intake, leading to positive energy balance and weight gain (Duffey and Popkin 2011; AlEesa et al. 2015). Overall, sedentary behavior and consumption of energy-dense, nutrient-poor foods contribute to the diabetic metabolic profile and promote weight gain.

A.iii. Type 2 Diabetes: Glycemic Control

Glycemic control is a highly regulated process that involves various organs, tissues and hormones and is quite complex. Following the ingestion of glucose (such as a meal, or glucose
infusion), there is an increase in plasma glucose as glucose is absorbed into the blood via the gastrointestinal tract (Thorens 2015; Triplitt 2012). In a metabolically healthy person, the rise in plasma glucose triggers the release of insulin, which is produced and secreted by β-cells within the islets of Langerhans in the pancreas (Triplitt 2012). Insulin facilitates the transport of glucose from the blood into insulin-sensitive cells and tissues for glycolysis, or into the liver for storage as glycogen (Thorens 2015; Triplitt 2012). Insulin is also responsible for suppressing glucagon to inhibit gluconeogenesis in the kidney and liver (Thorens 2015; Triplitt 2012). In contrast, if blood glucose concentration decreases (such as what would occur with low energy intake), alpha cells within the islets of Langerhans in the pancreas secrete glucagon into the blood (Triplitt 2012). Glucagon will stimulate glucose output via glycogenolysis in the liver, and inhibit the action of insulin (Triplitt 2012). This process is highly regulated but due to a variety of genetic and lifestyle risk factors, can become unregulated, leading to the development of T2D.

A.iv. Type 2 Diabetes: Screening

As T2D is becoming more common and is projected to increase dramatically, it is critical to have strategies in place to screen and treat individuals who have T2D, and establish interventions for those at risk for the development of T2D. The 2018 Diabetes Canada Clinical Practice Guidelines recommends that screening be performed every 3 years in individuals who are 40 years of age and older, or more frequently for high-risk individuals (those with a family history of diabetes, history of prediabetes or gestational diabetes, abnormal blood lipid levels, hypertension, overweight, and/or obese) (Diabetes Canada Clinical Practice Guidelines 2018; Ekoe et al. 2018). The first step in the screening process is the measurement of fasting levels of plasma glucose and/or glycated hemoglobin (HbA1c). If these levels indicate risk (Table 1), then a 2-hour 75g oral glucose tolerance test (OGTT) is recommended where a fasted and 2-hour
postprandial glucose measurement are completed for further assessment of glucose tolerance. Furthermore, an elevated random plasma glucose measurement can be performed at any time (fasted or postprandial), and can be used to diagnose prediabetes or diabetes. The accepted values for each screening method for the diagnosis of prediabetes and diabetes were released by the World Health Organization and the International Diabetes Foundation are included in Table 1 (World Health Organization 2006a). One of the four blood tests must be done to confirm the diagnosis of T2D, and should be repeated for confirmation.

Table 1: Diagnostic criteria for prediabetes and type 2 diabetes as outlined by the World Health Organization and the International Diabetes Foundation

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Prediabetes</th>
<th>Type 2 Diabetes</th>
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<tbody>
<tr>
<td>Fasting Plasma Glucose</td>
<td>6.0-6.9 mmol/L</td>
<td>≥7.0 mmol/L</td>
</tr>
<tr>
<td>2-hour Plasma Glucose</td>
<td>7-11.1 mmol/L</td>
<td>≥11.1 mmol/L</td>
</tr>
<tr>
<td>Random Plasma Glucose</td>
<td></td>
<td>≥11.1 mmol/L</td>
</tr>
<tr>
<td>Glycated Hemoglobin</td>
<td>6.0-6.4%</td>
<td>≥6.5%</td>
</tr>
</tbody>
</table>

The development, progression, and complications of T2D vary between individuals. Overall, poor glycemic control can have negative implications on health, and it is important to support the treatment and management in each individual to help with the worsening of symptoms and improve glycemic control. Although various strategies for the management of T2D and prediabetes can be recommended, the most common is lifestyle modification through the implementation of physical activity and dietary changes. The World Health Organization global strategy on diet, physical activity and health recommends at least 30 minutes a day of moderate intensity physical activity for adults as well as the incorporation of a healthy diet to reduce the risk of diabetes and other chronic health conditions (World Health Organization
Although physical activity should be incorporated into everyday life, poor nutrition can negatively affect the benefits of physical activity. There are a variety of alterations that can be made to the diet to improve nutritional quality for adults living with diabetes, but understanding how to make permanent modifications following the outlined dietary recommendations can be challenging, and hard to maintain. Therefore, the incorporation of simple healthy foods that help in the risk reduction of T2D could make a significant contribution to at risk and type 2 diabetic populations. This could improve nutritional strategies to help reduce the risk for developing T2D in various populations.

**A.v. Type 2 Diabetes: Diet for Management and Prevention**

Diet has been identified as a major risk factor for T2D development. The quantity of food, and the nutritional quality of food are determinants of human health. Using diet as a tool for the prevention and management of diabetes aims to achieve optimal blood glucose concentrations to prevent, delay and help with the treatment of diabetes related complications. While physical activity is critical in the management of T2D, it is also of equal importance to integrate a dietary strategy to reduce the risk of T2D development.

Diabetes Canada released 13 recommendations for the treatment and management of diabetes through nutritional intervention to improve quality of life and improve associated complications from diabetes (Diabetes Canada Clinical Practice Guidelines 2018). Recommendations outlined in the document include nutrition education from dieticians to encourage correct macronutrient distribution, emphasis of selecting carbohydrate food sources that are low on the glycemic index to optimize glycemic control, and implementing dietary patterns such as a Mediterranean style diet, and vegetarian eating patterns (Diabetes Canada Clinical Practice Guidelines 2018). Recommendation 12.4. from the Clinical Practice Guidelines
encourages the incorporation of dietary pulses, including lentils to improve glycemic control (Diabetes Canada Clinical Practice Guidelines 2018).

**B. Pulses: Lentils**

**B.i. Lentils**

Pulses are the edible dried seeds of legumes and are limited to crops harvested as dry grains. The United Nations Food and Agriculture Organization recognizes 11 types of pulses and some of the varieties include lentils, dried peas, chickpeas, and dried beans (Food and Agriculture Organization of the United Nations 1994; Pulse Canada 2018). Pulses are high in fibre and protein, and have low glycemic index values, which make them an ideal option for the prevention and management of diabetes. In particular, lentils are recommended in the Canadian Food Guide (Health Canada 2011), American Food Pyramid (United States Department of Agriculture 2005), and the American Dietary Guidelines (United States Department of Health and Human Services and U.S. Department of Agriculture 2015) as part of a healthy diet. Furthermore, Diabetes Canada Clinical Practice Guidelines established that the incorporation of dietary pulses such as lentils should be consumed by those living with diabetes to help with the regulation of glucose (Diabetes Canada Clinical Practice Guidelines 2018).

Lentils are regarded for their micronutrient and macronutrient composition (The Food Health Relationship 2013). Lentils are high in fibre, high in protein and are a complex carbohydrate source (The Food Health Relationship 2013). Half of a cup of cooked lentils contains approximately 12 g of protein, 4-10 g of fibre, and 0 g of cholesterol, *trans* fat, saturated fat, and sodium. Furthermore, lentils are also high in calcium, iron, potassium, folate and manganese, making them a nutritious food source (Hunt 2003).
Lentils are widely available in the market and can be categorized by colour and size. The three major classifications by colour include green lentils, red lentils, and specialty lentils. Green lentils can be cultivated in small, medium or large seed size and 75% of available green lentils are large seeded. Red lentils are typically dehulled and split, and can be found in large, small or extra-small market classes. The specialty market class refers to lentils that are not red or green and contain varieties such as the Beluga lentil, French green lentil, and Spanish brown lentil (Saskatchewan Pulse Growers 2018a). The Laird green lentil and the Eston split red lentil are the most readily available lentils in the market, and are widely produced in Canada (Saskatchewan Pulse Growers 2018b).

Canada is the largest producer and exporter of lentils, with over 95% of the world’s lentil production occurring in Saskatchewan (Agriculture and Agri-food Canada 2017). In 2016, green lentils brought in $659 million from exports while red lentils procured $1.4 billion (Pulse Convention 2017). The highest consumers of lentils worldwide include India, China, and the United States (Canadian Agri-Food Trade Alliance 2015).

C. Lentils and Glycemic Response: Human Studies

People with T2D can help prevent hyperglycemia by making dietary changes, such as the incorporation of lentils into the diet (Diabetes Canada Clinical Practice Guidelines 2018). Lentils have been studied for their effects on improving and regulating the glycemic response. Human clinical trials have been conducted to better understand the role of lentils in modulating glycemic response with both acute and chronic consumption. Furthermore, research has also been conducted on both healthy, and diabetic populations to determine the benefits of lentils on the glycemic response. Researchers have examined the effects of lentils independently, and in various food matrices to determine their viability as a potential dietary approach to glycemic
control for healthy and diabetic populations. Lentils can be easily incorporated into a typical diet, but the quantity of lentils that need to be consumed to effect significant blood glucose lowering abilities has yet to be determined. Nonetheless, human clinical trials have been conducted to determine the blood glucose lowering potential of lentils. Presented below is a review of the literature that examines the effects of both acute and chronic lentil consumption on glycemic measures in healthy and diabetic populations.

C.i. Acute Human Intervention Clinical Trials

C.a.i. Acute Studies of Whole Lentils in Healthy Participants (Appendix A)

The acute effects of lentils on postprandial blood glucose and insulin response have been studied using various study designs and methods. Furthermore, the majority of studies have used lentils and other pulse products in combination with other meals. These studies will be examined below.

The acute glycemic effects of lentils were first examined by Jenkins et al. (1982) in a randomized crossover study conducted at the University of Toronto. Seven healthy adults (5 men, 2 women) aged 26 ± 3 years, consumed 4 breakfasts of varying composition on different study days over an 8 to 12-week time period. Participants arrived to the University of Toronto after a 12 hour fast, and had a fasted finger prick blood sample taken to measure blood glucose. The participants then consumed the following breakfast meals within 15-20 minutes; Breakfast 1 which consisted of either whole boiled lentils (226 g dry weight) with butter and tomatoes (lentil breakfast), Breakfast 2 which was 280 g of whole wheat bread with cottage cheese and tomatoes (wholemeal bread breakfast), or Breakfast 3 which was 70 g of whole wheat bread with cottage cheese and tomatoes (¼ bread breakfast). Breakfast 4 (slow bread breakfast) was identical to Breakfast 2 (contained 280 g of whole wheat bread with cottage cheese and tomatoes) but was
consumed in small pieces over 4 hours instead of 15 to 20 minutes. All breakfasts were served with tea and were matched for fluid volume. Subsequent finger prick blood samples were taken at 15, 30, 45, 60, 90, 120 and 180 minutes following breakfast, and a standardized test lunch was then served. Participants had 20 minutes to consume whole wheat bread, low fat cottage cheese, tomato, marmite and banana for lunch, and had follow up finger prick blood samples at 0, 15, 30, 45, 60, 90 and 120 minutes after lunch. The 0-2 hour blood glucose area under the curve (AUC) after breakfast was significantly lower with the lentil, ¼ bread and slow bread breakfast compared to the wholemeal bread breakfast. The 0-2 hour blood glucose AUC following lunch was significantly lower for the lentil and ¼ bread breakfast, but not the slow bread breakfast compared to the wholemeal bread breakfast. Furthermore, the peak rise in glucose was significantly lower following breakfast consumption of lentils, the ¼ bread and slow bread breakfasts compared to the wholemeal bread treatment, but only for the lentil and ¼ bread treatments following lunch. The absolute blood glucose mean was significantly reduced at 30 minutes, with a 70% reduction in the peak glucose rise, and a 73% reduction in the 2-hour blood glucose AUC was observed in the lentil breakfast compared to the wholemeal bread breakfast. Insulin levels following breakfast were significantly reduced at 30 and 120 minutes in the lentil breakfast compared to the wholemeal bread breakfast and the peak rise in insulin was significantly reduced by 65% in the lentil breakfast compared to the wholemeal bread breakfast. Therefore 226 g of whole lentils (dry weight), as part of meal, were able to reduce blood glucose and insulin AUC immediately, and after a second meal when compared to wholemeal bread. Similarly, the peak rise in blood glucose and insulin were lower following the consumption of lentils compared to wholemeal bread.
Wong *et al.* (2009) also studied the acute effect of lentils, as well as other pulses (chickpeas, navy beans and yellow peas) on glycemic measures in healthy men in a randomized crossover design. Fifteen healthy men aged 18-35 consumed whole lentils (451 g), and other pulses (chickpeas, yellow peas, and navy beans) compared to white bread or water to determine the effects on glycem response. All treatments and controls were served in tomato sauce with water to equalize a 575 mL serving volume. Participants arrived at the University of Toronto after a 10-12 hour over night fast, and blood glucose was determined from a finger prick blood sample. The treatment or control meal was served, and participants were asked to consume the meal within 10 minutes. Blood samples were then taken by finger prick again at 15, 30, 45, 60, 90 and 120 minutes after consumption of the treatment, after which an *ad libitum* pizza meal was served. Lentils and chickpeas caused a significantly lower blood glucose concentration over the first hour (15, 30, 45, and 60 minutes) compared to the other pulses and white bread. Blood glucose AUC was significantly lower for lentils, chickpeas and yellow peas compared to white bread. Therefore 451 g of lentils served in tomato sauce (equalized to 575 mL volume) was able to reduce blood glucose concentration over the first hour, and blood glucose AUC when compared to white bread.

To further determine if pulses, including lentils can improve glycemic response, Mollard *et al.* (2011) conducted another study at the University of Toronto to determine the first and second meal effects of different varieties of pulses on blood glucose. This randomized crossover study recruited healthy male participants (n=25), age 21.3 ± 0.5 years, with a healthy BMI (21.6 ± 0.3 kg/m²). Participants consumed 332.9 g of whole lentils, 222.8 g of chickpeas, 375.6 g of yellow peas (amounted to 1.5-2.0 cups of pulses) mixed with macaroni and tomato sauce to match for 600 kilocalories, or a control meal of macaroni and cheese. Participants arrived to their
study visit fasted, had a blood sample collected by finger prick for analysis of blood glucose, consumed their treatment meal within 20 minutes, and then had subsequent finger prick blood samples at 20, 40, 60, 80, 110, 140, 200 and 260 minutes. An *ad libitum* pizza meal was served at 260 minutes, and blood glucose was again measured from finger prick blood samples at 280, 300, 320 and 340 minutes. Incremental area under the curve (iAUC) was calculated cumulatively as well as for both the pre and post-pizza meals for blood glucose. Blood glucose significantly increased at a slower rate to 20 minutes for chickpea, lentil and yellow pea macaroni compared to macaroni and cheese. Furthermore, at 140 minutes after consumption, blood glucose was significantly lower for the lentil macaroni compared to macaroni and cheese. Blood glucose concentration was significantly lower for lentil and chickpea macaroni at 280 minutes compared to macaroni and cheese. There were no significant differences for blood glucose AUC for the pre-pizza meal (0-260 minutes) between treatments, but the post-pizza meal blood glucose AUC (260- 340 minutes) was significantly lower for lentils and macaroni and chickpeas and macaroni compared to yellow peas and macaroni, but not macaroni and cheese. Overall, consumption of 332.9 g of lentils mixed with macaroni to yield 600 kcal reduced blood glucose concentration at 20 and 140 minutes, and 280 minutes following consumption of a pizza meal in healthy men compared to macaroni and cheese. Blood glucose AUC following the pizza meal was also reduced in healthy men for lentils and macaroni compared to yellow peas and macaroni.

Similar methodology from Mollard *et al.* (2011) was repeated for another study conducted by Mollard *et al.* (2012a). This study investigated if acute consumption of pulse containing meals had an impact on glycemic response, and measures of satiety at a later meal (which will not be summarized here). This study was a randomized crossover design that included 24 healthy men with a BMI of 22.8 ± 1.4 kg/m² and an age of 23.3 ± 3.6 years. After a
10-12 hour overnight fast, participants consumed a standardized breakfast at home (Cheerios™, 2% milk, orange juice and coffee or tea) and arrived at the laboratory 4 hours later. Upon arrival at the University of Toronto nutrition lab, participants had a baseline blood sample collected by finger prick for the measurement of blood glucose. Participants were then given the treatment meal (containing whole chickpea or lentil or navy bean or yellow pea mixed with macaroni in tomato sauce) or the control meal (macaroni in tomato sauce) *ad libitum*, and were instructed to consume the meal over 18 minutes and continue eating the treatment or control meal until comfortably full. Both meals were matched for caloric content per 100 g, but consumed the meals *ad libitum*. Following meal consumption, blood glucose was measured at 20, 40, 60, 110, 140, 200 and 260 minutes from finger prick blood samples. An *ad libitum* pizza meal was provided at 260 minutes and post-pizza meal blood glucose was measured at 280, 300, 320, and 340 minutes. The lentil treatment significantly reduced blood glucose at 20, 40, 80 and 200 minutes compared to the macaroni control, and at 60 minutes compared to yellow peas. Blood glucose AUC (cumulatively over 0-340 minutes) was significantly lower for the lentil, chickpea, navy bean and yellow pea meal compared to macaroni control. Furthermore, blood glucose AUC for the pre-pizza meal (0-260 minutes) was significantly lower for lentils, chickpeas and navy beans, but not yellow peas compared to macaroni in tomato sauce. Therefore, *ad libitum* consumption of whole lentils mixed with macaroni in tomato sauce (at 44% energy in a meal) was able to reduce blood glucose at 20, 40, 80 and 200 minutes compared to macaroni in tomato in 24 healthy males. Furthermore, blood glucose AUC was reduced for the lentils mixed with macaroni cumulatively (0-340 minutes), and before pizza consumption (0-260 minutes).

Mollard *et al.* (2014) conducted another study to determine if acute consumption of whole pulses (lentils, chickpeas, navy beans and yellow peas) would affect blood glucose
measures, and appetite (not summarized here) after a standard meal was consumed at 2 hours. Fifteen healthy men (age 22.5 ± 0.8 years) with a BMI of 22.9 ± 0.4 kg/m² were recruited to receive 1 of 5 isocaloric treatments in a randomized crossover study design. Treatments included whole chickpeas (222.8 g), whole lentils (332.9 g), whole navy beans (240.5 g), whole yellow peas (375.6 g) and white bread (116.6 g) and were served with tomato sauce. Participants arrived after a 10-12 hour fast and baseline blood glucose was measured from a finger prick blood sample. The treatment was consumed within 10 minutes, and blood samples were taken again by finger prick at 15, 30, 45, 75 and 135 minutes after the start of treatment consumption and were analyzed for blood glucose. A fixed pizza meal was served at 135 minutes for 15 minutes, and blood glucose was analyzed from blood samples collected at 150, 165, 195 and 210 minutes. Lentils in tomato sauce significantly lowered blood glucose at 15, 30, 45, and 75 minutes pre-pizza, and at 150 and 165-minutes post-pizza consumption when compared to white bread. Lentils in tomato sauce resulted in a significantly lower blood glucose AUC before the pizza meal was consumed (0-135 minutes) and following the pizza meal (150- 210 minutes) compared to white bread. Therefore, the consumption of 332.9 g of whole lentils in combination with tomato sauce reduced blood glucose concentration and blood glucose AUC before and after the pizza meal when compared to white bread in 15 healthy men.

In summary, the studies investigated above examined if the acute consumption of whole lentils served in food (lentils with butter and tomato, lentils in tomato sauce, or lentils mixed with macaroni and tomato sauce) in varying doses (451 g, 332.9 g or 226 g) in healthy adults would improve glycemic measures such as blood glucose concentration and blood glucose AUC compared to various control treatments. Overall, it was found that lentils were beneficial for the lowering of blood glucose, and improved blood glucose AUC compared to controls. It is
important to note that the dose of lentils consumed in many of the studies described above ranged from 1.5-2.0 cups of cooked lentils.

**C.a.ii. Acute Studies of Blended and Pulse Powders in Healthy Participants (Appendix B)**

This section will summarize studies that were conducted to determine if pulses, including lentils were beneficial on glycemic measures when they were no longer in their whole form. The following studies investigated lentils in their whole form compared to lentils that were in a blended or powdered form.

The study conducted by Anguah et al. (2014) studied 12 healthy men and women aged 28 ± 10 years with a BMI of 23.3 ± 3.1 kg/m². A randomized, crossover study design was used to determine if blended lentils (84.6 g), whole lentils (84.6 g) or no lentils (control) in a breakfast burrito in combination with a capsule (double-blind) containing α-galactosidase or placebo (lactose) could reduce blood glucose. Participants arrived at the Department of Nutrition Science at Purdue University after a standardized dinner (chicken and broccoli with macaroni) and a 12-hour over night fast. A fasted finger prick blood glucose measurement was taken, and the breakfast burritos and capsule were served with 240 mL of water and consumed within 15 minutes. Subsequent finger prick blood samples were collected for blood glucose measurements at 20, 40, 60, 90, 120 and 180 minutes. The study examined if blood glucose and blood glucose AUC were reduced in the lentil burritos compared to the control, and further explored whether the enzyme supplement interacted with the glucose response. Blood glucose at 40 minutes was significantly lower for the blended and the whole lentil burritos compared to the no lentil burrito. Blood glucose AUC over the first half (0-90 minutes) and total (180 minutes), but not the second half (90-180 minutes) was significantly lower for the blended (but not the whole) lentil burrito compared to the no lentil burrito. Overall, this study found that 84.6 g of blended lentils in a
breakfast burrito was able to reduce blood glucose concentrations at 40 minutes, and lower blood glucose AUC over 180 minutes compared to the burrito that did not contain lentils in healthy adults. There were no significant findings for the burrito with whole lentils for blood glucose AUC, but blood glucose at 40 minutes following treatment consumption was lower compared to the burrito containing no lentils.

Anderson et al. (2014) also focused on processed pulses, in the form of powders to examine if they would have the same benefits as previously seen in whole pulses in attenuating the blood glucose response in healthy adults. For the lentil portion of the study, 12 healthy males aged 22.2 ± 0.9 years with a BMI of 23.2 ± 0.4 kg/m² were recruited to participate in a randomized, crossover design. After a 10-12 hour fast, participants consumed a standardized breakfast (Honey Nut Cheerios™, 2% milk, and orange juice) and arrived at the lab 4 hours later for a baseline finger prick blood glucose measurement. Participants were then given 15 minutes to consume one of the treatments (183.8 g of whole canned green lentils, 183.8 g of pureed canned green lentils, or 47.7g of Eston lentil powder) or the control of whole wheat flour with 250 mL of water. All treatments were mixed into tomato sauce, and were matched for available carbohydrates. Postprandial blood glucose was measured at 15, 30, 45, 60, 90 and 120 minutes, and participants consumed an ad libitum pizza meal, and post-second-meal blood glucose was measured at 140, 155, 170, 185 and 200 minutes. The mean blood glucose concentration over 120 minutes was significantly lower for all lentil treatments compared to the whole wheat flour control. Furthermore, at 15 and 30 minutes, blood glucose concentration was significantly lower for whole and pureed lentil treatments (but not the lentil powder) compared to the whole wheat flour, but only the lentil powder was significantly lower than the whole wheat flour at 90 minutes. The mean blood glucose concentration following the pizza meal (140-200 minutes) was
not significantly different among any of the lentil treatments or the whole wheat flour control. Blood glucose AUC over 0-120 minutes was significantly reduced by the whole lentils and the lentil powder (but not the pureed lentils) compared to the whole wheat flour. The blood glucose AUC following the pizza meal was not affected by treatment. Therefore, the consumption of 183.8 g of whole, 183.8 g of pureed or 47.7 g of powdered lentils in combination with tomato sauce was beneficial on glycemic measures. Mean blood glucose was lower for all lentil treatments over 0-120 minutes, and blood glucose AUC over the same time period was lower for whole and powdered lentils (but not pureed) when compared to whole wheat flour in tomato sauce.

In summary, this section reviewed studies that examined various forms of lentils, including whole, blended, pureed and powdered in doses of 84.6 g to 183.8 g for their ability to modulate glycemic measures in healthy adults. These doses can be translated into approximately ½ to 1½ cups of lentils in breakfast burritos or with tomato sauce. Benefits were seen for blood glucose AUC for ½ cup of blended lentils over 0-90 minutes in a breakfast burrito compared to a burrito that did not contain lentils. Similarly, blood glucose AUC over the first 120 minutes was reduced for whole and powdered lentils with tomato sauce compared to whole wheat flour. Furthermore, throughout both studies, improvements in blood glucose concentration at a single time point were observed for all lentil treatments when compared to the control. Therefore, lentils in various forms in doses from ½ to 1½ cups have been shown to improve acute glycemic response measures in healthy adults.

C.b. Acute studies of Whole lentils in Adults with Diabetes (Appendix C)

Acute consumption of lentils has been shown to improve measures of glycemic response in healthy individuals. However, the management and improvement of glycemic measures is also
important to apply to adults with diabetes to improve the management of their disease, reduce the cost to the healthcare system, and to improve patient quality of life. The following studies have looked at the effects of pulse consumption, including lentils, on glycemic measures in adults with diabetes.

The study conducted by Coulston et al. (1984) identified the glucose, insulin and gastric inhibitory polypeptide responses to 4 treatment meals that were matched for protein and fat, but varied in the type of carbohydrate (baked potato, rice, spaghetti or lentils) in 8 adults (age 57 ± 2 years) diagnosed with type 2 diabetes and treated with sulfonylureas or diet. The study was a randomized crossover design, and each participant consumed all 4 treatments which contained 66% of energy from the test carbohydrate over a 2-week time period. It was reported that the lentil meal had 49.9 g of lentils, but it did not indicate if this was dry weight, or weight after cooking. The day of a study visit, participants were instructed to consume a standardized breakfast (white toast, margarine, fruit juice or tea or coffee). Four hours after breakfast (around noon), participants arrived for their study visit and had a baseline blood sample drawn by venous puncture for analysis of glucose, insulin and gastric inhibitory polypeptide. The test meal (containing bread, turkey, margarine, oil, lettuce and the test food) was served to the participants, and consumed within 20-25 minutes and subsequent blood samples were collected at 30, 60, 120 and 180 minutes. There were no significant differences in glucose, insulin or gastric inhibitory polypeptide between participants who were being treated with sulfonylureas or diet, and were therefore analyzed as one group. The AUC for plasma glucose and insulin over 180 minutes were significantly higher for the baked potato test meal compared to the lentil, spaghetti and rice meals, which were not different from each other. Gastric inhibitory polypeptide AUC over 180 minutes was significantly lower for the lentil test meal compared to spaghetti, rice and potato.
The study by Coulsten et al. (1984) demonstrated that lentils providing 66% energy in a meal can reduce plasma glucose and insulin in 8 adults with diabetes compared to a potato meal. Similarly, gastric inhibitory polypeptide was also reduced by the lentil meal compared to the spaghetti, rice and potato meals in the same population.

