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Postprandial glycemic response of whole peas and lentils and their flours in adults with type 2 diabetes

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**Postprandial glyceic response of whole peas and lentils and their flours
in adults with type 2 diabetes**

by

Mariel Camacho-Arriola

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Donna Winham, Co-major Professor
Stephanie Clark, Co-major Professor
Joey Talbert

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2020

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DEDICATION

I would like to dedicate this thesis to my mom - my first and favorite educator in my life. You instilled in me an appreciation for education and a continual pursuit of growth opportunities. This is possible because of your support throughout the years. Thank you for your love.

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NOMENCLATURE

T2DM	Type 2 Diabetes Mellitus
GI	Glycemic Index
TDF	Total Dietary Fiber
IDF	Insoluble Dietary Fiber
SDF	Soluble Dietary Fiber
SDS	Slowly Digestible Starch
RDS	Rapidly Digestible Starch
RS	Resistant Starch
IPAQ	International Physical Activity Questionnaire
FFQ	Food Frequency Questionnaire
BMI	Body Mass Index
iAUC	Incremental Area Under the Curve
ANOVA	Analysis of Variance

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ABSTRACT

With an increasing diabetes problem, foods that benefit our health are more important than ever. Whole peas, lentils, and other dry grain pulses yield lower postprandial glycemic responses in adults with type 2 diabetes. Additionally, pea and lentil flours are increasingly used in products to improve nutrition quality and functionality. However, the impact of pulse flours on blood glucose versus the whole form of the pulse requires more research. This 5x5 cross-over clinical trial seeks to address this research gap.

The glycemic effects of 5 treatment meals containing $\frac{1}{2}$ cup whole pulse and dry weight equivalent of flour, or a Glucola control beverage, were tested in adults with type 2 diabetes. Venous blood samples were collected at baseline and at 30, 60, 90, 120, 150, and 180 minutes post-meal consumption and analyzed for glucose concentrations. Significant mean differences when comparing treatment, time, and treatment x time interactions were found, with whole pulses exhibiting better glycemic effects than the control treatment. Responses to pulse flour meals were not always significantly different from the control treatment, but usually different from the whole pulses.

There may be several explanations for this related to nutrition and physiological properties of the pulses. Future research should include investigating the mechanisms behind possible differences between pulses and pulse forms. There should be caution when incorporating pulse flours into products and claiming similar health benefits as whole pulses.

CHAPTER 1. INTRODUCTION

Pulses are a dry staple in several parts of the world, except for the United States. In response to lack of consumption, efforts have been made to promote pulses. Many avenues of promotion focus on the health benefits of pulses, which are impressive. Their high fiber and protein content make them a beneficial food for health conditions, such as type 2 diabetes. This health benefit is one of the most widely recognized for whole pulses.

Since 2005, the Dietary Guidelines for Americans have recommended increased pulse consumption due to their high nutrition content and health benefits. Application-based efforts to increase pulse consumption include using pulse flours in products otherwise conventionally made with traditional grains. As we diversify the uses of pulses by using it in novel forms, it is necessary to consider the implications of nutritional and structural changes to the pulses on the observed health benefits. This research investigates the health benefits of whole pulses and pulse flours as it relates to the glycemic response, a daily concern for adults with type 2 diabetes.

Thesis Goals

The main objective of this research was to determine the glycemic response of whole peas and lentils versus their flour equivalents as part of a meal in adults with type 2 diabetes (T2DM).

Goal 1: Determine the postprandial glycemic response to whole peas and lentils in comparison to flour counterparts.

Goal 2: Use equivalent amount of flours to whole pulses on a dry weight basis based on a standard serving size (1/2 cup).

Goal 3: Match available carbohydrates (CHO) for all treatments.

Goal 4: Approach the project by highlighting connections between the food science and human nutrition disciplines.

Thesis Hypotheses

It was hypothesized that: 1) all pulse treatments would result in a lower postprandial glycemic response than a control treatment; 2) whole pulses would lower the glycemic response more than flours, and 3) there would be no difference when comparing whole peas and whole lentils, and comparing pea flour and lentil flour.

Thesis Organization

To understand the importance of pulses, their processing, and their health benefits associated with T2DM, a literature review provides background and the need for the current research project. An extensive methods section follows and details information on the pulse flours, test procedures, and data analysis. The results from this study are presented in Chapter 4. The thesis closes with a summary of findings, conclusions, and suggestions for future work. Resources used throughout this study are included in the appendices.

CHAPTER 2. REVIEW OF LITERATURE

Pulses

Definition

Pulses are an important food staple and include four main commercial crops in the United States: beans, peas, lentils, and chickpeas (USA Dry Pea & Lentil Council, n.d.). Pulses are members of the Leguminosae or Fabaceae family of plants, which are noted for their high nutrition content as a food, and their ability to fix nitrogen in the soil from a sustainable agriculture perspective. Other common legume crops include soybeans, peanuts, alfalfa, and fresh peas and beans. Pulses are a sub-classification of legumes, that are exclusively harvested for their dry seeds.

The United Nations Food and Agriculture Organization (FAO) declared 2016 the International Year of Pulses to highlight the essential role of these crops in sustainable food systems (Food and Agriculture Organization of the United Nations [FAO], 2016). The FAO recognizes 11 commonly consumed pulse classes: dry beans, dry broad beans, dry peas, chickpea, dry cowpea, pigeon pea, lentil, Bambara groundnut, vetch, lupins, and other minor pulses (FAO, n.d.). There are several varieties within these classes, totaling more than 80 pulse species (Tiwari et al., 2011).

Recommended Intake of Pulses

A weekly serving of 1½ cups of pulses is recommended by the United States Department of Agriculture (USDA) and United States Department of Health and Human Services (HHS) for a healthy eating pattern for adults (United States Department of Health and Human Services & [HHS] & United States Department of Agriculture [USDA], 2015). However, the intake recommended changes depending on dietary patterns, gender, and caloric needs, ranging from 1

to 3 cups per week for adults (USDA & HHS, 2015). In the United States, pulses are a part of the protein-rich (meat and alternatives) and the vegetable food groups (USDA & HHS, 2015). One serving of pulses is equivalent to 1 serving of vegetables or two ounces of meat (USDA, n.d.). A serving size of pulses is $\frac{1}{2}$ cup of cooked pulses or $\frac{1}{4}$ cup raw pulses prior to cooking (USDA, n.d.; HHS & USDA, 2015).

On a daily basis, at least $\frac{1}{2}$ cup cooked pulses improve diet quality and result in higher nutrient intakes (Mitchell et al., 2009; Mudryj et al., 2012). This amount ($\frac{1}{2}$ cup or 100 g) is the recommended international standard and provides nutrients which are commonly under consumed by several age-sex groups (Marinangeli et al., 2017). Pulses add fiber, protein, iron, and a number of other minerals to the diet (Mudryj et al., 2012). The nutrition profile of pulses has been shown to be beneficial for non-communicable diseases such as cardiovascular disease and diabetes (Duranti, 2006). Further details on the nutritional profile of pulses is discussed later.

Current Consumption

This food group has been consumed for at least 10,000 years (Leterme & Muñoz, 2002). Globally, pulses are the second most important food group, with cereal grains being first (Tiwari et al., 2011). Pulse consumption per capita, as well as the consumption growth rate, is double in developing countries compared to developed countries (Akibode & Maredia, 2012). The total consumption of pulses in developing countries is over 10 kg/capita/year, while it is below 3 kg/capita/year in developed countries (Akibode & Maredia, 2012).

Though they are a staple in many regions of the world (Winham et al., 2008; Sokhansanj & Patil, 2003), pulse consumption is low in the Western diet (Akibode & Maredia, 2012). In 2018, the per capita availability of dry beans was 9.62 pounds per person in the United States (USDA Economic Research Service, 2019), but availability does not necessarily translate to actual consumption. In 2015, the USDA reported consumption of the vegetable group, with

legumes as a subgroup. Legumes made up just 6% of vegetable consumption (USDA, 2016). The USDA also reported average weekly pulse consumptions of 0.8 to 1.1 cups for adult males aged 19 or older, falling short of the recommended 1.5 to 3.0 cups (dependent on age group) (HHS & USDA, 2015). Likewise, females fell short of the recommended 1.0 to 2.0 cups for adult female age groups (19 years or older), with an average weekly consumption of 0.5 to 0.7 cups (HHS & USDA, 2015). As noted earlier, a ½ cup is a standard serving size for (cooked) pulses (USDA, n.d.; HHS & USDA, 2015). Based on these estimates from national survey data, it is likely that that most Americans do not eat a ½ cup daily serving of pulses according to their weekly patterns. Pulse processing, discussed in the *Pulse Processing* section of this thesis, is vital in efforts to increase pulse consumption.

Sustainability

Pulses are an attractive crop for sustainability as well. Globally, there is a push for reduced meat consumption, with growing interest in plant proteins. Compared to meat products, pulses have a higher nutrient value, as well as a lower cost, per 100 kcal (Drewnowski, 2010). In addition to being a non-meat protein source, pulses have a biological nitrogen fixation ability (Hossain et al., 2016). These leguminous crops have a symbiotic relationship with rhizobial bacteria in soil and can fixate nitrogen. Through this ability, less inorganic N-fertilizer is used, which can mitigate detrimental environmental effects, such as water pollution from run-off, the release of greenhouse gases into the atmosphere, and our food production system's carbon footprint (Hossain et al., 2016; Gan et al., 2011; Harrison, 2011). Pulses are also a viable alternative to ethanol fuel, with bacteria in the roots producing butanol (Tigunova et al., 2013).

Nutrition

Both cereal grains and pulse crops have similar contents of total carbohydrates, fat, and B vitamins. However, traditionally used cereal grains, such as wheat, corn, rice, barley, and oats,

are limited in their nutrition (Frohlich et al., 2014). On the other hand, nutrients that set pulses apart from other grains are their higher amounts of protein, iron, folate, and minerals (Singh, 2017), which make pulses a viable alternative to these traditional ingredients (Frohlich et al., 2014).

Pulses are a vital food group, especially in the context of the nutrition transition and diabetes, with their plethora of health benefits. These two terms will be defined in the following *Diabetes* section of this thesis. Drewnowski and Rehm (2013) determined that pulses are a plant-based food with one of the highest nutritional values per dollar, as well as among the vegetables with the lowest cost per gram.

Pulses have a complementary relationship with whole grain cereal crops, in terms of composition, anti-inflammatory properties, and impact on the gut microbiome (Awika et al., 2018). Pulses can also be a complementary protein source due to their lysine content, which is low in cereal grains (Erbersdobler et al., 2017). Considering this, the implementation of pulses into whole grain cereal products is highly recommended (Akiwa et al., 2018).

Overall, pulses have several nutritional properties that make them attractive for product development (Mazumdar et al., 2016). Nutritional properties include high protein, high fiber, low glycemic index, and gluten-free and vegetarian status (Rohwer, 2015; Foschia et al., 2017; Rizkalla et al., 2002). Pulses are also high in the micronutrients folate, iron, zinc, and potassium (Winham et al., 2008). Several nutrient content claims could be made for pulses (100 g cooked serving) regarding their macronutrients, minerals, and vitamins (Marinangeli et al., 2017). The major health benefit of pulses is the control and management of several diseases like cardiovascular disease and type 2 diabetes (Duranti, 2006).

Composition

While pulses overall are known for their nutritional content, there are differences in composition among family, species, and even within market classes. The protein, carbohydrate, and lipid composition of pulses varies greatly (Hall et al., 2017). There are also differences in chemical composition between and within species (Rochfort & Panozzo, 2007). Additionally, differences in varieties, seed composition, physical properties, and storage conditions are known to affect the cooking quality (Gubbels & Ali-Khan, 1991; Gubbels et al., 1985; Kaur et al., 2009; Sefa-Dedeh & Stanley, 1979; Singh et al., 2004).

Pulses included in the study

Our study examines Hampton cultivar dry peas (*Pisum sativum*) and Avondale cultivar lentils (*Lens culinaris*) from the 2017 crop year. These two pulse varieties were chosen because they were of economic importance in the USA Dry Pea & Lentil Council 2015 Strategic Plan.

Hampton dry peas are very hardy and are the first field pea in the United States to be resistant to two prominent aphid-vectored virus diseases: pea enation mosaic virus and bean leafroll virus (Suszkiw, 2015). This variety bred by Dr. Rebecca McGee is also resistant to fungal disease pathogens (Suszkiw, 2015). Additionally, the cultivar has a high yield potential compared to commercial cultivars (Suszkiw, 2015). This, along with its disease resistance, make it a particularly appealing cultivar to grow and work with (Suszkiw, 2015).

Avondale lentils were also bred by Dr. McGee and offer disease resistance like the Hampton peas. This variety was bred to improve Richlea lentils, which have high yields but are vulnerable to the fungi ascochyta (Cahill Seeds, n.d.). Ascochyta blight results in a loss of seed and yield and is resistant to frequently applied fungicides (Markell et al., 2008). The Avondale cultivar answers the call for a high yield disease resistant lentil (Cahill Seeds, n.d.; Pulse USA, n.d.).

The 2017 Northern Pulse Growers Association (NPGA) U.S. Pulse Quality Survey findings (Northern Pulse Growers Association [NPGA], 2018) on Hampton dry peas and Avondale lentils is summarized in Table 2.1. The composition of peas and lentils appears very similar, except for the higher starch content in lentils. The Avondale variety had the highest starch content compared to all other lentil cultivars observed in the 2017 U.S. Pulse Quality Survey (NPGA, 2018). The most abundant component for both the 2017 lentils and peas was starch (NPGA, 2018), which other previous work on lentils and peas found as well (Li & Ganjyal, 2017). Although this study only looks at two specific cultivars, it is important to note that variety will influence some characteristics, such as protein, starch and ash content (Wang & Daun, 2006; Wang et al., 2009). Postharvest factors, including processing and cooking methods, can also affect nutrition such as the protein composition (Wang et al., 2009).

Table 2.1. 2017 U.S. Pulse Quality Survey Summary Findings

Property	Avondale lentils	Hampton peas
Moisture	9.3%	8.3%
Ash	2.5%	2.6%
Fat	2.1%	1.8%
Protein	22.3%	23.1%
Starch	46.9%	42.1%

Diabetes

The Nutrition Transition

The 20th and 21st century saw major shifts in diet from legumes, vegetables, and fruits to more refined and processed foods, which is a part of a complex historical phenomenon, termed the nutrition transition (Popkin, 2015). This shift started in the mid-1900s and is now seen in all regions and countries (Popkin et al., 2012). The nutrition transition involves both dietary and physical activity changes that promote inactivity and unhealthy eating habits (Mattei et al.,

2015). The transition is attributed to changes caused by globalization and urbanization (Mattei et al., 2015). More specific factors include access to low-cost food, survival no longer being dependent on physical activity, and technology (Cohen, 2008; Khush, 2003; O'Keefe et al., 2011).

Western diets are associated with the nutrition transition, as this diet typically consists of refined carbohydrates, added sugars, fats and animal-source foods (Popkin et al., 2012). Dietary patterns such as this are attributed to a rise in non-communicable diseases, like T2DM and cardiovascular diseases (Popkin, 2015). For example, meat-based protein sources in excess can aggravate postprandial hyperglycemia due to the high calorie and saturated fat content (O'Keefe & Bell, 2007; Jakulj et al., 2007).

Metabolic conditions, such as T2DM, are attributed to our diets, and rightfully so. A main modifiable risk factor for T2DM is diet (Ventura et al., 2009). Diets with minimally processed foods, such as the Mediterranean diet, are recommended for optimal postprandial glucose levels, as well as a reduced risk of chronic diseases (Lichtenstein et al., 2006; Mente et al., 2009). Pulses can meet our recommended dietary needs, as they are minimally processed compared to refined food items and offer nutrients that the Western diet often lacks (O'Keefe et al., 2008; Popkin, 2015).

Type 2 Diabetes Mellitus (T2DM)

Diabetes is defined by the American Diabetes Association (ADA) as a metabolic condition involving impaired insulin action and β -cell insulin secretion, resulting in elevated blood glucose (American Diabetes Association [ADA], 2000). Blood glucose is directly increased by the consumption of carbohydrates, around 10 minutes after starting a meal due to carbohydrate absorption (ADA, 2001). Clinical diabetes is described as fasting hyperglycemia,

along with increased hepatic glucose production (ADA, 2000). T2DM involves a loss of beta-cell insulin secretion, with a correlation to developed insulin resistance (ADA, 2019).

Several complications are associated with diabetes, including retinopathy, neuropathy, amputations, and cardiovascular disease (ADA, 2014). The ADA recognizes T2DM as a heterogeneous disease, meaning that it can be caused by several factors, including genetic and environmental (ADA, 2019).

A fasting postprandial glucose value of ≥ 126 mg/dL (7.0 mmol/L) is considered in the diabetic range. Individuals with T2DM have high fasting blood glucose due to a lack of regulation of hepatic glucose production (Blaslov et al., 2018). Contributing mechanisms to this are glucose precursors and free fatty acid oxidation (Blaslov et al., 2018). Other important factors are related to glucagon and insulin: an enhanced or decreased sensitivity, respectively (Blaslov et al., 2018).

Hemoglobin A1c (HbA1c)

A hemoglobin A1c (HbA1c) of $\geq 6.5\%$ (11.1 mmol/L) after an oral glucose tolerance test is also a criterion for the diagnosis of diabetes. HbA1c estimates average blood sugar levels for the past two to three months (ADA, n.d.). It measures how much hemoglobin in the body is glycosylated or covered with sugar (Mayo Clinic, 2018).

In adults without diabetes, either normal glycemic or with pre-diabetes, high HbA1c levels are associated with the development of metabolic syndrome (Kang et al., 2015; Veeranna et al., 2011). Metabolic syndrome is a combination of risk factors T2DM and cardiovascular disease (O'Neill & O'Driscoll, 2015). Additionally, high HbA1c levels are also associated with chronic kidney disease for adults without diabetes (Kang et al., 2015).

HbA1c is a common measure of glycemic control, serving as an indicator of diabetes management (Yu et al., 2010). Elevated HbA1c levels are linked to insulin resistance (Kang et al., 2015). For adults with diabetes, high HbA1c levels are associated with strokes, coronary heart disease, and cardiovascular disease (Chen et al., 2015). Overall, high HbA1c levels are detrimental to health status.

Prevalence of Type 2 Diabetes (T2DM)

Based on numbers from the ADA and the International Diabetes Federation (IDF), T2DM makes up a significant portion (90-95%) of diabetes cases globally (ADA, 2014; IDF, 2019). The estimated number of undiagnosed people with T2DM is alarming: one-third to one-half of the population with T2DM are unaware of their condition (IDF, 2019). Certain factors leading to the development of T2DM include obesity, increasing age, ethnicity, and family history (IDF, 2019). In addition, epigenetics play a key role when looking at the nutritional influences on developmental periods such as the intrauterine or early childhood periods (Fernandez-Twinn et al., 2019). The growing prevalence of T2DM is a marker of the epidemiologic transition in which the major causes of death are no longer communicable diseases, but instead non-communicable diseases (Omran, 2005).

Treatment Methods for Diabetes

Pharmacologic Methods

Type 2 diabetes is often treated with pharmacotherapy, as diet and exercise may not be enough to combat the progressive disease. However, there are several factors that influence treatment, so patients will often follow a multi-drug plan to address multiple health concerns (Grant et al., 2004). Table 2.2 summarizes the various oral and injectable drugs approved for treatment, modified from work by Kahn et. al (2014). As evident in the table, there are numerous medications available for people with type 2 diabetes. Insulin is a widely-known treatment,

though it is not initially needed for type 2 diabetes management (ADA, 2014). Other viable treatments in place of insulin are diet and exercise or another type of oral treatment (ADA, 2014). However, treatment is dependent on the severity of the disease.

This study allowed short/rapid acting metformin (biguanide antidiabetic) and Trulicity (GLP-1 receptor). This decision was based on the profiles of the medications, relating to their consistency (discussed further in *Study Population* in Chapter 3). Metformin enhances insulin sensitivity as its primary mode of action, thereby reducing hepatic gluconeogenesis and glycogenolysis (Rena et al., 2017; Rodbard et al., 2007). Metformin is the drug therapy of choice for T2DM. It is a first choice due to several reasons: its efficacy, cardiovascular and metabolic effects, and ability to pair with other drugs in combination therapy (Rojas & Gomes, 2013). Side effects of metformin are mainly of the gastrointestinal nature (Rena et al., 2017). When used in monotherapy, metformin adds an insignificant risk of hypoglycemia (Rena et al., 2017). The ADA recommends metformin first and then supplemental agents only if glycemic targets are not met (Inzucchi, et al., 2012).

Trulicity is injected once a week and is recommended as an add-on for a diet and exercise treatment approach (Smith et al., 2016). GLP-1 receptor agonists, such as Trulicity, utilize the incretin system to effectively improve glycemic control (Garber, 2011). Individuals with T2DM lack an effective incretin system, which normally utilizes hormones to lower blood glucose (Nauck & Meier, 2018; Smith et al., 2016). Trulicity's mechanism of action involves increased insulin secretion in response to elevated glucose, decreased glucagon secretion, delayed gastric emptying, and the activation of the GLP-1 receptor (Grunberger et al., 2012; Smith et al., 2016). GLP-1 receptors enhance insulin secretion and suppress glucagon secretion, resulting in a

lowered blood glucose (Druker, 2006). Side effects may include gastrointestinal discomfort, including nausea, vomiting, and constipation (Smith et al., 2016).

In addition to the side effects of medications, these drugs like Trulicity have a substantial cost. Even though metformin is the drug of choice recommended by the ADA (Inzucchi et al., 2012), a study found that 35% of patients were started on other drugs instead (Desai et al., 2012). These agents, including α -glucosidase inhibitors, thiazolidinediones, and dipeptidyl peptidase-4 inhibitors, are significantly more expensive than metformin. In 2012, metformin was \$116 for six (6) months, while these other drugs were \$677 for the same period (Desai et al., 2012). A review found that high healthcare costs were consistently linked to low T2DM medication adherence (Krass et al., 2014). In fact, it is recognized that there is a heavy medication cost burden on those with diabetes resulting from policy and health insurance (Krass et al., 2014). Because of the significant cost of pharmacotherapy, diet is an attractive alternative for treatment, if feasible.

Table 2.2. Approved medications for T2DM. Modified from Kahn, Cooper, Prato, 2014.

Agent	Delivery	Medication Names
Second-generation sulfonylurea antidiabetics	Oral	Glibenclamide (glyburide), gliclazide, glimepiride, glipizide
Biguanide antidiabetics	Oral	Metformin
Peroxisome proliferator-activated receptor γ agonists or thiazolidinedione antidiabetics	Oral	Pioglitazone, rosiglitazone
α -glucosidase inhibitors	Oral	Acarbose, miglitol, voglibose
Dipeptidyl peptidase-4 (DPP4) inhibitors	Oral	Alogliptin, linagliptin, saxagliptin, sitagliptin, vildagliptin
Sodium-glucose co-transporter 2 (SGLT2) inhibitors	Oral	Canagliflozin, dapagliflozin
Meglitinides (glinides)	Oral	Nateglinide, repaglinide
Bile-acid-binding resins	Oral	Colesevelam
Dopamine-receptor agonists	Oral	Bromocriptine
Islet amyloid polypeptide (amylin) analogues	Injectable	Pramlintide

Table 2.2. (continued)

Agent	Delivery	Medication Names
Glucagon-like peptide 1 (GLP-1) receptor agonists	Injectable	Exenatide (bydueron), liraglutide, lixisenatide, dulaglutide (trulicity)
Rapid-acting and short-acting insulin	Injectable	Soluble insulin (regular insulin), insulin aspart, insulin glulisine, insulin lispro, insulin zinc-amorphous (insulin semilente)
Intermediate-acting insulin	Injectable	Isophane insulin (NPH insulin), insulin zinc (insulin lente)
Long-acting insulin	Injectable	Insulin zinc–crystalline (insulin ultralente), insulin detemir, insulin glargine

Dietary Methods

A lifestyle intervention approach, which includes using the diet as a tool, is a universal recommendation for T2DM treatment (Aryangat & Gerich, 2010). One of the main benefits of a diet approach is a lowered economic burden on patients with T2DM. Unhealthy diets significantly contribute to the development of diabetes, as shown with prospective observational studies and randomized controlled trials (RCTs) (Ley et al., 2014). A focus on diet has been favorable for glucose levels. The Diabetes Prevention Program (DPP), which implements caloric restriction and exercise, reduced the development of diabetes in patients with impaired glucose tolerance by 58% (Knowler et al., 2002). For that study evaluating impaired glucose tolerance, a diet and exercise approach was favorable over metformin (Knowler et al., 2002).

Glycemic Response

Important parameters when considering diet as a treatment are glycemic index (GI), and glycemic response (GR). This thesis presents a brief overview of glycemic index (GI) for a better understanding of glycemic effects, though the study will focus on glycemic response.

The glycemic response (GR) describes the effects a food has on postprandial blood glucose. In other words, it is the change in postprandial blood glucose concentration after a meal

(Augustin, et al., 2015). With T2DM, it is critical to control elevated glycemic responses. The glycemic response is also referred to as postprandial glycemia (or postprandial glucose) (Jakobsen, et al., 2009). The postprandial glucose (PPG) will be discussed in the following section titled *Postprandial Glucose (PPG)*.

The glycemic index (GI) compares the ingestion of 50 g of oral glucose and a reference food item, using the incremental increase in the area under the postprandial glucose curves (O'Keefe et al., 2008). It is a GR that can be standardized for equal available carbohydrate (CHO) amounts (usually 50 g or 25 g), and it can also be a relative GR compared to a reference food item (Augustin et al., 2015). (Note that the term available CHO was first defined by McCance and Lawrence (1929) and refers to carbohydrates that are absorbed and digested in the small intestine). The GI is used as a food property that influences GR (Augustin et al., 2015; Bell et al., 2015) and is similar in populations with various metabolic statuses: normal glucose, hyperinsulinemic, and T2DM (Lan-Pidhainy & Wolever, 2011).

High GI foods are negatively associated with cardiovascular (CV) disease and type 2 diabetes (Beulens et al., 2007). High GI items may rapidly increase PPG and insulin demand (Ludwig, 2002; Willett et al., 2002). Conversely, a low GI diet could improve blood glucose control (Rovner et al., 2009). However, while GI may play a role in glucose, GR is more practical to evaluate. The GI lacks research to provide evidence-based claims (Dietary Guidelines Advisory Committee, 2010), which is why this study focuses on the GR. The GI methodology has flaws that may translate to an inaccurate use of it, including inadequate reference food testing and AUC calculations (Wolever, 2013). The GI can also be affected by various factors about foods such as processing and growing conditions, and this is still widely misunderstood (Wolever, 2013).

Pulses have glycemic lowering properties that address concerns with GI and GR. A low GI and GR is seen after consuming pulses (Rizkalla et al., 2002) due to the nature of their cell wall (Brummer et al., 2015) and starch (Singh, 2010). The cell wall is key in preventing starch degradation by digestive enzymes (Brummer et al., 2015). Further, digestive enzymes do not act as readily on pulse starches because of starch retrogradation, which makes the starches resistant to enzymes (Singh, 2010). Additionally, the glucose from starch is released slowly because of the dietary fiber and protein content in pulses. This is associated with the lower GI of pulses (Jenkins et al., 1983; Wolever et al., 1987). Pulses are also beneficial for overweight and obese adults at risk for metabolic syndrome (Mollard, Luhovyy, et al., 2012).

Postprandial Glucose (PPG)

Postprandial glucose (PPG) describes the glucose levels after eating (ADA, 2001). Estimates place the highest PPG peaks occurring within 1 hour (Esposito et al., 2008) or 1 hour and 15 minutes (Daenen et al., 2010) after a meal. Carbohydrate absorption and insulin and glucagon secretion impact PPG levels (ADA, 2001). For example, insufficient insulin levels are linked to elevated postprandial glucose levels since the insulin available is not enough to control the glucose (ADA, 2001).

Lower PPG levels are beneficial for those with metabolic disorders, as well as healthy individuals (Wood, 2007). Previous findings indicate PPG is important in overall glycemic control, as elevated PPG values can lead to cardiovascular (CV) complications (Cavalot et al. 2006; Fonseca, 2003; Mannucci et al., 2012). Oxidative stress induced by high PPG levels results in endothelial activation and dysfunction (Ceriello et al., 2004). These conditions promote the onset of cardiovascular disease (CVD), the leading cause of mortality for adults with T2DM (Ceriello et al., 2004; Roper et al., 2001).

High PPG is a predictor of CVD incidence, as well as of all-cause mortality (Mannucci et al., 2012; Takao et al., 2017). The risk for CVD mortality is more than doubled with diabetes (Dale et al., 2008). At a pre-diabetic postprandial level of 140 mg/dl, CV complications increase by 58% (Sasso, 2004). Further, a 1-mmol/L increase in mean 2-hour post-breakfast blood glucose leads to a 11% increase in CVD risk (Takao et al., 2017).

Elevated PPG also has a relationship with postprandial hyperlipidemia and inflammation. Hyperlipidemia can magnify the effects of postprandial hyperglycemia and is associated with insulin resistance (Ceriello et al., 2005; Dilley et al., 2007). Glucose leads to inflammation via the Krebs cycle (Monnier et al., 2006). Free radicals are produced by single-electron transfer, a result of a surplus of the reduced form of nicotinamide adenine dinucleotide (NAD). This is induced by the presence of glucose and free fatty acids (O'Keefe & Bell, 2007). This relationship between inflammation and postprandial hyperglycemia is seen in nondiabetic and diabetic individuals alike (Brownlee & Hirsch, 2006). Other complications induced by hyperglycemia include diabetic retinopathy (Mannucci et al., 2012).

Considering PPG is an independent risk factor for cardiovascular complications, it is a better indicator than fasting plasma glucose (FPG) for glycemic control (Fonseca, 2003; Cavalot et al., 2006; Gerich, 2003; Mannucci et al., 2012). Several studies have found PPG two hours after lunch was predictive of cardiovascular complications, as well as all-cause mortality. They did not find a similar correlation for fasting glucose (Cavalot et al., 2011, Nakagami et al., 2006). Postprandial glucose also plays an important role in regulating and achieving target HbA1c levels, important for diabetes diagnosis and treatment (Woerle et al., 2007).

Easily digestible foods will result in higher PPG levels compared to foods that are not digestible as readily (O'Keefe et al., 2008). Pulses have slowly digestible starch and resistant

starch (RS), which contributes to slow digestion (Jenkins, Wolever, Taylor, Barker et al., 1980; Jenkins et al., 1982; Brand et al., 1990; Guillon & Champ, 2002). Other factors that contribute to slow digestibility of pulses include proteins and the protein-starch matrix. Antinutrient factors also contribute to this effect on glucose levels, which includes enzyme inhibitors, phytates, lectins, and saponins (Thorne et al., 1983; Jenkins & Jenkins, 1995; Singh et al., 2017).

Glycemic Response

Relationship with Pulses

Dietary Fiber

Pulses are a known source of dietary fiber. Dietary fiber are carbohydrates resistant to digestion and absorption (Muir, 2019). While available carbohydrates are digested and absorbed, dietary fiber makes it past the small intestine and is either fermented in the large bowel or excreted as feces (Cummings & Stephen, 2007). The amount of dietary fiber will differ with pulse varieties (Wang et al., 2009). The two main dietary fiber classes include indigestible polysaccharides and oligosaccharides. Long-chain carbohydrates, such as nonstarch polysaccharides and resistant starch (RS), are indigestible polysaccharides. Conversely, short-chain carbohydrates are oligosaccharides and include fructo-oligosaccharides and galactooligosaccharides (Muir, 2019). One of the mechanisms explaining the beneficial glycemic effects of fiber is viscosity, which delays absorption and thereby influences the glycemic response (Muir, 2019). Fiber is known to decrease a meal's glycemic load (Raninen et al., 2011), which will lower the glycemic response. The dietary fiber in pulses consists of indigestible oligosaccharides, soluble fiber, insoluble fiber, and RS (Brummer et al., 2015).

Soluble and insoluble fiber

In addition to an indigestible polysaccharides or oligosaccharides classification, fiber can also be classified as soluble or insoluble. This classification is based on chemical properties of

the fiber components, in reference to water as the solvent (Raninen et al., 2011; Anderson et al., 2009). Insoluble fibers are not fermented to the same degree in the colon due to their bulking action, and include wheat bran and cellulose (Anderson et al., 2009; Dikeman & Fahey, 2006). Soluble fibers are fermented in the colon (Anderson et al., 2009) and are further classified as viscous (or fermentable) or nonviscous (Chutkan et al., 2012). Soluble viscous fibers are usually derived from psyllium husk but also include pectin (Anderson et al., 2009; Chutkan et al., 2012), while inulin and wheat dextrin are examples of soluble nonviscous fibers (Chutkan et al., 2012). However, the classification is fluid, as fibers can have both soluble and insoluble properties (Chutkan et al., 2012).

Soluble viscous fiber reduces the glycemic response (Wood, 2007). Foundational work from Jenkins and colleagues (1978) found that gastric emptying and glucose absorption is delayed by viscous fibers. Throughout the years, various studies have shown that barley and oat soluble fiber lower the glycemic responses in healthy adults (Granfeldt et al., 2008; Hlebowicz et al., 2008; Kim et al., 2009). More evidence is established on the effects of soluble fiber on glycemic control, but this does not rule out the benefits of insoluble fiber. Weickert and colleagues (2006) found that insoluble cereal fiber could improve insulin sensitivity in overweight and obese women.

Positive glycemic effects are not limited to cereal grains. The soluble fibers of pulses have demonstrated protective effects against metabolic syndrome and T2DM (Babio et al., 2009; Steenburg et al., 2009; Villegas et al., 2008). The cell wall polysaccharides of pulses contain large amounts of pectin (soluble viscous fiber) and proteins (Vogel, 2008). The pectin, along with protein and phenolic compounds, are linked to the disruption of the cell wall structure and release of bioactive compounds (Awika et al., 2018). In addition, pulses have fewer phenolate

ester cross-linkages than cereal crops. This, along with the presence of pectin, is likely to affect the fermentation of dietary fiber (Awika et al., 2018). This is of importance as the carbohydrates in pulses are normally rapidly fermented and used by colon bacteria (Henningsson et al., 2001).

In pulses, the quick fermenting fiber results in a quick release of phenolics (Awika et al., 2018). The importance of these phenolic compounds for glucose metabolism will be discussed in the *Phenolic acid* section. The effects of phenolic acid, along with the cell wall integrity, influence the glycemic response.

While pectin is a soluble fiber, the majority of dietary fiber in whole pulses is insoluble fiber (Hall et al., 2017; Tosh & Yada, 2010). The hulls contain more insoluble fiber (Singh et al., 2017; Frohlich et al., 2014), while the cotyledons have higher soluble fiber, along with oligosaccharides, SDS and RS (Singh et al., 2017). Pea fiber consists of 63-92% insoluble fiber (Martín-Cabrejas et al., 2003; de Almeida Costa et al., 2006).