The study conducted by Bornet et al. (1987) in France examined insulin and glycemic index of six starch rich foods in 18 adults (age: 57 ± 2, BMI: 27.9 ±1.1 kg/m²) with type 2 diabetes and treated with either diet or oral anti-diabetic drugs. Each participant consumed 3 test meals on 3 consecutive days; the starchy food alone (50 g of available carbohydrate), the same starch food in a mixed meal (matched for 50 g of available carbohydrate, 24 g of protein and 20 g of fat), and an oral glucose tolerance test (50 g of glucose). The six starch rich foods included kidney beans (n=3), green lentils (n=3), rice (n=3), spaghetti (n=3), instant potato (n=3), and white bread (n=3). Participants arrived in the morning, after a 12 hour fast. Similarly to Coulsten et al. (1984), blood samples were drawn by venous puncture before consumption of the test meal, and at 30, 60, 90, 120, 150 and 180 minutes after the start of the test meal and measured for plasma glucose and plasma insulin. A total of 3 participants consumed 225 g of green lentils alone, in a mixed meal, and an OGTT and were all standardized for 50 g of available carbohydrates. The glycemic index was not significantly different among the starch rich foods when eaten alone or in a mixed meal, however, lentils had a significantly lower glycemic index compared to white bread and potato. The insulin indices were significantly increased when starch rich foods were eaten in a mixed meal, but the insulin index did not significantly differ when the starch rich foods were eaten alone. Lentils and beans had the lowest insulin indices compared to the other four starchy foods eaten alone, but no statistical comparison was made between foods.
Therefore, 225 g of green lentils can be incorporated into the diet to benefit glycemic endpoints independently or within mixed meals in adults with diabetes.

Research conducted by Akhtar et al. (1987) used a randomized crossover design in 14 healthy men (age: 35.2 ± 1.54 years; BMI: 23.6 ± 0.55 kg/m²) and 14 men with type 2 diabetes (age: 52.5 ± 2.46 years; BMI: 24.3 ± 1.13 kg/m²) to determine the effect of traditional Pakastani meals, including two lentil meals (containing 89.3 g of lentils dry weight) on blood glucose. Participants arrived after an overnight fast, had a fasted finger prick blood sample and consumed in random order a meal of: bread + egg, bread + lentils, bread + grams, bread + moong, bread + mash, rice + lentils or, rice + moong (all meals matched for 50 g of available carbohydrates) and served with 250 mL of milk over 10 minutes. Postprandial finger prick blood samples were taken at 30, 60, 90, 120, 150, and 180 minutes. Fasted blood glucose in healthy men varied significantly between treatments, with rice + lentils having the highest fasted value compared to bread + eggs, rice + moong, and bread + lentils, while bread + lentils had a lower fasted value compared to bread + grams, and rice + lentils. Blood glucose AUC in healthy men was significantly reduced with bread + lentils compared to bread + mash, bread + egg, and rice + moong, and for rice + lentils compared to rice + moong. In men with diabetes, blood glucose AUC was significantly reduced with bread + lentils compared to bread + egg and both rice containing meals; the rice + lentils meal was significantly lower than rice + moong. The peak glucose concentration in healthy men was not different between lentil meals, and both were significantly lower compared to rice + moong. Peak glucose concentration in men with diabetes was lower for bread + lentils compared to bread + mash, bread + eggs, and both rice meals. This study demonstrated that 89.3 g (dry weight) of lentils, which amounts to approximately 1 cup
cooked, in meals with either bread or rice reduced blood glucose AUC compared to moong and mash controls when mixed in normal Pakistani meals in a healthy and diabetic population.

In summary, the studies examined above looked at the effects of whole lentils in varying doses (ranging from 49.9 g, 89.3 g (dry weight) and 225 g) alone, and mixed in meals on glucose and insulin measures in adults with type 2 diabetes. All studies demonstrated improvements in glycemic measures including all 3 studies showing benefits in blood glucose, and 2 of the 3 studies seeing improvements in insulin from lentil consumption compared to other starchy controls. Overall, lentils in varying doses from ½ cup to 3 cups cooked were beneficial in adults with diabetes for the management of blood glucose.

C.ii. Chronic Human Intervention Trials

Chronic, or longer term clinical trials are important in nutrition research to determine the effects consumption of treatments over time and to further understand the benefits associated with consumption of the same food products in acute human intervention trials. The following chronic intervention trials investigated the effect of consumption of pulses, including lentils on glycemic measures.

C.ii.a. Chronic Studies of Powdered Lentils in Healthy Adults (Appendix D)

It has been determined that acute consumption of lentils can help with the improvement in post prandial blood glucose control; however, it is also important to examine whether chronic consumption of lentils also results in improvements in glycemic measures in healthy adults. The following study has investigated the chronic consumption of lentils in healthy adults to identify the impact on glycemic measures.
The study conducted by Cryne et al. (2012) incorporated 100 g of pulses (including chickpeas, lentils or peas) in a spray dried pulse powder or 50 g of potato flakes into the diet of 21 healthy males (age: 28.1 ± 5.87 years; BMI: 25.2 ± 3.54 kg/m²) to examine their effects on biomarkers of type 2 diabetes risk. The double-blind, randomized, crossover study included four 28-day treatment periods, each separated by a 28-day washout period. Fasted blood samples were collected on days 1 and 29 of each treatment and were analyzed for glycemic measurements (fasting plasma glucose, insulin and homeostatic assessment model of insulin resistance (HOMA-IR)). There were no significant differences in plasma glucose, insulin or HOMA-IR between treatments at day 1 or day 29. Overall, the authors concluded that spray-drying pulse powders consumed for 28 days was not an effective dietary strategy to help with glycemic control management in healthy male adults.

In summary, this section examined the effects of 100 g of lentil powder on glycemic measures including plasma glucose, plasma insulin, and HOMA-IR in healthy males for 29 days as reported by Cryne et al. (2012). No significant differences were found between treatments on days 1 or 29. Further research should be conducted in this area to see if a larger dose, a different form, or a longer duration would be beneficial for glycemic measures in healthy males.

C.iib. Chronic Studies of Whole Lentils in Adults at risk for Diabetes (Appendix E)

As mentioned previously, the global incidence of diabetes is expected to increase dramatically by 2040 to 642 million people, and of these, many cases are preventable with diet, and exercise interventions (International Diabetes Federation 2015). Therefore, it is critical to identify whether chronic lentil consumption can reduce risk factors for T2D to prevent progression of at risk populations from developing T2D. The following studies have examined
the effect of chronic lentil consumption in at risk populations on glycemic measures and the development of T2D.

Mollard et al. (2012b) studied adults with Metabolic Syndrome (MetS) which is defined by the International Diabetes Federation as a person with central obesity (defined by waist circumference) plus any two of the following four factors: elevated fasting plasma glucose (or insulin resistance), triglycerides, blood pressure, or reduced high-density lipoprotein cholesterol (HDL cholesterol) and MetS is associated with an increased risk for cardiovascular disease (International Diabetes Federation 2006). Mollard et al. (2012b) studied 40 overweight and obese (BMI: 32.8 ± 0.7 kg/m²) men and women aged 45.5 ± 1.0 years who were randomized to a pulse group (including lentils, chickpeas, navy beans and yellow peas) that consumed 5 cups of pulses a week (n=19), or an energy restricted diet that reduced caloric intake by 500 kcal/day (n=21), for 8 weeks. Participants arrived fasted at the University of Toronto at weeks 1, 4, 8 and had a fasted blood sample for measurement of glucose, and insulin. At weeks 1 and 8, a 75 g oral glucose load tolerance test was also administered and blood measurements were taken at 0, 10, 20, 30, 60, 90 and 120 minutes. HbA1c was significantly decreased from weeks 1 to 8 within both the pulse group, and the energy restricted group, but there was no significant difference between treatments (p= 0.09). Fasted glucose and insulin were not significantly different within or between treatments. Blood glucose AUC was significantly decreased by 20.1% within the pulse group from weeks 1 to 8 and by 5.6% within the energy restricted group. Furthermore, insulin AUC also decreased from weeks 1 to 8 in female participants in the energy restricted group by 4.8% and in the pulse group by 13.9%, and in males by 24.2% in the energy restricted group, however insulin AUC increased by 27.3% in the pulse group. Therefore, the consumption of 5 cups of pulses a week for 8 weeks in adults at risk for the development of T2D improved
HbA1c, and blood glucose compared to baseline; however, there were no significant differences between the pulse and the energy restricted groups.

Similarly to Mollard et al. (2012b), Saraf-Bank et al. (2016) studied a population at risk for the development of T2D. A total of 26 participants who were first degree relatives to a patient diagnosed with T2D (but otherwise healthy) were recruited for the study. In this randomized, crossover design, participants consumed a legume-enriched diet (consisting of $\frac{1}{2}$ cup of lentils in 12 prepackaged bags as well as 12 prepackaged bags containing $\frac{1}{2}$ cup of pinto beans) in which they were directed to consume 4 of these bags (or 2 cups of legumes) a week. The other group followed their habitual diet, and were given advice to try to incorporate healthier food options. Each diet was eaten over a 6 week period, separated by a 2-week washout period. Fasting blood was collected at the start and end of each intervention period for analysis of blood glucose and HbA1C. Overall, there were no significant differences in fasting blood glucose or HbA1c from baseline to week 6 within the legume-enriched diet. There was no significant change in fasting blood glucose, but there was an increase in HbA1c from baseline to week 6 on the habitual diet, although it did not reach significance. The authors concluded that a $\frac{1}{2}$ cup serving size of legumes 4 times a week for a 6-week duration may not have been enough to produce changes in glycemic measures in a healthy population that is at risk for the development of T2D.

In summary, the two studies above investigated the effects of pulses, which include lentils, on glycemic response measures in adults at risk for the development of T2D. The studies incorporated either 2 or 5 cups of pulses and had differing effects on glucose measures. A dose of 5 cups a week was able to cause a reduction in HbA1c and fasted blood glucose, while 2 cups a week was not able to produce the same effects.
C.ii.c. Chronic Studies of Whole Lentils in Adults with Diabetes (Appendix F)

In providing guidance for regular consumption of lentils as an adjunct to management and prevention of T2D it is important to examine the available evidence. This section will examine the effect of chronic lentil consumption on adults who are clinically diagnosed with diabetes, to determine if consumption can improve glycemic measures.

The study by Jenkins et al. (2012) recruited 121 clinically diagnosed men and women with type 2 diabetes. Participants were randomized to consume a 3-month diet of either a low glycemic index legume diet that encouraged intake of 1 cup a day of legumes including lentils, chickpeas or beans (n=61; age: 58 ± 1.3 years; BMI: 31.4 ± 0.9 kg/m²) or a high wheat fibre control where consumption of whole wheat and whole grain carbohydrate sources was encouraged (n=58 age: 61 ± 1.0 years; BMI: 29.0 ± 0.7 kg/m²). Participants visited the research center at the University of Toronto at baseline, 2, 4, 6, 8, 10, and 12 weeks and a fasted blood sample was collected for analysis of blood glucose and HbA1c. The low glycemic index legume diet caused a significant reduction in fasted blood glucose and HbA1c from baseline to week 12, and to a significantly greater extent than the high wheat fibre diet.

Similarly to Jenkins et al. (2012), the study conducted by Hosseinpour-Niazi et al. (2015) included adult men and women who were overweight and diagnosed with type 2 diabetes. A total of 31 participants were recruited (age: 58.1 ± 6.0 years; BMI: 27.7 ± 0.7 kg/m²) from Iran for a randomized cross over study. Eligible participants had a 2-week run in period where they consumed their usual diet but without legumes. Participants were then randomized to either the control group that consumed the therapeutic lifestyle changes (TLC) diet with no legumes, or the TLC diet with the addition of legumes (6 weekly servings or 3 cups of cooked lentils, chickpeas, or beans) for 8 weeks each separated by a 4-week washout period. At baseline, and upon
completion of the diet (week 8), participants arrived after a 12-14 hour overnight fast and blood samples were drawn to measure fasted serum glucose and insulin. Firstly, fasted serum glucose and fasted insulin were significantly reduced when comparing from baseline to week 8 in both dietary interventions. Furthermore, fasted serum glucose and insulin were significantly decreased after 8 weeks of consuming the legume TLC diet compared to the legume-free TLC diet. Therefore, it was shown that the addition of 3 cups a week of legumes, including lentils, significantly improved fasted serum glucose and insulin over 8 weeks in overweight adults with type 2 diabetes compared to the TLC diet with no legumes.

The study conducted by Shams et al. (2008) investigated whether 30 adults age 50.2 ± 3.8 years and a BMI of 28.9 ± 4.1 kg/m² with type 2 diabetes would see improvements in blood glucose with the consumption of 50 g of cooked lentils. The randomized crossover study included group A, who consumed their usual diet and restricted legume intake (control), and group B who replaced 30 g of bread and 20 g of cheese with 50 g of cooked lentils and 6 g of canola oil into the isocaloric breakfasts for 6 weeks. Participants had fasted plasma glucose measurements taken at baseline, and upon the completion of each diet at week 6. Fasted plasma glucose was significantly lower from baseline to 6 weeks with the lentil diet compared to the control treatment. Therefore, the addition of 50 g of cooked lentils once a day for 6 weeks can improve blood glucose in adults with diabetes.

In summary, the studies examined above investigated if chronic consumption of lentils could improve glycemic measures including fasted blood glucose, fasted plasma glucose, fasted insulin and HbA1c in adults with type 2 diabetes. The studies ranged in length from 6 weeks, 8 weeks, and 3 months, and in dose from 50 g a day, 1 cup a day, and upwards to 3 cups a week. Regardless of dose or duration of intervention, all of the studies examined above demonstrated
improvements from baseline to the end of the study with consumption of lentils in adults with type 2 diabetes. Therefore, the chronic consumption of lentils can be used for the management of type 2 diabetes, but an effective dose needs to be more easily defined in order to guide consumption levels for the public.

C.iii. Meta-analysis of Acute Lentil Consumption in Adults with Diabetes

The benefit of meta-analyses is their use of statistical methods to summarize the effect found in numerous studies researching common categories. The pooled analysis is able to strengthen the power across studies to determine the effects of research in particular research topic. This section summarizes the results of a meta-analysis conducted by Augustin et al. (2012).

A meta-analysis by Augustin et al. (2012) examined 38 acute clinical trials conducted between 1983 and 2003 that examined the effect of acute pulse consumption on postprandial blood glucose response in adults with diabetes (both type 1 and type 2 diabetes). Treatments were included if they contained lentils, chickpeas, beans or split peas compared to matched quantities of carbohydrate containing control meals. The meta-analysis showed that all types of pulses examined (lentils, chickpeas, beans and split peas) significantly reduced postprandial glucose levels by 50%, when compared to equal carbohydrate containing, commonly consumed control foods. The authors concluded that pulses, including lentils were able to reduce postprandial glucose levels by 50% in adults with diabetes. This study did not examine what dose was able to elicit a reduction in glucose levels by 50%, and further studies should be conducted to solidify the quantity of pulses. Therefore, the consumption of pulses including lentils, improved postprandial blood glucose levels by 50% compared to equal amounts of carbohydrate containing foods in adults with diabetes.
C.iv. Summary of Lentils and Glycemic Response: Human Studies

In summary, the above review has analyzed the effects of pulses including lentils, in adults with and without diabetes, on various glycemic measures including fasting and postprandial glucose and insulin, as well as fasting HbA1c. Research on acute lentil consumption has looked at whole lentils incorporated into mixed meals (breakfast burrito, macaroni, and tomato sauce), and as pulse powders in both adults with diabetes, and in healthy adults. The amount of lentils served to participants has ranged from 49.9 g, upwards to 332.9 g and many studies did not differentiate if the amount was the dry weight or the weight after cooking. Furthermore, research has been conducted on the chronic consumption of whole pulses (including lentils), and powdered lentils in healthy adults, in adults with diabetes, and in adults at risk for the development of diabetes. In these studies, intake of pulses, including lentils ranged from 50 g per day, upwards to 5 cups per week and the length of consumption also varied from 29 days to 3 months. Lastly, the meta-analysis combined many of these studies to summarize an improvement in glycemic measures in acute consumption of pulses, including lentils in adults with diabetes, but the study did not report the dose of pulses that demonstrated an effect in improving glycemic measurements.

Overall, human studies have investigated the impact of lentils on glycemic measures in healthy adults, adults at risk for diabetes, and adults with diabetes in various quantities. In these studies, the amount of lentils consumed ranged in dose from 49.9 g per day, 1 cup per day, to 5 cups per week which can be translated to a range of approximately ½ cup per day to an upper level of 5 cups per week. A majority of the studies investigated have demonstrated an acute glycemic effect of lentils at ½ cup, but the literature is not consistent at studying this serving size measurement, and many studies do not indicate if the amount of lentils served is the dry weight...
or the weight after cooking. Therefore, it needs to be determined if lower serving sizes of lentils, such as \( \frac{1}{4} \) cup have the same benefits on glycemic measures as \( \frac{1}{2} \) cup of lentils. If lower serving sizes of lentils still carry the same benefits as larger quantities, consumers would be able to achieve the health benefits for glycemic measures without having to consume as large amounts.

Furthermore, the rising prevalence of obesity, and type 2 diabetes has been linked to unhealthy lifestyle choices, such as lack of exercise, and an increase in consumption of energy-dense foods which alter metabolic responses (Aller et al. 2011). The intake of these commonly consumed starchy foods are high in carbohydrates and tend to be low in fibre which rapidly increases blood glucose, increases insulin secretion, and over time can lead to insulin resistance (Aller et al. 2011). Examples of these foods include white pasta, white potato, and white rice, which have been examined in the literature above, and are still commonly consumed starchy foods in Canada (Canadian Community Health Survey 2017). Although corn was not analyzed in the literature outlined above, corn is also one of the most commonly consumed starchy foods as reported in the Canadian Community Health Survey, and is frequently consumed on its own, in food and cereal products, and as high fructose corn syrup (Canadian Community Health Survey 2017; National Corn Growers Association 2018). Therefore, it is important to consider multiple starchy foods that the general public consumes when communicating the effects of eating them on their health.
Chapter II. STUDY RATIONALE, PURPOSE, OBJECTIVES, and HYPOTHESIS

A. Study Rationale

Strategies need to be identified for the management and prevention of T2D in Canada, and globally as T2D prevalence and diagnosis is increasing. Although there are many non-modifiable risk factors for the development of T2D such as age, ethnicity, and genetics, there are also many modifiable risk factors, such as exercise and dietary interventions that can be incorporated for the prevention, delay, and management of blood glucose for T2D and its associated complications. Dietary strategies such as the incorporation of foods that are nutrient dense, high in fibre, contain complex carbohydrates, and are low on the glycemic index can be utilized to improve and manage glycemic measures.

Previous research on the acute effects of lentil consumption on various measures of postprandial glycemic response has demonstrated improvements in various populations including healthy adults and those with type 2 diabetes. As mentioned previously, the amount of lentils consumed in these studies range widely, the form and study meal that the lentils are served in are variable, and the glycemic measures reported differ between studies, including insufficient data on insulin. Therefore, a clinical trial that examines the effects of consumption of lentils independently compared to matched serving sizes of starchy foods in a healthy population can help clarify if lentils are beneficial in smaller quantities.

B. Study Purpose, Objectives and Hypotheses

The purpose of this study was to determine if consumption of two varieties of lentils (Laird green and Eston split red) in half-cup and quarter-cup serving sizes would significantly improve glycemic response measures compared to four commonly consumed starchy foods (corn,
macaroni, white potato and white rice) in healthy adults. The specific objectives, and hypotheses of this research were:

1. To determine the acute effect of Laird green and Eston split red lentils consumed in a half-cup serving size on postprandial blood glucose and insulin compared with the same serving size of starchy controls (corn, macaroni, white potato and white rice) in healthy adults.
   a. It was hypothesized that a half-cup serving of the Laird green and Eston split red lentil would significantly reduce blood glucose, and would not significantly change plasma insulin compared to the starchy controls (corn, macaroni, white potato and white rice).

2. To determine the acute effect of Laird green and Eston split red lentils consumed in a quarter-cup serving size on postprandial blood glucose compared with the same serving size of starchy controls (corn, macaroni, white potato and white rice) in healthy adults.
   b. It was hypothesized that the quarter-cup serving of the Laird green and Eston split red lentil would significantly reduce blood glucose compared to the starchy controls (corn, macaroni, white potato and white rice).
Chapter III. METHODS

A. Study Approvals and Training

A.i. Research Ethics Board Approval

A University of Guelph Research Ethics Board (REB) application and appendices were completed and submitted in August 2016 for a full board review. Upon completion of the revision process, REB approval was received on November 17, 2016 with annual renewal and included a certificate (Appendix G) and an approval number (REB#16AU001).

A.ii. Biohazardous Materials Certification

An application was submitted to the University of Guelph Biosafety Committee for the approval to work with biohazardous materials. After submitting the necessary application and the safe handling protocol, the study received a Biohazard Permit (H-254-13-18-11) that was valid from October 26, 2016 until November 30, 2018 (Appendix H).

A.iii. Clinical Trials.gov Registration

Information regarding the study protocol including the description, design, interventions, outcome measures, eligibility criteria and contact information were submitted to the protocol registration and results system at Clinicaltrials.gov. Recruitment status, was updated throughout the duration of the study. The clinicaltrials.gov identifier ID was NCT02940158.

A.iv. Study Researcher Training

Research personnel that were performing finger prick blood sampling were trained by a certified phlebotomist, and were certified in first aid and CPR. Each member of the research team completed HNRU and HHNS Research Safety Training, online College of Biological Science (CBS) Laboratory Safety and WHMIS courses, and the online Tri-council policy
Statement (TCPS2) Course on Research Ethics (CORE). Research members were also provided with metabolic kitchen training, and blood handling and safety training from the HNRU manager and phlebotomists in the HNRU.

**B. Study Design**

Two studies were completed. The first study examined the effects of half-cup serving sizes of six treatments (green lentils, red lentils, corn, macaroni, white potato, white rice) on postprandial blood glucose and insulin, and the second study examined the effects of the same treatments in quarter-cup serving sizes on postprandial blood glucose. Both studies utilized a randomized crossover design that consisted of 6 study visits separated by washout periods of 3-7 days (Figure 1). All study visits occurred in the HNRU at the University of Guelph and were conducted between November 28, 2016 and January 8, 2018. A study email account was set up to facilitate all communication and scheduling.

![Figure 1: Overview of study design for the half-cup and quarter-cup studies.](image)

**C. Study Participants**

**C.i. Inclusion and Exclusion Criteria**

Healthy adults were included in this study with inclusionary and exclusionary criteria established (Table 2) to minimize confounding variation from as many sources as possible.
Table 2: Participant inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Participant Inclusion Criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult males and females 18-40 years old</td>
<td>Eighteen years of age is the age of a consenting adult, and &gt;40 years old is consistent with the Public Health Agency of Canada’s identification of increased age as a risk factor for T2D (Public Health Agency of Canada 2009).</td>
</tr>
<tr>
<td>BMI 20- 30 kg/m²</td>
<td>There is a strong correlation between high BMI and the development of T2D, as larger body mass &gt; 30 kg/m² is associated with insulin resistance (Onyeseom et al. 2013; Kahn 1994; Sue and Sara 1993).</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participant Exclusion Criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes (fasting blood glucose ≥7.0 mmol/L, or 2-hour blood glucose ≥11.1 mmol/L post-75g oral glucose tolerance test (OGTT))</td>
<td>The values indicated represent the clinical classification of diabetes as outlined by Diabetes Canada using fasted and 2-hour OGTT values (Punthakee and Goldenberg 2018).</td>
</tr>
<tr>
<td>Impaired Glucose Tolerance (fasting glucose between 6.1-6.9 mmol/L, or 2-hour blood glucose between 7.8-11.0 mmol/L post-75g OGTT)</td>
<td>The values outlined are used to define and diagnose impaired glucose or pre-diabetes by Diabetes Canada in their clinical classification document (Punthakee and Goldenberg 2018).</td>
</tr>
<tr>
<td>Blood pressure &gt;140/90 mmHg</td>
<td>Hypertension is a cardiometabolic risk factor that is also largely found with glucose intolerance or diabetes as outlined by the Clinical Practice Guidelines from the International Society of Hypertension for hypertension management (Weber et al. 2014).</td>
</tr>
<tr>
<td>Major medical condition including a history of AIDS or hepatitis</td>
<td>Major medical conditions may require medication that can alter study endpoints, and blood diseases may endanger study personnel.</td>
</tr>
<tr>
<td>Medical or surgical event requiring hospitalization within 3 months of randomization</td>
<td>Study treatments or study protocol may be contraindicated in people who have recently undergone surgery or a major medical event. Such events, their required medications, or associated complications may alter the study endpoints.</td>
</tr>
<tr>
<td>Any medication use except stable dose (3 months) of oral contraceptives, blood pressure or statin meds</td>
<td>Use of medications may alter study endpoints.</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>Tobacco use is associated with an increased risk for T2D</td>
</tr>
</tbody>
</table>
(Willi et al. 2007).