Starch

Starch is a substantial carbohydrate in pulses, making up anywhere from 22% to 45% of pulses (dry weight) (Hoover et al., 2010). Three types of starch will be presented: slowly digestible starch (SDS), rapidly digestible starch (RDS), and resistant starch (RS). Englyst and colleagues (1992) measured the time periods for the glucose release corresponding to these types of starch. They defined RDS as the amount of glucose released after 20 minutes, SDS was between 20 and 180 minutes hydrolysis, and RS as the total starch minus amount of glucose within 180 minutes hydrolysis (Englyst et al., 1992). In other words, RS is the amount of starch undigested after 180 minutes (Chung et al., 2009).

While starch is likely important for glycemic control, the mechanism probably lies with digestive enzyme accessibility and not the structural features of starches (Xiong et al., 2018).

Xiong and others (2018) found structural features such as crystallinity, melting temperatures and

enthalpy change had no correlation to starch digestion kinetics. They found digestion was instead mediated by enzyme accessibility for whole pinto beans, garbanzo beans, green-split peas, and black-eyed peas. The authors proposed physical structures of the cell walls and/or pulse protein matrix modulate starch digestion, by way of enzyme accessibility. This was shown with the discovery of dextran, an enzymatic reaction by-product, in the cellular protein matrix (Xiong et al., 2018).

Slowly digestible starch (SDS) and Rapidly digestible starch (RDS)

The starch in pulses may contribute to their low GI nature, as they are slowly digestible (Rizkalla et al., 2002). The low in vitro starch digestibility value (ISDV) and SDS content of pulses make them ideal for individuals with T2DM (Chung, Shin, et al., 2008; Lehmann & Robin, 2007; Madhusudhan & Tharanathan, 1996, McCrory et al., 2010). The low ISDV is attributed to higher amylose content (Madhusudhan & Tharanathan, 1996, McCrory et al., 2010).

Whereas SDS results in a slow increase of PPG over time, RDS has the opposite effect on blood glucose. Fast and high peaks are seen with RDS (Lehmann & Robin, 2007), and this fraction is responsible for the sudden blood glucose peaks after eating a food (Chung et al., 2009). Certain processes, such as autoclaving, may convert RDS into RS (Kasote et al., 2014). However, one of the major findings from that *in vitro* starch digestibility study was that both RS and RDS are converted to SDS with autoclaving (Kasote et al., 2014).

In a study looking at peas, lentils and chickpeas, lentils had the higher SDS and lower RDS content of the three (Chung, Liu, et al., 2008). In another study looking at corn, peas and lentils, some of those authors found peas had more RDS than lentils. That study also found lentils had more SDS than peas, and peas had more RS than lentils (Chung et al., 2009). SDS and RS are linked to beneficial glycemic effects.

Resistant starch (RS)

Resistant starch, an indigestible polysaccharide, has four categories: RS1, RS2, RS3, and RS4 (Yadav et al., 2010). RS1 includes physically entrapped starch, RS2 describes ungelatinized starch, RS3 is retrograded starch, and RS4 is chemically modified starch (Yadav et al., 2010). For this study, RS1 and RS2 are likely to be the RS forms of interest due to the structural implications on digestive enzyme accessibility. Physically entrapped starch of RS1 will affect the role amylolytic enzymes play in digestion, and the structure of RS2 makes this form unavailable to the enzymes (Yadav et al., 2010).

Pulses have higher resistant starch values than cereal and tuber crops (Yadav et al., 2010). This may be explained three-fold: starch enveloped in intact tissue/cell structures, high amylose content, and a large amount of viscous soluble fiber (Yadav et al., 2010). In particular, the cell wall has a protective effect for RS, and is the likely mechanism behind high levels (Brummer et al., 2015). The resistant starch found in all pulses limits the accessibility of α -glucosidase enzymes and will likely result in a lower PPG response (Dhital et al., 2016; McCrory et al., 2010). α -glucosidases cannot act on this fraction of starch, and it remains undigested until it reaches the colon (McCrory et al., 2010). This enzyme does not readily digest resistant starch due to its starch-protein matrix (McCrory et al., 2010).

In a sample of eight different pulses, including the four main types of pulses (beans, peas, lentils, and chickpeas), green lentils had the lowest resistant starch content (Brummer et al., 2015). When paired with rice powder, pulse powders made of lentil, pigeon pea, mung bean, or chickpea increased the RS of the rice-pulse mixture (Kumar et al., 2018).

Resistant starch is affected by processing such as boiling, and this effect will be discussed in the *Pulse Processing* section. Other processes, like retrogradation reduce RS content through the conversion into RDS (Chung, Shin, et al., 2008).

Phenolic acid

When looking at green peas (*Pisum sativum*) and green lentils (*Lens culinaris*), they have some distinguishing characteristics. Of all the pulses, peas have the most phenolic acid (Hall et al., 2017). Phenolic acid plays a significant role in glucose metabolism. These polyphenols inhibit α -glucosidase and α -amylase, enzymes that digest dietary carbohydrates to glucose (Lin et al., 2016). Phenolic acid is likened to the action of metformin, by stimulating glucose uptake (Prabhakar et al., 2009). A mode of action may be glucose transporter interference, resulting in a reduction in enterocyte glucose absorption (McCrorry et al., 2010).

Studies Using Pulses

This section on glycemic response studies using pulses is divided in the following six ways: studies using only pulses that were whole (not part of a meal), studies looking at pulses as a part of a meal, studies using both whole and flour, and studies looking at pulses only in flour form. The section ends with a discussion on glycemic response work on T2DM, and a summary of the glycemic response studies presented.

Whole pulses only

Jenkins and colleagues were pioneers for glycemic response studies using pulses. In 1980, they reported lentils and soya beans (also known as soy beans) raised blood glucose by 30% of the responses seen from wholemeal bread (Jenkins, Wolever, Taylor, Ghafari et al., 1980). All the treatments had equal amount of carbohydrates (50 g), and the subjects had normal glucose or diabetes (type unspecified). All meals were served with skinned tomatoes for palatability. The authors speculated that these effects were due to the rate of digestion attributed to foods. Later, some of those same authors also hypothesized that the low glycemic response was not because of increased insulin secretion, and thus were due to the properties of the foods (Jenkins, Wolever, Taylor, Barker, et al., 1980).

That same year, Jenkins and colleagues also reported on the glycemic response of whole pulses compared to other carbohydrate containing foods, such as pasta, breakfast cereal, and grains (Jenkins, Wolever, Taylor, Barker, et al., 1980). In this study, thirty-five (35) foods were tested in a group of 25 subjects (15 men, 10 women), with eleven of those meals being a legume. The eight legumes classified as a dry legume meal (and were boiled) were butter beans, haricot beans, kidney beans, soy beans, black-eyed peas, chickpeas, marrowfat peas (also known as green mature peas), and lentils. There were three additional legume meals: fresh frozen peas, baked haricot beans, and canned soy beans. The legumes, along with some of the other meals, were served with skinned tomato for palatability.

Key findings from this study were that all the eight dried legumes produced lower mean blood glucose responses, lower mean peak rises in glucose concentration, and a lower incremental area under the glucose curve (iAUC) than all the other foods (Jenkins, Wolever, Taylor, Barker, et al., 1980). The authors noticed that the effects were similar among all the legumes, with two exceptions: the green peas elicited a high response and soy beans elicited a low response. In the context of pulses, soy beans are not included in this group. It is still noteworthy that all the legumes produced lower PPG values than the other treatments. It is also noteworthy that the green peas produced the highest PPG of the other pulses (regardless of including or excluding the soy beans). With the three additional legume meals (fresh frozen pea, canned baked beans, and canned soy beans), the authors observed a few things as well. The fresh frozen peas produced a similar PPG response as the dried peas, but was not statistically similar to the other dried legumes. Although similar, the authors theorized there would be differences in the peas' carbohydrates, expecting more starch and less sugar in the marrowfat peas because of its maturity. The baked beans elicited a higher glycemic response than the dried haricot beans. They

also observed that the canned soy beans did not differ from the plain dried soy beans. These observations have implications on the effects pulse processing has on the glycemic response, which has later been explored over the years. Finally, the authors recognized that fiber content and type of starch would have an impact on the glycemic response.

As part of a meal

Winham, Hutchins, and Melde (2007) found that incorporation of pulses into high GI meals did not have any significant effects on the GR. This randomized study was the first to look at GR of pulses as a part of a meal. The authors used pinto beans, black-eyed peas, and navy beans in the form of a spread with a bagel (a high-glycemic index item). A placebo was used as the control spread. The spreads were prepared with a food processor. Twelve healthy, insulin-sensitive adults completed this study (aged 20 to 65). The serving sizes were a low-dose (1/2 cup) and a high dose (1 cup). Their findings were that there was a significant effect based on time for both dosages, but not when looking at a time x treatment interaction. They also did not find a significant difference between a low-dose or a high-dose of pulses. This suggests pulses have beneficial glycemic effects in any quantity past ½ cup. They theorized that processing the pulses into a spread damaged the cell wall and may have impacted the GR of the pulse treatments paired with a high-GI item (i.e., bagel). This theory is a relevant research question, as many publications have now looked at the effects of pulse processing on the glycemic response. The authors further noted that the rapid digestion of the pulse spreads resulted in an absence of significant net glucose changes, which would not be seen with whole pulses.

Another study also explored this concept of blending pulses. Researchers from Purdue University looked at meals with either whole or blended lentils (Anguah et al., 2014). Twelve healthy men and women aged 18 to 55 completed six test days. The serving size for the lentil meals were approximately ½ cup and were served in a burrito. The control was a burrito without

lentils, and there were two additional treatments consisting of an enzyme supplement and a placebo capsule. Comparing the whole lentil and blended lentil meals, the blended lentils did not significantly increase the PPG levels more than the whole lentil meal. The study emphasized that the meals were nutritionally identical, so differences are likely due to the physical form of the pulses. The authors highlighted either as blended or whole may be ways to increase pulse consumption, but that this should be explored with other pulses before applying this observation across all pulse types. Additionally, these findings were both in agreement and in contrast with other previous work on the physical form of lentils (O'Dea & Wong, 1983; Jenkins et al., 1982). This shows the variability in glycemic response studies.

While Winham and colleagues (2007) did not find significant differences when the pulses were paired with a bagel in normoglycemic individuals, findings from 2017 by Winham and colleagues contrast this. Winham, Hutchins, and Thompson (2017) found that the GR improved when pulses were paired with another high GI food, rice. The 3x3 randomized cross-over study evaluated the GR of plain white rice, black beans with rice, and chickpea with rice meals. The meals were matched for 50 g available carbohydrates. Nine healthy women aged 18 to 65 completed the study. The rice-only and chickpea and rice meals were significantly different ($P = 0.047$), while the rice-only and black bean and rice meals had a trending difference. The authors also noted an extended effect on glucose levels from the black bean and rice meal compared to the other meals. The black bean and rice meal had a lower GR at 120 minutes postprandial, while the chickpea and rice meal had a positive effect only until 90 minutes. They hypothesized that the higher fiber and protein content of the black beans and rice meal translated to this observed effect.

Pulses have also been used in combination with pasta and tomato sauce. Mollard, Zykus, and others (2012) used pulse treatments (pulse, pasta, and tomato sauce) with 44% of the energy coming from the pulses. This translates to two cups or about 44.5 g of available carbohydrate. Pulses used were chickpeas, lentils, navy beans, and yellow peas. The control treatment was pasta and tomato sauce alone. Twenty-four healthy males aged 20 to 30 completed the study. The authors assessed the treatment effects at all the postprandial time points (total of 260 minutes) and several observations come from this. All pulse treatments had significantly lower PPG values than the control until 40 minutes postprandial. At 20 minutes, the navy beans elicited lower PPG values than the chickpeas. Then at the 60-minute time point, the lentils and navy beans were lower than the yellow peas. The navy beans continued to be lower than the yellow peas at 80 minutes. Additionally, at 80 minutes, the chickpeas and lentil treatments were lower than the yellow peas and control. Then at 110 minutes, the navy beans show a lower PPG than the control. Statistical significance from post hoc tests was not found at 140 minutes. Further out at 200 minutes, lentils and navy beans were lower than the control treatment. Finally, at 260 minutes, the only significance was seen with the navy beans. This treatment was lower than the chickpeas and the control treatment. This study noted that the yellow peas were distinctive from the other pulses with its high available CHO and low fiber content, which may explain why it was not significantly different from the control treatment between 60 and 260 minutes. A conclusion from this study was that pulses were able to provide glucose lowering properties even with the high carbohydrate meal (pasta) but these effects are dependent on pulse type.

Whole and pulse flours

Ramdath and colleagues (2018) investigated the PPG response of products or meals containing various forms of lentils, including boiled whole lentils, boiled lentil puree, roasted lentil flour and cooked spray-dried lentil flour. Adults aged 18 to 75 with healthy fasting glucose

levels were recruited for three different studies within the publication ($n = 10$ for each study). The amount of available carbohydrate for the meals in their first study was 50 g. The lentil-containing treatments for this study were compared to instant potato flakes and white bread. Tomato juice and oregano were added to the meals for palatability. The flour meals had higher incremental area under the curve (AUC) and relative glycemic response (RGR) values than the boiled cooked lentil meal. The authors found significant ($p < 0.05$) differences between the lentil-containing products and the instant potato flakes. They concluded that boiled whole lentils and boiled lentil puree were very similar in their glycemic properties, and that the spray-dried lentil flour elicited the highest glycemic response compared to all the other lentil treatments. Further, they hypothesized that the effects of spray-dried lentil flour were explained by resistant starch content, lower total dietary fiber, and smaller particle sizes.

Anderson and colleagues (2014) looked at various forms of lentils and their effects on the GR of healthy males aged 18 to 30 ($n = 12$). Whole canned green lentils, pureed canned green lentils, pre-cooked lentil powder, and whole wheat flour were used as treatments in this repeated-measures study. The meals were prepared with tomato sauce. The authors found that the PPG was not significantly affected by treatment, but they did find a significant main effect of time ($P < 0.0001$). They reflected that their findings differed from previous work and could be explained by differences in processing, such as drying time.

Pulse flours only

Fujiwara and colleagues (2017) used split yellow pea, split green pea, split green lentil, and split red flours (as well as other fractions such as pea fiber) in an *in vivo* and *in vitro* GI study. The pulse ingredients were incorporated into a variety of products, including pastas, breads, and muffins. Control products contained 100% wheat flour, while the pulse comparison contained up to 50% pulse flour. Protein content was similar among the flours, except for the

split yellow pea flour. Total starch was lowest for the split green pea flour, with the split green lentil flour at an intermediate level. The higher starch content of the lentil flour was in line with 2017 U.S. Pulse Quality Survey (NPGA, 2018). Nevertheless, all the pulse products saw reduced total starch content. The resistant starch was higher in the pulse products than the control, except for the granola bars. Ten healthy subjects (5 male, 5 female) aged 36 ± 14 years completed this study. Participants consumed five control and five pulse test meals. Some reduction in postprandial glucose peaks were found with pulse variants, though the power of the study did not provide statistical significance for GI or iAUC. Glycemic index reductions were found with the pulse-added products. The authors concluded that pulse ingredients led to positive reductions in GI. They reported a variation in results dependent on products and processing methods, with a recommendation to investigate pulse flours and processing further.

Kumar and colleagues (2018) investigated the glycemic response of rice powder paired with 0.05 g pulse powder. The study determined the glycemic response by GI *in vitro* methods, resistant starch estimations, and amylose content determination. Four types of pulses were made into pulse powder and mixed with the rice powder: pigeon pea, lentil, mung bean, and chickpea. The mung beans had the largest effect on the GI compared to rice-only samples, with the lentils and chickpeas having a lower effect. These results highlight the differences in glycemic properties by pulse type.

T2DM

Jenkins and others (1983) looked at the glycemic response of fifteen meals, including five pulses. The pulses tested were chickpeas, kidney beans, red lentils, romano beans, and black-eyed peas. The twelve adults (six men, six women) with diabetes of a mean age of 67 years participated in the study, in groups of five to seven. There were 19 sessions over the course of 5 months, and the average completion was 15 sessions. Diabetes type was not confirmed, but the

authors speculate that eleven individuals had T2DM, and the remaining one person had type 1 diabetes. The study's goal was to determine if there were differences between the foods, and if any of these differences could be attributed to food constituents such as fiber, sugar, or protein. When comparing values for foods consumed among the same groups of individuals, the mean glucose values, overall mean blood glucose peak rise, and AUCs for the pulses were lower than the other foods. Among the pulses, the kidney beans, chickpeas and lentils had lower PPG responses. Interestingly, spaghetti and rice meals did not differ significantly for AUCs and peak rises from the beans. The researchers discussed the possibility of food form and particle size playing a role in glycemic control, especially when considering the white bread and spaghetti. For the pulses, Jenkins et al. (1983) pointed out that several factors may be responsible for the beneficial glycemic profile of whole beans, including enzyme inhibitors, lectins, and phytates. The effects of carbohydrate type and processing on *in vitro* digestion were also presented as another factor. Finally, the authors compared the glycemic responses of their participants to a previous study looking at adults with normal glucose (Jenkin, Wolever, Taylor, Barker, et al., 1980), and they found similar patterns between the two populations.

Bornet and colleagues (1987) looked at six starch-rich foods consumed alone and part of mixed meals in adults with T2DM. Eighteen adults (12 men, 6 women) tested three of the meals containing either white bread, spaghetti, white rice, instant flaked potatoes, dried kidney beans, or dried lentils. The food items were tested three times. The GI for the lentils and beans were lowest compared to the other foods, with the kidney beans having the lowest GI. There were significant differences between potato GI and that of lentils ($P < 0.01$) and kidney beans ($P < 0.01$), and between the rice and kidney beans ($P < 0.05$). These results suggest that there are

differences with pulse type, with the kidney beans having better glycemic lowering responses than the lentils.

Schäfer and others (2003) looked at dried yellow peas and potatoes in adults with T2DM, who managed their diabetes with diet and not medications. Nine adults (6 men, 3 women, aged 48 to 75) completed the 3x3 crossover study. The three meals contained carrots, celery, and a meat and were meant to mimic a “normal mixed meal.” The meals were classified based on the carbohydrate source: only peas, peas (2/3 of carbohydrate source) and potatoes (1/3 of carbohydrate source), and only potatoes. The study found that the glycemic responses of the dried peas was a 1/3 of potatoes. However, the authors found that the combination of potatoes and peas did not present a significant effect on the digestion of the potato starch. One of the purposes of the study was to determine the place dried peas have in carbohydrate counting. A conclusion addressing that research questions was that 2/3 of the carbohydrate content for dried peas should be disregarded to avoid hypoglycemia or PPG values higher than expected, in the context of mixed meals.

Thompson, Winham, and Hutchins (2012) looked at pulses as part of a meal in adults with T2DM. Pinto beans, black beans, or red kidney beans were served with white long grain rice. Seventeen men and women with type 2 diabetes consumed these test meals, along with a control of rice only. All of the test meals gave 50 grams of available CHO. This study found that the mixed meal (high and low GI) produced intermediate responses, as expected from previous literature. Additionally, key findings were that the three market classes (pinto, black, red kidney) produced different glycemic responses compared to each other. Specifically, the authors found that although black beans had a lower fiber content, this meal still produced a lower glycemic response than the red kidney treatment. These authors also hypothesized that differences among

the pulses are likely due to variations in fiber fractions, as previous work shows carbohydrate content and GI is important with mixed meals (Wolever et al., 2006). In the discussion, Thompson et al. (2012) referenced previous work on fiber in kidney beans to offer explanations for PPG differences. The levels of indigestible starch may differ among the pulses, with red kidney beans having less than pinto and black beans (Ospina, 2000). This would have resulted in a faster digestion process for the kidney beans. Additionally, data from *in vitro* animal studies showed differences in soluble fiber and RS between red kidney and black beans. The red kidney beans contained lower amounts of soluble fiber and RS, which would have resulted in a slower digestion and lower PPG (Bednar et al., 2001).

Summary and Research Gaps

There are a few common observations in much of the existing literature on pulses and the glycemic response. Many of the studies reached a similar conclusion: pulses elicit varying glycemic responses dependent on pulse type. This study explores two pulse types for this reason. Additionally, digestion is a key mechanism that comes up in many of these studies, and is proposed as having a large influence on the glycemic response. The food properties and their interactions with digestion is recognized as an important factor in resulting glycemic responses. A consensus is that these properties vary from food to food, though exactly how is still debated.

Another theme is differences that are not related to nutrient content. In studies where the meals are nutritionally equivalent (based on basic nutrient analysis reporting carbohydrates, protein, fat, etc.), it is clear that differences in PPG values are due to other factors. For instance, processing, pulse type, fiber content, and starch are likely to influence PPG.

A few research gaps exist: flours with hulls retained, serving portions, information on adults with T2DM, and inconclusive results. First, in studies using pulse flours, dehulled (or split) flours are often used as pulse test meals. Dehulling (discussed in more detail in the

Dehulling section) is a conventional practice for pulse flour processing, but it results in a loss in fiber. The loss of fiber makes comparisons between whole pulses and pulse flours more difficult, as they will no longer be as nutritionally equal as possible. The difference in nutrient content adds another factor that may influence results. Additionally, dehulling has other effects that can negatively impact glycemic control, such as the reduction of phenolics (discussed in more detail in *Dehulling* section). Although it is important to know how commercial products behave, it is important to understand how the principle behind pulse flour processing (i.e., grinding), and not the reduction of fiber, and that might influence the glycemic response.

Secondly, serving portions are not always matched for available CHO in existing literature. There may be large variability in available CHO even within a single study. It is important for test meals to be similar in available CHO otherwise the focus becomes on nutritional differences.

There is substantially more work on adults with normal glucose than those with T2DM. However, the benefits of glycemic control are more significant in adults with poor glycemic control, rather than those with a normal metabolism (Livesey et al., 2008). The size of the effect of low GI diets is more impactful for people with type 1 or 2 diabetes. In a systematic review and meta-analysis, Livesey and colleagues (2008) found that increasing the amount of unavailable carbohydrates improved blood glucose more for people with poor glycemic control. Compared to research on adults with normal glucose, the population with T2DM is underrepresented. The literature also showcases the large variability in findings. Results are not always consistent with previous findings. These differences could be due to processing parameters, study design, or several other reasons. This variability demonstrates an expansive area of research yet to be investigated and/or the need to continue investigating. In order to one day reach a consensus,

whether it be regarding pulse type, digestion, processing, or other factors, there is a need for more scientifically sound studies on the glycemic response.

Pulse Processing

Whole pulses do not hold a lot of appeal in the context of the American diet. However, processing is a tool to promote the food group and increase consumption. An avenue to implement pulses in the American diet is through pulse flours or fractions, which is currently uncommon, especially for pea and lentil flour (Tiwari et al., 2011; USA Dry Pea and Lentil Council, 2010). In favor of peas and lentils, these pulses do not require as long of a cooking time as beans, and this is likely to appeal to pulse processors. Implementation can occur across several food groups: meat, snacks, baked goods, and cereals.

Notable functional properties include solubility, emulsifying, gelation, foaming, water-binding capacity, and mechanical shearing and heat stability (Foschia et al., 2017; Ettoumi & Chibane, 2015; Singh, 2017). The starch content of pulses make them acceptable ingredients for extrusion processing because the starch allows expansion, which is proportional to starch content (Frohlich et al., 2014, Linko & Linko, 1981). These vast properties of pulses make them an adaptable ingredient with several product development applications.

Food processing has effects on several functional properties of raw ingredients. The pulses in the present study were soaked, heated and then milled to a pulse flour, which are all forms of processing. This discussion will be limited to the impact of processing on digestion, as it potentially influences the GR.

Processing influences digestive enzyme accessibility, further influencing the digestion rate and extent. The disruption of native macrostructures increases starch digestion rate and extent as digestive enzymes have better access to the starches (Tappy et al., 1986; Würsch et al.,

1986). Further, the total starch content is affected by cooking: Brummer and colleagues (2015) found that cooked peas and lentils had lower total starch content than their raw forms.

Processing also affects antinutritional factors, such as lectins (Singh et al., 2017). Recall that lectins may have a positive association with glucose levels. Further, cooking and processing destroys amylase inhibitors, which normally reduce carbohydrate digestion through the inhibition of pancreatic amylase (McCrorry et al., 2010). Thus, the presence of α -amylase inhibitors is important for blood sugar control.

Naturally, pulses have a subsequent meal effect, in which consumption during a meal will affect the glucose values for later meals (Higgins, 2011). Processing, cooking, and milling may remove this effect or produce higher PPG values than expected (Higgins, 2011). Processing or cooking, such as canning, may also affect the GI of pulses (Wolever et al., 1987; Atkinson et al., 2008).

Effect on Resistant Starch

Since resistant starch may play a large role in glycemic control, the discussion on processing impacts will be limited to this factor. Certain processing may convert other forms of starch into RS. Kasote and colleagues (2014) found that autoclaving converted RDS into RS. However, they also found that autoclaving could also convert RS to SDS (Kasote et al., 2014). This study also observed that various forms of processing, such as cooking, splitting, soaking, and boiling, generally reduce resistant starch content of lentils and green peas (Kasote et al., 2014). This is due to a conversion into digestible starch induced by thermal processing, as well as enzymatic action (Pujolà et al., 2007). As mentioned earlier, resistant starch has positive effects on the GR via digestive enzyme accessibility (Dhital et al., 2016; McCrorry et al., 2010) and thus, a higher glycemic response is expected with less resistant starch present. However, another study looking at cooked lentils reported an increase in resistant starch (Wang et al.,

2009). In this study, total starch, IDF and TDF were also increased (Wang et al., 2009). Other studies looking at various beans (pinto, black beans, and chickpeas) also saw increased resistant starch content after boiling (Kutoš et al., 2003; Fabbri et al., 2016).

The catabolism of amylose inhibitors during boiling may explain decreases in resistant starch, while retrogradation of starch may explain an increase (Wang et al., 2010). Cooling allows for further retrogradation, and cooked pulses are likely to have an increase in resistant starch compared to fresh cooked pulses (Fabbri et al., 2016). The term fresh cooked pulses for that study refers to whole pulses that were dry and rehydrated by boiling, and were compared to pulses that were cooled for longer. In the study by Fabbri and colleagues (2016), pulses that were processed (canned as whole pulses or refried) were also higher in resistant starch than fresh cooked pulses. It is recognized that thermal processing effects on dietary fiber are dependent on several factors: pulse type, processing methods, duration, and analytical methods (Fabbri et al., 2016; Kutoš et al., 2003; Pujolà et al., 2007).

Pulse Flours

Pulse flour currently lacks a universal definition in comparison to the well-known wheat flour. The Food and Drug Administration defines wheat flours as powders in which 98% or more of it passes through 212 μm or less cloths (Food and Drug Administration [FDA], 2018). Many pulse flours used in academia would not meet this wheat flour classification (Thakur et al., 2019). In many cases, particle size for pulse flours is poorly defined (Thakur et al., 2019). Several milling studies describe pulse flour as pulse seeds that have been ground, with an openness to the degree of coarseness or fineness achieved (Thakur et al., 2019). This study follows that definition.

Brummer and colleagues (2015) compared whole pulses to ground raw pulse flours and saw differences related to starch. The grinding of the flours resulted in the cell walls opening and

a release of starch granules. Some of the starch granules remained stuck to cell wall fragments. When they cooked the flour with water, the presence of whole starch granules was minimal (Brummer et al., 2015).

With respect to the influence of the cotyledon cell structure on starch hydrolysis, Berg and colleagues (2012) used three different processing conditions on navy beans. The processing conditions were cooked whole then milled, milled then cooked, and cooked beans milled under extreme conditions. The levels of starch hydrolysis were 60%, 80 to 90%, and 70 to 80%, respectively. The second processing method served to disrupt the cell walls prior to cooking, while the third process served to intensely break the cell walls after cooking (Berg et al., 2012). This study showed that the highest starch hydrolysis occurred with the pulses that were first milled and then cooked. Cooking refers to boiling in this study. The authors concluded that even after boiling, the cell wall of whole pulses maintained their integrity and prevented starch degradation (Berg et al., 2012).

Pulse flours are conventionally made from dehulled seeds, which are lower in fiber compared to seeds with hulls (also called the seed coat) but are still sources of protein and starch. Flours made like this are called split flours. However, the incorporation of the seed coat is recommended as it would result in higher fiber, iron and calcium (Frohlich et al., 2014). Fractions from milled lentil flour are high in protein, which may be due to a lower initial seed hardness of lentils (Pelgrom et al., 2015). Large decreases in starch content were also seen in lentil flours that were soaked in water and then dried (Vidal-Valverde et al., 2002). Further, moisture content impacts pulse millability, with a lower moisture content resulting in an increase in flour yield, as well as a smaller particle size (Sakhare et al., 2014; Pelgrom et al., 2015).

Dehulling

Dehulling, a common practice in pulse processing, removes the outer seed coat (also known as the hull) from the cotyledons of the pulses (Patterson et al., 2017; Vishwakarma et al., 2018; Wood & Malcolmson, 2011). Pulses are soaked prior to dehulling, after the exterior matrix is softened from soaking (Thakur et al., 2019). Factors that influence dehulling include seed variety, size, shape, moisture content, and seed hardness (Vishwakarma et al., 2018).

Peas and lentils are some of the easiest pulses to dehull (Singh, 1995). Dehulling proportionally increases protein and starch content since the seed coat has little and is removed (Wang et al., 2008; Wang et al., 2009). However, it will result in a decrease in insoluble dietary fiber, as this is present in the seed coat (Wang et al., 2008; Hall et al., 2017).

The soluble dietary fiber content is also lower in dehulled seeds compared to whole, raw seeds (Wang et al., 2008). In lentils, dehulled seeds are lower in trypsin inhibitor activity, tannin, soluble dietary fiber, insoluble dietary fiber, total dietary fiber, and iron (Wang et al., 2008). Dehulled lentils have as high as 41% and 50% reductions in soluble and insoluble fiber, respectively (Wang et al., 2009). Dehulled peas can have as high as 27% and 46% reductions in soluble and insoluble fiber, respectively (Wang et al., 2008). When looking at pulse flours, dehulling typically results in a flour with an increased protein and starch content and a decreased insoluble and soluble fiber content (Vaz Patto et al., 2015; Wu & Nichols, 2005).

Dehulling also reduces phenolics, which inhibit α -glucosidase and α -amylase. Recall these are the enzymes that digest dietary carbohydrates to glucose (Lin et al., 2016). Therefore, a reduction of phenolics would lead to more carbohydrate digestion and an increased release of glucose into the blood. A 25-30% and 10-50% reduction in total phenolic content was reported for lentils and peas, respectively (Vidal-Valverde et al., 1994; Bishnoi et al., 1994; Alonso et al.,

1998; Han & Baik, 2008). In the case of α -amylase inhibitors, an increase was reported in lentils after dehulling (Shekib et al., 1988; Alonso et al., 2000).

Milling

Milling is a combination of dehulling, splitting and flour milling (Wood & Malcomson, 2011). Dehulling removes the seed coat, then splitting cleaves, and flour milling (or grinding) produces the final flour product (Wood & Malcomson, 2011). The outcomes of milling are size reduction, component separation, and stress-induced changes to physiochemical properties (Thakur et al., 2019). Flour particle size and functionality are interconnected, as size reduction enables food applications such as blending (Frohlich et al., 2014; Thakur et al., 2019).

Physicochemical changes may occur with starch, protein, and fiber as a result of milling. Studies have confirmed that roller milling results in high starch damage, considering it alone (Sakhare et al., 2014) and compared to other milling methods (Maskus et al., 2016). Particle size and degree of shearing have a profound impact on the starch damage caused by roller milling (Scanlon & Dexter, 1986). Particle size also has an impact on protein, with a study finding an increase in soluble protein with finer flour (Kerr et al., 2001). Differences in dietary fiber components like hydration and porosity (Dogan et al., 2018) also exist between coarsely milled and finely milled flours (Daubenmire et al., 1993; Dogan et al., 2018).

Milling also impacts the cell wall, which influences starch hydrolysis and glucose release rates. Starch hydrolysis was compared in the cells of intact and mechanically damaged pulse seeds (Dhital et al., 2016). The digestive enzymes were found outside of intact cells and inside the broken cells. The broken cells had a faster starch digestion as the enzymes had better access to the starch. Pulse flour was proposed as having a faster digestion than cooked whole legumes, due to the differences in the cell wall (Dhital et al., 2016). Processing whole pulses into smaller particle sizes (i.e., flours) also results in faster glucose release rates (Luhovyy et al., 2017). A

gradual release of glucose results in a lower postprandial glycemic response, as opposed to rapidly digested and absorbed carbohydrates (Jenkins et al., 1981; O'Keefe et al., 2008).

Roller Milling

This study's pea and lentil flours were produced with two types of milling, one being a roller mill. Roller milling is an automated milling method that may be used for the production of pulse flours (Tiwari et al., 2011). This technology was introduced in the 1870s (Thakur et al., 2019). It breaks up particles through compressive stress, shear, and friction (Scanlon & Dexter, 1986). This mill is set up with a set of two rollers and simultaneously completes the grinding and screening steps (Tiwari et al., 2011). The first crushes the seed and removes the seed coat, and the second uses screens to pass desired particle sizes through (Tiwari et al., 2011).

Roller milling has been shown to reduce total dietary fiber (TDF) and insoluble dietary fiber (IDF) in lentils and peas from their raw seeds to their flours (Dalgetty & Baik, 2003). Meanwhile, the soluble dietary fiber (SDF) did not change significantly (Dalgetty & Baik, 2003). Fractions obtained by roller milling for lentils and peas had 87% and 89% fiber, respectively (Dalgetty & Baik, 2003; Dalgetty & Baik, 2006).

Hammer Milling

The hulls of the pulses were milled in a hammer mill. Hammer mills is a type of impact mill that is commonly used to produce very fine flour from hard seed coated pulses (Thakur et al., 2019). It can be used on a variety of materials though, including soft, medium-hard, and hard (Hixon et al., 1990). It uses impact as its force to break up samples (Thakur et al., 2019), which means it uses collisions between the wall and the powder particles to mill (Pelgrom et al., 2013). With this technology, the presence (or lack) of a screen is influential on the particle shape distributions obtained (Scanlon & Lamb, 1995).

For faba beans, hammer milling resulted in pulses flours that were superior in functional properties (such as total phenolic content and resistant starch) to other milling methods like screw crushing or jet milling (Limsangouan & Isobe, 2009). Conversely, hammer milling may lead to lower cell wall content depending on processing parameters used (Maaroufi et al., 2000). Additionally, one of the drawbacks of this high-speed fine milling technology is that more starch damage can occur (Kerr et al., 2000). However, a study found that roller milling resulted in more starch damage than hammer milling (Maskus et al., 2016).

CHAPTER 3. MATERIALS AND METHODS

Study Design

This study utilizes a randomized 5x5 crossover study design with five different meals (Glucola control beverage, whole lentils meal, lentil flour meal, whole peas meal, and pea flour meal). The Iowa State University Institutional Review Board approved recruitment of human subjects for the study and all participants provided written, informed consent (IRB #17-191) (Appendix A). Each day was separated by two days to one week, and lasted about 4 hours (starting around 7 a.m. and finishing at approximately 11 a.m. or 12 p.m. depending on start time). All testing occurred at the Nutrition and Wellness Research Center at the Iowa State University Research Park (Ames, IA). Participants were given a timetable with study procedures and payment schedule (Appendix B) detailing each step of the study, as well as instructions on study protocols (Appendix C).