<table>
<thead>
<tr>
<th>Probiotic supplement use</th>
<th>Use of probiotics may alter study endpoints.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary fibre supplement use</td>
<td>Use of fibre supplement may alter study endpoints.</td>
</tr>
<tr>
<td>Natural Health Products (NHPs) used for glycemic control</td>
<td>Use of NHPs may alter study endpoints.</td>
</tr>
<tr>
<td>Pulse consumption &gt;4 servings per week</td>
<td>To reduce confounding variables, individuals that consumed more than 4 servings of pulses a week were excluded to prevent alterations in glycemic endpoints.</td>
</tr>
<tr>
<td>Food allergy or non-food life-threatening allergy</td>
<td>To maximize the safety of participants, any individual with food allergy or life threatening allergy was excluded.</td>
</tr>
<tr>
<td>Pregnant or breastfeeding</td>
<td>To maintain safety of participants and their children, any women who were known to be pregnant or breastfeeding were excluded.</td>
</tr>
<tr>
<td>Alcohol consumption (&gt;14 drinks/week or &gt;4 drinks/sitting)</td>
<td>Alcohol consumption above these levels increases the risk of long-term health complications and may interfere with study endpoints (Butt et al. 2010).</td>
</tr>
<tr>
<td>Recent or intended significant weight loss or gain (&gt;4 kg in previous 3 months)</td>
<td>Weight loss/gain can alter metabolism and may affect study endpoints.</td>
</tr>
<tr>
<td>Elite athletes</td>
<td>Elite athletes have shown to have improved glucose metabolism, and studies have demonstrated those with T2D can improve glucose metabolism with intense physical activity (Causa et al. 2005; Little et al. 2011; Hood et al. 2011).</td>
</tr>
<tr>
<td>Shift worker</td>
<td>Studies have demonstrated that shift workers are at an increased risk for T2D due to altered circadian rhythm and altered glucose metabolism (Pan et al. 2011). Shift work also limited the availability to participate in the study.</td>
</tr>
</tbody>
</table>
C.ii. Sample Size Calculation

A sample size calculation was completed for both the half-cup and quarter-cup serving size studies on the primary endpoint of fasting blood glucose. The calculation was done with the help of an online sample size calculator (http://hedwig.mgh.harvard.edu/sample_size/size.html). The variables included an alpha level of 0.05, a power level of 80% (beta), a reduction of blood glucose by 20% as per Health Canada’s guidelines indicate that a reduction of postprandial blood glucose by 20% is considered a significant benefit for blood glucose response (Health Canada Draft Guidance Document on Food Health Claims Related to the Reduction in Post-Prandial Glycemic Response 2013). The coefficient of variation for intra-individual variation in glucose incremental AUC (iAUC) used was 23% (Wolever 2006). Using the aforementioned variables and a t-distribution, a total of 18 participants were required to detect at least a 20% difference in iAUC for the half-cup study. The quarter-cup study required a larger sample size due to the expected lower blood glucose iAUC, and higher variance in blood glucose response. Therefore, a total of 24 participants were required to detect at least a 20% difference in iAUC for the quarter-cup study. It was decided that it would be clearer if both studies reported on the same number of participants, and therefore 24 participants were used as the target sample size for both the half-cup and quarter-cup studies, and to account for attrition, a total of 26 participants were recruited for each study.

C.iii. Participant Recruitment

Participant recruitment was completed using a variety of methods for the half-cup and quarter-cup serving studies, as summarized in Table 3. Recruitment took place starting in November 2016, and finished in December 2017. Recruitment posters (Appendix I) (approximately 150 posters) were placed around the University of Guelph campus, and around
the community in shopping malls, sports facilities, gyms, libraries, grocery stores, and community centers. Recruitment email advertisements were also distributed to various departments at the University of Guelph and word of mouth was also used.

Table 3: Enrollment of participants by recruitment method

<table>
<thead>
<tr>
<th>Method/Location of Recruitment</th>
<th>Enrolled Participants (Half-Cup)</th>
<th>Enrolled Participants (Quarter-Cup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment flyer on campus</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Word of mouth</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Previous participant in HNRU (flyer/ email)</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Recruitment flyer in community</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Recruitment email</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

C.iv. Participant Screening

Participant screening included a 3-step process and is summarized in Table 4.

Table 4: Summary of measurements completed in the screening process

<table>
<thead>
<tr>
<th>Screening</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening-1</td>
<td>Brief eligibility questionnaire</td>
</tr>
<tr>
<td>Screening-2</td>
<td>Height</td>
</tr>
<tr>
<td></td>
<td>Non-fasted body weight</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
</tr>
<tr>
<td></td>
<td>Blood pressure</td>
</tr>
<tr>
<td></td>
<td>Expanded eligibility questionnaire</td>
</tr>
<tr>
<td>Screening-3</td>
<td>Fasted body weight</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
</tr>
<tr>
<td></td>
<td>Waist circumference</td>
</tr>
<tr>
<td></td>
<td>Blood pressure</td>
</tr>
<tr>
<td></td>
<td>Oral glucose tolerance test: fasted 2-hour blood glucose</td>
</tr>
</tbody>
</table>
Screening-1 involved a brief eligibility questionnaire that was completed over the phone. A researcher asked questions to determine if the highest priority, easiest to rule out eligibility criteria was met including age, height and body weight, BMI, food allergies, and pulse consumption (Appendix J). If the potential participant was deemed eligible after Screening-1, they progressed to Screening-2 which was conducted at the Human Nutraceutical Research Unit (HNRU) at the University of Guelph. At Screening-2, the potential participant signed a consent form (Appendix K) and completed a more detailed eligibility questionnaire (Appendix L). Body measurements were also completed including height (measured on a stadiometer (SECA Portable Stadiometer 214, Hanover, MD, USA)), non-fasted body weight using an electronic scale (SVI-200F, Acculab Sartorius Group, Edgewood, NY, USA) (participants stood on the scale without shoes or bulky clothing), and blood pressure (measured in duplicate using a blood pressure monitor (Omron® Digital Blood Pressure Monitor, HEM-907 XL, Omron Healthcare Inc., Burlington, ON, Canada)). If the potential participant was eligible and remained interested, they were invited back to the HNRU for Screening-3. At Screening-3, potential participants signed a consent form (Appendix M) and had measurements of fasted body weight, fasted blood pressure and waist circumference (measured with a measuring tape placed on the top of the individual’s hipbone and wrapped around the waist (World Health Organization 2008) after the participants were instructed to remove any excess clothing, and to stand upright). They then had a fasted blood sample collected by finger prick, consumed a 75g carbohydrate beverage (Trutol 75 g orange beverage, ThermoFisher Scientific, Mississauga, ON, Canada) within 10 minutes and had another blood sample collected by finger prick 2 hours later. All samples were immediately analyzed for blood glucose in duplicate using a Nova Biomedical Statstrip Glucose Hospital Meter (Ref #53634, Nova Biomedical Canada Ltd, Mississauga, ON, Canada). If the participant
was still eligible and interested after completing the screening process, they were invited to the HNRU for a study orientation.

At the study orientation, participants were provided with a study handbook (Appendix N) that contained information to help them through the study. The study handbook included sections that provided information about the study researchers, the purpose of the research, and other information that the participants would need to know prior to enrollment in the study. A summary of information located in the study handbook can be found in Table 5. A study coordinator reviewed the study handbook with each participant and answered all their questions. The study orientation concluded with a review and signing of the study consent form (Appendix O). Once enrolled into the study, each participant was assigned a participant ID and a study binder to organize their study documents.

Table 5: Summary of study handbook

<table>
<thead>
<tr>
<th>Section of Handbook</th>
<th>Summary of Content</th>
</tr>
</thead>
</table>
| Study Welcome       | - A welcome letter that describes the purpose of the study handbook, and described their contribution to research as a participant.  
                      - A thank you note for their participation and commitment to the study. |
| Contact Information | - An introduction to the research team including the study director and coordinator.  
                      - The location of the HNRU. |
| Research Summary    | - A description of the study design and the purpose.  
                      - A statement of how their participation will contribute to the literature and particularly a substantiation for a health claim. |
| Study Treatments    | - A summary of all the treatments that would be consumed at each study visit, and their nutritional information.  
                      - The details for treatment consumption (consume within 10 minutes with 250 mL |
**Study Visit Activities**
- A detailed list with instructions for what is required for before and during every study visit.

| Study Measurements | - A list of measurements that would be taken at each study visit including the procedure for each measurement (finger prick blood samples, body weight, blood pressure). |
| Compensation and Feedback | - Acknowledgement that participants would receive a cheque in the amount of $400 for completion of the study and a payment confirmation sheet would need to be signed.  
  - A note indicating that participants will receive their individual results upon completion of the study. |

**AD. Study Treatments**

**D.i. Study Treatments: Description**

The study treatments included two varieties of lentils along with 4 starchy controls consumed in either half-cup or quarter-cup serving sizes. Serving size was the focus of both studies and so the treatments were matched for serving size (half-cup or quarter-cup) after cooking. The lentil varieties included the Laird green lentil, and the Eston split red lentil and were provided by the Alliance Grain Traders (Regina, Saskatchewan, Canada). The starchy controls included frozen peaches and cream corn (Presidents Choice®, Brampton, ON, Canada, L6Y 5S5, purchased at Zehrs Markets), white elbow traditional macaroni (Italpasta®, Brampton, ON, Canada, L6T 4E5, purchased at Zehrs Markets), instant mashed potato (Idahoan original mashed potatoes®, Idahoan foods LLC, Lewisville, ID, USA, 83431, purchased at Zehrs Markets), and white rice (Selection®, St. Paul, MN, USA, 55130, purchased at Food Basics). The nutritional composition of the treatments is summarized in Table 6.
Table 6: Nutritional composition of each cooked treatment matched for half-cup and quarter-cup serving sizes\textsuperscript{1,2,3,4}.

<table>
<thead>
<tr>
<th></th>
<th>Green Lentils\textsuperscript{1,2}</th>
<th>Red Lentils\textsuperscript{1,2}</th>
<th>Corn\textsuperscript{1,2}</th>
<th>Macaroni\textsuperscript{1,2}</th>
<th>White Potato\textsuperscript{2,3}</th>
<th>White Rice\textsuperscript{1,2}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amount (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>100.0</td>
<td>120.0</td>
<td>80.0</td>
<td>54.6</td>
<td>128.0</td>
<td>84.0</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>50.0</td>
<td>60.0</td>
<td>40.0</td>
<td>27.3</td>
<td>64.0</td>
<td>42.0</td>
</tr>
<tr>
<td><strong>Energy (kcal)\textsuperscript{6}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>161 ± 0.2</td>
<td>152 ± 0.3</td>
<td>103 ± 0.2</td>
<td>68.9 ± 0.1</td>
<td>65.6</td>
<td>140 ± 0.2</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>80.8 ± 0.1</td>
<td>76.1 ± 0.2</td>
<td>51.5 ± 0.08</td>
<td>35.8 ± 0.07</td>
<td>32.8</td>
<td>70.2 ± 0.09</td>
</tr>
<tr>
<td><strong>Protein (g)\textsuperscript{5}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>9.88 ± 0.01</td>
<td>8.88 ± 0.05</td>
<td>2.87 ± 0.02</td>
<td>2.23 ± 0.02</td>
<td>1.73</td>
<td>1.45 ± 0.03</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>4.94 ± 0.01</td>
<td>4.44 ± 0.03</td>
<td>1.44 ± 0.01</td>
<td>1.16 ± 0.01</td>
<td>0.87</td>
<td>0.73 ± 0.02</td>
</tr>
<tr>
<td><strong>Fat (g)\textsuperscript{5}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>0.15 ± 0.00</td>
<td>0.24 ± 0.01</td>
<td>2.30 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>0.08</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>0.07 ± 0.00</td>
<td>0.12 ± 0.01</td>
<td>1.15 ± 0.01</td>
<td>0.02 ± 0.00</td>
<td>0.04</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)\textsuperscript{6}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>26.3 ± 0.01</td>
<td>25.1 ± 0.05</td>
<td>15.8 ± 0.03</td>
<td>13.6 ± 0.01</td>
<td>12.6</td>
<td>32.0 ± 0.04</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>13.2 ± 0.01</td>
<td>12.5 ± 0.03</td>
<td>7.90 ± 0.01</td>
<td>7.10 ± 0.01</td>
<td>6.31</td>
<td>16.0 ± 0.02</td>
</tr>
<tr>
<td><strong>Dietary Fibre (g)\textsuperscript{6}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>5.55 ± 0.1</td>
<td>2.71 ± 0.07</td>
<td>3.99 ± 0.1</td>
<td>1.07 ± 0.03</td>
<td>1.56</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>2.78 ± 0.1</td>
<td>1.36 ± 0.03</td>
<td>2.00 ± 0.05</td>
<td>0.56 ± 0.01</td>
<td>0.78</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td><strong>Available Carbohydrate (g)\textsuperscript{4}</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>13.8 ± 0.06</td>
<td>16.1 ± 0.05</td>
<td>11.3 ± 0.04</td>
<td>15.5 ± 0.1</td>
<td>12.27</td>
<td>20.4 ± 0.00</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>6.91 ± 0.03</td>
<td>8.06 ± 0.03</td>
<td>5.63 ± 0.02</td>
<td>8.08 ± 0.05</td>
<td>6.14</td>
<td>10.2 ± 0.00</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data is presented as mean ± SE, unless otherwise indicated.
\textsuperscript{2}Data provided by Agriculture and Agri-food Canada.
\textsuperscript{3}Data was analyzed in singlet.
\textsuperscript{4}Available Carbohydrate calculated from Total Starch – Resistant Starch + Sugars (galactose + glucose + sucrose + fructose).
\textsuperscript{5} Analyzed by methods outlined from the University of Arkansas.
\textsuperscript{6} Analyzed by Maxxam Analytics.
D.ii. Study Treatments: Preparation

Study treatments were prepared in the HNRU’s metabolic kitchen according to standardized cooking protocols (Table 6). Cooking protocols included the preparation method, amount of water, cooking time and rest time. All researchers had kitchen orientation training, and were trained on how to cook each treatment. The cooking protocols for the half-cup and quarter-cup serving size treatments were identical, with the final step of each protocol to weigh out the designated amount of treatment for each serving size.

Table 7: Summary of cooking instructions for treatments for the half-cup and quarter-cup treatments

<table>
<thead>
<tr>
<th></th>
<th>Green Lentils&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Red Lentils&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Corn</th>
<th>Macaroni</th>
<th>White Potato</th>
<th>White Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation Method</td>
<td>Black &amp; Decker rice cooker</td>
<td>Black &amp; Decker rice cooker</td>
<td>Microwave</td>
<td>Pot on stovetop</td>
<td>Microwave</td>
<td>Black &amp; Decker rice cooker</td>
</tr>
<tr>
<td>Amount of Water (g)</td>
<td>170.0</td>
<td>125.0</td>
<td>26.0</td>
<td>228.0</td>
<td>180.0</td>
<td>110.0</td>
</tr>
<tr>
<td>Cooking Time (min)</td>
<td>25</td>
<td>10</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Rest Time (min)</td>
<td>5</td>
<td>5</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>1</sup>Lentils were tested for doneness when 8 out of 10 lentil seeds were squeezed between the thumb and forefinger and had little to no resistance

D.iii. Study Treatments: Randomization and Blinding

Study treatments were randomized using an online randomizer (https://www.randomizer.org) which created 24 unique sets of orders of the 6 treatments for each of the half and quarter-cup studies. Each treatment was assigned a number between 1 and 6 (1=green lentils; 2=red lentils; 3=corn; 4=macaroni; 5=white potato; 6=white rice) and the order of treatment consumption as per the the research randomizer was transferred to an excel
spreadsheet for each participant. Data collection flow sheets were filled in with the order of
treatment randomization prior to a participant’s enrollment in the study.

Although treatments were assigned a code (GL=green lentils; RL=red lentils; Corn=corn;
Mac=macaroni; WP=white potato; WR=white rice), blinding was not possible as participants
would be able to identify each treatment at the time of consumption. All treatments were served
as whole food, and therefore were easily identifiable. The decision to keep the treatments as
whole food was to mimic how these foods are typically consumed in Canada and increases
generalizability of the study results.

D.iv. Study Treatments: Consumption

Study treatments were served to participants warm on a tray with utensils, a napkin and
250 mL of bottled water (Figures 2-7). After the fasted blood sample, the tray was brought over
to the participant at the table they were sitting at, and they were instructed to consume the
treatment within 10 minutes, and to consume the water within 1 hour. A timer was started when
the participant took their first mouthful of food, and the time was recorded.
Figure 2: Serving tray containing the green lentil treatment. The tray includes 250 mL of water, utensils, a napkin, and a half-cup serving of green lentils.
Figure 3: Serving tray containing the red lentil treatment. The tray includes 250 mL of water, utensils, a napkin, and a half-cup serving of red lentils.
Figure 4: Serving tray containing the corn treatment. The tray includes 250 mL of water, utensils, a napkin, and a half-cup serving of corn.
Figure 5: Serving tray containing the macaroni treatment. The tray includes 250 mL of water, utensils, a napkin, and a half-cup serving of macaroni.
Figure 6: Serving tray containing the white potato treatment. The tray includes 250 mL of water, utensils, a napkin, and a half-cup serving of white potato.
Figure 7: Serving tray containing the white rice treatment. The tray includes 250 mL of water, utensils, a napkin, and a half-cup serving of white rice.

E. Data Collection

E.i. Study Visit Schedule

Study visits occurred at the HNRU in the morning and participants were instructed to avoid all food and beverages for 10-12 hours (except water the morning of to help facilitate blood sampling), and to avoid pulses, alcohol, unusual physical activity, and over-the-counter medications for 24 hours before the study visit. Participants were also instructed to follow their usual lifestyle including maintaining their usual eating and physical activity routines, and not start any new dietary approaches or new NHPs during each of the two studies. Data collection flow sheets (Appendix P) were created for each study visit that allowed researchers to record
measurements and data (body weight, blood pressure, and blood glucose) at each study visit. The study visit data collection schedule is summarized in Figure 8.

![Figure 8: Data collection schedule for each study visit. Red blood drops = time points (minutes) where blood samples were collected by finger prick.]

**E.ii. Body Measurements**

Body weight was measured in duplicate at each study visit after participants emptied their pockets and stood on an electronic scale (SVI-200F, Acculab Sartorius Group, Edgewood, NY, USA) without shoes or bulky clothing. Body weight was averaged and recorded to the nearest 0.1 kg. BMI was then calculated as body weight (kg)/height (m²), using the height measurement from Screening-2.

Blood pressure was measured at each study visit after participants remained seated quietly for 15 minutes. Blood pressure was measured in duplicate (Omron® Digital Blood Pressure Monitor, HEM-907 XL, Omron Healthcare Inc., Burlington, ON, Canada).

**E.iii. Blood sample collection and analysis**

Upon arrival at the HNRU, a researcher began setting up the blood collection tray that was used in the data collection (Figure 9). Blood samples were collected by finger prick at time...
points 0 (fasted) and 15, 30, 45, 60, 90 and 120 minutes after the start of the treatment consumption. Blood was collected into a 500 µL BD Vacutainer containing 1.0 mg potassium ethylene diamine tetraacetic acid (K₂EDTA) tubes, labelled with participant ID and time point. Before each blood sample, participants warmed their hands in heating pads (Model 731AO-CN, Sunbeam, Brampton, ON, Canada) for a minimum of 5 minutes until the participant indicated that their hand was sufficiently warm and then a tourniquet was placed on their forearm. Participants were also asked to lower their hand below the table and to open and close their hand into a fist repeatedly before each finger prick, to increase blood pooling. An alcohol swab was used to sterilize the finger, and after the finger was dry, a contact activated lancet (Ref #366594; 1.5mmx 2.0mm blade, BD Diagnostics, Franklin Lakes, NJ, USA) was placed against the finger with the blade perpendicular to the lines of the finger until it activated. A piece of gauze (model #A2103-CH; 3’x3’ 4 ply, AMD-RITMED, Lachine, QC, Canada) was used to wipe away the first drop and the remaining blood was collected in the K₂EDTA tube. The first drop was wiped away since participants were unable to wash their hands between each finger prick and a study demonstrated that when unable to wash hands, wiping with an alcohol swab and wiping the first drop and using the second drop of blood reduced variability in blood glucose compared to using the first drop from unwashed hands by 10% (Hortensius et al. 2011). The hand was massaged in order to help with blood flow and formation of blood drops until the tube was filled to the 500 µL mark. Following the collection of blood, the tourniquet was removed, and a piece of gauze was applied to the finger prick and pressure was applied to allow for the blood to clot. A fabric bandage (model #03026; 2.2 cm x7.6 cm latex free non-stick absorbent pad; Safe Cross First Aid, Toronto, ON, Canada) was applied when the participant was comfortable doing so.
Figure 9: Blood collection tray prepared for blood sampling. The tray includes K₂EDTA blood tube (labelled with participant ID and time point) secured in tray, fabric bandage, alcohol wipes, lancets, gauze, and tourniquet.

After each blood collection, the tube was capped and transported to the lab by another member of the study team. Blood was inverted slowly in the K₂EDTA tube 8 times, as per manufacturing instructions. Blood samples were analyzed immediately for blood glucose in duplicate using a Nova Biomedical Statstrip Glucose Hospital Meter (Ref #53634, Nova Biomedical Canada Ltd, Mississauga, ON, Canada). Blood was pipetted onto the statstrip which was then fed into the glucometer to enable measurement and display of the glucose concentration. If the blood glucose values were >0.3 mmol/L apart, a third measurement was
taken and the 2 values that were within 0.3 mmol/L of each other were selected to be averaged. If the third concentration was within range of both glucose measurements, the two glucose values were determined after analysis of the subsequent blood sample. The coefficient of variation for the Nova Biomedical Statstrip Glucose Hospital Meter was 7.03%, and 7.17% for the half-cup and quarter-cup serving size respectively (n=144 measurements; 24 participants at 6 study visits each). The remainder of the blood was centrifuged (Model Allegra x-22R Centrifuge, Beckman Coulter, Mississauga, ON, Canada) at 3000 rpm at 4°C for 10 minutes and plasma was micropipetted into 1.2 mL Corning Cryovials (Model C430658, Corning Inc., Corning, NY, USA) and stored at -80 °C for later analysis of insulin.

Plasma insulin was measured in duplicate using an enzyme linked immunosorbent assay (ELISA) (Mercodia, Insulin ELISA, Ref #10-1113-01, Uppsala, Sweden) according to the manufacturer protocol. A total of 25 plates were utilized, which had an inter-assay variability for the controls of 6.65%, and an intra-assay variability of all duplicate samples of 6.82%.

Table 8 contains a summary of the processes for blood sampling collection, processing and storage for blood glucose and plasma insulin.
Table 8: Blood sample collection, processing and storage summary

<table>
<thead>
<tr>
<th>Study Measure</th>
<th>Time Point</th>
<th>Blood Collection Tube</th>
<th>Blood Collection, Processing, and Storage Instructions</th>
</tr>
</thead>
</table>
| Blood Glucose | 0, 15, 30, 45, 60, 90, 120 minutes | 500 µL K₂EDTA microtainer microtube | - Collect blood into microtube and invert slowly 8 times  
- Pipette blood onto statstrip in duplicate until blood glucose values are within 0.3 mmol/L.  
- Average the values within 0.3 mmol/L |
| Plasma Insulin | 0, 15, 30, 45, 60, 90, 120 minutes | 500 µL K₂EDTA microtainer microtube | - Following glucose analysis, centrifuge microtube at 3000 rpm at 4°C for 10 minutes  
- Aliquot plasma into cryovial and store at -80°C for later analysis  
- Follow manufacturer protocol for ELISA analysis of insulin |

F. Data and Statistical Analysis

All data was entered into Microsoft Excel™ spreadsheets, and double checked at time of entry by a second study member. Blood glucose and plasma insulin iAUC were calculated using the trapezoid rule (Brouns et al. 2005) and was inserted into the Microsoft Excel™ spreadsheet. Relative glycemic response was calculated by dividing blood glucose iAUC of the starchy controls (corn, macaroni, white potato and white rice) by the blood glucose iAUC of the green and red lentil treatments, and was multiplied by 100. The relative insulinemic response was calculated by dividing the plasma insulin iAUC of the starchy controls (corn, macaroni, white potato and white rice) by the plasma insulin iAUC of the green and red lentil treatments, and was multiplied by 100. Maximum concentration (Cmax) over the 7 time points was determined using the max formula in Microsoft Excel™. Time to maximum concentration (Tmax) was identified by visual inspection in accordance with the Cmax value. Insulinogenic index was calculated for the half-cup study using the method by Wolever et al. 2008, as the rise in insulin from 0 to 30
minutes divided by the rise in glucose from 0 to 30 minutes. Any insulinogenic index values that were negative were taken to be 0.

All statistical analysis was performed using Statistical Analysis System (version 9.4, Cary, NC, USA) with p<0.05 considered statistically significant. Data was examined for normality using box plots and stem leaf diagrams. For each of the half-cup and quarter-cup studies, summary statistics were generated for blood glucose and plasma insulin at each time point within each treatment, blood glucose 2-hour iAUC, Cmax and Tmax.