Study Population

Adults aged 24-75 with type 2 diabetes were recruited from Ames and neighboring communities to participate in the study. Recruitment methods involved the use of half-page and full page fliers (Appendix D, Appendix E), newspaper ads, newsletters and e-mail or listserv (Craigslist) announcements. Participant eligibility was determined through online screening (www.surveymonkey.com) and/or oral interviews in person or over the phone using a list of screening questions.

At the time of study consent, participants were instructed on all study forms and selected a commercial frozen dinner that served as their last meal on pre-test days. Available options were Marie Callender's meals (ConAgra Brands, Chicago, IL, USA): Roasted Turkey and Stuffing, Honey Roasted Turkey Breast, Chicken Teriyaki, or Salisbury Steak (with cheesy broccoli and

cauliflower or with mac and cheese). Optional snacks included: dark chocolate Milano cookies (Pepperidge Farm, Norwalk, CT, USA) or Honey Maid Teddy Grahams (Nabisco, East Hanover, NJ, USA). The optional snacks were offered at the time of meal selection in case participants felt their frozen dinner would not be satiating enough. These measures were in place to account for the second-meal effect, where a first meal can continue to affect the glycemic response at the second meal (Higgins, 2011).

Additionally, participants filled out a medical history questionnaire (Appendix F) and Food Frequency Questionnaire (FFQ) (Appendix G) to obtain information on medical conditions and dietary habits at baseline. The medical history questionnaire provided a method to check for any underlying medical conditions that may affect study participation. Information obtained from the FFQ was used to compare food consumption to the U.S. Dietary Guidelines. Questions on demographics were also presented at the end of the FFQ.

All participants were physician-diagnosed with T2DM at least 4 months prior to starting the study. Recruited participants managed diabetes with metformin (also known as Glucophage, Gluformin, or Carbophage), GLP-1 receptor agonists, (specifically Trulicity, which is a once-a-week injectable drug), or diet and exercise. Metformin was allowed in short/rapid acting because of its short-term effect, as well because of its well-perceived and documented profile as a medication. Trulicity was allowed because it has a long-lasting background effect on glucose metabolism due to its weekly injections and would lead to more consistency over time compared to daily medications. Other inclusion criteria included body mass index (BMI) 22-39.9 kg/m², HbA1c% $\leq 10\%$, and ability to walk on own and feed oneself.

Participants were ineligible if any of the following applied to them: smoker (cigarettes and/or e-cigarettes), known food allergy or intolerance (to beans, peas, lentils, gluten, eggs,

dairy, wheat, tomatoes, soy, or peanuts), pregnant/lactating, diagnosed with a gastrointestinal disease (other than gastroesophageal reflux disease), 10% or more weight change within the past 6 months, on a salt- or sodium-restricted diet, and taking excluded medications. Excluded medication classes included: insulin, sulfonylurea, thiazolidinedione antidiabetics, meglitinides (glinides), α -glucosidase inhibitors, or DDP-4 Inhibitors. These medications are known to affect glucose control, such as by lowering HbA1C, PPG and FPG (Fonseca, 2003). Further, these medications were excluded to minimize potential modes of action at work. Other medications not known to affect glucose or insulin metabolism were allowed if current treatment dosages had been for ≥ 6 months, and medication dosage was not to be altered during the study period. Participants were excluded from further participation if they were unable to adhere to the study protocol after starting the study.

Sample Size

The sample size required for a power level of 80% and a type 1 error probability (α) of 0.05 was determined using a shifted-t distribution. This method is suitable for small sample sizes (De Winter, 2013), which could describe the population with T2DM in Ames. It was determined that a minimum of eleven (11) adults with T2DM was adequate to test our study hypotheses. Appendix H shows the equation used.

Materials

Pulses

Whole pulses

Green peas (*Pisum sativum*, var. Hampton) and green lentils (*Lens culinaris*, var. Avondale) from the 2017 crop year were sourced by the USA Dry Pea & Lentil Council. Varieties were grown within 5 miles of each other in Great Falls, MT, USA. Select properties and characteristics from the 2017 U.S. Pulse Quality Survey are previously discussed in *Pulses*

included in the study in Chapter 2. Nutrient analysis on the pulses by Eurofins Scientific Incorporated Nutrition Analysis Center (Des Moines, IA, USA) are presented later in this section under *Analysis*.

Pulse flour production

Pea and lentil samples were processed into flours at North Dakota State University (NDSU), Fargo, ND by Dr. Clifford Hall. The study's pulses were not dehulled, in contrast to common commercial practices for pulse flour (Patterson et al., 2017). The nutritional differences between a commercial lentil flour (from Harvest Innovations) and this study's flours are presented in Table 3.1. The difference between the two flours in fiber and available CHO content is striking. The commercial lentil flour has 4 grams of fiber, and the study lentil flour has 19 grams. Due to the lower fiber content, the commercial flour has substantially more available CHO than the study lentil flour. The lower fiber is due to the removal of the hulls, which contain a large portion of the fiber in pulses.

Table 3.1. Nutrient differences between commercial and study flours.

Attribute	Harvest Innovations dehulled green lentil flour (commercial)	North Dakota State University green lentil whole flour (study)
Calories (kcal)	363	361
Total Carb. (%)	62.06	60.35
Fiber (%)	4.40	19.10
Avail. CHO (%)	57.66	41.25
Protein (%)	25.51	24.78
Fat (%)	1.39	2.22

Each step was applied individually to the pulses (the peas and lentils were not combined). First, pulses were soaked overnight at 25°C in water (10 parts water 1 part pulse). Soaking was done for an easy removal of the seed coat, through softening the pulse's exterior matrix (Thakur et al., 2019). Second, pulses were drained over a 40 mesh sieve (Gilson Inc., Lewis OH), with any material passing through the screen discarded. Then, pulses were placed on perforated baking pans in single layers (approximately 0.45 kg per tray). Heat treatment was completed in a Baxter OV300G Mini Rotating Rack Convection Oven (Baxter Manufacturing Co., Orting WA) set at 149°C for 18 minutes (lentil) or 33 minutes (peas). Next, the pulses were mixed at five minute intervals until the end of their heating time.

After the mixing step, the heat-treated pulses were milled in a 2-step system with a roller mill (roll stands by Creason, Wichita, KS; rolls by Buhler AG, Uzwil, Switzerland). The first pass dehulled the pulses using a roller mill, and the second pass milled the cotyledon. Specific weight ratios are not available. The first pass was at 0.7 kg/min feed rate with corrugated rolls (8 % spiral, 0.1 mm land, 8.9 flutes per cm, 0.254 mm roll gap) using sharp to sharp action and a front/back roll speed differential of 1:2.5 (resulted in hull fraction and cotyledon fraction). The second pass, which was at 0.3 kg/min feed rate with smooth rolls (0.038 mm roll gap) and a 1:1.23 front/back roll speed differential was done on the cotyledon fraction. Hulls obtained from the break roll (first pass in the roller mill) were subsequently milled in a hammer mill (Model DASO6, Fitzpatrick, Elmhurst, IL) at 102 m/s hammer speed, and 0.838 mm diameter screen aperture.

After milling, the hulls were fed back into the pulse samples and the pulses were sifted through 80 mesh and 100 mesh sieves. The particle sizes for the flours are shown in Table 3.2. An 80-mesh sieve was used for above 177 µm, and a 100-mesh sieve was used for the other

particle size ranges. Particle size classifications are widely variable in literature (Clouett et al., 1986; Daubenmire et al., 1993; Indira & Bhattacharya, 2006; Singh et al., 2015; Thakur et al., 2019, Wu & Nichols, 2005; Zucco et al., 2011), so the flours used in this study could be classified as coarse or fine.

Table 3.2. Percentage Division of Flour Particle Size

Particle Size	Lentil Flour (%)	Pea Flour (%)
Above 177 μm	3.4	5.1
150-176 μm	28.5	61.6
149 μm and smaller	61.1	33.3

Whole vs. flour equivalency calculations

Portions for whole pulses and pulse flours were matched to be equivalent to $\frac{1}{2}$ cup dry weight serving of pulses (USDA, n.d.; HHS & USDA, 2015). The calculations were made based on the percentage of moisture compared to dry solids per 100 grams. Values used were from the proximate analysis provided by Eurofins Scientific Incorporated (Des Moines, IA) and all weights were in grams. A $\frac{1}{2}$ cup serving of each whole pulse treatment was determined. The dry solid weight per 100 grams of each whole pulse and pulse flour was calculated using Eq. 1. The amount of dry whole pulse solids per 1 cup was calculated using Eq. 2. The flour equivalency to the whole pulses was calculated using Eq. 3. The amount of flour required for an equivalent $\frac{1}{2}$ cup dry weight serving to the whole pulse counterpart was derived from Eq. 3. The resulting calculations for equivalent amounts are listed in Table 3.3. Calculations with the actual test values are shown in Appendix I.

$$\text{Dry solid weight (g)} = 100 \text{ (g)} - \text{sample moisture (g)} \quad (\text{Eq. 1})$$

$$\frac{\text{dry whole pulse solids (g)}}{1 \text{ cup}} = \frac{\text{grams of cooked whole pulse}}{1 \text{ cup}} \times \frac{\text{dry solid weight (g)}}{100 \text{ g}} \quad (\text{Eq. 2})$$

$$\frac{\text{Flour (g)}}{1 \text{ cup}} = \frac{\text{dry pulse solids per 1 cup}}{\text{dry solid weight of flour}} \quad (\text{Eq. 3})$$

Table 3.3. Pulse Equivalent Amounts

Pulse Type	Whole (g)	Flour (g)
Pea	76.00	32.81
Lentil	80.00	40.13

Analysis

Cell wall analysis was completed at Michigan State University (East Lansing, MI) by Amber Bassett (data not shown). Additionally, proximate, starch, and mineral analysis were performed. Proximate analysis of test foods (whole lentil, lentil flour, whole pea, pea flour, spaghetti sauce, and wheat bread) was conducted by Eurofins Scientific Incorporated Nutrition Analysis Center (Des Moines, IA, USA) using standard methods for total fat (AOAC 954.02), ash (AOAC 942.05), crude protein/total nitrogen (AOAC 992.15; AOAC 990.03), moisture (AOAC 925.09), total starch (AOAC 996.11), total dietary fiber (TDF, AOAC 991.43), calories (CFR – Atwater calculation) and total carbohydrates (CFR 21-calculation). Available carbohydrates was derived from the calculation: avCHO = total carbohydrates – TDF. Two samples were analyzed for each food item. Moisture contents were used to determine serving size in calculations (Appendix I). Proximate analysis was also provided by Dr. Hall’s laboratory.

Nutrient Analysis

The nutrient composition values per 100 grams based on Eurofins data are shown in Table 3.4. For 100 g of cooked lentils or peas, nutrient content claims can be made on the fiber (Marinangeli et al., 2017). In other regions, including Australia, Canada, Europe, nutrient content claims can be made on protein and fiber for 100 g of all types of cooked pulses (beans, lentils, chickpeas, peas) (Marinangeli et al., 2017).

Energy, total carbohydrates and fat were similar among the four pulse treatments. The treatments containing lentils were both higher in protein than the pea containing treatments. The flour meals had slightly more fat than the other treatments, but other than that there are no striking contrasts comparing them nutritionally to the treatments. However, when we compare a flour treatment to its whole counterpart, there are a few notable differences. Differences between the pea flour and whole pea meals include a higher fat content, larger weight, and lower fiber content for the pea flour meal. Differences between the lentil flour and whole lentil treatments include a higher fiber content, slightly higher protein, larger weight, and lower CHO for the lentil flour treatment. Notably, all pulse treatments contained fiber, protein, and fat, while the control treatment only contained CHO.

Table 3.4. Nutrient Composition of Test Meals

Characteristics	Glucola control	Whole lentil	Lentil flour	Whole pea	Pea flour
Total weight (g)	215	295.6	339.0	295.6	335.7
Pasta Sauce (g)	--	166.6	166.6	166.6	166.6
Bread (g)	--	49.0	49.0	49.0	49.0
Pulse (g)	--	80.0	40.1	76.0	32.8
Water (g)	--	--	83.3	--	83.3
Sugar (g)	--	--	--	4.0	4.0
Energy (kcal)	200	391	392	382	383
Total Carbohydrate (g)	50	66.8	66.0	68.2	67.7
Fiber (g)	0	11.6	13.9	12.3	11.5
Available CHO (g)	50	55.2	52.1	55.9	56.3
Pasta Sauce (g)		15.6	15.6	15.6	15.6
Bread (g)		20.0	20.0	20.0	20.0
Pulse (g)	-	19.6	16.6	16.3	16.7
Sugar		--	--	4.0	4.0
Protein (g)	0	17.0	17.6	13.8	13.8
Fat (g)	0	6.1	6.3	5.9	6.3

kcal = kilocalorie, g = grams

Starch Analysis

Starch analysis was also performed by Dr. Clifford Hall's laboratory. A comparison of total starch values from Eurofins and Dr. Hall's laboratory are presented in Table 3.5. Total starch values from Eurofins showed the following: raw lentils (38.8%), boiled lentils (15.8%), lentil flour (42.2%), raw peas (40.8%), boiled peas (14.1%), and pea flour (36.9%). This data shows boiling resulted in a decrease in total starch for both pulse samples. Based on this information, theoretically the order of total starch from highest to lowest in our test meal samples was theoretically: lentil flour, pea flour, boiled lentils, and boiled peas. For the purpose of this thesis, these are the values used in discussions regarding total starch, though data from North Dakota State University is still presented in this methodology section. Conversely, based on total starch information from Dr. Hall's laboratory, the pea flour had higher total starch than the lentil flour. These values may vary based on cooking methods applied for the analytical analysis. Recall that based on the 2017 U.S. Pulse Quality Survey (NPGA, 2018), the Avondale lentils had a higher total starch content than the Hampton peas. Under this observation, the Eurofins data is aligned with the survey. This study's own test procedures would need to be taken into consideration for the total starch calculations, which is an area future work can explore.

Resistant starch values were provided by Dr. Hall's laboratory and determined using a Megazyme RS kit (Megazyme, Ireland) procedure. Resistant starch values from Dr. Clifford Hall's lab (Table 3.6) show that the content increased from raw peas to pre-cooked pea flour (14.4% to 27.5%) and decreased from raw lentils to pre-cooked lentil flour (29.1% to 16.9%). This aligns with previous findings by Brummer and colleagues (2015), who found that green lentils had the lowest RS compared to peas, beans, red lentils, and chickpeas.

Table 3.5. Total starch analysis by Eurofins and NDSU.

Sample	Eurofins	NDSU
Raw pea	40.8	43.0
Boiled peas	14.1	n/a
Pea flour	36.9	44.9
Raw lentil	38.8	41.1
Boiled lentils	15.8	n/a
Lentil flour	42.2	40.7

Table 3.6. Resistant starch analysis of pulses (NDSU).

Sample	Total Starch (%)	Starch (% of total CHO)	Resistant Starch (%)	Resistant Starch (% of total starch)
Raw pea	43.0	61.9	14.4	23.3
Pre-cooked pea flour	43.9	64.7	27.5	42.5
Raw lentil	41.1	62.7	29.1	46.4
Pre-cooked lentil flour	40.7	62.8	16.9	26.9

Mechanisms behind an increase or a decrease in resistant starch were discussed earlier in the *Pulse Processing* section of Chapter 2. The increase seen in the peas may be related to starch retrogradation, while a decrease may be due to amylose inhibitors (Wang et al., 2010). A decrease in resistant starch is expected, though the peas saw an increase. Additionally, other work using lentils saw an increase with cooking or dehulling compared to raw lentils (Wang et

al., 2009). This study's lentils do not show this pattern, possibly due to the presence of the hull, variety, and test procedures.

Mineral Analysis

Mineral analyses of the pulses were provided by Dr. Hall's laboratory. The mineral content of the samples is shown in Table 3.7. Many of minerals presented are important for glucose metabolism through several mechanisms (Martini et al., 2010). Several of these minerals have been proposed as reducing the risk of T2DM or improving metabolic functions associated to diabetes, including calcium, magnesium, potassium, and sodium (Martini et al., 2010). Of the minerals presented, nutrient content claims in the United States could be made for 100 g cooked lentils on their iron, phosphorus, and zinc content (Marinangeli et al., 2017). Similar claims cannot be made in the United States for 100 g cooked peas (Marinangeli et al., 2017).

When comparing within a pulse type (i.e., raw pea compared to pre-cooked pea flour), there are differences in calcium, iron, magnesium, potassium, phosphorous, selenium, and sodium according to physical form. Copper, manganese, and zinc remain similar between pulse forms. Potassium content decreases from raw pea to pea flour but increased in the lentils. Selenium decreased with both pulse types according to form (raw whole to pre-cooked flour). Calcium, iron, magnesium, and phosphorous content increases with changes in form (whole pulse to flour). In a previous study looking at chickpea, pigeonpea, urd bean, mung bean, and soybean, a different effect was seen with some of these minerals. The publication reported minimal effects from processing (autoclaving, roasting, germination, and fermentation) on calcium, magnesium and iron (Chitra et al., 1996). However, the increases seen in the pulse flours may be explained by bioavailability. Sandberg (2002) reported legumes generally have poor bioavailability because of antinutrient factors. These factors, including lectin, raffinose,

oligosaccharides, polyphenols and phytate, can lower nutrient bioavailability, as well as digestibility (Sandberg, 2002).

In the case of dehulling, this practice reduces phenolics (Lin et., 2016). While the study flours' hulls were reinserted into the final product, a milling pass did remove the seed coat from the pulses so it may have reduced the phenolic content. Mentioned earlier, polyphenols inhibit digestive enzymes α -glucosidase and α -amylase (Lin et al., 2016), and thus their presence would have a positive impact on glycemic control. Further, processing can reduce lectins (Singh et al., 2017). The milling processes may have made some of these minerals more bioavailable by reducing antinutrient factors.

Comparing the flours to each other, the pea flour had more calcium, iron, magnesium, and selenium than the lentil flour. On the flip side, lentil flour had more potassium, phosphorous, sodium, and zinc than the pea flour. Information in the following paragraphs is presented on the effects of calcium, magnesium, potassium, and sodium (as sodium chloride) in relation to glucose metabolism.

Table 3.7. Mineral analysis of pulses.

Mineral	Raw pea	Pre-cooked pea flour	Raw lentil	Pre-cooked lentil flour
Calcium (mg/kg)	512	675	293	373
Copper (mg/kg)	6	6	8	6
Iron (mg/kg)	41	76	41	67
Magnesium (mg/kg)	748	758	640	690
Manganese (mg/kg)	7	5	8	8
Potassium (mg/kg)	6162	5677	5947	6026

Table 3.7. (continued)

Mineral	Raw pea	Pre-cooked pea flour	Raw lentil	Pre-cooked lentil flour
Phosphorous (mg/kg)	2789	2847	2477	3102
Selenium ($\mu\text{g/kg}$)	246	236	248	225
Sodium (mg/kg)	120	110	132	199
Zinc (mg/kg)	34	33	36	36

Calcium metabolism is linked to the effects that vitamin D has on glucose metabolism, namely a vitamin D deficiency. Vitamin D deficiency indirectly promotes glucose intolerance, altered insulin secretion and T2DM because of calcium metabolism (Palomer et al., 2008). In an animal model looking at vitamin D and calcium, dosages of these two resulted in increased insulin secretion and decreased plasma glucose (Cade & Norman, 1987), which is beneficial for glucose metabolism. Further, a randomized controlled trial showed a decreased fasting glucose in individuals with impaired glucose tolerance with vitamin D and calcium (Pittas et al., 2007). Calcium alone can worsen insulin resistance, if the intracellular calcium concentration is increased because of reduced intracellular magnesium (Barbagallo et al., 2003).

Lopez-Riadura and colleagues (2004) found that magnesium supplementation for 4 to 16 weeks resulted in a decreased fasting plasma glucose in adults with T2DM. Magnesium is a second messenger for oxidative glucose metabolism (i.e., insulin action) (Martini et al., 2010), and a deficiency results in impaired insulin action through defective insulin receptor activity or increased intracellular calcium (Barbagallo et al., 2003). Insulin resistance is consequently worsened when either of these events occur (Barbagallo et al., 2003).

Potassium has been shown to have a negative correlation with plasma glucose (Zillich et al., 2006). This might be due to reduced insulin secretion, which results in impaired glucose tolerance (Rowe et al., 1980).

Lastly, sodium chloride may decrease insulin sensitivity by stimulating plasma renin secretion when present in low quantities (Townsend & Zhao, 1994; Townsend et al., 2007). On the flip side, plasma renin activity is inhibited when there is increased sodium chloride, in efforts to maintain blood pressure (Townsend & Zhao, 1994; Townsend et al., 2007).

There are interesting relationships between various minerals and glucose metabolism. However, the focus of this study was not on the minerals so conclusions based on this factor may be limited. For instance, mineral analysis was not completed on the cooked (boiled) pulses and the bioavailability of these minerals is unknown in this study. Nonetheless, this brief discussion highlighted some of the differences in mineral content between the pulse flours and proposed mechanisms behind those minerals' effects on glucose control, the impact of processing going from raw to flour, and brought up the possibility of the role antinutrients play in mineral content.

Test Meal Content

Participants received a total of five test treatments (four pulse treatments and one control treatment). The control treatment was a glucose tolerance beverage, Glucola® 50 g (Thermo Fischer Scientific, Waltham, MA, USA). All test meals had an approximate 50 g of available carbohydrates (actual nutrient composition in Table 3.4). The masses for each of the pulses were: 40 g lentil flour, 33 g pea flour, 80 g whole lentils, and 76 g whole peas. Pulse treatments were served with 49 g or approximately 1 slice of Pepperidge Farm Honey Whole Wheat Grain bread (Pepperidge Farm, Norwalk, CT, USA) and 167 g of Classico traditional spaghetti sauce (The Kraft Heinz Company, Glenview, IL, USA). The whole pea and pea flour meals had an additional 4 g of sugar (company, location). Both the bread slice and sugar were used in order to

meet the desired available carbohydrate content (~50 g). The spaghetti sauce was used to improve palatability and serve as a simple food matrix to reduce confounding influences from multiple food ingredients. The pulse flour treatments had 83 g (1/3 cup of water) added for palatability.

Test Meal Preparation

Test treatments were prepared in the kitchen of the NWRC. All ingredients were also stored in the building's kitchen. All ingredient weights were measured using a Mettler PC 4000 scale (Mettler Toledo, Columbus, OH, USA). Actual weights during meal preparation were recorded by staff. The control treatment of 50 g Glucola was stored in a walk-in cooler at 4°C. The unopened 296 mL bottle was inverted slowly 2-3 times to assure mixing of contents, then all contents were poured into a drinking glass and immediately served to the participant. No other food besides bottled water was served with the Glucola beverage.

The whole peas and whole lentils were soaked (~16 h and ~2 h, respectively) at room temperature (20C) in reverse osmosis (RO) water in a 1:3 ratio. Water from the soaked pulses was then discarded and the pulses were rinsed under cold running water. This is done to reduce oligosaccharides, which are correlated to gastrointestinal discomfort, such as flatulence (Wang et al., 2007). Several studies show reduction from soaking (Han & Baik, 2006; Agbenorhevi et al., 2007; Tajoddin et al., 2010), and this process may involve two mechanisms by which oligosaccharides are reduced. The proposed mechanisms are the activation of α -galactosidase which breaks down oligosaccharides (Rakshit et al., 2015), or the water absorbed causes the oligosaccharides to dissolve and leach out of the pulses (Han & Baik, 2006; Agbenorhevi et al., 2007).

In a saucepan, RO water was set to medium to high heat on a Southbend commercial gas range (Southbend, Fuquay-Varina, NC, USA). The whole pulse was added once the water reached boiling point (100°C). Whole peas simmered for approximately 1 hour, and whole lentils simmered for approximately 12 minutes. All whole pulses were tested subjectively for tenderness by sampling. If pulses were very, moderately or slightly hard, then they were cooked longer. The ideal tenderness was between slightly hard and slightly soft. Very soft or mushy pulses were not served to participants.

All pulse treatments were also served with toasted bread and mixed in spaghetti sauce. A full slice of bread was weighed to 49 g, cut in half diagonally and toasted in a Proctor Silex toaster (Hamilton Beach Brands, Glen Allen, VA, USA) at the medium setting for approximately two minutes. The end pieces of the loaves were not used due to their small sizes. If the weight of the full slice was slightly less than 49 g, a small portion of a smaller slice was cut off to meet the weight. The small piece was not toasted and was instead mixed into the sauce mixture. If the weight was slightly more, a small portion of a corner on the slice was trimmed off and discarded. The toasted slice was served on a plate next to the bowl containing the treatment meal.

Unopened jars of spaghetti sauce were kept at room temperature (20°C) in a pantry. Opened jars were stored in a walk-in cooler at 4°C. The jar was first shaken, then the sauce was stirred with a knife or long spoon. The digital scale with a clean small white bowl was tared to zero. The sauce was then scooped into the bowl and weighed out to 166.6 g. The sauce was covered with a small white plate and set aside until the rest of the meal's ingredients were prepared. Heating of the sauce was the final step in meal preparation.

In a separate bowl, 166.6 g of Classico traditional spaghetti sauce was heated in a 1300W Panasonic microwave (Panasonic, Kadoma, Osaka, Japan) at 100% power, with a lid to prevent

moisture loss. The sauce was heated on high power level for 15 seconds, and then stirred. This was repeated for a total of 3 times. For pulse flour treatments, 83.3 g of RO water was heated in the microwave for 1 minute. The pulse flour was weighed out and mixed with the RO water and 166.6 g of spaghetti sauce.

Forms

Medical history questionnaire

Participants completed a medical history questionnaire, providing a list of their medications and supplements, as well as a detailed list of any previous medical conditions (Appendix F).

Food frequency questionnaire

A Food Frequency Questionnaire (FFQ), also called a dietary assessment screener in our study, was given to participants to complete at their screening visit (Appendix G). It asked the participants how often they ate certain foods. With this information, we gathered the number of servings of fruits and vegetables they consume a day, along with their grams of fiber, and the percentage of fat that makes up their diet (Block et al., 2000). The FFQ is a validated dietary assessment tool, with its dietary intakes correlating with type 2 diabetes (Yang et al., 2010; Hu et al., 2001).

Food log

For each pre-test day, participants filled out 24-hour dietary recalls (food logs) (Appendix J) and staff reviewed these with participants on the morning of test days. Food scales and measuring cups were provided to participants at their request or if the quality of their food logs depended on it. These food logs were to monitor and control for any drastic changes to dietary habits throughout the study, as well as compliance with dietary restrictions required by the study (on pre-test days), such as food intake consistency, no caffeine and fasting.

Physical activity

Participants completed the International Physical Activity Questionnaire (IPAQ) form (Appendix K) at their screening visit and on each of the five test days. The self-report short form asked the participants how many hours they spent sitting, and how many days and hours they spent walking and doing moderate and vigorous physical activity. This ensured participants did not participate in any moderate or vigorous activity the day before testing nor start a new exercise routine that would increase their metabolism. Moderate-intensity exercise, described as “walking briskly,” results in a half reduction of postprandial glucose levels. These effects are evident when 90 minutes of moderate-intensity exercise is performed in a 2-hour range before or after a meal (O’Keefe & Bell, 2007; Levine, 2007). Physical activity categories on the IPAQ were high (approximately one hour or more per day of at least moderate intensity), moderate (approximately half an hour on most days of at least moderate intensity), and low (none of the criteria for moderate or high levels is met). The IPAQ offers moderate and vigorous activity definitions according to the physical effort required and observed changes from normal breathing. A meta-analysis (Kim et al., 2013) found the overall effect sizes of the IPAQ were medium-sized (ranging from 0.32-0.49) for walking, total moderate physical activity (TMPA), vigorous physical activity (VPA), and total physical activity (TPA). Moderate physical activity (MPA) had a small-sized effect size of 0.27 (Kim et al., 2013). The total effect sizes were calculated according to Cohen’s guideline (Kim et al., 2013).

Data Collection Procedure

Pre-testing protocol

Participants were required to obtain adequate sleep (7-9 hours) (Hirshkowitz et al., 2015) on nights prior to test dates. They were instructed to refrain from caffeine intake, such as caffeinated beverages, foods or medications. Other dietary restrictions included alcohol

consumption and other pulses for the 24-hour period prior to the morning testing appointment. In addition, participants were asked to refrain from moderate or vigorous exercise (as defined by the IPAQ) 24 hours prior to test dates. Participants could take pre-approved medications including metformin up until the night before testing. If using injectable medication like Trulicity, the day of dosage was consistent throughout the study. Unless circumstances warranted, participants were not allowed to take medications until after testing completion.

Participants were required to fast for 12 hours preceding their testing time. Their frozen meal and optional snack had to be consumed prior to fasting as their last meal consumption. Once their fasting period began, they were only allowed to drink plain water. After completing their testing for the day, the pre-testing protocols no longer applied and participants resumed their normal activities and dietary habits.

Participant arrival

Upon arrival for test days, participants were asked a series of questions to assess protocol compliance regarding fasting, diet, exercise, and sleep. After this, they had their anthropometric measurements taken (blood pressure and weight), followed by a fasting blood draw by a trained nurse or phlebotomist. Physical activity restrictions on pre-test days continued until the end of a test visit. For the test day morning (approximately 7 a.m. to 12 p.m. at the latest), participants refrained from excessive physical activity (i.e., leisurely walking) and occupied themselves at tables with laptops or books.

Anthropometric measurements

The anthropometric measurement methods are summarized in Appendix L.

Height, waist circumference, weight

At the initial screening visit, height measurements and waist circumferences were taken.

The height measurements were taken using a wall-mounted stadiometer and the waist

circumference with a retractable fiberglass tape measurer. Measurements were taken to the nearest 0.1 cm by trained staff. Waist circumference was measured at the iliac crest according to NHANES Anthropometry Procedures Manual (Centers for Disease Control and Prevention, 2017). These measurements were only taken at baseline for body mass index calculations.

Body weight was also measured using methods from NHANES Anthropometry Procedures Manual (Centers for Disease Control and Prevention, 2017) and the Fitness & Metabolism Unit of the NWRC in Ames, IA, US (report not shown). Body weight was measured to the nearest 0.1 kg at the screening visit and/or at the start of each test day using a Detecto digital scale (Webb City, Missouri). Light clothing was allowed, though shoes, sweaters, or jackets were removed. After the scale was zeroed, participants stepped onto the platform with their arms at their sides and looked straight ahead. This was repeated once more to obtain a total of two readings which were averaged.

Body mass index (BMI)

The body mass index (BMI) was calculated at baseline with measured height and weight measurements using the Metric System. The following formula was used: weight (kg)/[height(m)]². The resulting calculation used to account for centimeters as the common unit of height measurement: [weight (kg) / height (cm) / height (cm))] * 10,000. Both the formula and calculation are used by the Centers for Disease Control and Prevention (Centers for Disease Control and Prevention [CDC], 2014).

Blood pressure

Blood pressure was measured in a seated position at all visits using an Omron automatic digital blood pressure monitor (Omron Healthcare, Inc., Lake Forest, IL, USA) after 5 minutes of sitting. The arm was in a relaxed position with the antecubital fossa facing the researcher and the participant's arm resting on a table.

Test meal procedure

Fasting blood draw and meal intake

A trained nurse collected blood at fasting for baseline glucose values. The *Blood Handling and Analysis* section details blood tests completed at fasting. After this blood draw, participants ate their meal treatment. Participants were allowed to drink bottled water during all of their meals. Meal start and meal end time were recorded. Each participant ate the test meal in an isolated dining room and avoided electronics while eating to decrease distractions. Participants were expected to consume the entire test meal in a timely fashion (within 15 minutes).

Post-meal procedure

Blood collection

Blood samples were collected via catheter or venipuncture by trained nurses or phlebotomists at the fasting state and postprandial at 30, 60, 90, 120, 150, and 180 minutes. The post-treatment blood draws began after the subject had finished consuming the treatment meal. Blood collection protocols were followed according to a Standard Operating Procedure (SOP) for Obtaining and Working with Human Blood Samples (Appendix M), produced by Jeanne Wempe Stewart, Assistant Scientist II at Iowa State University (Ames, IA). Blood tests performed are detailed in the *Blood Handling and Analysis section*.

Preparation for new test day

Prior to leaving the testing site, participants were given a new food log form to complete for their next test day. They were also given a new frozen meal and dessert (if selected) to take home for the next pre-test day.

Data Handling

Microsoft Excel 2016 (Microsoft, Redmond, WA, USA) was used for data input for all study measurements (i.e., dietary information, demographics, glucose values). SPSS Statistics software version 25.0 for Windows (IBM Corporation, Somers, NY, USA) was used for statistical analyses using imported Excel datasets. ESHA Food Processor® SQL Software (version 11.3, ESHA Research, Salem, OR, USA) was used for food log data input and to run macronutrient summary reports from participant food logs. Nutrition Quest (Nutritionquest Inc., Berkeley, CA) is an online assessment tool that was used to input FFQ information and generate summary reports on servings and amounts consumed (percentages or servings) of fat, fruits and vegetables, and fiber. Continuous variable data are reported as mean and standard errors of the mean.

Descriptive Analysis

Descriptive statistics were used for participant demographics, medical parameters (i.e., triglycerides), food frequency questionnaires, food logs, and physical activity.

ANOVA

A multivariate analysis of variance (MANOVA) for repeated measures with time and diet as factors was used to evaluate differences in glucose measures between the five meal treatments. A one-way analysis of variance (ANOVA) was used to compare means for glucose net change, iAUC, and dietary intake. Tukey HSD post-hoc test was used to identify mean differences among treatments and between time points, among iAUC and time points, and among macronutrients and treatments or test days. P-values less than 0.05 were considered statistically significant. A 95% confidence interval was used for post-hoc analysis.

Blood Handling and Analysis

Blood was drawn by trained nurses and was handled by trained study staff. Handling included inverting the test tubes at least 10 times to prevent hemolysis, transporting the tubes in a sterile lab cart to the NWRC laboratory, following proper centrifuging procedures, and transferring test tubes into appropriate tubes on weekends when the blood would not be collected that same day.

Laboratory tests

Materials (tubes and requisition forms) were provided by Quest Diagnostics Incorporated (Secaucus, NJ, USA). Wood Dale, IL location. Two types of tubes were used: Serum Separator Tubes® (SST) or Ethylenediaminetetraacetic acid (EDTA) lavender tubes. Blood in SST tubes were centrifuged in a Horizon Model mini E (Quest Diagnostics Inc., Secaucus, NJ, USA) at 3348 RPM for 10 minutes or a Horizon model 642 E (Quest Diagnostics Inc., Secaucus, NJ, USA) at 3378 RPM for 15 minutes, both at 22C. The EDTA tubes did not require centrifugation. The EDTA tubes were used for the collection of anticoagulated whole blood, necessary for Complete Blood Count (non-fasting) and HbA1c (non-fasting) blood tests. The SSTs were used for the collection of serum, necessary for the Lipid Panel (fasting) test. Quest Diagnostics also performed the blood analysis with a one-to-three day turnaround for results. For the Lipid Panel, the low-density lipoprotein cholesterol (LDL-C) was calculated using the Martin-Hopkins calculation. These blood tests illustrate overall health. Serum glucose was collected all time points (fasting or 0 min, 30 minutes postprandial up to 180 minutes).

iAUC

The incremental area under the curve (iAUC) of the blood glucose was used to compare the effect of the test meals. Timepoint differences between fasting and post-treatment glucose concentrations were determined and iAUC calculations were completed using the trapezoidal

rule (Winham et al., 2017). Values below fasting were ignored for the iAUC calculations, as is customary (Ramdath et al., 2018; Winham et al., 2017; Ventura et al., 2009). The fasting value was subtracted from the postprandial values before the iAUC calculations (Vakkilainen et al., 2002). The iAUC for blood glucose was assessed between 0-60, 60-120, and 120-180 minutes for participants. ANOVA was also used, which is described earlier.