Blood glucose and plasma insulin were compared among time points within each treatment using repeated measures analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. Blood glucose and plasma insulin were compared among treatments at each time point using repeated measures analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. To compare postprandial blood glucose and plasma insulin response among treatments, blood glucose iAUC and Cmax were compared among treatments using repeated measures ANOVA followed by the Tukey’s test for multiple comparisons. Blood glucose Tmax frequencies were compared among treatments using the Chi-square test.
Chapter IV. RESULTS

A. Participant Flow

Participant flow for the half-cup and quarter-cup studies throughout Screening-1, Screening-2, Screening-3, randomization, and study completion is summarized in Figure 10. A total of 74 interested individuals completed Screening-1 of which 17 were identified as ineligible to continue in the screening process. Of the 57 individuals who were still interested and completed Screening-2, a total of 12 were deemed ineligible. The remaining 45 individuals who were still interested completed Screening-3 and 6 were identified as ineligible leaving 39 individuals who were eligible and willing to participate. A total of 24 participants were randomized into the half-cup and quarter-cup studies, 9 of which completed both studies. There were no participant exclusions or drop-outs during either the half-cup or quarter-cup studies.
Figure 10: CONSORT diagram summarizing participant flow through screenings, randomization and completion of the half-cup and quarter-cup studies.
**B. Participant Baseline Characteristics**

Participant characteristics at baseline for the half-cup and quarter-cup studies are summarized in Table 9. Participants included more females than males with a total of n=15 females and n=9 males and n=16 females and n=8 males for the half-cup and quarter-cup studies, respectively. Participants on average were 24.5 and 23.8 years old, had an average BMI of 23.3 kg/m² and 23.3 kg/m² and had an average fasted blood glucose of 4.7 and 4.6 mmol/L, and an average systolic blood pressure of 113 mmHg and 117 mmHg, and an average diastolic blood pressure of 68 mmHg and 72 mmHg for the half-cup and quarter-cup studies, respectively.
Table 9: Baseline participant characteristics for the half-cup (n=24) and quarter-cup (n=24) studies\textsuperscript{1,2}

<table>
<thead>
<tr>
<th></th>
<th>Half-Cup</th>
<th>Quarter-Cup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)</td>
<td>n = 9 males and 15 females</td>
<td>n = 8 males and 16 females</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24.5 ± 0.9</td>
<td>23.8 ± 0.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.01</td>
<td>1.70 ± 0.02</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67.9 ± 1.6</td>
<td>67.5 ± 2.3</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>23.3 ± 0.5</td>
<td>23.3 ± 0.6</td>
</tr>
<tr>
<td>Fasted blood glucose (mmol/L)</td>
<td>4.7 ± 0.1</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 2.1</td>
<td>117 ± 2.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68 ± 1.3</td>
<td>72 ± 1.3</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are mean ± SE unless otherwise indicated.
\textsuperscript{2}Abbreviations used: BMI= body mass index.
C. Participant Characteristics During the Study

Participant characteristics during the half-cup cup and quarter-cup study are summarized in Table 10. There were no significant differences in body weight, BMI, systolic or diastolic blood pressure between treatments in either the half-cup or the quarter-cup study.
Table 10: Participant characteristics for the half-cup (n=24) and quarter-cup (n=24) studies$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>Green Lentil</th>
<th>Red Lentil</th>
<th>Corn</th>
<th>Macaroni</th>
<th>White Potato</th>
<th>White Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>68.1 ± 1.6</td>
<td>68.2 ± 1.6</td>
<td>66.9 ± 2.0</td>
<td>68.1 ± 1.5</td>
<td>68.2 ± 1.5</td>
<td>68.2 ± 1.6</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>67.4 ± 2.3</td>
<td>67.6 ± 2.4</td>
<td>67.6 ± 2.3</td>
<td>67.4 ± 2.3</td>
<td>67.8 ± 2.3</td>
<td>67.6 ± 2.3</td>
</tr>
<tr>
<td><strong>BMI (kg/m$^2$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>23.4 ± 0.5</td>
<td>23.4 ± 0.5</td>
<td>22.9 ± 0.6</td>
<td>23.4 ± 0.5</td>
<td>23.4 ± 0.5</td>
<td>23.4 ± 0.5</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>23.3 ± 0.6</td>
<td>23.3 ± 0.6</td>
<td>23.3 ± 0.6</td>
<td>23.3 ± 0.6</td>
<td>23.3 ± 0.6</td>
<td>23.3 ± 0.6</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>116 ± 1.7</td>
<td>116 ± 2.6</td>
<td>116 ± 1.8</td>
<td>115 ± 1.9</td>
<td>115 ± 1.7</td>
<td>115 ± 2.1</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>116 ± 1.6</td>
<td>115 ± 2.0</td>
<td>118 ± 2.0</td>
<td>119 ± 2.0</td>
<td>117 ± 1.9</td>
<td>117 ± 1.8</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-Cup</td>
<td>71 ± 1.4</td>
<td>69 ± 1.8</td>
<td>70 ± 1.3</td>
<td>70 ± 1.2</td>
<td>70 ± 1.5</td>
<td>69 ± 1.7</td>
</tr>
<tr>
<td>Quarter-Cup</td>
<td>73 ± 1.2</td>
<td>72 ± 1.4</td>
<td>72 ± 1.4</td>
<td>74 ± 1.5</td>
<td>73 ± 1.7</td>
<td>71 ± 1.4</td>
</tr>
</tbody>
</table>

$^{1}$Data are mean ± SE.
D. Half-Cup Study: Postprandial Glycemic Response

D.i. Half-Cup Study: Blood Glucose and Plasma Insulin Time Point Comparisons Within Treatments

Postprandial blood glucose and plasma insulin response over 120 minutes following consumption of a half-cup serving of green lentils, red lentils, corn, macaroni, white potato, and white rice are presented in Figure 11a and Figure 11b, respectively. A comparison of time points within each treatment showed significant changes in blood glucose (Figure 12a) and plasma insulin (Figure 12b) (p<0.05). For green lentils, blood glucose increased from baseline to peak at 30 minutes and gradually decreased but did not completely return to baseline at 120 minutes (Figure 12a.i.). Similarly, plasma insulin increased from baseline to peak at 15 minutes and gradually decreased, but did not completely return to baseline at 120 minutes (Figure 12b.i.). For red lentils, blood glucose increased from baseline, but did not peak until 120 minutes (Figure 12a.ii.). Plasma insulin also increased from baseline and peaked early at 15 minutes and then decreased until 90 minutes when it increased and remained increased at 120 minutes (Figure 12b.ii.). For corn, both blood glucose (Figure 12a.iii.) and plasma insulin (Figure 12b.iii.) increased from baseline and peaked at 30 minutes, and then decreased until it returned to baseline at 60 minutes where it remained at 120 minutes. For macaroni, blood glucose (Figure 12a.iv.) and plasma insulin (Figure 12b.iv.) both increased from baseline until they both peaked at 30 minutes, and then decreased to return to baseline at 120 minutes. For white potato, blood glucose (Figure 12a.v.) and plasma insulin (Figure 12b.v.) both increased from baseline until they both peaked at 30 minutes, and returned to baseline at 60 minutes where glucose remained until 120 minutes but insulin decreased past baseline at 90 and 120 minutes. For white rice, blood glucose (Figure 12a.vi.) and plasma insulin (Figure 12b.vi.) both increased from baseline
to peak at 30 minutes, and then both decreased until glucose returned to baseline at 90 minutes and remained there at 120 minutes while insulin returned to baseline at 120 minutes.

Figure 11a: Postprandial blood glucose time point curves over 120 minutes following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), and white rice (WR). Data are mean ± SE; n=24.
Figure 11b: Plasma insulin time point curves over 120 minutes following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), and white rice (WR). Data are geometric mean ± 95% confidence interval; n=24.
i. Half-Cup: Green Lentil (n=24)  

![Graph of postprandial blood glucose time point curves for green lentil.](image)

ii. Half-Cup: Red Lentil (n=24)  

![Graph of postprandial blood glucose time point curves for red lentil.](image)

iii. Half-Cup: Corn (n=24)  

![Graph of postprandial blood glucose time point curves for corn.](image)

iv. Half-Cup: Macaroni (n=24)  

![Graph of postprandial blood glucose time point curves for macaroni.](image)

v. Half-Cup: White Potato (n=24)  

![Graph of postprandial blood glucose time point curves for white potato.](image)

vi. Half-Cup: White Rice (n=24)  

![Graph of postprandial blood glucose time point curves for white rice.](image)

Figure 12a: Postprandial blood glucose time point curves over 120 minutes following consumption of a half-cup serving of green lentil (i), red lentil (ii), corn (iii), macaroni (iv), white potato (v), and white rice (vi). Values with different letters are significantly different (p<0.05). Data are mean ± SE.
i. Half-Cup: Green Lentil (n=24)

![Graph for Green Lentil]

ii. Half-Cup: Red Lentil (n=24)

![Graph for Red Lentil]

iii. Half-Cup: Corn (n=24)

![Graph for Corn]

iv. Half-Cup: Macaroni (n=24)

![Graph for Macaroni]

v. Half-Cup: White Potato (n=24)

![Graph for White Potato]

vi. Half-Cup: White Rice (n=24)

![Graph for White Rice]

Figure 12b: Postprandial plasma insulin time point curves over 120 minutes following consumption of a half-cup serving of green lentil (i), red lentil (ii), corn (iii), macaroni (iv), white potato (v), and white rice (vi). Values with different letters are significantly different (p<0.05). Data are geometric mean and 95% confidence intervals.
D.ii. Half-Cup Study: Blood Glucose and Plasma Insulin iAUC Treatment Comparisons

Postprandial glycemic response following a half-cup serving of green and red lentils compared to four starchy controls were compared using iAUC for blood glucose (Figure 13a) and plasma insulin (Figure 13b). Blood glucose iAUC was significantly lower following consumption of a half-cup serving of green or red lentils (which were not different) compared to macaroni ($p<0.0001$ and $p<0.005$, respectively), white potato ($p<0.0001$ for both), and white rice ($p<0.0001$ for both), but not corn ($p=0.66$ and $p=0.99$, respectively). There were also significant differences in blood glucose iAUC among the starchy controls with corn significantly lower than macaroni ($p=0.01$), white potato ($p<0.0001$), and white rice ($p<0.0001$); and macaroni significantly lower than white potato ($p=0.01$) and white rice ($p=0.005$). Plasma insulin iAUC was significantly lower following consumption of a half-cup serving of green or red lentils (which were not different) compared to white potato ($p=0.0003$ and $p<0.0001$, respectively) and white rice ($p=0.0002$ and $p<0.0001$, respectively), but not corn ($p=0.88$ and $p=0.24$, respectively). Plasma insulin iAUC was also significantly lower for red lentils compared to macaroni ($p=0.007$), but was not significantly different for green lentils compared to macaroni ($p=0.15$). There were also significant differences in the plasma insulin iAUC among the starchy controls with corn significantly lower than white potato and white rice ($p=0.01$ for both).
Figure 13a: Blood glucose incremental area under the curve (AUC) following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). Values with different letters are significantly different (p<0.05). Data are mean + SE; n=24.
Figure 13b: Plasma insulin incremental area under the curve (AUC) following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). Values with different letters are significantly different (p<0.05). Data are mean + SE; n=24.
D.iii. Half-Cup Study: Blood Glucose and Plasma Insulin Cmax Treatment Comparisons

Postprandial glycemic response following a half-cup serving of green and red lentils compared to four starchy controls were compared using Cmax for blood glucose (Figure 14a) and plasma insulin (Figure 14b). Blood glucose Cmax was significantly lower following consumption of a half-cup serving of green or red lentils (which were not different) compared to corn, macaroni, white potato, and white rice (p<0.0001 for all). There were also significant differences in blood glucose Cmax among the starchy controls with corn and macaroni (which were not different) significantly lower than white potato and white rice, and white rice significantly lower than white potato (p<0.0001 for all). Plasma insulin Cmax was significantly lower following consumption of a half-cup serving of green or red lentils (which were not different) compared to corn, macaroni, white potato, and white rice (p<0.0001 for all). There were also significant differences in plasma insulin among the starchy controls with corn and macaroni (which were not different) significantly lower compared to white potato (p<0.0001 for both) and white rice (p=0.0002 and p=0.003, respectively).
Figure 14a: Blood glucose maximum concentration (Cmax) following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). Values with different letters are significantly different (p<0.05). Data are mean + SE; n=24.
Figure 14b: Plasma insulin maximum concentration (Cmax) following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). Values with different letters are significantly different (p<0.05). Data are mean + SE; n=24.
D.iv. Half-Cup Study: Blood Glucose and Plasma Insulin Tmax Treatment Comparisons

Postprandial glycemic response following a half-cup serving of green and red lentils compared to four starchy controls were compared using Tmax for blood glucose (Table 11a) and plasma insulin (Table 11b). Blood glucose Tmax was significantly different among the study treatments (p<0.0001). Blood glucose Tmax most frequently occurred at 30 minutes for the green lentils, at 120 minutes for the red lentils and at 30 minutes for the starchy controls corn, macaroni, white potato, and white rice (Table 10a). Plasma insulin Tmax was also significantly different among the half-cup study treatments (p<0.0001). Plasma insulin Tmax most frequently occurred at 15 minutes for green and red lentils, and at 30 minutes for the starchy controls corn, macaroni, white potato, and white rice (Table 11b).
Table 11a: Blood glucose Tmax following consumption of a half-cup serving of green lentil, red lentil, corn, macaroni, white potato, and white rice

<table>
<thead>
<tr>
<th>Tmax (minutes)</th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
<th>Corn (n=24)</th>
<th>Macaroni (n=24)</th>
<th>White Potato (n=24)</th>
<th>White Rice (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>5 (20.8%)</td>
<td>4 (16.7%)</td>
<td>7 (29.2%)</td>
<td>4 (16.7%)</td>
<td>0 (0%)</td>
<td>2 (8.33%)</td>
</tr>
<tr>
<td>30</td>
<td>12 (50.0%)</td>
<td>6 (25.0%)</td>
<td>17 (70.8%)</td>
<td>14 (58.3%)</td>
<td>23 (95.8%)</td>
<td>16 (66.7%)</td>
</tr>
<tr>
<td>45</td>
<td>5 (20.8%)</td>
<td>2 (8.33%)</td>
<td>0 (0%)</td>
<td>5 (20.8%)</td>
<td>1 (4.17%)</td>
<td>5 (20.8%)</td>
</tr>
<tr>
<td>60</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
</tr>
<tr>
<td>90</td>
<td>0 (0%)</td>
<td>4 (16.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>120</td>
<td>2 (8.33%)</td>
<td>8 (33.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

1Data are n (%).
Table 11b: Plasma insulin Tmax following a half-cup serving of green lentil, red lentil, corn, macaroni, white potato, and white rice

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
<th>Corn (n=24)</th>
<th>Macaroni (n=24)</th>
<th>White Potato (n=24)</th>
<th>White Rice (n=24)</th>
</tr>
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<tbody>
<tr>
<td>15</td>
<td>12 (50.0%)</td>
<td>8 (33.3%)</td>
<td>10 (41.7%)</td>
<td>3 (12.5%)</td>
<td>7 (29.2%)</td>
<td>7 (29.2%)</td>
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<tr>
<td>30</td>
<td>6 (25.0%)</td>
<td>7 (29.2%)</td>
<td>13 (54.2%)</td>
<td>13 (54.2%)</td>
<td>17 (70.8%)</td>
<td>12 (50.0%)</td>
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<tr>
<td>45</td>
<td>4 (16.7%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
<td>7 (29.2%)</td>
<td>0 (0%)</td>
<td>5 (20.8%)</td>
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<td>60</td>
<td>0 (0%)</td>
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<tr>
<td>90</td>
<td>1 (4.17%)</td>
<td>3 (12.5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>120</td>
<td>1 (4.17%)</td>
<td>6 (25.0%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

1Data are n (%).
D.v. Half-Cup Study: Blood Glucose and Plasma Insulin Treatment Comparisons Within Time Points

Postprandial blood glucose and plasma insulin response over 120 minutes following consumption of a half-cup serving of green lentils, red lentils, corn, macaroni, white potato, and white rice is presented in Figure 15a and Figure 15b, respectively. Blood glucose (Figure 15a) and plasma insulin (Figure 15b) significantly differed between the green lentils and the starchy controls at various time points. At 15 minutes, blood glucose was significantly lower following consumption of green lentils compared with corn (p<0.0001), macaroni (p=0.01), white potato (p<0.0001), and white rice (p<0.0001). Plasma insulin was also significantly lower following consumption of green lentils compared with corn (p=0.01), white potato (<0.0001) and white rice (p=0.0006). At 30 minutes, blood glucose was significantly lower following consumption of green lentils compared with corn, macaroni, white potato, and white rice (p<0.0001 for all). Plasma insulin was also significantly lower following consumption of green lentils compared with corn (p=0.0008), macaroni (p<0.0001), white potato (p<0.0001), and white rice (p<0.0001). At 45 minutes, blood glucose was significantly lower following consumption of green lentils compared with macaroni (p=0.0004), white potato (p<0.0001), and white rice (p<0.0001). Plasma insulin was also significantly lower following consumption of green lentils compared with macaroni, white potato, and white rice (p<0.0001 for all). At 60 minutes, blood glucose was significantly lower following consumption of green lentils compared with macaroni (p=0.03) and white rice (p<0.0001). Plasma insulin was also significantly lower following consumption of green lentils compared with white rice (p=0.002). At 90 minutes, blood glucose was significantly lower following consumption of green lentils compared with white rice (p=0.002), but blood glucose was significantly higher for green lentils compared to white potato (p=0.01). Plasma insulin was also significantly higher following the consumption of green lentils compared to corn.
and white potato (p<0.001 respectively). At 120 minutes, blood glucose and plasma insulin were significantly higher following consumption of green lentils compared to corn (p=0.001 and p=0.0001, respectively) and white potato (p=0.008 and p<0.0001).

Blood glucose (Figure 15a) and plasma insulin (Figure 15b) also significantly differed between the red lentils and the starchy controls at various time points. At 15 minutes, blood glucose was significantly lower following consumption of red lentils compared with corn (p=0.0005), white potato (p<0.0001), and white rice (p<0.0001). Plasma insulin was also significantly lower following consumption of red lentils compared with corn (p=0.01), white potato (p<0.0001), and white rice (p=0.0008). At 30 minutes, blood glucose was significantly lower following consumption of red lentils compared with corn, macaroni, white potato, and white rice (p<0.0001 for all). Plasma insulin was also significantly lower following consumption of red lentils compared with corn (p=0.0007), macaroni (p<0.0001), white potato (p<0.0001), and white rice (p<0.0001). At 45 minutes, blood glucose was significantly lower following consumption of red lentils compared with macaroni (p=0.002), white potato (p<0.0001), and white rice (p<0.0001). Plasma insulin was also significantly lower following consumption of red lentils compared with macaroni (p=0.0001), white potato (p<0.0001), and white rice (p<0.0001). At 60 minutes, blood glucose was significantly lower following consumption of red lentils compared with white rice (p<0.0001). Plasma insulin was also significantly lower following consumption of red lentils with macaroni (p=0.005) and white rice (p<0.0001). At 90 minutes, blood glucose and plasma insulin were significantly higher following consumption of red lentils compared with corn (p<0.0001 and p<0.0001, respectively) and white potato (p<0.0001 and p<0.0001, respectively). At 120 minutes, blood glucose and plasma insulin were significantly higher following consumption of red lentils compared with corn (p<0.0001 and p<0.0001,
respectively), macaroni (p<0.0001 and p=0.002, respectively), and white potato (p<0.0001 and p<0.0001, respectively).

Blood glucose also significantly differed between the green lentils and the red lentils. At 90 minutes, blood glucose was significantly lower following consumption of green lentils compared to red lentils (p=0.01). At 120 minutes, blood glucose was significantly lower following consumption of green lentils compared to red lentils (p=0.001). Plasma insulin did not significantly differ following the consumption of the green lentils compared to the red lentils at any time point.
Figure 15a: Postprandial blood glucose response following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). * indicates that blood glucose for half-cup of GL is significantly lower than one or more of the starchy controls at the denoted time point (p<0.05).
◊ indicates that blood glucose for half-cup of RL is significantly lower than one or more of the starchy controls at the denoted time point (p<0.05). + indicates that blood glucose for half-cup of GL is significantly lower than RL at the denoted time point (p<0.05). Data are mean ± SE; n=24.
Figure 15b: Postprandial plasma insulin response following consumption of a half-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). * indicates that plasma insulin for half-cup of GL is significantly lower than one or more of the starchy controls at the denoted time point (p<0.05). ◇ indicates that plasma insulin for half-cup of RL is significantly lower than one or more of the starchy controls at the denoted time point (p<0.05). Data are geometric mean ± 95% confidence interval; n=24.
D.vi. Half-Cup Study: Blood Glucose and Plasma Insulin iAUC Relative Glycemic Response

Postprandial blood glucose iAUC relative glycemic response (RGR) and plasma insulin iAUC relative insulinemic response for the half-cup serving of green lentils, red lentils, corn, macaroni, white potato, and white rice is presented in Tables 12a and 12b, respectively. For the half-cup serving of green lentils, the blood glucose RGR of corn, macaroni, white potato and white rice was 352, 550, 825 and 618%, respectively, indicating a respective percent increase of 252, 450, 725, and 518% in the blood glucose response. Blood glucose RGR significantly differed among some of the starchy controls with corn lower than white potato (p=0.02), but not from macaroni (p=0.61) or white rice (p=0.36). Furthermore, macaroni, white potato, and white rice were not significantly different from each other. The relative insulinemic response for the half-cup serving of green lentils for corn, macaroni, white potato and white rice was 166, 288, 388 and 434%, respectively, indicating a respective percent increase of 65.9, 188, 288 and 334% in the insulinemic response. Relative insulinemic response significantly differed among some of the starchy controls with corn lower than white potato (p=0.0001) and white rice (p<0.0001), but not macaroni (p=0.07). Furthermore, macaroni was lower than white rice (p=0.02), but not white potato (p=0.18), and white potato and white rice were not significantly different from each other.

For the half-cup serving of red lentils, the blood glucose RGR of corn, macaroni, white potato, and white rice was 148, 252, 331, 317%, respectively, indicating a respective percent increase of 47.6, 152, 231, and 217% in the blood glucose response. Blood glucose RGR significantly differed among some of the starchy controls with corn lower than white potato (p=0.001) and white rice (p=0.003), but not from macaroni (p=0.12). Furthermore, macaroni, white potato, and white rice were not significantly different from each other. The relative insulinemic response for the half-cup serving of red lentils for corn, macaroni, white potato and
white rice was 186, 246, 399, and 437%, respectively, indicating a respective percent increase of 86.4, 146, 299, and 337% in the insulinemic response. Relative insulinemic response significantly differed among some of the starchy controls with corn lower than white potato (p=0.03) and white rice (p=0.009), but not macaroni (p=0.86). Furthermore, macaroni, white potato, and white rice were not significantly different from each other.
Table 12a: Blood glucose iAUC relative glycemic response for the half-cup serving size of green and red lentils compared to the starchy controls

<table>
<thead>
<tr>
<th></th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (%)</td>
<td>352 ± 131&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148 ± 22.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Macaroni (%)</td>
<td>550 ± 226&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>252 ± 80.0&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Potato (%)</td>
<td>825 ± 376&lt;sup&gt;b&lt;/sup&gt;</td>
<td>331 ± 74.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Rice (%)</td>
<td>618 ± 185&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>317 ± 55.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are mean ± SE.
<sup>2</sup>Values with different letters within a column are significantly different.
<sup>3</sup>Relative glycemic response was calculated by dividing blood glucose iAUC of the starchy controls (corn, macaroni, white potato, and white rice) by the blood glucose iAUC of the green and red lentil treatments, and multiplying by 100.

Table 12b: Plasma insulin iAUC relative insulinemic response for the half-cup serving size of green and red lentils compared among the starchy controls

<table>
<thead>
<tr>
<th></th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (%)</td>
<td>166 ± 28.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>186 ± 58.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Macaroni (%)</td>
<td>288 ± 57.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>246 ± 49.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Potato (%)</td>
<td>388 ± 70.6&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>399 ± 122&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Rice (%)</td>
<td>434 ± 97.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>437 ± 151&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are mean ± SE.
<sup>2</sup>Values with different letters within a column are significantly different.
<sup>3</sup>Relative insulinemic response was calculated by dividing plasma insulin iAUC of the starchy controls (corn, macaroni, white potato, and white rice) by the plasma insulin iAUC of the green and red lentil treatments, and multiplying by 100.
D.vii. Half-Cup Study: Insulinogenic Index

Insulinogenic index for the half-cup serving size of green lentils, red lentils, corn, macaroni, white potato and white rice are presented in Table 13. The insulinogenic index did not significantly differ between either the green or red lentils and any of the starchy controls (corn, macaroni, white potato and white rice).

Table 13: Insulinogenic indices of green lentils, red lentils, corn, macaroni white potato and white rice for the half-cup study

<table>
<thead>
<tr>
<th></th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
<th>Corn (n=24)</th>
<th>Macaroni (n=24)</th>
<th>White Potato (n=24)</th>
<th>White Rice (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulinogenic Index (pmol/mmol)</td>
<td>69.6 ± 18.0</td>
<td>58.8 ± 12.2</td>
<td>45.0 ± 8.04</td>
<td>56.5 ± 8.34</td>
<td>47.1 ± 4.80</td>
<td>51.2 ± 4.93</td>
</tr>
</tbody>
</table>

\(^1\)Data are mean ± SE.
E. Quarter-Cup Study: Postprandial Glycemic Response

E.i. Quarter-Cup Study: Blood Glucose Time Point Comparisons Within Treatments

Postprandial blood glucose response over 120 minutes following consumption of a quarter-cup serving of green lentils, red lentils, corn, macaroni, white potato, and white rice are presented in Figure 16. A comparison of time points within each treatment showed significant changes in blood glucose (Figure 17) (p<0.05). For green lentils, blood glucose increased from baseline to peak at 15 minutes, and gradually decreased until it had a secondary spike at 90 minutes, and then returned to baseline at 120 minutes (Figure 17.i.). For red lentils, blood glucose increased from baseline to peak at 30 minutes, but did not return to baseline over 120 minutes (Figure 17.ii.). For corn, blood glucose increased from baseline and peaked at 30 minutes, and then decreased until it returned to baseline at 45 minutes where it remained at 120 minutes (Figure 17.iii.). For macaroni, blood glucose increased from baseline and peaked at 30 minutes, and gradually decreased until it returned to baseline at 60 minutes where it remained until 120 minutes (Figure 17.iv.). For white potato, blood glucose increased from baseline and peaked at 30 minutes, and then decreased until it returned to baseline at 60 minutes where it remained at 120 minutes (Figure 17.v.). For white rice, blood glucose increased from baseline and peaked at 30 minutes, and then decreased until it returned to baseline at 45 minutes where it remained at 120 minutes (Figure 17.vi.).
Figure 16: Postprandial blood glucose time point curves over 120 minutes following consumption of a quarter-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), and white rice (WR). Data are mean ± SE; n=24.
i. Quarter-Cup: Green Lentil (n=24)  

ii. Quarter-Cup: Red Lentil (n=24)  

iii. Quarter-Cup: Corn (n=24)  

iv. Quarter-Cup: Macaroni (n=24)  

v. Quarter-Cup: White Potato (n=24)  

vi. Quarter-Cup: White Rice (n=24)  

Figure 17: Postprandial blood glucose time point curves over 120 minutes following consumption of a quarter-cup serving of green lentil (i), red lentil (ii), corn (iii), macaroni (iv), white potato (v), and white rice (vi). Values with different letters are significantly different (p<0.05). Data are mean ± SE; n=24.
E.ii. Quarter-Cup Study: Blood Glucose iAUC Treatment Comparisons

Postprandial glycemic response following a quarter-cup serving of green and red lentils compared to four starchy controls were compared using iAUC for blood glucose (Figure 18). Blood glucose iAUC was significantly lower following consumption of a quarter-cup serving of green lentils compared to red lentils (p=0.05), white potato (p=0.001), and white rice (p=0.04). There were no significant differences in blood glucose iAUC in the quarter-cup serving size for red lentils compared to the starchy controls or among the starchy controls.

Figure 18: Blood glucose incremental area under the curve (AUC) following consumption of a quarter-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). Values with different letters are significantly different (p<0.05). Data are mean + SE; n=24.
E.iii. Quarter-Cup Study: Blood glucose Cmax Treatment Comparisons

Postprandial glycemic response following a quarter-cup serving of green and red lentils compared to four starchy controls were compared using Cmax for blood glucose (Figure 19). Blood glucose Cmax was significantly lower following consumption of a quarter-cup of green or red lentils (which were not different) compared to corn (p<0.0001 and p=0.05, respectively), white potato (p<0.0001 for both), white rice (p<0.0001 and p=0.0003) and for green lentils compared to macaroni (p=0.0002), but not red lentils. There were also significant differences in blood glucose Cmax among the starchy controls with corn and macaroni (which were not different) significantly lower compared to white potato (p=0.0006 and p<0.0001, respectively).

![Figure 19: Blood glucose maximum concentration (Cmax) following consumption of a quarter-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). Values with different letters are significantly different (p<0.05). Data are mean + SE; n=24.](image-url)
E.iv. Quarter-Cup study: Blood glucose Tmax Treatment Comparisons

Postprandial glycemic response following a quarter-cup serving of green and red lentils compared to four starchy controls were compared using Tmax for blood glucose (Table 12). Blood glucose Tmax was significantly different among the study treatments (p=0.006). Blood glucose Tmax most frequently occurred at 15 minutes for green lentils and 30 minutes for red lentils and at 15 or 30 minutes for the starchy controls corn, macaroni, white potato, and white rice (Table 13).