CHAPTER 4. RESULTS & DISCUSSION

Study Population

A total of 111 residents in the greater Ames area expressed interest in the clinical trial (Figure 4.1). Of the 111 individuals, 94 did not meet inclusion criteria or did not initiate further contact. Common reasons for ineligibility included disqualifying medication use (medications not approved for this study), issues with blood draws, and time commitment concerns. Seventeen individuals enrolled during the allocation phase. Of the seventeen individuals, two declined to participate further due to medication changes or time commitment. Four individuals were discontinued from the study after starting due to non-compliance, medical reasons, or time commitment. Eleven participants (7 men and 4 women) successfully completed all five test days.

Demographics

Descriptive statistics at study entry for the 11 participants are shown in Table 4.1. All participants self-identified as Caucasian. The mean HbA1c value was 6.48% +/- 0.2 and indicated a good or excellent control of diabetes by meeting a common HbA1c target range of under 7% for adults (ADA, 2019). Wong and colleagues (2012) examined results from the 2003-2006 U.S. National Health and Nutrition Examination Surveys and found that 58% of the cohort of adults with T2DM had an A1c of <7%. Most of the participants in this study also met that goal, as shown with the mean HbA1c value of 6.48%. The majority of participants ($n = 9$) used metformin to manage their diabetes, with the remaining two using dietary methods and/or physical activity. Based on this, and the number of ineligible interested persons on disqualifying medications, there appears to be a tendency to medicate T2DM, rather than promote diet and exercise as treatment methods. In fact, the prescription of exercise for T2DM is not very common (Hordern et al., 2012).

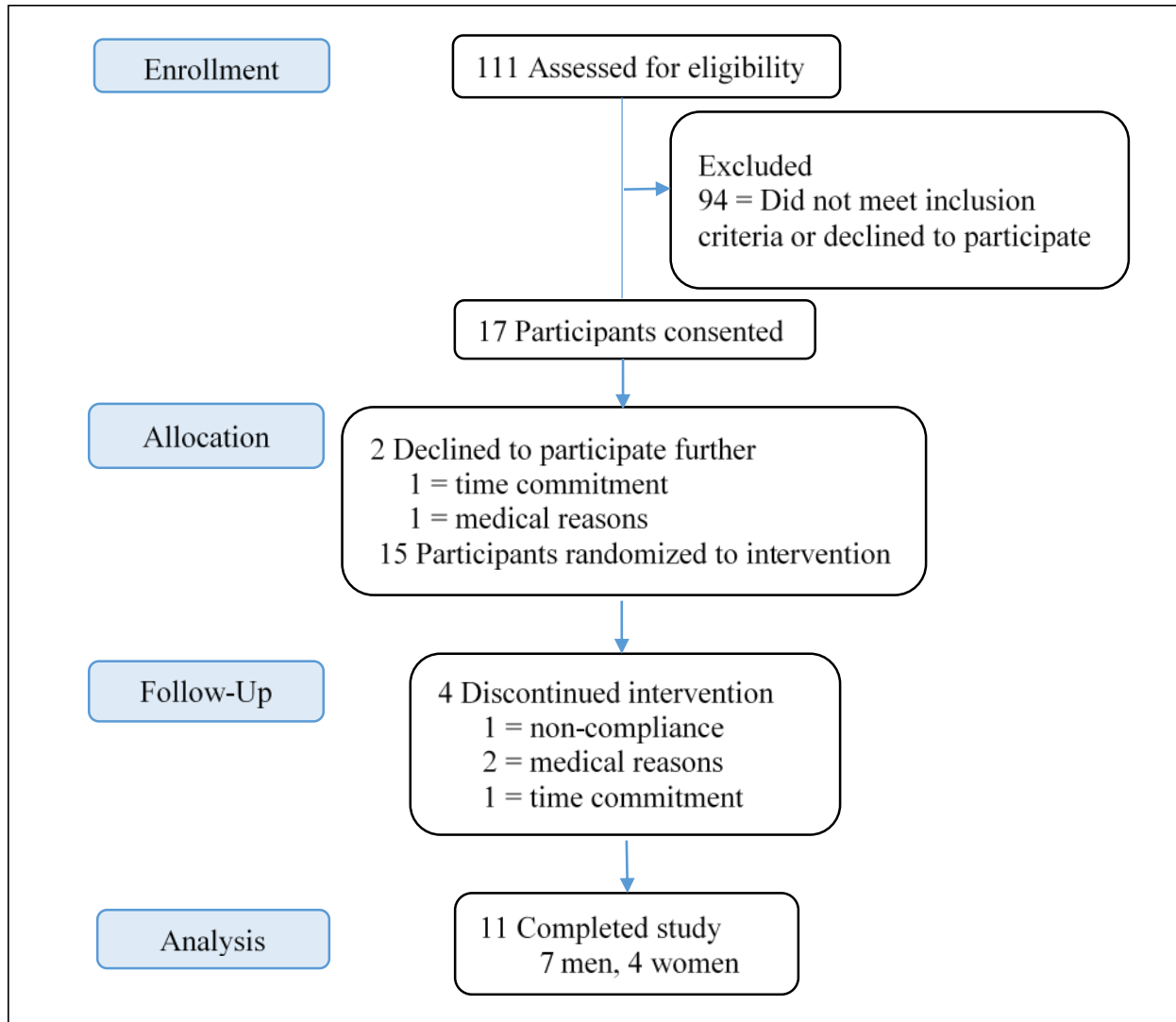


Figure 4.1. Consort flow diagram for clinical trial participants

All participants were in the overweight range or higher, as defined by the CDC (2017). Mean BMI was in the obese class 1 category (30.0-34.9) (CDC, 2017). A one-way ANOVA showed that body weight and BMI did not significantly vary between the five test days for participants (data not shown). These demographics are similar to previous findings from the publication by Wong and colleagues (2012). Wong et al. (2012) found just 13.9% of the adults with T2DM met a BMI goal of $< 25 \text{ kg/m}^2$. If looking at $< 30 \text{ kg/m}^2$ as a goal instead, 43.1% of the adults met that goal (Wong et al., 2012). Our study results show that none of the participants

were under $< 25 \text{ kg/m}^2$, while 18% were under $< 30 \text{ kg/m}^2$. While much lower than the other authors' findings, these results still show a low rate of meeting BMI targets.

Three participants (27%) had normal waist circumferences (below 88.9 cm or 35 inches for women, below 101.6 cm or 40 inches for men), and eight (73%) had large waist circumferences (past the mentioned cut offs). National Institutes of Health references were used for waist circumference classifications (National Institutes of Health [NIH], n.d.). Compared to findings from Wong and colleagues (2012), slightly more participants in this study met a normal waist circumference goal, though the rate is still low. The authors reported only 17.7% of the adults in the cohort met their gender-specific waist circumference target (Wong et al., 2012).

Four (36%) participants had normal HDL cholesterol (HDL-C) levels (above 50 mg/dL for women or above 40 mg/dL for men), and seven (64%) had low levels (below reference values). The 36% is much lower than the findings from Wong et al. (2012), who saw 56.4% of adults met their gender-specific HDL-C goals. However, mean low density lipoprotein cholesterol (LDL-C) levels were 83.5 mg/dL, which meets the LDL-C goal of $< 100 \text{ mg/dl}$ (Wong et al., 2012).

Five (45.5%) participants had normal triglyceride values (below 150 mg/dL), and six (54.5%) had high values (equal to or greater than 150 mg/dL). These results are much higher than those reported by Wong and colleagues (2012), who found that 25.8% of the adults with T2DM had triglycerides under 150 mg/dL. Standards from the National Institutes of Health were used to interpret triglyceride and HDL-C levels (NIH, n.d.).

Overall, our participants are similar to expectations for HbA1c, waist circumference, and LDL-C targets (Wong et al., 2012). The results for BMI were negatively different from previous findings, and HDL-C and triglycerides results were positively different (Wong et al., 2012).

Conclusions from Wong and colleagues (2012) were that less than half of U.S. adults with T2DM met the goals for HbA1c, LDL-C or BMI individually. However, when looking at composite goals for HbA1c, blood pressure, and LDL or BMI, just one-tenth or less met those goals (Wong et al., 2012). In general, our results show the majority of participants are not meeting ideal classifications, except for Hb1Ac and LDL-C.

Table 4.1. Descriptive characteristics of participants

Variable	Mean \pm SEM	Range
Age (yrs)	55.1 \pm 4.3	(29.0-71.0)
Weight (kg) ¹	101.1 \pm 5.5	(80.0-133.4)
Height (cm) ¹	173.4 \pm 3.0	(150.5-188.2)
BMI (kg/m ²) ¹	33.4 \pm 1.0	(28.0-37.9)
HbA1c (%)	6.5 \pm 0.2	(5.4-8.4)
Total Cholesterol (mg/dL)	157.1 \pm 10.9	(101.0-236.0)
LDL-C (mg/dL) ²	83.5 \pm 10.5	(30.0-157.0)
HDL-C (mg/dL)	43.3 \pm 3.0	(34.0-65.0)
Triglycerides (mg/dL)	203.9 \pm 31.0	(86.0-411.0)
Hemoglobin (g/dL)	13.6 \pm 0.5	(11.2-13.6)

¹Values obtained at study entry. ²Baseline values obtained for $n = 10$ due to high triglycerides. BMI = body mass index, HbA1c = hemoglobin A1c, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol.

Findings from Dietary Intake and Physical Activity Measures

Food Frequency Questionnaire

Table 4.2 shows information obtained from the Food Frequency Questionnaire compared to the U.S. Dietary Guidelines for Americans (HHS & USDA, 2015). The majority of participants did not meet the recommendations: above recommended calories from fat, and below recommended fruit and vegetable servings and fiber intake. These results are not surprising. Even in patients who do follow a diet treatment plan for T2DM, there is a low adherence to dietary approaches (King et al., 2009). Considering the nutrition transition seen in the U.S. (less whole grains, fruits, vegetables and fiber consumed), the study results coincide

with this documented dietary shift. These findings emphasize the need for the incorporation of wholesome foods for better health, such as pulses. Pulses meet the call for many nutrients lacking in the U.S. diet.

Table 4.2. Percentage of Participants Meeting U.S. Dietary Guidelines

Dietary Guideline Recommendation	Total (11)	Male 64% (7)	Female 36% (4)
30% or less of calories from fat			
Percent calories from fat %			
Less than 30%	0% (0)	0% (0)	0% (0)
30-35% average	36% (4)	29% (2)	50% (2)
36-40% high	55% (6)	57% (4)	50% (2)
40-50% very high	9% (1)	14% (1)	0% (0)
5-9 servings of fruits/vegetables per day			
Servings of fruits & vegetables:			
5 or more per day	9% (1)	14% (1)	0% (0)
Less than 5 per day	91% (10)	86% (6)	100% (4)
At least 20 grams of fiber per day			
Dietary fiber intakes			
20+ grams per day	9% (1)	14% (1)	0% (0)
Less than 20 grams per day	91% (10)	86% (6)	100% (4)

24-hour Food Logs

Macronutrient composition of dietary intake 24 hours prior to test days was analyzed (Table 4.3). A one-way ANOVA showed no significant mean differences. While the main purpose of these food logs was to monitor and control the foods consumed on pre-test days, we can also make observations on macronutrients consumed. The information from the food logs could also be used in the future to determine the glycemic load of the day. The means of notable

nutrients were: 2014 kcal, 199 g of carbohydrates, 3668 mg of sodium, and 21 g of fiber. Since the majority of participants were moderately active (detailed below in the *Physical Activity* section), the recommended calorie intake for adults 18 or older is between 1,800 and 2,600 kcal, dependent on specific age-gender groups (HHS & USDA, 2015). The variability of limits within age-gender groups make the mean caloric intake difficult to interpret.

However, other limits are easier to interpret. The mean carbohydrate intake (g) of the study participants exceeded the Recommended Daily Allowance (RDA) for carbohydrates, which is 130 g (HHS & USDA, 2015). The sodium consumed was much more than the Upper Limit (UL) < 2,300 mg per day for people 14 years or older (HHS & USDA, 2015). The amount of fiber consumed by the participants was less than the recommended grams (ranging from 22.4 to 33.6 grams, dependent on age-gender group) (HHS & USDA, 2015). These findings give similar conclusions as the FFQs, showing that the U.S. eating patterns follow the nutrition transition phenomena (suboptimal diet quality).

Macronutrient composition of evening meals consumed prior to test days was analyzed (Table 4.4). A one-way ANOVA showed no significant mean differences in any macronutrients prior to the test days. The mean caloric intake was 465.1 kcal, and mean carbohydrate intake was 56.3 g. The evening meal provided 23% of the day's calories, 27% of the carbohydrate intake, and 38% of the fiber intake. Some of this fiber may have come from non-starchy vegetables, depending on the frozen meal selected.

Table 4.3. Macronutrient composition of dietary intake 24 hours prior to test days¹

Nutrient	Glucola control	Whole lentil	Lentil flour	Whole pea	Pea flour
Energy (kcal)	2013.9 ± 293.7 (992.3-4510.1)	1884.3 ± 312.1 (967.3-4838.3)	1827.5 ± 233.6 (913.1-3757.9)	2027.5 ± 305.8 (920.6-4820.3)	1927.6 ± 310.1 (784.8-4820.3)
Carbohydrate (g)	198.6 ± 20.8 (102.5-272.7)	192.3 ± 19.2 (94.2-293.8)	186.1 ± 18.9 (75.5-269.8)	194.5 ± 18.3 (75.9-277.0)	185.7 ± 20.0 (65.6-277.0)
Available CHO (g)	178.1 ± 19.7 (89.5-248.0)	173.1 ± 17.8 (84.1-258.0)	167.6 ± 18.1 (65.6-248.4)	174.6 ± 17.2 (66.0-243.1)	167.9 ± 18.7 (57.5-243.1)
Total fiber (g)	20.5 ± 2.2 (9.5-35.9)	19.1 ± 2.4 (8.0-35.9)	18.5 ± 2.3 (9.9-30.9)	19.8 ± 2.0 (9.9-33.9)	17.9 ± 2.4 (8.1-33.9)
Total fat (g)	92.8 ± 19.3 (31.0-260.5)	82.5 ± 19.7 (39.6-271.5)	82.6 ± 15.2 (44.6-222.3)	95.4 ± 19.1 (44.2-271.1)	88.0 ± 19.5 (35.0-271.1)
Saturated fat (g)	29.9 ± 5.1 (9.7-59.4)	26.0 ± 4.8 (8.9-65.9)	27.1 ± 4.9 (9.7-52.6)	31.1 ± 5.0 (13.1-65.6)	29.2 ± 4.4 (16.0-65.6)
Cholesterol (mg)	362.6 ± 77.5 (50.0-801.1)	302.1 ± 68.2 (95.0-674.1)	291.1 ± 59.8 (65.0-661.9)	335.7 ± 69.5 (46.7-667.4)	311.0 ± 56.6 (125.4-667.4)
Sodium (mg)	3668.0 ± 449.0 (1922.7-6789.3)	3137.0 ± 366.6 (819.2-5590.8)	2971.6 ± 357.9 (1120.7-4800.9)	3200.8 ± 415.2 (1001.1-5785.8)	3187.5 ± 401.1 (1638.8-5880.6)
Potassium (mg)	2418.0 ± 263.7 (1032.7-3694.3)	2107.6 ± 317.9 (950.5-4389.3)	1919.5 ± 319.2 (181.7-3953.2)	2290.3 ± 284.3 (977.7-4239.3)	1853.9 ± 314.1 (545.9-4239.3)
Protein (g)	102.7 ± 21.1 (52.3-299.7)	98.7 ± 23.9 (57.2-332.6)	94.2 ± 15.0 (52.4-228.5)	105.2 ± 24.7 (57.1-345.6)	103.5 ± 24.9 (55.9-345.6)

¹ All values are means ± standard error of the mean (SEM) (range)

kcal =kilocalorie, mg=milligram, g=gram

Table 4.4. Macronutrient composition of evening meals consumed prior to test days¹

Characteristics	Value	Range
Energy (kcal)	465.1 ± 12.8	(320.0-808.8)
Carbohydrate (g)	56.3 ± 1.7	(31.0-87.4)
Available CHO (g)	48.7 ± 1.7	(24.0-75.4)
Total fiber (g)	7.6 ± 0.6	(7.0-21.25)
Total fat (g)	16.0 ± 0.9	(4.5-37.8)
Saturated fat (g)	5.3 ± 0.4	(1.0-19.1)
Cholesterol (mg)	56.4 ± 2.2	(35.0-116.8)
Protein (g)	25.7 ± 0.4	(23.0-35.5)

¹ All values are means ± standard error of the mean (SEM) (range)
kcal =kilocalorie, mg=milligram, g=gram

Physical Activity

Over one-third (39.6%) of self-reported forms throughout the study duration showed participants performed a moderate level of physical activity. This was followed by 35.8% of forms reporting high physical activity levels, and 24.5% reporting low physical activity. None of the participants engaged in high or moderate physical activity on the day before testing, as instructed. The reported levels of physical activity match national survey findings. Nationally, the majority of adults (< 30%) do not meet physical activity guidelines (HHS & USDA, 2015). Additionally, for adults following an exercise treatment plan for T2DM, this approach has the lowest adherence compared to diet or medication (World Health Organization, 2003; King et al., 2009).