Table 14: Blood glucose Tmax following consumption of a quarter-cup serving of green lentil, red lentil, corn, macaroni, white potato, and white rice

<table>
<thead>
<tr>
<th>Tmax (minutes)</th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
<th>Corn (n=24)</th>
<th>Macaroni (n=24)</th>
<th>White Potato (n=24)</th>
<th>White Rice (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>9 (37.5%)</td>
<td>6 (25.0%)</td>
<td>16 (66.7%)</td>
<td>8 (33.3%)</td>
<td>12 (50.0%)</td>
<td>9 (37.5%)</td>
</tr>
<tr>
<td>30</td>
<td>7 (29.2%)</td>
<td>7 (29.2%)</td>
<td>7 (29.2%)</td>
<td>10 (41.7%)</td>
<td>12 (50.0%)</td>
<td>12 (50.0%)</td>
</tr>
<tr>
<td>45</td>
<td>1 (4.17%)</td>
<td>3 (12.5%)</td>
<td>0 (0%)</td>
<td>4 (16.7%)</td>
<td>0 (0%)</td>
<td>2 (8.33%)</td>
</tr>
<tr>
<td>60</td>
<td>5 (20.8%)</td>
<td>2 (8.33%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>90</td>
<td>1 (4.17%)</td>
<td>3 (12.5%)</td>
<td>1 (4.17%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
</tr>
<tr>
<td>120</td>
<td>1 (4.17%)</td>
<td>3 (12.5%)</td>
<td>0 (0%)</td>
<td>1 (4.17%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

1Data are n (%).
E.v. Quarter-Cup Study: Blood Glucose Treatment Comparisons Within Time Points

Postprandial blood glucose response over 120 minutes following consumption of a quarter-cup serving of green lentils, red lentils, corn, macaroni, white potato, and white rice is presented in Figure 20. Blood glucose significantly differed between the green lentils and the starchy controls at various time points (Figure 20). At 15 minutes, blood glucose was significantly lower following consumption of green lentils compared with corn (p<0.0001), macaroni (p=0.01), white potato (p<0.0001), and white rice (p<0.0001). At 30 minutes, blood glucose was significantly lower following consumption of green lentils compared with corn (p=0.02), macaroni (p=0.03), white potato (p<0.0001), and white rice (p<0.0001). At 45 minutes, blood glucose was significantly lower following the consumption of green lentils compared with macaroni (p=0.03). For the remainder of the time points (60, 90 and 120 minutes), blood glucose was not significantly different for green lentils compared to the starchy controls.

Blood glucose (Figure 20) also significantly differed between the red lentils and the starchy controls at various time points. At 15 minutes, blood glucose was significantly lower following consumption of red lentils compared with corn (p=0.0004), white potato (p<0.0001), and white rice (p=0.008). At 30 minutes, blood glucose was significantly lower following the consumption of red lentils compared with white potato (p<0.0001) and white rice (p=0.007). At time points 45, 60 and 90 minutes, blood glucose was not significantly following the consumption of red lentils compared with the starchy controls. At 120 minutes, blood glucose was significantly higher following the consumption of red lentils compared with corn (p=0.004).

Blood glucose did not significantly differ following the consumption of green lentils compared to red lentils at any of the time points over 120 minutes.
Figure 20: Postprandial blood glucose response following consumption of a quarter-cup serving of green lentil (GL), red lentil (RL), corn (Corn), macaroni (Mac), white potato (WP), or white rice (WR). * indicates that blood glucose for quarter-cup of GL is significantly lower than one or more of the starchy controls at the denoted time point (p<0.05). ◇ indicates that blood glucose for quarter-cup of RL is significantly lower than one or more of the starchy controls at the denoted time point (p<0.05). Data are mean ± SE; n=24.
E.vi. Quarter-Cup Study: Blood glucose iAUC Relative Glycemic Response

Postprandial blood glucose iAUC RGR for the quarter-cup serving of green lentils, red lentils, corn, macaroni, white potato, and white rice is presented in Table 14. For the quarter-cup serving of green lentils, the blood glucose RGR of corn, macaroni, white potato and white rice was 364, 440, 551 and 433%, respectively, indicating a respective percent increase of 264, 340, 451, and 333% in the blood glucose response. Blood glucose RGR did not significantly differ among the starchy controls.

For the quarter-cup serving of red lentils, the blood glucose RGR of corn, macaroni, white potato and white rice was 193, 132, 246 and 214%, respectively, indicating a respective percent increase of 93.2, 31.9, 146, and 114% in the blood glucose response. Blood glucose RGR significantly differed among some of the starchy controls with macaroni lower than white potato (p=0.02), but not from corn (p=0.53) or white rice (p=0.14). Furthermore, corn, white potato and white rice were not significantly different from each other.

Table 15: Blood glucose iAUC relative glycemic response for the quarter-cup serving size of green and red lentils compared to the starchy controls\textsuperscript{1,2,3}

<table>
<thead>
<tr>
<th></th>
<th>Green Lentils (n=24)</th>
<th>Red Lentils (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (%)</td>
<td>364 ± 110</td>
<td>193 ± 67.9\textsuperscript{a,b}</td>
</tr>
<tr>
<td>Macaroni (%)</td>
<td>440 ± 158</td>
<td>132 ± 28.4\textsuperscript{a}</td>
</tr>
<tr>
<td>White Potato (%)</td>
<td>551 ± 156</td>
<td>246 ± 70.8\textsuperscript{b}</td>
</tr>
<tr>
<td>White Rice (%)</td>
<td>433 ± 140</td>
<td>214 ± 75.7\textsuperscript{a,b}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data are mean ± SE.
\textsuperscript{2}Values with different letters within a column are significantly different.
\textsuperscript{3}Relative glycemic response was calculated by dividing blood glucose iAUC of the starchy controls (corn, macaroni, white potato, and white rice) by the blood glucose iAUC of the green and red lentil treatments, and multiplying by 100.
Chapter V. DISCUSSION

The primary purpose of this research was to determine the effects of two serving sizes (half-cup and quarter-cup) of two lentil varieties (Laird green and Eston split red) on acute postprandial blood glucose response compared to matched serving sizes of four commonly consumed starchy foods (corn, macaroni, white potato and white rice) in healthy adults. Participants completed a 3-phase screening to confirm they did not have diabetes or impaired glucose tolerance, were healthy, and were comfortable with finger prick blood samples. This research was organized into two studies (half-cup and quarter-cup serving sizes) that both used a randomized, crossover study design. Participants consumed either a half-cup or quarter-cup serving size of two varieties of lentils (Laird green or Eston split red), or four commonly consumed starchy foods (corn, macaroni, white potato and white rice), for a total of 6 study visits separated by washout periods of 3-7 days. This study adds to the limited literature regarding the effects of smaller serving sizes on the acute postprandial blood glucose and plasma insulin responses in healthy adults. As Canada is the largest producer and exporter of lentils, it is valuable to determine if lentils can attenuate the postprandial blood glucose and plasma insulinemic response in smaller quantities so this knowledge can be communicated with the general public, as only 13.1% of North Americans are currently consuming pulses on a given day (Saskatchewan Pulse Growers 2018a; Mudryj et al. 2012). Furthermore, this study compared matched serving sizes of lentils to four commonly consumed starchy foods including corn, macaroni, white potato and white rice on the postprandial blood glucose and insulin response because they are reported in the Canadian Community Health Survey as the most frequently consumed starches (Statistics Canada 2017). Therefore, the relevance of this study is high and is important for adults with diabetes, at risk for diabetes, and for healthy adults to further enhance
knowledge on the health benefits of lentils compared to foods already widely accepted in the diet.

A. Participant Flow

The study determined via a sample size calculator that a total of 18, and 24 participants were required for the half-cup and quarter-cup serving size studies, respectively. For the purpose of reporting, it was determined that it would be clearer if both studies reported on 24 participants. Therefore, a total of 24 participants were recruited for both the half-cup and quarter-cup serving size studies. There were no participant exclusions or drop-outs during either the half-cup or quarter cup study, therefore the attrition rate was 0%. Similar acute clinical studies examining lentil interventions did not report an attrition rate (Mollard et al. 2012a; Mollard et al. 2014; Wong et al. 2009), but 4 acute studies did report attrition rates of 0 (Anderson et al. 2014), 0 (Jenkins et al. 1982), 11.1 (Mollard et al. 2012b) and 14.3% (Anguah et al. 2014). The low rate of attrition of the current study may be due to the relationship that the researchers built with the participants to ensure that all participants felt researchers were professional and engaged in their safety and comfort during the study. Furthermore, the 3-step screening process required two in-person screening visits to confirm participants were interested, the study orientation established that all participants understood the study and what they would need to do and this allowed them to ask questions to ensure they felt comfortable with the protocol of the study visits before they even started. Lastly, the study visit reminder emails prompted participants to consume their pre-study visit dinner and arrive fasted the following morning which served as a reminder, and helped further communication with participants.
**B. Participant Characteristics**

The current study used a 3-phase screening process to ensure participants were healthy men and women 18-40 years old that met all study inclusionary and exclusionary criteria. In order to do this, screening-1 and 2 inquired about lifestyle factors such as medical conditions, smoking status, pulse consumption, medication and natural health product use, pregnancy, and shift work, and screening-2 required body measurements such as blood pressure and height and weight for the determination of BMI. Screening-3 was an oral glucose tolerance test that assessed fasted blood glucose and two-hour blood glucose concentrations following consumption of a 75 g glucose beverage, Trutol. This process also confirmed that participants were comfortable with receiving finger pricks. Overall, the 3-phase screening process removed participants who were at an increased risk of T2D, or had other medical conditions, to determine eligible participants who were healthy for this study.

Other studies that recruited healthy adult participants in acute clinical trials included Jenkins *et al.* 1982 and Anguah *et al.* 2014 who studied healthy men and women, and Wong *et al.* 2009, Mollard *et al.* 2011, Mollard *et al.* 2012a, Mollard *et al.* 2014, Anderson *et al.* 2014, who studied only healthy men. Furthermore, the present study recruited adults between the ages of 18-40 years and this was similar to Anguah *et al.* 2014; Anderson *et al.* 2014; Wong *et al.* 2009; Mollard *et al.* 2014, who recruited participants starting at age 18, while Mollard *et al.* 2011 started recruiting at age 20. The upper limit of age recruitment also differed among studies, with Mollard *et al.* 2011; Mollard *et al.* 2012a and Anderson *et al.* 2014 excluding adults greater than 30 years, Wong *et al.* 2009 at 35 years, and Anguah at 55 years of age. The present study recruited participants with a BMI range of 20-30 kg/m² while 5 studies had a range of 20-25
kg/m² (Wong et al. 2009; Mollard et al. 2011; Mollard et al. 2012a; Mollard et al. 2014; Anderson et al. 2014) and Anguah et al. 2014 had a range of 18.5-40 kg/m².

Participants in the current half-cup and quarter-cup studies had an average age of 24.5 and 23.8 years, a BMI of 23.3 and 23.3 kg/m², a systolic blood pressure of 113 and 68 mmHg, and a diastolic blood pressure of 68 and 72 mmHg, respectively, indicating that the participants were within the acceptable age, BMI and blood pressure range for this study. It is also important to note that the participant characteristics did not significantly change from baseline, or between treatments.

C. Postprandial Glycemic Response
C.i. Blood Glucose and Plasma Insulin iAUC Treatment Comparison

Following a half-cup serving size, blood glucose iAUC was significantly lower following consumption of green and red lentils (which were not different) compared to macaroni, white potato, and white rice, but not corn. Similarly, plasma insulin iAUC was significantly lower following consumption of a half-cup serving of green or red lentils (which were not different) compared to white potato, and white rice, but not corn, and for red (but not green) lentils compared to macaroni. Blood glucose iAUC was significantly lower following consumption of a quarter-cup serving of green lentils compared to red lentils, white potato and white rice and there were no significant differences between red lentils and the starchy controls.

Similar research that reported a reduction in blood glucose iAUC included a study that examined the effects of 451 g of lentils mixed into tomato sauce in 15 healthy men compared to other pulses and white bread, and found that lentils had the lowest blood glucose iAUC of all treatments (Wong et al. 2009); 44% of energy from lentils mixed with macaroni and tomato sauce resulted in a lower blood glucose iAUC cumulatively (0-340 minutes, including a pizza
meal) and from 0-260 minutes in 24 healthy men (Mollard et al. 2012a); 332.9 g of lentils in
tomato sauce in 15 healthy men reduced blood glucose iAUC 0-135 minutes following
consumption, and 150-210 minutes following the post-pizza meal (Mollard et al. 2014); blended
lentil (≥ ½ cup) containing burritos reduced blood glucose iAUC over 180 minutes compared to
the non-lentil containing burrito in 12 healthy adults (Anguah et al. 2014); 183.8 g of whole
lentils or pureed lentils in tomato sauce reduced blood glucose AUC from 0-120 minutes in 12
healthy men (Anderson et al. 2014). This was further supported in a meta-analysis by Augustin
et al. that reviewed 38 clinical trials that examined adults with diabetes, and was substantiated in
a review by Ramdath et al. 2016.

Blood glucose iAUC accounts for the blood glucose response over 120 minutes and
neglects values that drop below fasting, and is an important measure to relay as elevated iAUC
values can be indicative of a higher risk of T2D and other non-communicable diseases (Lunde et
al. 2012). Hyperglycemia, and subsequent insulin resistance from mismanagement of blood
glucose can be modified with lifestyle alterations including body weight management,
incorporation of exercise, and dietary strategies (Lunde et al. 2012). The evidence found by the
current study and previous studies have demonstrated that consumption of lentils reduced blood
glucose iAUC independently, and within mixed meals in various portion sizes. Overall,
consumption of a half-cup of green and red lentils was efficacious in reducing blood glucose
iAUC, and further showed that green lentils can reduce iAUC in a quarter-cup serving size in
healthy adults.

The plasma insulin iAUC has not been as thoroughly investigated, but findings for the
reduction of plasma insulin iAUC after consumption of lentils was demonstrated in 40 obese or
overweight men and women that consumed 5 cups of pulses (including lentils) a week for 8
weeks, although the results were only seen in women (Mollard et al. 2012b). In contrast, the study that examined the effects of 25 g of available carbohydrates from lentils in combination with white potato or white rice (which added an additional 25 g of available carbohydrates) observed a reduction in plasma insulin iAUC for all three lentils mixed with white potatoes compared to white potatoes alone, but this was not observed for white rice compared to any of the lentil varieties mixed with white rice (Moravek et al. 2018). The observed difference may be due to the plasma insulin response for white rice which did not increase as much as white potato, and therefore the lentils in combination with white rice reduced the plasma insulin iAUC, but it did not reach significance due to the blunted plasma insulin response of just white rice alone. The analysis of plasma insulin should be included in future studies to further increase the evidence supporting lentils in their ability to reduce iAUC. Plasma insulin iAUC is an important measure to assess if the incorporation of foods intended to reduce blood glucose also cause hyperinsulinemia which increases the output of insulin to maintain glucose homeostasis (Fonseca 2009). Elevated insulin secretion (as an attempt to compensate for increased blood glucose concentration) can lead to decreased ability of beta-cells to produce insulin and can lead to insulin resistance; over time this will lead to hyperglycemia and the development of T2D (Fonseca 2009). The evidence from the current study supports that the acute consumption of green and red lentils in a half-cup serving size can reduce plasma insulin iAUC compared to matched serving sizes of starchy controls.

C.ii. Blood Glucose and Plasma Insulin Cmax Treatment Comparisons

This study also examined the maximum concentration that each treatment peaked at in both the half-cup and quarter-cup serving size for blood glucose and for plasma insulin for the half-cup serving size. Blood glucose Cmax and plasma insulin Cmax were significantly lower
following consumption of a half-cup serving size of green or red lentils (which are not different) compared to corn, macaroni, white potato and white rice. Blood glucose Cmax was significantly lower following consumption of a quarter-cup of green or red lentils (which were not different) compared to corn, white potato and white rice, and for green lentils compared to macaroni, but not red lentils.

The observed reduction in blood glucose Cmax has been previously reported in the literature in a study that analyzed the glycemic response of 12 healthy men to whole lentils served in tomato sauce at a dose of 25g of available carbohydrates (Anderson et al. 2014) (which amounts to approximately 1 cup of lentils as reported by Ramdath et al. 2016); 44% energy density from lentils mixed with macaroni in tomato sauce in 15 healthy men (Mollard et al. 2012a; 25 g of available carbohydrates from lentils mixed with 25 g of available carbohydrates from white potato, or from lentils mixed with white rice in 24 healthy men and women (Moravek et al. 2018); and 226 g (dry weight) of boiled lentils mixed into a breakfast meal (containing lentils, butter and tomato) in 7 healthy men and women (Jenkins et al. 1982). These studies have used controls that were similar to the current study, with macaroni, white potato and white rice being investigated, although the quantity of lentils provided to participants was higher than the present study. Of the studies outlined above, Moravek et al. 2018 is the only study that reported the plasma insulin Cmax, which was significantly lower for all three lentil varieties (large green, small green and split red lentils) when mixed with white potato compared to white potato alone. This finding was similar to the present study where plasma Cmax was significantly lower for green and red lentils compared to white potato in both the half-cup and quarter-cup serving size. However, the plasma insulin Cmax was not significantly lower following consumption of white rice mixed with all three lentil varieties compared to white rice alone, which was not observed in
the present study. One potential difference in the observed finding might relate to how the study treatments were consumed. The study by Moravek et al. 2018 provided participants with a total of 50 g of available carbohydrates from the starches alone, or 25 g from the starch plus 25 g from the lentils. In that study, the white potato served alone had a very high plasma insulin Cmax, and the lentils were able to help attenuate the increase peak in plasma insulin when combined with the potato (Moravek et al. 2018). Conversely, the white rice did not reach as high of a peak compared to white potato, so the lentils mixed with white rice were unable to elicit the same effects to reduce the plasma insulin Cmax significantly (Moravek et al. 2018). Whereas in the present study, all treatments were served independently and this may have allowed the green and red lentils to have caused the observed decrease in plasma insulin Cmax in the half-cup serving size when compared to white rice. The present study demonstrated that a half-cup of green and red lentils can reduce blood glucose and plasma insulin Cmax compared to all starchy foods, and that a quarter-cup serving can reduce blood glucose Cmax. The present study adds to the previous evidence that lentils should be incorporated into the diet as part of evidence-based nutrition for the management of blood glucose, for the prevention of diabetes. Nutrition-based therapy for the prevention and management of diabetes includes maintaining blood glucose levels in normal ranges, and preventing the onset of insulin resistance to prevent hyperglycemia and reduce the risk of complications of diabetes, such as microvascular and macrovascular complications (Diabetes Canada Clinical Practice Guidelines 2018; Fowler 2011). Therefore, these findings demonstrate that lentils in a small serving size can reduce the peak glucose and insulin concentration and ultimately help maintain blood glucose and insulin within healthy ranges.
C.iii. Blood Glucose and Plasma Insulin Tmax Treatment Comparisons

The time to reach maximum concentration was examined in the current study for the half-cup and quarter-cup serving size for blood glucose, and for the half-cup serving of plasma insulin. For the half-cup serving size, blood glucose Tmax most frequently occurred at 30 minutes for green lentils, 120 minutes for red lentils, and at 30 minutes for corn, macaroni, white potato and white rice. Likewise, Tmax for plasma insulin for the half-cup serving size occurred most frequently at 15 minutes for green and red lentils, and at 30 minutes for corn, macaroni, white potato and white rice. Blood glucose Tmax for the quarter-cup serving size most frequently occurred at 15 minutes for green lentils, 30 minutes for red lentils, and at either 15 or 30 minutes for the starchy controls corn, macaroni, white potato and white rice.

Although previous research depicts graphs with the average blood glucose concentration at each time point, majority of studies do not report the frequency that the maximum concentration occurs at for each time point. Upon reviewing the literature and estimating the peak blood glucose concentrations, Tmax occurred at 40 minutes in 24 healthy men consuming macaroni and cheese and lentils with macaroni (Mollard et al. 2012a); 30 minutes for whole lentils, pureed lentils and blended lentils in tomato sauce and at 30 minutes for the control whole wheat flour in tomato sauce in 12 healthy men (Anderson et al. 2014); 45 minutes and 30 minutes for the lentil breakfast and whole bread breakfast, respectively, for blood glucose Tmax, and at 45 minutes for both meals for plasma insulin Tmax in 4 healthy adults (Jenkins et al. 1982); 30 minutes for white rice, white potato, and all lentil varieties for both blood glucose and plasma insulin Tmax in 24 healthy adults (Moravek et al. 2018); 15 minutes for blood glucose Tmax for both white bread and lentils served in tomato sauce in 15 young men (Wong et al. 2009). These results align with the findings of the present study conducted, although the lentils
in this study were served independently, and in two serving sizes. The red lentils in the half-cup serving size rebounded and increased the blood glucose concentration at 120 minutes, and this result was not observed in the other studies as they did not report the frequency of Tmax, and majority of the studies examined green lentil varieties.

The time to achieve the blood glucose maximum concentration is important to identify differences in the rate of glucose absorption between treatments. Tmax reflects the time that the maximum concentration occurred at and represents how quickly blood glucose is absorbed and cleared from the blood (Bellmann et al. 2017). The time to achieve maximum concentration relies on factors such as the food matrix, and the amount of carbohydrate within a food (Muller et al. 2018; Kuwata et al. 2016; Wong and Jenkins 2007). Consuming foods that can delay the time to reach maximum concentration, or reduce the amplitude of the blood glucose spike can help with the regulation of blood glucose in healthy adults, and in adults with diabetes (Muller et al. 2018; Kuwata et al. 2016; Wong and Jenkins 2007). Therefore, this study determined the frequency of Tmax for half-cup and quarter-cup serving sizes of green lentils, red lentils, corn, macaroni, white potato and white rice.

C.iv. Blood Glucose and Plasma Insulin Treatment Comparisons Within Time Points

Postprandial blood glucose and plasma insulin response following consumption of a half-cup and quarter-cup serving size of treatments were analyzed in the current study. For the half-cup serving size, the postprandial blood glucose and insulin response for green lentils and red lentils were significantly different compared to the starchy controls at various time points, and blood glucose was lower for green lentils compared to red lentils at 90 and 120 minutes, but there were no differences between the lentil varieties for plasma insulin at any of the time points. For the quarter-cup serving size, the postprandial blood glucose response for green and red
lentils were significantly different compared to the starchy control at various time points, and red and green lentils were not significantly different from each other.

Treatment comparisons within time points for blood glucose and plasma insulin were also analyzed by Jenkins et al. 1982 who studied 4 healthy adults that consumed 226 g of lentils within a breakfast meal and saw reductions between the lentil breakfast and the wholemeal bread breakfast at 30 minutes for blood glucose and at 30 minutes and 120 minutes for plasma insulin. Although the present study saw reductions in blood glucose and plasma insulin at 30 minutes, there was also observed decreases at more time points, including 15 minutes, but no significant reductions were found for plasma insulin at 120 minutes. These differences might be due to the test meals used in Jenkins et al. 1982 study which incorporated a whole meal approach that contained tomato and cottage cheese in combination with wheat bread which may have slowed the glycemic response by slowing down starch digestion and glucose absorption (Meynier et al. 2015; Singh et al. 2010) and prevented an increase at 15 minutes in the blood glucose response as seen in the controls from the present study. Mollard et al. 2011 had similar findings to the current study and examined the blood glucose response at each time point between macaroni and cheese, and lentils mixed with macaroni and cheese found that the incorporation of lentils resulted in a reduction at 20 minutes and 140 minutes; blood glucose was reduced at 40 minutes in 12 healthy adults for blended and whole lentils compared to the no lentil burrito (Anguah et al. 2014); 24 healthy adults consuming 50 g of available carbohydrates from white rice or lentils in combination with white rice had reductions in blood glucose at 15, 30, 45, 60 and 90 minutes, with no differences in plasma insulin, and reductions in blood glucose from consumption of white potato alone or in combination with lentils at 15, 30, 45, 60 and 90 minutes with a reduction in plasma insulin at 15, 30, 45, 60 and 90 minutes (Moravek et al. 2018). This supports
the findings in the current study that have shown reductions in both blood glucose and plasma insulin over 120 minutes, although the quantity of lentils served to the participants in the study outlined above are greater than the amount the participants consumed in the present study, and the consumption of lentils was mixed with other foods including bread, macaroni, white potato and white rice, whereas in the current study, the participants only consumed the assigned treatment independently.

The blood glucose and plasma insulin treatment comparisons at each time point allow the examination of concentration over the 120 minutes. This allows researchers to determine the points in which foods, such as lentils or the starchy controls, have differences in concentration at a given time point. The benefit of knowing when treatments vary in blood glucose or plasma insulin concentration at a given time allows foods to be compared directly to determine if one food can attenuate an increase in the response compared to a more commonly consumed food (Wachters-Hagedoorn et al. 2006; Leidy et al. 2011). This benefits consumers who can select lentils over different types of starchy food for their ability to elicit a lower blood glucose response, and encourages that both red and green lentils are effective at doing this, as demonstrated in the current study.

D. Glycemic Response Summary

Overall the glycemic response results are consistent with results that have been reported in the literature in healthy participants on the acute postprandial blood glucose response, blood glucose and plasma insulin iAUC, Cmax, Tmax and for the within time point treatment comparisons. This is the first study to examine the effects of green lentils, red lentils, corn, macaroni, white potato and white rice independently in half-cup and quarter-cup serving sizes. As well, the incorporation of corn as a starchy control was novel. The study design was planned
to determine if small serving sizes, such as a half-cup or quarter-cup could elicit blood glucose lowering abilities, and detect significant differences among the treatments in blood glucose and plasma insulin iAUC, Cmax and Tmax. This was achieved in the current study, and the results demonstrated improvements in glycemic response biomarkers in healthy adults for both the half-cup and quarter-cup serving sizes.

**E. Study Limitations**

There are some limitations to the current study that should be discussed regarding the study treatments and the study design.

The first limitation of the study relates to the treatments that were served. Lentils are commonly served as part of a mixed meal such as in soup, chilli, and curry rather than on their own. However, to minimize confounding variables from other food ingredients and to determine the glycemic effects of such small serving sizes, it was warranted to study the lentils and the starchy controls independently. Furthermore, when large amounts of lentils are incorporated into food products, it is challenging to determine the quantity of lentils that are being consumed by the individual. This way, we ensured that all participants consumed the exact same quantity of lentils to determine the effects on glycemic measure outcomes. Another limitation was the inability to match the treatments for their nutritional composition including, protein, fat and available carbohydrates. It is important to note, however that the core purpose of this study was to match the treatments for serving size, therefore matching for nutritional composition was not possible. Furthermore, serving size comparisons were used to enhance the translatability of the results to the general public who are more familiar with the concept of cup-based serving sizes than the concept of matching for available carbohydrates.
Another limitation to the study was the lack of blinding of the study treatments for both the participants and the researchers. This inability to blind was unavoidable, as the study was examining the effects of whole foods including green lentils, red lentils, corn, macaroni, white potato and white rice, and therefore the study team preparing the food, and the participants consuming the food could visually identify what the meal was and could not be blinded. Although participants were aware of what treatment they were receiving, all measurements acquired, including blood glucose and plasma insulin were objective measurements and could not have been influenced by knowing what treatment they were consuming.

The current study was conducted on healthy adults between the ages of 18-40 years, which is not reflective of the growing population that has T2D. As this is the first study to examine the effects of two varieties of lentils on the postprandial blood glucose response compared to multiple starchy controls, it is important to minimize the amount of confounding variables in the study population. Furthermore, in order to substantiate a potential function claim for lentils and the reduction of the postprandial glycemic response, Health Canada’s Draft Guidance Document on food claims related to the reduction in postprandial glycemic response requires the study population to be adult individuals who are healthy (Health Canada Draft Guidance Document 2013). Therefore, the inclusion of healthy adults instead of at risk, or adults with T2D is justified for the current study.

One last limitation for the current study was the use of the Nova Biomedical Statstrip Glucose Hospital Meter instead of the gold standard YSI glucose analyzer. Studies in the literature have used YSI (Jenkins et al. 1982) and others have used glucometers (Anderson et al. 2015; Anguah et al. 2014; Mollard et al. 2012a; Mollard et al. 2014; Wong et al. 2009) so for the current study the Nova Biomedical Statstrip Glucose Hospital Meter was selected. A glucometer
was selected for the study as it is convenient to use, and finger prick blood samples measured via a glucometer is currently the method in which individuals living with diabetes use and are familiar with. This enhances the applicability of the study to the general public, as they can understand that the measurements were taken on an instrument that they have seen, and some may have used, versus the unfamiliarity with YSI and how it works. Furthermore, one study conducted by Ekhlaspour et al. 2017 examined the accuracy of 17 commercially available glucometers and compared the outputs to YSI to determine the mean absolute relative difference for blood glucose. It was found that the Nova Biomedical Statstrip glucose analyzer had the second lowest mean absolute relative difference when compared to YSI, and was only one of two glucose meters that met the accuracy outlines for International Organization for Standardization 2013, demonstrating that the Statstrip glucose analyzer is accurate, and warranted for use in the current study (Ekhlaspour et al. 2017; International Organization of Standardization:15197. 2013). Additional research demonstrated that during a 6-hour glucose clamp study comparing YSI to the Nova Biomedical Statstrip glucose analyzer for determination of blood glucose and accuracy, 100% of the Nova Biomedical Statstrip glucose analyzer values fell in the accurate zone when using YSI values as a reference and was reported by the authors as being accurate and acceptable, although it required triplicate or quadruplicate values to be analyzed as duplicates should not exceed 5 mg/dl (or 0.3 mmol/L) difference (Rabiee et al. 2010). In the current study, this protocol was followed, and any values greater than 0.3 mmol/L apart had an additional sample of blood run for the determination of the blood glucose concentration.

Overall, the limitations of the current study were related to the study treatments such as consuming the treatments independently and not in mixed meals, the inability to match nutritional composition or were related to the study design including the inability to blind
participants and researchers, the recruitment of healthy adults, and the selection to use the Nova Biomedical Statstrip glucose analyzer. As outlined above, these decisions were unable to be avoided given the justifications above.

**F. Study Strengths**

There are numerous strengths of the current study including the design, the study treatments, and data collection. Firstly, this study was a randomized, crossover design that consisted of participants consuming all 6 study treatments in either the half-cup or quarter-cup serving size, with a 3-7 day washout period in between. The randomized order in which participants received the treatment helped eliminate potential bias from researchers and participants. The crossover study design is a strength as each participant served as their own control which minimized inter-individual variation from intrinsic differences between study participants. The washout period of 3-7 days between study visits allowed any effects of the treatments to return to baseline before consuming another treatment, and this was demonstrated by the lack of statistical significance between fasted values for each study treatment. Furthermore, the washout period allowed the finger pricks to heal in between study visits. Participants were also asked to consume a pre-study dinner that would be consumed the night before a study visit that would be consistent each time. Participants advised researchers what this meal would be, and this reduced possible confounders in the glycemic response between each study visit. The pre-study sample size calculation that was done was another strength of the current study and was done to detect a minimum reduction of 20% for the postprandial blood glucose response with a power of 80% and significance was set at 5%. Therefore, the study design was well planned to account for reducing potential confounding variables including randomization, the crossover study design, the washout period, and the sample size calculation
and was used to determine the effects of half-cup and quarter-cup serving sizes of two varieties of lentils on the acute postprandial blood glucose and insulin response compared to multiple starchy controls.

The study treatments provided to participants were also a strength of the study. The study treatments included two varieties of lentils (Laird green lentil and the Eston split red lentil), and four starchy foods (corn, macaroni, white potato and white rice). Canada is the largest producer of lentils, with red and green lentils being the most commonly cultivated varieties, therefore research on the effects of the aforementioned lentil types is warranted (Agriculture and Agri-food Canada 2008). The starchy foods that were selected are the most commonly consumed starches as indicated in the Canadian Community Health Survey (Statistics Canada 2017), and macaroni, white potato and white rice have been previously studied in the literature as comparisons (Mollard et al. 2011; Mollard et al. 2012a; Anguah et al. 2014; Bornet et al. 1987). Although corn has not been used as a control treatment in the literature, the addition of corn as a comparison is novel and is a strength to this study as it has the most similar structure and matrix to lentils, and is frequently consumed by the Canadian population (National Corn Growers 2018; Statistics Canada 2017). The half-cup and quarter-cup serving sizes that the participants consumed for the treatments are another strength of the study, as all treatments were matched for serving size. The benefits of lentils were observed at a quarter-cup serving size, and no other study (to the researchers’ knowledge) has examined a serving size as small as a quarter-cup. The observed benefits of improving glycemic measures by consuming lentils can be easily conveyed by encouraging the consumption of a half-cup or a quarter-cup to the general public, and allows the translation of this information to be easily understood.
The screening method that was employed in this study was another strength. The 3-phase screening process allowed participants time to understand the study protocols and procedures, and to ask questions to ensure they understood what was expected of them before, and during a study visit. This improved the communication between study researchers, and participants, and helped minimize attrition rates as all participants felt comfortable with the procedure when they enrolled in the study. The oral glucose tolerance test that was conducted at the third screening served useful to determine if the participant had blood glucose measurements that fell within the accepted healthy ranges, and helped prepare the participant for a study day. When the participant arrived for the oral glucose tolerance test, they were fasted, were asked the pre-study questions, had body measurements and a fasting finger prick taken, consumed Trutol within 10 minutes, and had a subsequent finger prick at 2 hours. All of these protocols are employed during a study visit, and therefore helped prepare the participant for what a normal visit would look like, and allowed them to ask questions to make sure they were completely comfortable with the process. Since the study recruited healthy adults, the results are representative of the Canadian population who is composed of both males and females, as many studies in the literature have just examined men (Wong et al. 2009; Mollard et al. 2011; Mollard et al. 2012a; Mollard et al. 2014; Anderson et al. 2014; Akhtar et al. 1987; Cryne et al. 2012). The screening methods used allowed the inclusion of healthy adults that is representative of the Canadian population who can benefit from consumption of lentils in their day to day lives.

Another strength of the current study was the analysis of the variety of glycemic measures. Previous studies have measured the postprandial blood glucose and insulin response, iAUC, Cmax, comparisons at time points, RGR, and the insulinogenic index, but not all studies have reported on all of them. Furthermore, this study analyzed all the glycemic measures for the
half-cup and quarter-cup serving size, and the insulinemic measures for the half-cup serving size. The analysis of blood glucose and insulin is a strength, as it helps further solidify that lentils are effective at reducing the postprandial blood glucose response and the mechanism is not by increasing insulin production, but rather it reduces the postprandial insulin response, plasma insulin iAUC and the Cmax compared to the starchy controls.

An additional strength of the study is the detailed measures that were taken for data collection, and verification through the entire study duration. All researchers that were involved in this study were trained, and worked together as a team to complete the data collection by following the procedures that were outlined by the study coordinators. The procedures that were outlined included training for all interactions with participants such as screening and study visit protocols to ensure that all data was collected completely and correctly as outlined in study flow-sheets. Flow-sheets allowed researchers to follow step by step how to walk a participant through a study visit, including emailing a detailed reminder the day before, ensuring all questionnaire information and body measurements were taken, and the flow-sheets had spots to record data with additional space for notes. Finger prick blood samples were taken by 3 trained M.Sc. students who practiced their technique thoroughly and as outlined in the HNRU SOP protocols. The analysis of glucose was measured in duplicate on the same Nova Biomedical Statstrip Glucose Hospital Meter and was performed by one researcher on a given day to minimize variation. All insulin ELISA assays were completed by the same individual to minimize inter-individual variation in plating technique, and all protocols for the assay were followed as outlined in the SOP from Mercodia. All data entry was entered into excel files and was then verified by a different researcher to confirm the validity of the data entered, and any
discrepancies were noted and reviewed. This process ensured that all data was accurate, complete and adds to the strength of the data collection and analysis for the results of the study.

**G. Future Research**

There are various directions that can be further investigated for lentil research in human clinical trials. Some of the directions that the studies can go can include more acute and chronic clinical trials in various populations on glycemic and insulinemic measures, implementing a standardized treatment serving size, and developing more commercially available lentil products.

This study examined the effects of green and red lentils on the acute postprandial blood glucose and plasma insulin response compared to matched serving sizes of four commonly consumed starchy foods in healthy adults. As this study was focused on the acute effects, in healthy adults, the next possible translation would be to examine the effects in different populations, including in adults at risk for T2D and in adults with T2D. Previous studies have examined the glycemic effects of adults with T2D, but the quantity of lentils investigated ranged (49.9 g, 89.3 g and 225 g), the form of how the lentils were consumed varied, and the glycemic measures were inconsistent (Coulsten et al. 1984; Bornet et al. 1987; Akhtar et al. 1987) including measuring plasma glucose or blood glucose. Although these measurements are variable, all of these studies above observed improvements in the plasma glucose, insulin and blood glucose measurements. Future research should use consistent glycemic measurements to help further encourage the consumption of lentils for the improvement of glycemic measures, and the analysis of insulin regularly can confirm that lentils are effective without inducing a hyperinsulinemic state. Furthermore, there are no current studies (to the authors knowledge) that have examined the acute effects of lentil consumption of glycemic and insulinemic measures in adults at risk for T2D, including those with pre-diabetes. This research would help advance the
knowledge of lentils as a dietary strategy for the prevention of T2D, as worldwide, an estimated 193 million people are living with undiagnosed diabetes (Chatterjee et al. 2015). The addition of lentils into one’s lifestyle is relatively easy, and by demonstrating that the addition of smaller serving sizes, such as a half-cup or quarter-cup, can be effective would be beneficial for an at-risk population.

As well, future research should aim to assess if the addition of lentils into a diet are an effective lifestyle intervention for the management of T2D by conducting chronic studies. Chronic studies would be an effective next step to determine the duration of consumption, and what population can best benefit from lentil intake. Previously, studies have examined the incorporation of pulses into the diet for adults at risk for T2D (Mollard et al. 2012; Saraf-Bank et al. 2016) and adults with T2D (Jenkins et al. 2012; Hosseinpour et al. 2015; Shams et al. 2008) for various durations of time, but majority of these studies have looked at pulses which include beans, chickpeas, peas and lentils, as opposed to just lentils. Focusing solely on lentils can help confirm the physiological relevance of lentils and their ability to lower glycemic and insulimetic markers.

Additionally, future studies should focus on implementing a standard treatment serving size of lentils such as half-cup and quarter-cup. Although the literature has demonstrated the benefits of lentils in different adult populations (at risk, with diabetes, and healthy) the amount of lentils served in a study has varied widely, and the method of reporting consumption has been variable. The literature typically reports the amount of lentils served to participants in grams, but this information is challenging to transfer to the general public that typically uses cup-based serving sizes in the preparation of meals. Due to reporting in grams instead of cup-based serving sizes, consumers are unable to use the literature to make informed decisions on the importance of
lentils in the improvement of glycemic measures, and therefore do not understand their health benefits. New strategies need to be communicated to the general public, and to lentil consumers to ensure they understand the benefits of lentils and to further their knowledge and education on the amount that should be consumed to elicit these benefits. Transferring this information into serving sizes would enhance consumer’s knowledge and understanding of how much lentils to consume, how often to consume it, and studies that focus on implementing a standard serving size for the analysis of glycemic measures would help to establish a comprehensive literature background that is easy to understand.

Another direction for future studies would be to consider the development of food products that can incorporate half-cup and quarter-cup servings of lentils. The addition of a half-cup or quarter-cup serving size of lentils into a food product will ensure that consumers are receiving an amount of lentils that has been demonstrated to improve glycemic measures, and is an effective way to increase consumption of lentils to people who are unaware of the benefits of lentils. For the purpose of this study, participants consumed green and red lentils plain, and on their own so the effects on the glycemic response could be investigated, but developing food products with lentils incorporated into them could enhance their appeal and taste to consumers.

**H. Summary and Conclusions**

The research presented investigated the effects of green and red lentils in half-cup and quarter-cup serving sizes on the acute postprandial blood glucose and plasma insulin response compared to multiple starchy controls in healthy adults. The design was a randomized, crossover study consisting of 6 study visits with a 3-7 day washout period in between where participants consumed green lentils, red lentils, corn, macaroni, white potato and white rice in either half-cup
or quarter-cup serving sizes. This study demonstrated that the consumption of green and red lentils in both the half-cup and quarter-cup study can improve some glycemic measures.

The first hypothesis was that the half-cup serving of green and red lentils would significantly reduce blood glucose and would not significantly change plasma insulin compared to the starchy controls (corn, macaroni, white potato and white rice). For blood glucose, the hypothesis was partly accepted as blood glucose iAUC was significantly lower for green and red lentils compared to macaroni, white potato and white rice (but not corn) and blood glucose Cmax was significantly lower for both green and red lentils compared to corn, macaroni, white potato and white rice. For plasma insulin, the hypothesis was rejected as plasma insulin iAUC was significantly lower for green and red lentils compared to white potato and white rice (but not corn), and in addition red lentils were significantly lower compared to macaroni. The plasma insulin Cmax for both green and red lentils were significantly lower compared to corn, macaroni, white potato and white rice.

The second hypothesis of this research was that the quarter-cup serving of green and red lentils would significantly reduce blood glucose compared to the starchy controls (corn, macaroni, white potato and white rice). This hypothesis was rejected for blood glucose iAUC for green lentils as only two of the starchy controls (white potato and white rice) were significantly lower, and red lentils were not significantly different from any of the starchy controls (corn, macaroni, white potato and white rice). Furthermore, blood glucose Cmax was significantly lower for green lentils compared to corn, macaroni, white potato and white rice (which is in line with the hypothesis), while red lentils were significantly lower compared to corn, white potato and white rice (but not macaroni).
Overall, this research supports the acute consumption of red and green lentils in half-cup and quarter-cup serving sizes for the reduction of postprandial blood glucose and plasma insulin response compared to multiple starchy controls matched for serving size in healthy adults. Future research is needed to determine the effect of smaller serving sizes, such as half-cup and quarter-cup in participants with T2D in acute studies, and in longer duration studies for the prevention of T2D, and the management of blood glucose and plasma insulin. In summary, this research supports the incorporation of green and red lentils in half-cup and quarter-cup serving sizes for the reduction and management of glycemic and insulinemic measures.
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Sue RW, Sara LA. Nutrition and diet therapy. 7th (ed), Mosby. USA 1993.


Wong JMW, Jenkins DJA. Carbohydrate digestibility and metabolic effects. J Nutr. 2007; 137(11):2539S-2546S.


### Appendix A: Summary of Human Clinical Trials that Examined the Glycemic Effects of Acute Consumption of Whole Lentils in Healthy Adults

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenkins, D. J. A. et al.</td>
<td>Slow release dietary carbohydrate improves second meal tolerance. Am J Clin Nutr. 1982; 35(6), 1339–1346.</td>
<td>n=7 Healthy men and women Age: 26 ± 3 years Healthy body weight</td>
<td>Lentil breakfast: 226 g (dry weight) of boiled lentils, 11 g butter, 150 g tomatoes Wholemeal bread breakfast: 280 g of wholemeal bread, 213 g low fat cottage cheese, 150 g of tomatoes ¼ bread breakfast: 70 g of wholemeal bread, 213 g low fat cottage cheese, 150 g of tomatoes Slow bread breakfast: 280 g of wholemeal bread, 213 g low fat cottage cheese, 150 g of tomatoes (note: eaten over 4 hours) Breakfasts were matched for protein, fat and carbohydrate Standardized lunch: 150 g wholemeal bread, 60 g low fat cottage cheese, 100g tomato, 15 g Marmite, 150 g</td>
<td>Blood glucose AUC (0-2 hour breakfast and 0-2 hour lunch) Peak rise in glucose Insulin AUC (0-2 hour breakfast and 0-2 hour lunch) Peak rise in insulin</td>
<td>Blood glucose AUC (0-2 hour breakfast) was lower for the lentil, ¼ bread, and slow bread treatments compared to the wholemeal bread treatment Blood glucose peak rise was lower for the lentil and the slow bread treatments compared to the wholemeal bread for 0-2 hours after breakfast, and for the lentil treatment 0-2 hours after</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Participant Characteristics</td>
<td>Study Treatments</td>
<td>Glycemic Endpoints</td>
<td>Significant Effects of Lentil Treatment(s)</td>
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<tr>
<td>Wong, C. L. <em>et al.</em></td>
<td>Randomized crossover</td>
<td>n=15, Healthy men, Age: 18-35 years (average age was not reported), BMI of 20-25 kg/m² (average BMI was not reported)</td>
<td>Chickpeas (341 g) in a tomato based sauce, Lentils (451 g) in a tomato based sauce, Navy beans (359 g) in a tomato based sauce, Yellow peas (491 g) in a tomato based sauce, White bread (235 g) in a tomato based sauce, Water control, All meals were equalized to a volume of 575 mL</td>
<td>Blood glucose at each time point over two hours, Blood glucose AUC</td>
<td>Blood glucose for lentils and chickpeas was lower than the other pulses and white bread over the first hour, Blood glucose AUC was lower for lentils, chickpeas and yellow peas compared to white bread</td>
</tr>
</tbody>
</table>


Form: Whole lentils served in a tomato sauce

- skinned banana

- Insulin was lower at 30 and 120 minutes in the lentil treatment compared to the wholemeal bread treatment
- Insulin peak rise was reduced by the lentil treatment compared to the wholemeal bread treatment
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment(s)</th>
</tr>
</thead>
</table>
| Mollard, R. C. et al. | First and second meal effects of pulses on blood glucose, appetite, and food intake at a later meal. Appl Physiol Nutr Metab. 2011; 36(5), 634-642. | • Randomized crossover  
• 4 treatments  
• Weekly study visits | • Chickpeas (222.8 g) and macaroni with tomato sauce  
• Lentils (332.9 g) and macaroni with tomato sauce  
• Yellow peas (375.6 g) and macaroni with tomato sauce  
• Macaroni and cheese | • Blood glucose at each time point  
• Blood glucose AUC pre-pizza (0-260 minutes)  
• Blood glucose AUC post-pizza (280-340 minutes) | • Blood glucose response was lower for the chickpea, lentil and yellow pea macaroni compared to macaroni and cheese  
• Blood glucose was lower for lentil and chickpea macaroni at 280 minutes compared to macaroni and cheese  
• Blood glucose AUC was lower post-pizza meal for the lentils and macaroni and chickpeas and |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment(s)</th>
</tr>
</thead>
</table>
• 5 treatments  
• Washout periods of 1 week between treatments | • n=24  
• Healthy men  
• Age: 23.3 ± 3.6 years  
• BMI: 22.8 ± 1.4 kg/m² | • Chickpeas and macaroni with tomato sauce  
• Lentils and macaroni with tomato sauce  
• Navy beans and macaroni with tomato sauce  
• Yellow peas and macaroni with tomato sauce  
• Macaroni and tomato sauce  
• All treatments provided 6280.2 kJ, with 44% of energy from pulses- and were consumed *ad libitum*  
• *Ad libitum* pizza meal was served | • Blood glucose at each time point pre-pizza (0-260 minutes), and post-pizza (280-340 minutes) meal  
• Cumulative (0-340 minutes), pre-(0-260 minutes) and post- (280-340 minutes) pizza blood glucose AUC | • The lentil treatment reduced blood glucose at 20, 40, 80 and 200 minutes compared to the macaroni control, and at 60 minutes compared to yellow peas  
• Cumulative Blood glucose AUC (0-340 minutes) was lower for lentils (and all pulse treatments) compared to macaroni and tomato sauce  
• Pre-pizza meal (0-260 minutes) blood glucose AUC was lower for lentils (and chickpeas and navy beans) compared to macaroni and |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatments</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment(s)</th>
</tr>
</thead>
</table>
• 5 treatments  
• Washout periods of 1 week | • n=15  
• Healthy men  
• Age: 22.5 ± 0.8 years  
• BMI: 22.9 ± 0.4 kg/m² | • Chickpeas (222.8 g) in a tomato sauce  
• Lentils (332.9 g) in a tomato sauce  
• Navy beans (240.5 g) in a tomato sauce  
• Yellow peas (375.6 g) in a tomato sauce  
• White bread (116.6 g) dipped in tomato sauce  
• Fixed pizza meal was served | • Blood glucose at each time point  
• Blood glucose AUC (0-135 minutes) and after pizza meal (150-210 minutes) | • Lentils in tomato sauce lowered blood glucose concentrations at 15, 30, 45, 75, 150, and 165 minutes compared to white bread.  
• Lentils in tomato sauce lowered blood glucose AUC pre-pizza meal (0-135 minutes) and post-pizza meal (150-210 minutes) compared to white bread |
### Appendix B: Summary of Human Clinical Trials that Examined Glycemic Effects of Acute Consumption of Various Forms of Lentils in Healthy Adults

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
</table>
• Double-blinded for capsule (either α-galactosidase or placebo)  
• Placebo controlled  
• 6 treatments  
• 3-5 day washout period between study days | • n=12  
• Healthy men and women  
• Age: 28 ± 10 years  
• BMI: 23.3 ± 3.1 kg/m² | • Blended lentils (≥ ½ cup) in a burrito with α-galactosidase capsule  
• Blended lentils (≥ ½ cup) in a burrito with placebo capsule  
• Whole lentils (≥ ½ cup) in a burrito with α-galactosidase capsule  
• Whole lentils (≥ ½ cup) in a burrito with placebo capsule  
• Burrito with no lentils and α-galactosidase capsule  
• Burrito with no lentils with placebo capsule | • Blood glucose over 180 minutes  
• Blood glucose AUC over 180 minutes and from 0-90 minutes, and 90-180 minutes. | • Blood glucose was lower at 40 minutes for the blended and whole lentil burritos compared to the no lentil burrito  
• Blood glucose AUC was lower over the first half (0-90) and total (0-180) minutes for the blended lentil burrito compared to the no lentil burrito |
| **Anderson, G.H. et al.** The acute effect of commercially available pulse powders on postprandial glycaemic responses in | • Randomized crossover  
• 4 treatments  
• Washout periods of 1 week | • n=12  
• Healthy men  
• Age: 22.2 ± 0.9 years  
• BMI: 23.2 ± 0.4 kg/m² | • Whole canned green lentils (183.8 g) in tomato sauce  
• Pureed canned green lentils (183.8 g) in tomato sauce  
• Eston lentil powder (47.7 g) in tomato sauce  | • Blood glucose concentration at each time point (0-120 minutes) and (140-200 minutes)  
• Blood glucose AUC  | • The mean blood glucose concentration was lower for all lentil treatments, compared to the whole wheat flour control from 0-120 |
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>healthy young men. Br J Nutr. 2014; 112(12): 1966-1973.</td>
<td></td>
<td></td>
<td>• Whole wheat flour (39.4 g) in tomato sauce</td>
<td></td>
<td>minutes • Blood glucose at 15 minutes was lower for whole and pureed lentils compared to the whole wheat flour • Blood glucose at 90 minutes was significantly lower for lentil powder compared to whole wheat flour • Blood glucose AUC from 0-120 minutes was lower for the whole lentils and powdered lentils compared to whole wheat flour</td>
</tr>
</tbody>
</table>

Form: Whole, pureed or powdered lentils served in tomato sauce
### Appendix C: Summary of Human Clinical Trials that Examined the Glycemic Effects of Acute Consumption of Whole Lentils in Adults with Type 2 Diabetes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coulston, A. et al.</strong></td>
<td>• Randomized crossover</td>
<td>• n=8</td>
<td>• Baked potato</td>
<td>• Plasma glucose concentration over 180 minutes</td>
<td>• Plasma glucose was lower for the lentil test meal compared to the potato test meal over 180 minutes</td>
</tr>
<tr>
<td>Effect of source of dietary carbohydrate on plasma glucose. Insulin and gastric inhibitory polypeptide responses to test meals in subjects with noninsulin-dependent diabetes mellitus. Am J Clin Nutr. 1984; 40: 965-970.</td>
<td>• 4 treatments</td>
<td>• Men and women with type 2 diabetes</td>
<td>• Rice</td>
<td>• Plasma insulin concentration over 180 minutes</td>
<td>• Plasma insulin was lower for the lentil test meal compared to the potato test meal over 180 minutes</td>
</tr>
<tr>
<td></td>
<td>• 2 weeks</td>
<td>• Age: 59 ± 2.0 years</td>
<td>• Spaghetti</td>
<td>• Plasma Gastric Inhibitory polypeptide (GIP) over 180 minutes</td>
<td>• Plasma GIP was lower for the lentil test meal compared to the other 3 test meals over 180 minutes</td>
</tr>
<tr>
<td></td>
<td>• Length of washout period between study visits was not reported</td>
<td>• BMI: 27.3 ± 0.6 kg/m²</td>
<td>• Lentils (49.9 g, not indicated if dry weight or cooked)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• All 4 meals above were matched for protein and fat, and the carbohydrate source provided 66% energy</td>
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<tr>
<td><strong>Bornet, F.R. J. et al.</strong></td>
<td>• Randomized crossover</td>
<td>• n=18</td>
<td>• Starch-rich foods consumed alone (white bread, spaghetti, white rice, instant potato flakes, dried kidney)</td>
<td>• Plasma glucose concentration over 180 minutes</td>
<td>• The GI for lentils was lower over 180 minutes compared to potato and bread</td>
</tr>
<tr>
<td>Insulinemic and glycemic indexes of six starch-rich foods taken alone</td>
<td>• 6 test foods, 6 test meals and oral glucose tolerance</td>
<td>• Men and women with type 2 diabetes</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Age: 57 ± 2</td>
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<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Participant Characteristics</td>
<td>Study Treatment</td>
<td>Glycemic Endpoints</td>
<td>Significant Effects of Lentil Treatment</td>
</tr>
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<tr>
<td>and in a mixed meal by type 2 diabetes. Am J Clin Nutr. 1987; 45: 588-595. Form: Whole green lentils alone, and in combination with butter and cheese</td>
<td>test (OGTT) • 3 study visits on 3 consecutive days • Participants consumed the same carbohydrate source for their study visits</td>
<td>years • BMI: 27.9 ± 1.1 kg/m²</td>
<td>beans, dried green lentils 225 g) • Starch-rich foods combined in a meal (white bread, spaghetti, white rice, instant potato flakes, dried kidney beans, dried green lentils) with butter and cheese: standardized for 50 g of available carbohydrates • OGTT</td>
<td>180 minutes • Glycemic AUC over 180 minutes • Insulin AUC over 180 minutes • Glycemic index (GI) • Insulinemic index was increased in all mixed meals compared to the starch meal alone • The insulinemic index was lower for lentils and beans compared to the other starch rich foods (significance not reported)</td>
<td></td>
</tr>
<tr>
<td>Akhtar, M.S. et al. Blood glucose responses to traditional Pakastani dishes taken by normal and diabetic patients. Nutr Res. 1987; 7: 696-706. Form: Whole lentils incorporated into a meal with bread or rice</td>
<td>• Randomized crossover • 7 test meals • Duration of study not reported • Length of washout period between study visits not reported</td>
<td>n=28 • 14 men with diabetes Age: 52.5 ± 2.46 years • BMI: 24.3 ± 1.13 kg/m² • 14 healthy men • Age: 35.2 ± 1.54 years • BMI: 23.6 ± 0.55 kg/m²</td>
<td>• Bread + egg • Bread + grams • Bread + lentils (89.3 g dry weight) • Bread + moong • Bread + mash • Rice + lentils (89.3 g dry weight) • Rice + moong • All meals were standardized for 50 g of available carbohydrate</td>
<td>• Blood glucose concentration at each time point over 180 minutes • Blood glucose AUC over 180 minutes for the men with diabetes and the healthy men • Peak glucose concentration for each test meal • Fasted blood glucose in healthy men was higher for the rice + lentils compared to the rice +moong • Blood glucose AUC was lower in the healthy men for the bread + lentils compared to bread + mash and bread + eggs • Blood glucose AUC and peak glucose</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Participant Characteristics</td>
<td>Study Treatment</td>
<td>Glycemic Endpoints</td>
<td>Significant Effects of Lentil Treatment</td>
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</tbody>
</table>

- Blood glucose AUC and peak glucose concentration in healthy men was lower for rice + lentils compared to rice + moong
- Blood glucose AUC in men with diabetes was lower for bread + lentils compared to bread + eggs
- Blood glucose AUC in men with diabetes was lower for rice + lentils compared to rice + moong
Appendix D: Summary of Human Clinical Trials that Examined the Glycemic Effects of Chronic Consumption of Powdered Lentils in Healthy Adults

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cryne, C. N. et al.</strong></td>
<td>Spray-dried pulse consumption does not affect</td>
<td>• Randomized crossover</td>
<td>• 100g of chickpea powder</td>
<td>• Plasma glucose</td>
<td>• None</td>
</tr>
<tr>
<td></td>
<td>cardiovascular disease risk or glycemic control in</td>
<td>• Double-blind</td>
<td>• 100g of Laird green</td>
<td>• Serum insulin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>healthy males.</td>
<td>• 4 treatments</td>
<td>lentil powder</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Food Res Int. 2012; 48: 131-139.</td>
<td>• 28 days x 4 treatments</td>
<td>• 100g of green pea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Form: Lentil powder</td>
<td>• 28-day washout period between treatments</td>
<td>• n=21</td>
<td>powder</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>• Healthy men</td>
<td>• 50 g of instant potato</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Age: 28.1 ± 5.87 years</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• BMI: 25.2 ± 3.54 kg/m²</td>
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</tbody>
</table>


## Appendix E: Summary of Human Clinical Trials that Examined the Glycemic Effects of Chronic Consumption of Whole Lentils in Adults at Risk for Diabetes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mollard, R.C. et al.</strong> Regular consumption of pulses for 8 weeks reduces metabolic syndrome risk factors in overweight and obese adults. Br J Nutr. 2012; 108: S111-S122.</td>
<td>• Randomized • 2 treatments • 8 weeks</td>
<td>• n=40 (total) • n=21 for energy restricted diet • n=19 for pulse diet • Men and women with 3 Metabolic Syndrome risk factors • Age: 45.5 ± 1.0 years • BMI: 32.8 ± 0.7 kg/m²</td>
<td>• Pulse diet (included lentils, chickpeas, yellow peas and navy beans) at 5 cups per week • Energy restricted diet (decrease energy intake by 500 kcal per day) • 75 g Trutol at study visits 1, 4, and 8</td>
<td>• Fasted blood glucose concentration (weeks 1, 4 and 8) • Fasted blood insulin concentration, (weeks 1, 4 and 8) • HbA1c • Blood glucose and insulin AUC following glucose load over 2 hours (week 1, and 8)</td>
<td>• HbA1c decreased within both groups from week 1 to week 8 • Blood glucose AUC was lower in the pulse group compared to the the energy restricted group from week 1 to week 8</td>
</tr>
</tbody>
</table>

<p>| <strong>Saraf-Bank, S. et al.</strong> Legume-enriched diet on Cardiometabolic risk factors among individuals at risk for diabetes: a crossover study. J Am Coll Nutr. | • Randomized crossover • 2 treatments • 14 weeks (6 weeks of diet #1, washout, and 6 weeks of diet #2) • Washout period of 2 weeks | • n=26 • Participants were first degree relatives with someone diagnosed with T2D • Age: 50 ± 6.58 years | • Legume-enriched diet: 24 packs of legumes (12 lentils and 12 pinto beans containing ½ cup) • Habitual diet: dietary recommendations | • Fasting blood glucose • HbA1c | • None |</p>
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016; 35(1): 31-40.</td>
<td>Form: Whole lentils incorporated into a diet</td>
<td>• BMI: 28.84 ± 0.83 kg/m²</td>
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</tbody>
</table>
Appendix F: Summary of Human Trials that Examined the Glycemic Effects of Chronic Consumption of Whole Lentils in Adults with Diabetes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Participant Characteristics</th>
<th>Study Treatment</th>
<th>Glycemic Endpoints</th>
<th>Significant Effects of Lentil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Jenkins, D. J. A. et al.</strong></td>
<td>Randomized, parallel-arm</td>
<td>n=121 (total)</td>
<td>Low GI diet (1 cup per day of lentils, chickpeas or beans)</td>
<td>HbA1C</td>
<td>HbA1c was lower from baseline to week 12 in the low GI diet group</td>
</tr>
<tr>
<td></td>
<td>2 treatments</td>
<td>Adults with diabetes</td>
<td>High wheat fibre diet (consumption of whole wheat and whole grain carbohydrate foods)</td>
<td>Fasted blood glucose concentration</td>
<td>Fasted blood glucose was lower from baseline to week 12 in the low GI diet group</td>
</tr>
<tr>
<td></td>
<td>12-week duration</td>
<td>n=61 for low GI diet</td>
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<tr>
<td></td>
<td></td>
<td>Age: 58 ± 1.3 years</td>
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<tr>
<td></td>
<td></td>
<td>BMI: 31.4 ± 0.9 kg/m²</td>
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<tr>
<td></td>
<td></td>
<td>n=58 for high wheat fibre diet</td>
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<tr>
<td></td>
<td></td>
<td>Age: 61 ± 1.0 years</td>
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<tr>
<td></td>
<td></td>
<td>BMI: 29.0 ± 0.7 kg/m²</td>
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<tr>
<td><strong>Hosseinpour-Niazi, S. et al.</strong></td>
<td>Randomized crossover</td>
<td>n=31</td>
<td>Legume-free TLC diet</td>
<td>Fasted blood glucose</td>
<td>Fasted blood glucose was lower from baseline to week 8 in the legume TLC diet</td>
</tr>
<tr>
<td></td>
<td>2 treatments</td>
<td>Men and women with type 2 diabetes</td>
<td>Legume-based TLC diet (6 servings or 12 cups of legumes including lentils, chickpeas and beans a week)</td>
<td>Fasted serum insulin</td>
<td>Fasted serum insulin was lower from baseline to week 8 in the legume TLC diet</td>
</tr>
<tr>
<td></td>
<td>20 weeks (8 weeks of diet #1, washout, 8 weeks of diet #2)</td>
<td>Age: 58.1 ± 6.0 years</td>
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<td></td>
<td></td>
<td>BMI: 27.7 ± 0.6 kg/m²</td>
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<td></td>
<td>4-week washout</td>
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<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Participant Characteristics</td>
<td>Study Treatment</td>
<td>Glycemic Endpoints</td>
<td>Significant Effects of Lentil Treatment</td>
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<tr>
<td>Form: Whole lentils (and other legumes)</td>
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<tr>
<td><strong>Shams. H. et al.</strong> Effects of cooked lentils on glycemic control and blood lipids of patients with type 2 diabetes. ARYA Atheroscler. 2008; 4(1): 1-5.</td>
<td>• Randomized crossover</td>
<td>• n=30</td>
<td>• Normal diet with the restriction of legume intake</td>
<td>• Fasted plasma glucose</td>
<td>• Fasted plasma glucose was lower from baseline to week 6 with the lentil diet, compared to the legume-free diet</td>
</tr>
<tr>
<td>Form: Whole lentils at breakfast</td>
<td>• 2 treatments</td>
<td>• Men and women with type 2 diabetes</td>
<td>• Normal diet plus 50 g of cooked lentils and 6 g of canola oil (in substitution of 30 g of bread and 20 g of cheese) at breakfast</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>• 15 weeks (6 weeks of diet #1, washout, 6 weeks of diet #2)</td>
<td>• Age: 50.2 ± 3.8 years</td>
<td>• BMI: 28.9 ± 4.1 kg/m²</td>
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<tr>
<td></td>
<td>• 3-week washout</td>
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Appendix G: University of Guelph Research Ethics Board Certificate

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

APPROVAL PERIOD: November 17, 2016
EXPIRY DATE: November 17, 2017
REB: NPES
REB NUMBER: 16AU001
TYPE OF REVIEW: Full Board
PRINCIPAL INVESTIGATOR: Duncan, Alison (amduncan@uoguelph.ca)
DEPARTMENT: Human Health & Nutritional Sciences
SPONSOR(S): Saskatchewan Pulse Growers
TITLE OF PROJECT: Lentils Minimum Effective Dose Study

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: L. Vallis
Date: November 17, 2016

L. Vallis
Chair, Research Ethics Board-NPES
Appendix H: University of Guelph Biohazardous Materials Certificate

UNIVERSITY OF GUELPH

BIOSAFETY COMMITTEE

BIOHAZARD PERMIT

PRINCIPAL INVESTIGATOR: ALISON DUNCAN

DEPARTMENT: HUMAN HEALTH AND NUTRITIONAL SCIENCES

TITLE OF PROJECT:
The Lentils Minimum Effective Dose Study: The effects of acute consumption of different serving sizes of lentils on post-prandial blood glucose and insulin in healthy adults.

NUMBER: H-254-13-18-11

LOCATION: Bldg. 88, Rm. FS143; Bldg. 70, Rm. 302

APPROVED FOR THE PERIOD: 2016 October 26 TO 2018 November 30

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

Approved:
Chair, Biosafety Committee

Approved:
University Biosafety Officer

Date: Oct 26, 2016

Date: November 23, 2016

(Amended)
Appendix I: Recruitment Poster

LMED Study
Lentils Minimum Effective Dose Study

*Males and females 18-40 years old are needed for a nutrition study on the effects of consuming varying doses of lentils on type 2 diabetes risk*

This study will involve:

- Two screening visits
- Six 2.5-hr morning study visits over a 6-wk period, which will involve:
  - Consuming a small amount of lentils, corn, macaroni, white potato or white rice,
  - Having periodic finger prick blood samples 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#16AU001)

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

lmed@uoguelph.ca or 519-824-4120 x58081
Appendix J: Screening-1 Questionnaire

LMED Study REB#16AU001 Researcher: __________ Date: _______ Time: ________  
Participant Screening ID: __________

**The Lentils Minimum Effective Dose Study**

The effects of acute consumption of lentils in varying doses on post-prandial blood glucose and insulin in healthy adults

**Screening-1 Questionnaire**

First name of caller: ___________  Sex: _____

Contact info: Record in Confidential Participant List _____  Best way to communicate: ______

1. How did you hear about the study? ______________________

2. What is your date of birth (month/year)? ________

3. How tall are you? ___________

4. How much do you weigh? _____   BMI: ________ kg/m²

5. Have you recently experienced significant weight loss/gain (>4kg in past 3months)?  YES  NO

6. Do you smoke?  YES  NO

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

8. Are you a shift worker?  YES  NO

9. Do you have any medical conditions?  YES  NO
   a. If YES, please describe ____________________________

10. Are you currently taking any medications or NHPs (probiotics, fibre supp., etc)?  YES  NO
    If YES, what are they?

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

11. Do you have any food or other allergies?  YES  NO
    a. If YES, please describe ____________________________

12. Do you consume pulses on a weekly basis?  YES  NO
    a. If YES, how many servings do you consume ___________(1 serving=½ cup)
13. Do you consume alcohol?  
   YES  NO  
   a. If YES, how many drinks per week? _____ How many typically in one sitting? _____  
      (1 drink = 12oz beer, 5oz wine, 1.5oz hard liquor)  
14. Are you an elite athlete/involved in regular intense physical activity?  
   YES  NO  
15. Are you comfortable with having a researcher prick your finger to provide blood samples?  
   YES  NO  
16. The study visits are in the morning after a 10-12 hour fast, would this be OK?  
   YES  NO  
17. As part of this study you will be required to visit the University of Guelph for  
   approximately 2.5 hours on 6 different occasions, over a period of 6 weeks.  
   The study visits will occur in the morning hours and occur roughly once a week.  
   Can your schedule accommodate these visits?  
   YES  NO  

If caller meets the study eligibility requirements, describe the study to them and answer any questions  
they have. If they are still interested in the study, set up a Screening-2 visit where they will fill out a more  
detailed questionnaire, body measurements, and learn more about the study. Arrange to send potential  
participant a map to the HNRU and provide information about parking at the meters.  
If the caller does not meet the eligibility requirements, remind them that the data collected today will be  
destroyed according to protocol.  

Study Description: For the Lentils Minimum effective Dose study we want to compare your blood levels of  
glucose and insulin after you consume a meal of lentils, macaroni, rice, potato, or corn in either ½ or ¼ cup  
servings. For this, we will have you come in for each visit fasted (no food/drink except water for 10-12 hours)  
where we will take a blood sample by finger prick, and you will then eat whichever treatment meal you are  
assigned that day. Afterwards, we will take blood samples by finger prick at 7 different time points over 2  
hours. The study also includes body weight measurements and short questionnaires throughout the 2-hour  
period. You will be provided with some snacks before leaving.  

Throughout the study we will ask you to maintain your typical lifestyle. This means maintaining your usual  
diet, physical activity and natural health product routines, unless your physician tells you otherwise, and then  
please inform us.  

Participant Screening ID: ________  

HNRU Directions:  
- Next to the Animal Science and Nutrition building (ANNU)  
- In the Food Science building – walk down McGilvray St. (between Food Science and Pathobiology  
   buildings)  
- HNRU sign  
- Head through 3 doorways  
- Parking meters along McGilvray Street and behind Food Science building
Appendix K: Screening-2 Consent Form

CONSENT TO PARTICIPATE IN RESEARCH

The Lentils Minimum Effective Dose Study
The effect of acute consumption of different serving sizes of lentils on postprandial blood glucose and insulin in healthy adults.

Screening-2 Study Visit

INTRODUCTION
You are being asked to participate in screening for a research study directed by Professor Alison Duncan of the Department of Human Health and Nutritional Sciences (HHNS). Results of this research will contribute to the thesis of University of Guelph MSc. student Brittany MacPherson and the research activities of HHNS M.Sc. and B.Sc. students at the University of Guelph. This research is funded by Saskatchewan Pulse Growers in collaboration with Agriculture and Agri-food Canada. Please note that the study sponsor has a commercial interest in research showing that lentils can improve health.

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

Brittany MacPherson, BSc.
University of Guelph Study Coordinator
MSc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or Email: bmacpher@uoguelph.ca

Wesley Newton, BSc.
University of Guelph Study Coordinator
MSc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or Email: wnewton@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

RESEARCH PURPOSE
The overall purpose of this research is to explore the ability of lentils to reduce risk of type 2 diabetes, which is a disease that is occurring more and more frequently in people of all ages. Changes in eating habits can provide inexpensive and convenient ways to control blood sugar and reduce the risk of developing type 2 diabetes and one possible way to do this is by consuming pulses such as lentils, peas, beans and chickpeas.

The purpose of this study is to determine the effects of lentils in two varied doses to determine which dose is more effective at controlling blood sugar and insulin levels.
directly after consumption. This information will improve our understanding of the role of lentils in type 2 diabetes management and prevention, as well as helping formulate new food products with lentils.

This study will examine 2 varieties of lentils consumed in 2 serving sizes (½ cup and ¼ cup) and compare them to 4 starchy foods including, potato, corn, macaroni and white rice, for a total of 6 study visits. A total of 18 participants will consume ½ cup serving size of the study treatments and a total of 24 participants will consume ¼ cup serving size of the study treatments. At each study visit, participants’ blood will be sampled by finger prick before and periodically for 2 hours after consuming the study treatment. Blood samples will be analyzed for glucose and insulin.

Before this study is started, participants must be fully screened to ensure they meet the study criteria. This Screening-2 Study Visit is part of this process and will include a screening questionnaire and body measurements, which are all described below.

**STUDY PROCEDURES**
This Screening-2 Study Visit will take approximately 30 minutes and 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). If you choose to volunteer to participate, you would be asked to do the following:

- In a private area, have your height, body weight and blood pressure measured.
- Complete the Screening-2 questionnaire that will gather information about your medical history, dietary habits and lifestyle habits.
- Learn more about the study and have your questions answered.

**POTENTIAL RISKS AND DISCOMFORTS**
Every effort to ensure your comfort and safety will be made during this screening. The following describes how we will work to minimize potential discomfort:

- 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. Screening ID numbers will be assigned to all participants and names will not be included on any study forms that include study data.
- In the unlikely event of a study-related injury, study staff from the University of Guelph will engage appropriate emergency response to assist in your care.

**POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY**
If you take part in this screening step, you will have the benefit of gaining experience in 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 helpful recommendations for pulse consumption as well as the development of new food products that aid in reducing the risk of type 2 diabetes.

**PAYMENT FOR PARTICIPATION**
You will not receive any compensation for participating in this Screening-2 Study Visit.

**COSTS FOR PARTICIPATION**
There is no direct cost for participating in this Screening-2 Study Visit. However, you will be responsible for covering any costs related to ensuring you are able to attend your scheduled study visits (i.e. gas money, parking fees, public transportation fees, childcare fees, etc.).

**CONFIDENTIALITY**
Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. The only individuals who will have access to identifying data will be the study coordinator: Prof. Alison Duncan, and the graduate student study investigators: Brittany MacPherson and Wesley Newton. All participants will be assigned a screening ID number which will be used on all study documents. Your name will never be used in communicating any aspect of the study. Records will be kept on a password-protected desktop computer and/or in a locked file cabinet in Prof. Duncan’s lab at the University of Guelph. 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 and blood samples, will be stored until after publication of the study, in accordance with the guidelines. If you are not eligible for this study, your screening data will be destroyed to maintain confidentiality.

If requested, direct access to your research records for this study will be granted to study monitors, auditors, the University of Guelph Research Ethics Board, and regulatory authorities for the verification of study procedures and/or data. Your confidentiality as a study participant will not be violated during this process, to the extent permitted by applicable laws and regulations. By signing this written study 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 participate in this screening process, 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. The investigator may withdraw you from this research if it becomes necessary to do 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 or willingness to stay in this study. You will be given a copy of this consent form.

RIGHTS OF RESEARCH PARTICIPANTS
This project has been reviewed by the Research Ethics Board for compliance with federal guidelines for research involving human participants. If you have any questions regarding your rights and welfare as a research participant in this study (REB#16AU001) please contact: Director, Research Ethics; University of Guelph at reb@uoguelph.ca; 519-824-4120 ext 56606. You do not waive any legal rights by agreeing to take part in this study.

SIGNATURE OF RESEARCH PARTICIPANT
I have read the information provided for the study “The Lentils Minimum Effective Dose Study: The effect of acute consumption of different serving sizes of lentils on postprandial blood glucose and insulin in healthy adults." Screening-2 Study Visit" as described herein. My questions have been answered to my satisfaction, and I agree to participate. I have been given a copy of this form.

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

LMED Study  Participant Screening ID: __________
REB#16AU001  Researcher: __________  Date: ________  Time: ________

Lentils Minimum Effective Dose Study
The effects of acute consumption of varying doses of lentils on post-prandial blood glucose and insulin levels in healthy adults

Screening 2 Questionnaire

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.

DIET-RELATED QUESTIONS:

18. Do you have any food or other allergies?  YES NO
   a. If YES, please describe ____________________________________________

19. Are you on a special diet?  YES NO
   a. If YES, please describe ____________________________________________

20. Do you consume pulses regularly?  YES NO
   a. If YES, how many servings do you consume per week? ________ (1 serving=½ cup)

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

22. Do you consume caffeine (coffee, tea, pop, energy drinks, supplements)? YES NO
   If so, how much per day? __________________

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

24. Completion of brief 24-hour dietary recall (below). 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

Date of Birth: ___________ Age ___________

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

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

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

28. Are you a shift worker?  YES  NO

29. Do you have, or have had, any of the following health conditions?

<table>
<thead>
<tr>
<th>Health Condition</th>
<th>Currently Have</th>
<th>Have Had in Past</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
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<tr>
<td>Type 1 Diabetes</td>
<td></td>
<td></td>
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<tr>
<td>Type 2 Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired Liver Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired Kidney Function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celiac Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crohn’s Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative Colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritable Bowel Syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression or Anxiety</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

30. Are there any other health conditions you have or have had?  YES  NO
   If YES, what are they? ___________________________________________________________________

31. Are you currently taking any prescription medications?  YES  NO
   If YES, please complete the following table:

<table>
<thead>
<tr>
<th>Medication</th>
<th>Purpose</th>
<th>Start Date (month/year)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>
32. Have you taken antibiotics in the last 3 months?  
   If YES, when did you stop using them? ________________  
   YES  NO

33. Have you had any surgeries or medical events in the last 3 months?  
   If YES, what are they? ________________  
   YES  NO

34. Do you use any over-the-counter medications, including pain relievers (i.e. Tylenol)?  
   If YES, what are they, and how often? ________________  
   YES  NO

35. Do you take vitamin, mineral or herbal supplements (natural health products)?  
   YES  NO  
   If YES, please complete the following table:

<table>
<thead>
<tr>
<th>Natural Health Product</th>
<th>Purpose</th>
<th>Start Date (month/year)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

36. 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

37. Do you exercise?  
   Can you describe your exercise? ________________  
   How often and how intense? ________________  
   YES  NO

38. 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: ____________________________________________

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

40. Do you have any issues with having your blood taken by finger prick?  
   YES  NO
41. The study visits are in the morning after a 10-12 hour fast, would this be OK?  YES  NO

42. As part of this study you will be required to visit the University of Guelph for approximately 2.5 hours on 6 different occasions over a period of 6 weeks. The study visits will occur in the morning hours and occur roughly once a week. Can your schedule accommodate these visits?  YES  NO

43. Are there particular weekdays you prefer to have study visits?

MONDAY  TUESDAY  WEDNESDAY  THURSDAY  FRIDAY

44. Is there a particular weekday that you absolutely could NOT have a study visit?

MONDAY  TUESDAY  WEDNESDAY  THURSDAY  FRIDAY

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

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

If YES, please expand briefly __________________________________________

____________________________________________________________________

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

CONSENT TO PARTICIPATE IN RESEARCH

The Lentils: Minimum Effective Dose Study
The effect of acute consumption of different serving sizes of lentils on postprandial blood glucose and insulin in healthy adults.

Screening-3 Study Visit

INTRODUCTION
You are being asked to participate in screening for a research study directed by Professor Alison Duncan of the Department of Human Health and Nutritional Sciences (HHNS). Results of this research will contribute to the thesis of University of Guelph MSc. student Brittany MacPherson and the research activities of HHNS M.Sc. and B.Sc. students at the University of Guelph. This research is funded by Saskatchewan Pulse Growers in collaboration with Agriculture and Agri-food Canada. Please note that the study sponsor has a commercial interest in research showing that lentils can improve health.

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

Brittany MacPherson, 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: bmacpher@uoguelph.ca

Wesley Newton, BSc
University of Guelph Study Coordinator
MSc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or Email: wnewton@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: amducan@uoguelph.ca

RESEARCH PURPOSE
The overall purpose of this research is to explore the ability of lentils to reduce risk of type 2 diabetes, which is a disease that is occurring more and more frequently in people of all ages. Changes in eating habits can provide inexpensive and convenient ways to control blood sugar and reduce the risk of developing type 2 diabetes and one possible way to do this is by consuming pulses such as lentils, peas, beans and chickpeas.

The purpose of this study is to determine the effects of lentils in two varied doses to determine which dose is more effective at controlling blood sugar and insulin levels.
directly after consumption. This information will improve our understanding of the role of lentils in type 2 diabetes management and prevention, as well as helping formulate new food products with lentils.

This study will examine 2 varieties of lentils consumed in 2 serving sizes (½ cup and ¼ cup) and compare them to 4 starchy foods including, potato, corn, macaroni and white rice, for a total of 6 study visits. A total of 18 participants will consume ½ cup serving size of the study treatments and a total of 24 participants will consume ¼ cup serving size of the study treatments. At each study visit, participants’ blood will be sampled by finger prick before and periodically for 2 hours after consuming the study treatment. Blood samples will be analyzed for glucose and insulin.

Before this study is started, participants must be fully screened to ensure they meet the study criteria. This Screening-3 Study Visit is part of this process and will include an oral glucose tolerance test and body measurements, which are all described below.

**STUDY PROCEDURES**

This Screening-3 Study Visit will take approximately 2.5 hours 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). If you choose to volunteer to participate, you would be asked to do the following:

- Arrive to the study visit having avoided any food and beverages (except water which is encouraged) for 12 hours prior, and having avoided alcohol, over-the-counter medication, pulse consumption, and unusual/vigorous exercise for 24 hours prior.

- In a private area, have your body weight, waist circumference, and blood pressure measured.

- Have a fasted blood sample collected by finger prick:
  - In a private area, your hand will be wrapped in an electric blanket to warm it up and improve circulation.
  - An alcohol wipe will be used to sterilize your finger tip.
  - A fully-trained researcher will prick one of your fingers with a finger prick device that has a small retractable blade. The device will be lightly pressed against your finger to activate and the blade will not be visible at any point during the procedure. This type of procedure is used on a daily basis by people living with diabetes and checking their blood glucose.
  - Your finger will be pressed gently to allow a small amount of your blood to be collected into a small tube.
Gauze will be placed on your finger tip and you will be asked to apply pressure and your finger will be wrapped with a bandage.

The blood sample will be analyzed for blood glucose.

To ensure your 2-hour blood glucose is in a healthy range, we will next conduct an Oral Glucose Tolerance Test (OGTT), which is a commonly done test to screen for type 2 diabetes. This will involve the following:

You will consume a chilled Trutol orange beverage (296 mL). This beverage tastes like orange pop and contains the following ingredients: Dextrose, Water, Citric Acid, Sodium benzoate, Natural orange flavouring, FD&C Yellow No.6 and Red No.40

You will stay in the Human Nutraceutical Research Unit for 2 hours during which time you can watch TV, work on your computer, or read.

At the 2-hour time point, we will collect a second blood sample by finger-prick which will be analyzed for blood glucose.

You will immediately be provided with snacks as you will be hungry. Snack choices will include juice, cheese, cookies, fruit cups and granola bars.

During this study visit, you can ask any questions you have to learn more about the study and ensure you are fully aware of all the study procedures you would be involved in.

POTENTIAL RISKS AND DISCOMFORTS

The following summarizes the potential risks and how we will work to minimize them:

All body measurements will be completed by a trained study coordinator in a private area and according to set standard operating procedures.

A fully trained study researcher will collect blood samples by finger prick. There is a chance that this process could cause you some momentary discomfort like a prick of a needle and your finger tip may be bruised and/or feel tender the next day. Consuming plenty of water the night before and the morning of (up to one glass one hour prior to the study visit) which can facilitate blood sampling and will minimize bruising.

You will experience hunger during the 2 hours since all you would have consumed is the Trutol orange beverage. We will provide you with snacks immediately after the 2 hours.

All information obtained about you will be collected privately and kept confidential. Screening ID numbers will be assigned to all participants and names will not be included on any study forms that include study data.
• In the unlikely event of a study-related injury, study staff from the University of Guelph will engage appropriate emergency response to assist in your care.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY
If you take part in this screening step, you will have the benefit of gaining experience in 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 helpful recommendations for pulse consumption as well as the development of new food products that aid in reducing the risk of type 2 diabetes.

PAYMENT FOR PARTICIPATION
You will not receive any payment for participation in this Screening-3 Study Visit.

COSTS FOR PARTICIPATION
There is no direct cost for participating in this Screening-3 Study Visit. However, you will be responsible for covering any costs related to ensuring you are able to attend your scheduled study visits (i.e. gas money, parking fees, public transportation fees, child care fees, etc.).

CONFIDENTIALITY
Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. The only individuals who will have access to identifying data will be the study coordinator, Prof. Alison Duncan, and the graduate study investigators: Brittany MacPherson and Wesley Newton. All participants will be assigned a screening ID number which will be used on all study documents. Your name will never be used in communicating any aspect of the study. Records will be kept on a password-protected desktop computer and/or in a locked file cabinet in Prof. Duncan’s lab at the University of Guelph. 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 and blood samples will be stored until after publication of the study, in accordance with the guidelines. If you are not eligible for this study, your screening data will be destroyed to maintain confidentiality.

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

PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you don’t want to answer. The investigator may withdraw you from this
research if it becomes necessary to do 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 or willingness to stay in this study. You will be given a copy of this consent form.

**RIGHTS OF RESEARCH PARTICIPANTS**

This project has been reviewed by the Research Ethics Board for compliance with federal guidelines for research involving human participants. If you have any questions regarding your rights and welfare as a research participant in this study (REB#16AU001) please contact: Director, Research Ethics; University of Guelph at reb@uoguelph.ca; 519-824-4120 ext 56606. You do not waive any legal rights by agreeing to take part in this study.

**SIGNATURE OF RESEARCH PARTICIPANT**

I have read the information provided for the study “The Lentils Minimum Effective Dose Study: The effect of acute consumption of different serving sizes of lentils on postprandial blood glucose and insulin in healthy adults. “Screening-3 Study Visit” as described herein. My questions have been answered to my satisfaction, and I agree to participate. I have been given a copy of this form.

<table>
<thead>
<tr>
<th>NAME OF PARTICIPANT</th>
<th>SIGNATURE OF PARTICIPANT</th>
<th>DATE</th>
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<tr>
<th>NAME OF WITNESS</th>
<th>SIGNATURE OF WITNESS</th>
<th>DATE</th>
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</table>
Appendix N: Study Handbook

The Lentils Minimum Effective Dose Study Handbook

A handbook of essential information to guide you through this study

Human Nutraceutical Research Unit (HNRU)
88 McGilvray Street, Guelph, Ontario, N1G 2W1
Food Science Addition, Room 144, University of Guelph
Phone: 519-824-4120 x53925
I. Study Welcome

Welcome to the Lentils Minimum Effective Dose (LMED) Study! We are very grateful that you have joined our team and are willing to dedicate your time in the following weeks to participate in this study.

Your participation in this study will be making an important contribution to advancing knowledge in nutritional science about the effects of varying serving sizes of lentils on blood glucose.

This handbook is a great one-stop resource that provides important information about the various aspects of our study, and you are encouraged to bring it with you to all of your Study Visits.

If at any time you have questions or concerns, please do not hesitate to contact us. We have included phone numbers and email addresses in the ‘Contact Us’ section of this handbook. We will work hard to make your experience as worthwhile and rewarding as possible.

We thank you again for participating in the LMed Study. We could not carry out this research without you!

Sincerely,

Alison M. Duncan, Ph.D., R.D.
Professor
Department of Human Health and Nutritional Sciences
University of Guelph
Ph: 519-824-4120 ext. 53416
amduncan@uoguelph.ca

Brittany MacPherson, B.Sc.
M.Sc Candidate
Department of Human Health and Nutritional Sciences
University of Guelph
Ph: 519-824-4120 ext. 58081
bmacpher@uoguelph.ca

Wesley Newton, B.Sc
M.Sc Candidate
Department of Human Health and Nutritional Sciences
University of Guelph
Ph: 519-824-4120 ext. 58081
wnewton@uoguelph.ca
II. Contact Information

All Study Visits will take place at the:
Human Nutraceutical Research Unit (HNRU)
88 McGilvray Street, Guelph, Ontario, N1G 2W1
Food Science Addition, Room 144, University of Guelph
Phone: 519-824-4120 x53925

The HNRU is part of the Department of Human Health and Nutritional Sciences at the University of Guelph. The HNRU is set up to run human studies designed to evaluate the health-related effects of value-added foods and natural health products. The HNRU runs several human studies for the natural health product industry but also allows for research to be conducted by the faculty from the Department of Human Health and Nutritional Sciences at the University of Guelph.

The LMED Study research team consists of the following people who are available for you to contact at any time during the study. The study coordinators will be checking the voicemails and emails daily and will get back to you as soon as possible.

<table>
<thead>
<tr>
<th>Name</th>
<th>Role</th>
<th>Email</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alison Duncan, Ph.D., R.D. (Professor)</td>
<td>Study Director</td>
<td><a href="mailto:amduncan@uoguelph.ca">amduncan@uoguelph.ca</a></td>
<td>519-824-4120 x53416</td>
</tr>
<tr>
<td>Brittany MacPherson, B.Sc. (M.Sc. Candidate)</td>
<td>Study Coordinator</td>
<td><a href="mailto:bmacpher@uoguelph.ca">bmacpher@uoguelph.ca</a></td>
<td>519-824-4120 x58081</td>
</tr>
<tr>
<td>Wesley Newton, (M.Sc Candidate)</td>
<td>Study Coordinator</td>
<td><a href="mailto:wnewton@uoguelph.ca">wnewton@uoguelph.ca</a></td>
<td>519-824-4120 x58081</td>
</tr>
<tr>
<td>B.Sc Student</td>
<td>Study Investigator</td>
<td>TBD.</td>
<td></td>
</tr>
</tbody>
</table>
III. Research Summary

The LMED Study is examining the acute glycemic response of two common varieties of lentils, in ¼ or ½ cup serving sizes, with 4 matched serving sizes of various starchy meals. The study has been approved by the Research and Ethics Board.

This human intervention study will be carried out in a randomized crossover design. This means that as a participant, you will consume 2 lentil meals, and 4 starchy meals within your 6-scheduled Study Visits. The four starchy meals include: corn, macaroni, white rice and potato. The order in which you consume each type of lentil and control in this study is randomized. An example of the various study treatments would be:

<table>
<thead>
<tr>
<th>Study Visit 1</th>
<th>Study Visit 2</th>
<th>Study Visit 3</th>
<th>Study Visit 4</th>
<th>Study Visit 5</th>
<th>Study Visit 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Rice</td>
<td>Red Lentil</td>
<td>Macaroni</td>
<td>Potato</td>
<td>Green Lentil</td>
<td>Corn</td>
</tr>
</tbody>
</table>

We are aiming for 18 participants to complete this study for the ½ cup dose and 24 participants for the ¼ cup serving. Study measurements will body measurements (height, body weight, waist circumference, blood pressure, heart rate) and blood measurements (glucose, insulin).

Overall, this research will contribute to the scientific literature in the form of published papers, a M.Sc. thesis, and M.Sc. coursework and B.Sc. research projects. This research is done in collaboration with Agriculture and Agri-food Canada under the direction of Dr. Dan Ramdath. Results of this research may be submitted to Health Canada providing substantiation for a proposed functional health claim that focuses on lentils.

We truly appreciate your participation in this study. Undoubtedly, the commitment that you make to this study will affect the scientific quality of the data that will be published, in addition to affecting future research in this area of nutrition and health. Thank you for joining our team to learn more about dietary strategies that can be used to help reduce the risk of chronic disease and improve the health of Canadians!
IV. Study Treatments

Over the course of this study, you will consume 6 study treatments. The study treatments include either two types of lentils: small red split lentil, or the small green lentil, which are common Canadian lentil varieties or four starchy foods including corn, macaroni, white potato and white rice. Each meal will be served with a glass of 250mL (1 cup) of water. Water can also be consumed freely in the final hour of your Study Visit.

Your study treatments will be prepared in the HNRU research kitchen, and served warm until you are ready to begin eating. Each study meal will be served on a tray accompanied by a glass of water (250mL), a fork and spoon for your convenience.

The study treatment consumption will go as follows:

- Following your fasted blood finger prick sample, you will continue to warm your hand as one of our study coordinators brings you your meal.
- As soon as you take your first bite, a timer begins that will count down from 15 minutes.
  - You will be asked to finish your meal in 10 minutes, or, when the timer indicates there are 5 minutes remaining.
  - This will allow enough time to relax and warm your hand in preparation for your next finger prick.

Here you will see a composition breakdown of all of the study treatments that you will be consuming throughout the study. This table shows the nutritional information for ½ cup serving size (so it will be half of this for the ¼ cup serving size).

<table>
<thead>
<tr>
<th></th>
<th>Corn (kcal)</th>
<th>White Rice (kcal)</th>
<th>Macaroni (kcal)</th>
<th>White Potato (kcal)</th>
<th>Lentils Green (kcal)</th>
<th>Lentils Red (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>92.0</td>
<td>102.0</td>
<td>104.0</td>
<td>106.0</td>
<td>162.0</td>
<td>160.0</td>
</tr>
<tr>
<td>Protein</td>
<td>2.6</td>
<td>2.2</td>
<td>3.8</td>
<td>2.6</td>
<td>13.0</td>
<td>13.6</td>
</tr>
<tr>
<td>Fat</td>
<td>1.0</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>18.2</td>
<td>22.2</td>
<td>20.4</td>
<td>24.4</td>
<td>31.0</td>
<td>30.2</td>
</tr>
<tr>
<td>Dietary Fibre</td>
<td>1.8</td>
<td>0.4</td>
<td>1.2</td>
<td>2.0</td>
<td>10.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Note: Values obtained from the USDA nutrient database; kcal=kilocalories.
All measurements are taken from consuming ½ cup of each food.
V. Study Visit Activities

You can complete the following table with your Study Visit dates and times.

<table>
<thead>
<tr>
<th>Study Visit</th>
<th>Study Visit Date and Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

All Study Visits will be held at the HNRU located at 88 McGilvray Street in room 144 of the Food Science Building at the University of Guelph (phone 519-824-4120 x53925).

Prior to each Study Visit, please remember to:

- Consume your pre-study dinner (your dinner should be consistent before each Study Visit).
- Avoid all food and beverages except water for 12 hours prior.
- Consume plenty of water the night before and morning of as it helps with blood sampling.
- Avoid alcohol for 24 hours.
- Avoid unusual or strenuous physical activity for 24 hours.
- Avoid over-the-counter medication for 24 hours.
- Avoid consuming pulses for 24 hours.

For the entire study, please remember to:

- Maintain your usual lifestyle, dietary and exercise habits.
- Do not start any new natural health products (NHPs)/dietary supplements, unless instructed to do so by your healthcare provider and then please inform the study coordinators.
- Inform the study coordinators if any of your medications change.
- Note that if you have to go on antibiotics for any reason please inform the study coordinators.
Summary of Study Visit Activities
The following detailed summary inventories what should be happening on every study day. Use this resource to remind yourself what you should be doing, or should bring, for any given Study Visit.

Study Visits 1-6 (2.5 hours):
- Arrive at the HNRU at your pre-scheduled appointment date and time.
- Report any changes in medications or health issues, and ask about any questions or concerns you may have.
- Have your body weight measured.
- Provide a fasted finger prick blood sample.
- Consume your study treatment (one of two lentil meals or four starchy meals) and 250mL of water within 10 minutes.
- Provide 6 periodic postprandial finger prick blood samples over 2 hours while you relax (you can work on your computer, read, etc.).
- Upon completion of all finger prick blood samples, remain seated to have blood pressure and heart rate measured.
- On Study Visit 6 (last one), complete a study exit questionnaire.
- Have snacks before you leave.

VI. Study Measurements

During the study you will have your body weight, waist circumference, blood pressure and heart rate measured, in addition to providing finger prick blood samples. You will also be asked to complete a sensory questionnaire the study days you consume lentils.

All measurements will be conducted by trained study coordinators in accordance with standard operating procedures established by the Department of Human Health and Nutritional Sciences at the University of Guelph, and the HNRU. The following describes how the various measurements will be taken.

Body Weight
Your body weight will be measured at every Study Visit. You will be asked to remove your shoes and pocket contents and then simply stand on a digital scale. This will occur in a private area. We ask that you maintain your typical dietary and exercise habits during this study and that you make no special attempts to gain or lose weight over the course of this study.

Blood Pressure and Heart Rate
Your blood pressure and heart rate will be measured at every Study Visit. The procedure will involve you resting quietly for 5 minutes. Your upper dominant arm will then be wrapped with a cuff and a digital measurement of your blood pressure and heart rate will be recorded. The measurements take only seconds to complete. The measurement will be repeated 2 times and the average recorded.
Finger Prick Blood Samples
You will have both fasted and post-prandial finger prick blood samples taken at all 6 Study Visits by a trained study coordinator. You will have an initial fasted finger prick blood sample taken first thing in the morning. Following the consumption of the study treatment and 250mL of water, you will then have a finger prick blood sample taken at 15, 30, 45, 60, 90, and 120 minutes. The finger prick blood collection method is widely used in clinical research and all study coordinators have experience in performing finger pricks. The initial prick will feel like a slight pinch and may cause slight discomfort. In the days following, you may experience slight sensitivity on the fingers that have been pricked.

VII. Compensation and Feedback
Reimbursement for study participation will be a cheque in the amount of $400 that will be provided to you upon completion of your final Study Visit #6. A study coordinator will provide you with a form to sign, confirming that you have received your compensation. Following completion of the study, you will be sent a confidential summary of your individual study results.
Appendix O: Study Consent Form

CONSENT TO PARTICIPATE IN RESEARCH

The Lentils Minimum Effective Dose Study
The effect of acute consumption of different serving sizes of lentils on postprandial blood glucose and insulin in healthy adults.

Study Consent Form

INTRODUCTION
You are being asked to participate in a research study directed by Professors Alison Duncan of the Department of Human Health and Nutritional Sciences (HHNS). The results of this research will contribute to the thesis of University of Guelph M.Sc. student Brittany MacPherson and to the research activities of HHNS M.Sc. and B.Sc. students at the University of Guelph. Since the lentils are already available in the market, there is no likelihood of commercialization of the study results. This research is funded by the Saskatchewan Pulse Growers and in collaboration with Agriculture and Agri-food Canada.

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

Brittany MacPherson, 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: bmacpher@uoguelph.ca

Wesley Newton, BSc.
University of Guelph Study Coordinator
MSc. Candidate, Dept. of Human Health & Nutritional Sciences, University of Guelph
Phone: 519-824-4120 x58081 or Email: wnewton@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

RESEARCH PURPOSE
The overall purpose of this research is to explore the ability of lentils to reduce risk of type 2 diabetes, which is a disease that is occurring more and more frequently in people of all ages. Changes in eating habits can provide inexpensive and convenient ways to control blood sugar and reduce the risk of developing type 2 diabetes and one possible way to do this is by consuming pulses such as lentils, peas, beans and chickpeas.

The purpose of this study is to determine the effects of lentils in two varied doses to determine which dose is more effective at controlling blood sugar and insulin levels.
directly after consumption. This information will improve our understanding of the role of lentils in type 2 diabetes management and prevention, as well as helping formulate new food products with lentils.

This study will examine 2 varieties of lentils consumed in 2 serving sizes (½ cup and ¼ cup) and compare them to 4 starchy foods including, potato, corn, macaroni and white rice, for a total of 6 study visits. A total of 18 participants will consume ½ cup serving size of the study treatments and a total of 24 participants will consume ¼ cup serving size of the study treatments. At each study visit, participants’ blood will be sampled by finger prick before and periodically for 2 hours after consuming the study treatment. Blood samples will be analyzed for glucose and insulin.

**STUDY PROCEDURES**

This study involves attending a total of 6 study visits that 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 study visits will each last 2.5 hours and will be separated by a break of 3-7 days. Throughout the duration of the study you will be asked to maintain your normal eating and physical activity routines. You will be asked not to start any new dietary approaches and to not start any new natural health products (unless instructed by a physician). The following describes in more detail what will happen at each of the 6 study visits.

- The day before each study visit, you will need to consume a dinner of your choice that you will also consume the day before each study visit throughout the study to be consistent. After consumption of your dinner meal, you should drink water, but should not consume any other drinks or any foods to ensure you are fasted for 10-12 hours before your study visit. Water is encouraged in the evening and especially in the morning of your study visit, as it helps the blood sampling. We also ask that you avoid alcohol, over-the-counter medication, unusual/strenuous exercise and consuming pulses for 24 hours prior to your study visit.

- On each study visit, you will have your body weight and blood pressure measured by a trained study coordinator in a private area.

- You will have a fasting blood sample collected by finger prick. This will involve:
  - In a private area, your hand will be wrapped in an electric blanket to warm it up and improve circulation.

  - An alcohol wipe will be used to sterilize your finger tip.

  - A fully-trained researcher will prick one of your fingers with a finger prick device that has a small retractable blade. The device will be lightly pressed against your finger to activate and the blade will not be visible at any point during the procedure. This type of procedure is used on a daily basis by people living with diabetes and checking their blood glucose.
Your finger will be pressed gently to allow a small amount of your blood to be collected into a small tube.

Gauze will be placed on your finger tip and you will be asked to apply pressure and your finger will be wrapped with a bandage.

The blood sample will be analyzed for blood glucose.

- You will consume one of the study treatments with 250 mL of water within 10 minutes. The study treatments are all single ingredient foods and will include either a ½ or ¼ cup serving of: lentil variety 1 (Easton small green lentil), lentil variety 2 (small red dehulled lentil) (no preservatives or additives in the lentils), corn (PC frozen; peaches and cream whole kernel corn) macaroni (Italpasta- elbows Tradizionale ingredients: durum wheat semolina, niacin, folic acid, ferrous sulfate, riboflavin, thiamine mononitrate. Allergens: contains wheat, may contain eggs), white potato (Idaho original mashed potato, ingredients: Idaho potatoes, mono and diglycerides, sodium acid pyrophosphate, sodium bisulfite, citric acid. Contains: sulphites) and white rice (Selection long grain white rice, ingredients: long grain white rice. May contain tree nuts and peanuts). All food will be prepared in the HNRU metabolic kitchen. The lentil varieties and white rice will be prepared in a black and decker rice cooker, the corn and macaroni will be cooked on the stove top in a pot, and the white potato will have boiled water from the microwave added to it.

- You will complete a brief sensory questionnaire regarding your overall liking of the study treatment (appearance, taste, aroma, flavour, texture).

- You will then have subsequent blood samples by finger prick (on different sides of different fingers) at 15, 30, 45, 60, 90, and 120 minutes following consumption of the study treatment. A total of 0.3 mL will be collected at each time point. During the 2 hours you will remain seated with minimal activity. 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. All blood samples will be analyzed for glucose and insulin and will be labeled with participant ID number for confidentiality. All samples will be stored in a lab freezer until the results are published.

- After the last blood sample, you will immediately be provided with some snacks including juice, cookies, fruit cups, cheese and granola bars.

- On your last Study Visit (Study Visit 6), you will be asked to complete a brief study exit questionnaire about 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. You also have the option of coming in person to pick up your study cheque rather than having it mailed.

**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). After publication, your individual data will be destroyed. 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
The following summarizes the potential risks and how we will work to minimize them:

- All body measurements will be completed by a trained study coordinator in a private area and according to set standard operating procedures.

- A fully trained study researcher will collect blood samples by finger prick. There is a chance that this process could cause you momentary discomfort like a prick of a needle and your finger tip may be bruised and/or feel tender the next day. Consuming plenty of water the night before and the morning of (up to one glass one hour prior to the study visit) which can facilitate blood sampling and will minimize bruising.

- You will experience hunger during the 2 hours since all you would have consumed is either a ½ or ¼ cup of food. We will provide you with snacks immediately after the 2 hours.

- All information obtained about you will be collected privately and kept confidential. Screening ID numbers will be assigned to all participants and names will not be included on any study forms that include study data.

- In the unlikely event of a study-related injury, study staff from the University of Guelph will engage appropriate emergency response to assist in your care.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY
If you participate in this research; you will have the benefit of gaining experience participating in a research study. You will receive a written summary of your individual study data. 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 different lentils to develop new food items that can aid in diabetes management and prevention.

PAYMENT FOR PARTICIPATION
You will be financially compensated for your time and effort for this study in the total amount of $400 which includes $67 for each of the 6 study visits. If you withdraw from the study before its completion, your compensation will be pro-rated accordingly. You will be asked to sign a document confirming that you have received your payment upon completion of the study however a SIN is not needed.
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, 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. The only individuals who will have access to identifying data will be Prof. Alison Duncan, and the graduate student study investigators Brittany MacPherson and Wesley Newton. All participants will be assigned a screening ID number which will be used on all study documents. Your name will never be used in communicating any aspect of the study. Records will be kept on a password-protected desktop computer and/or in a locked file cabinet in Prof. Duncan's lab. 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 and blood samples will be stored until after publication of the study results, in accordance with the guidelines.

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 it is necessary. 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.

RIGHTS OF RESEARCH PARTICIPANTS
This project has been reviewed by the Research Ethics Board for compliance with federal guidelines for research involving human participants. If you have any questions regarding your rights and welfare as a research participant in this study (REB#16AU001) please contact: Director, Research Ethics; University of Guelph at reb@uoguelph.ca; 519-824-4120 ext 56606. You do not waive any legal rights by agreeing to take part in this study.
SIGNATURE OF RESEARCH PARTICIPANT
I have read the information provided for the study “The Lentils Minimum Effective Dose Study: The effect of acute consumption of different serving sizes of lentils on postprandial blood glucose and insulin in healthy adults” 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>
</table>

<table>
<thead>
<tr>
<th>NAME OF WITNESS</th>
<th>SIGNATURE OF WITNESS</th>
<th>DATE</th>
</tr>
</thead>
</table>
Appendix P: Data Collection Flow Sheet

<table>
<thead>
<tr>
<th>LMed Study Flowsheet</th>
<th>Participant ID: _____</th>
<th>Researcher: ____</th>
<th>Date: ____</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study Day: Ser-1 Ser-2 Ser-3 Orient 1 2 3 4 5 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment: Lentil-Green Lentil-Red Corn Macaroni White Potato White Rice</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Study Visit 1 2 3 4 5 6**

1. Greet the participant, thank them for coming, confirm that they are fasted and ask if they need to go to the bathroom. Take them to Sampling Bay #2 and measure body weight.

2. While still in sampling bay ask them the following questions:

<table>
<thead>
<tr>
<th>Participant ID: _____</th>
<th>Study Visit: _____</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Body Weight (kg)</td>
<td>1 2</td>
<td></td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>1 2</td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>1 2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Have you consumed anything besides water for the past 10-12 hours?</th>
<th>Yes</th>
<th>No</th>
<th>Notes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you consumed any water this morning?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have any health issues to report or any changes in your medications or NHPs?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you avoided alcohol for 24 hours?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you avoided unusual or vigorous physical activity for 24 hours?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you avoided OTC medication for 24 hours?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you avoided consuming pulses for 24 hours?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you had any unusual events recently that might affect your participation (i.e. stress, insomnia, illness)?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How are you feeling today?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you have any questions or concerns?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Seat participant and allow them to heat their hand for at least 5 minutes.
4. Complete fasting finger prick blood sample and record details in the **Blood Collection Table**
5. Serve the participant their study treatment, and complete the following table:
6. Complete post-prandial blood samples and record in the following table:

### Blood Collection Table

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Target Time (clock time)</th>
<th>Actual Time (clock time)</th>
<th>Blood Glucose Statstrip #1 (mmol/L)</th>
<th>Blood Glucose Statstrip #2 (mmol/L)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>___</td>
<td>___</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>60 min</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Process samples according to LMed Blood Sample Processing Instruction Sheet.*

7. After last blood sample have participant relax for about 5 minutes, then measure blood pressure and heart rate (record in above table).

8. Provide participant with their choice of snacks.

9. Before the participant leaves, remind them to:
   - Maintain their habitual lifestyle, dietary and exercise habits, and to avoid any new dietary approaches throughout the entire study.
   - Avoid alcohol, unusual physical activity, over-the-counter medication and consuming pulses 24 hours before their next Study Visit.
   - If it is their last Study Visit 6, provide them with their study cheque and have them sign study payment form.