Individuals with normal waist circumferences were more likely to perform high levels of physical activity, reporting high physical activity 87% of the time and 13% for low activity. Individuals with large waistlines were more likely to perform low or moderate levels of physical activity, reporting moderate physical activity 50% of the time, followed by 34% and 16% of the time for low and high physical activity, respectively.

Postprandial glucose (PPG)

A general linear model analysis revealed significant interactions between time and diet as factors when evaluating differences in glucose values. There were no significant differences by gender, so the data were pooled for analysis. Wilks' Lambda test results were significant among time points ($P < 0.0005$) as well as when looking at the interaction between time points and treatment type ($P = 0.001$), meaning the specific time point affects net glucose change, and effects are seen when considering time and treatment together too. Means of net glucose change for treatment type across time points with a MANOVA (Tukey HSD) showed significant differences in mean when comparing the control treatment to the whole lentil ($P = 0.001$) and whole pea ($P = 0.012$) treatments, meaning there was an overall difference between whole pulses and the carbohydrate-heavy control when looking at all the mean net glucose changes for all treatments. However, there were no significant differences among the other treatments based on overall pooled means. This means that there was something attributed to the whole pulses that the pulse flours did not share, or at least not to the same extent to make the flours significantly different from the control treatment. One apparent nutritional difference between the whole pulses and flours was total starch content, with the flours having more total starch.

An additional nutritional difference may be related to the antinutrient content. Milling, specifically dehulling, and processing in general reduce polyphenols and lectins, both of which have normally positive effects on glycemic control when present (Lin et al., 2016; Singh et al., 2017). Reductions of these antinutrient factors may explain why the pulse flours were not statistically different from the control treatment and produced higher PPG values than the whole pulses (with the exception of pea flour being higher than the whole peas at 120 minutes).

Another difference is based on structure - the whole pulses should have intact cell walls, while the flours would not. The cell wall prevents starch degradation by digestive enzymes

(Brummer et al., 2015), and a damaged one, as in the flours, would mean more starch is digested and released into the blood. Additionally, dehulling also reduces phenolics (Lin et al., 2016), which would lead to the same outcome. The relationship between the cell wall and starch degradations explains why the whole pulses yielded a lower response, as there was less starch degradation in these meals. Despite any potential distinctions between the whole pulses and pulse flours, these were not significant enough to be different from the flours looking at this level (all the means of net glucose change for treatment type pooled across time points).

A profile plot (Figure 4.2) of the estimated marginal means of time points and treatments shows that the combination of treatment and time was not significant for the marginal means, meaning there was a clear influence from main effects of treatment and time, regardless of the time or the treatment. As expected, the treatment served, along with which time point is observed, translates to the net glucose changes seen.

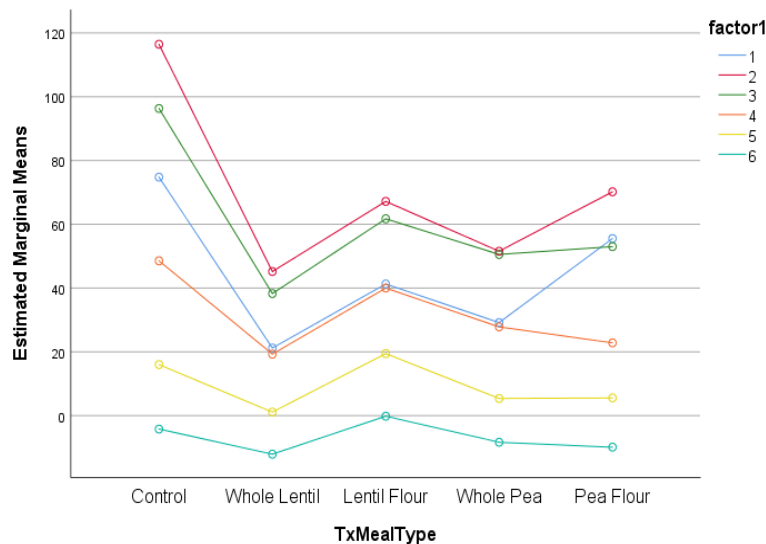


Figure 4.2. Estimated marginal means of time point and treatment type*

*factor1: postprandial time points. 1 = 30 minutes, 2 = 60 minutes, 3 = 90 minutes, 4 = 120 minutes, 5 = 150 minutes, 6 = 180 minutes.

Incremental changes in blood glucose for all treatments are presented in Table 4.5. Glucose levels <140 mg/dL after two hours indicate normal glucose tolerance, while levels ≥ 140 and <200 mg/dL indicate impaired glucose tolerance (ADA, 2000). All of the participants exhibited impaired glucose tolerance after two hours, as expected with type 2 diabetes, confirming their qualification for this study. Clinically significant changes in blood glucose have been set at a decrease equivalent to ≥ 1 mmol/l (Hordern et al., 2008). In a study involving pleural glucose concentration, a difference of 1.0 mmol/L or greater (at or above 18 mg/dL) were considered clinically significant because of potential influences on clinical management (Rahman et al., 2008). The changes in mean blood glucose for this study were greater than 18 mg/dL until 150 minutes postprandial, and thus are clinically relevant (Table 4.5). Variations among subjects may be explained by a faster gastric emptying rate of some participants (Sevenpiper et al., 2000).

Following a statistically significant interaction between time and treatment, a one-way ANOVA was performed for individual time points (Table 4.5). The results showed significance for 30 minutes ($P < 0.005$), 60 minutes ($P < 0.005$), and 90 minutes ($P = 0.006$) (Figure 4.3). This means that there were treatments that had notable effects at certain time points. Significance was lost starting at 120 minutes postprandial, indicating an effect on glucose metabolism under 120 minutes post meal consumption. A lack of significance starting at 120 minutes is not necessarily a negative observation. Elevated PPG has several negative implications on health, with the highest observed post-meal glucose peaks occurring within 1 hour and 15 minutes after eating a meal (Daenen et al., 2010). Because of the negative health implications with elevated PPG, the significant differences between the control and the pulses within the time period of interest (within 1 hour and 15 minutes) were clinically relevant.

Although significance was not continuous until 180 minutes, there were some noteworthy observations looking at the entire time span (0 to 180 minutes), regardless. The whole lentil trended lower than all other treatments for all time points, indicating there was some factor associated with whole lentils that was not present to the same extent (if at all) in the other pulses. The whole lentil treatment contained the largest amount of pulses (grams), though this likely does not explain this trend. The one distinction between the whole lentils and all the other treatments was that the lentils provided the most available CHO out of all the other pulse treatments. The lentils provided 19.6 g of available CHO, which the other pulses provided 16.3 to 16.7 grams. Comparing the whole peas to their flour counterparts, the whole pulses trended to yield lower PPG for all time points, with the exception of similar values between the two pea treatments at 150 and 180 minutes. The whole pea and pea flour treatments were similar nutritionally, with total carbohydrates, fiber, available CHO, protein, and fat. The SDS or RS content of the two may be similar, accounting for the similar glycemic responses at 150 and 180 minutes attributed to the time of starch digestion and amount.

Another noteworthy observation is made when comparing the two flours, lentil flour trended to yield lower PPG up until 60 minutes. The lentil flours had a higher total starch content than the pea flour (Eurofins data) and while information on specific type of starch (SDS, RDS, or RS) is unavailable, the lentil flour likely had more SDS that was absorbed and digested after 60 minutes than the pea flour. This coincides with the definition of SDS, as the term details the amount of starch hydrolyzed between 20 and 180 minutes (Englyst et al., 1992). If there was more SDS content in the lentil flours compared to the pea flours, it is a logical assumption that this type of starch explains the rising trend after 60 minutes. Further, these results suggest that the pea flour had more RDS than the lentil flours, since RDS is the amount of glucose released

after 20 minutes (Englyst et al., 1992). This could explain why the pea flour peaked higher at first compared to the lentil flour.

Results with a Tukey HSD test are shown in Table 4.5. At 30 minutes, significant differences were found comparing the control to the whole lentil ($P < 0.005$), lentil flour ($P = 0.001$), and whole pea ($P < 0.005$) treatments, but not the pea flour. The pea flour had the lowest pulse gram weight compared to the other pulse treatments, but there are no other differences based on the information at hand. Thus, an explanation for this may be due to one or several of the various factors that are still relatively unexplored (i.e., fiber, cell wall, etc.). For example, observations based on mineral content cannot be made, as this information is not available for the pulse meals (information was provided for raw whole pulses and the flours, not the boiled whole pulses), but this might be one of the mechanisms explaining nonsignificant differences between the pea flour and control.

Then at 30 minutes, the PPG after eating pea flour was significantly different from the whole lentil ($P = 0.001$) and the whole pea meals ($P = 0.014$). Based on the information available, differences are a lower pulse gram weight for pea flour treatments and higher total starch than the other two treatments. The amount of total starch may be a key mechanism in explaining this difference at 30 minutes, with RDS likely being released and digested with the pea flour meals. The pea flour was not significantly different from the lentil flour, which was likely due to similar total starch contents (compared to the whole pulses).

At 60 minutes, the control was significantly different from all treatments: whole lentil ($P < 0.005$), lentil flour ($P = 0.010$), whole pea ($P < 0.005$), and pea flour ($P = 0.02$). The pulse meals were different from the control treatment in virtually every way, except for the presence of available carbohydrates. The other nutrients present in pulses (i.e., protein, fiber) were most

likely responsible for the beneficial glycemic response at 60 minutes. These factors were not active at 30 minutes, suggesting they have a delayed, though still positive, effect. No significant differences were found among the pulse treatments. Again, this was likely because of their nutrient profiles being much more similar to each other than to the control treatment. At 90 minutes, the control was only significantly different from the whole lentil ($P = 0.003$) and whole pea ($P = 0.04$) treatments. The two main differences between the whole pulses and the flours were that the whole pulses have a higher pulse gram weight and were lower in total starch. The amount of total starch, along with some other unexplored factors (i.e., SDS, RS, physical structure, digestive enzyme accessibility), are possibly attributed to this observation at 90 minutes.

Table 4.5. Incremental changes in blood glucose for all treatments¹

	Glucola control	Whole lentil	Lentil flour	Whole pea	Pea flour
Fasting glucose (mg/dL)	129.5 ± 10.3	130.9 ± 10.7	134.0 ± 7.2	130.5 ± 8.9	128.8 ± 9.8
30 min glucose (mg/dL)	74.8 ± 9.0	21.2 ± 3.8***	41.3 ± 5.4**	29.2 ± 3.7***	55.6 ± 5.4
60 min glucose (mg/dL)	109.0 ± 8.8	45.2 ± 7.3***	67.2 ± 10.4*	51.6 ± 7.0***	70.2 ± 8.6*
90 min glucose (mg/dL)	87.8 ± 11.5	38.3 ± 7.3**	61.7 ± 10.1	50.6 ± 6.6*	53.0 ± 9.3
120 min glucose (mg/dL)	45.1 ± 9.8	19.3 ± 6.1	40.0 ± 9.8	27.8 ± 5.4	22.8 ± 10.0
150 min glucose (mg/dL)	12.6 ± 8.1	1.1 ± 5.5	19.5 ± 9.6	5.4 ± 5.1	5.6 ± 9.4
180 min glucose (mg/dL)	-7.6 ± 6.6	-12.1 ± 4.9	-0.2 ± 8.3	-8.4 ± 5.2	-9.9 ± 7.2

¹All values are means ± standard error of the mean.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Min=minutes, mg/dL=milligrams per deciliter

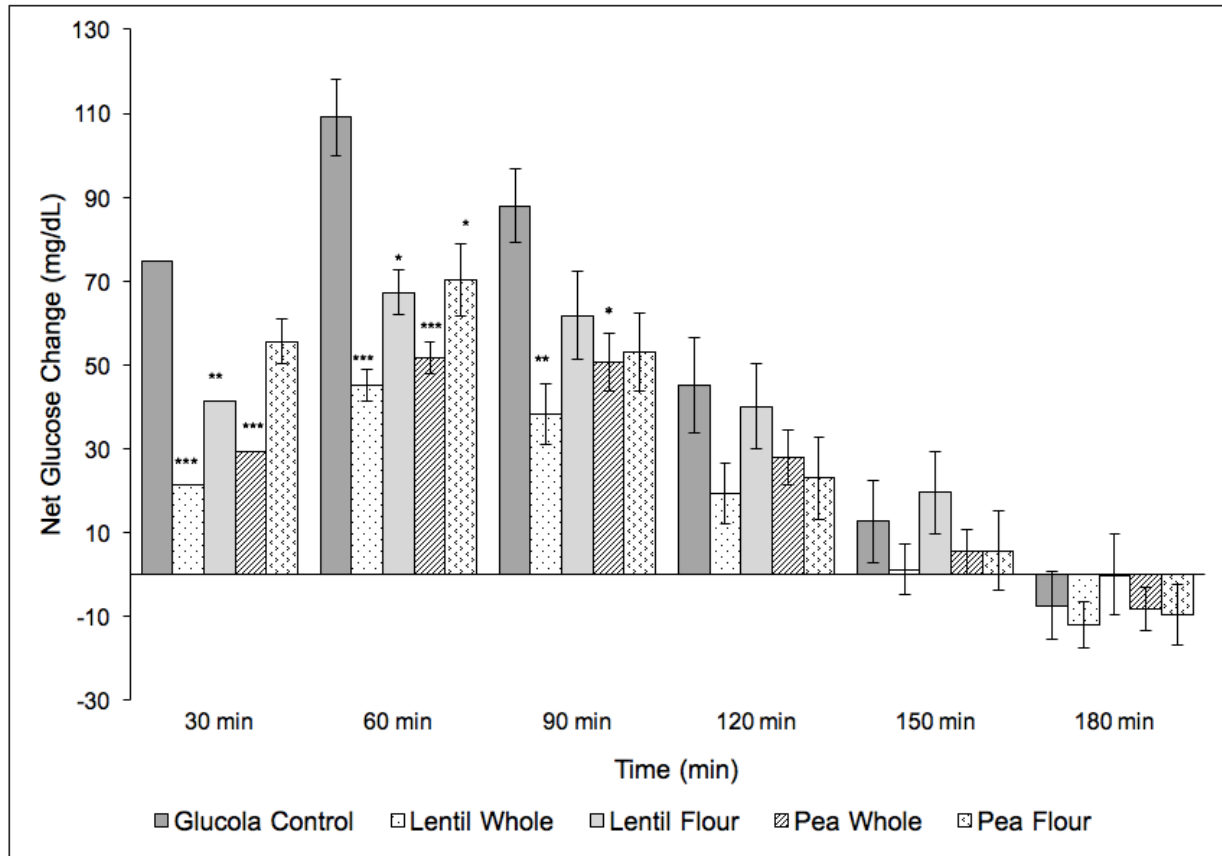


Figure 4.3. Effect of treatment on postprandial net glucose (n = 11)¹

¹All values are means \pm standard error of the mean (SEM).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Incremental areas under the curve for blood glucose were assessed between 0-60, 0-120 and 0-180 minutes postprandial (Figure 4.3). A one-way ANOVA showed significance ($P < 0.005$) between groups (treatments) for all the time increments. Table 4.6 shows the mean values for the time ranges. Post-hoc test Tukey HSD showed significant differences between the control mean and all pulse treatments at 0-60 minutes, with every P-values being < 0.0005 , except for the pea flour ($P = 0.017$). At this time period, the nutritional profiles of the pulses were likely the cause for this difference. Additionally, at this increment, the lentil flour and pea flour were not significantly different from each other, though the pea flour was different from the whole peas (P

= 0.043) and whole lentils ($P = 0.004$). The flours had similar nutrient profiles, except there was more fiber and protein in the lentil flour but this did not appear to be significant at 0-60 minutes. Both flours had high total starch and were fairly similar in terms of minerals. Another possible explanation is related to the similar milling processes applied to both flours. This logically has an impact on the physical structure of the pulses and makes them fundamentally different from the whole pulse. As for differences between the pea flour and whole pulses, the pea flour has a higher total starch content. However, the lentil flour had the highest total starch content, but was not significantly different from the whole pulses. Based on this, there must be something else (not total starch) in play with the pea flour that made it different from the whole pulses, but not the lentil flour. At 0-120 minutes, the control was significantly different from all pulse treatments (whole lentil, lentil flour, whole pea, and pea flour with P-values of <0.0005 , 0.006, <0.0005 , and 0.007, respectively). There were no significant differences among the pulse treatments. At this time period (0-120 minutes), the nutrients in the pulse meals were most likely the reason for the positive glycemic responses (compared to the control). At 0-180 minutes, the control treatment was significantly different from the pulse treatments (whole lentil, whole pea, and pea flour with P-values of <0.0005 , 0.002, and 0.040, respectively), except for the lentil flour. The lentil flour was not significantly different from any of the treatments. Some things that set the lentil flour apart from the other pulse treatments, and may explain why it was the only pulse treatment similar to the control at 0-180 minutes include that it had the highest fiber content (and thus the lowest available CHO), highest protein (though not considerably higher than the other pulse treatments), and highest total starch content. The fiber content was probably the reason why the lentil flour was similar to the control, but the difference was not significant enough to

make it different from the other pulses. There are likely other factors contributing to this observation.

Table 4.6. Postprandial areas under the curve for blood glucose^{1,2}

	Glucola Control	Whole Lentil	Lentil Flour	Whole Pea	Pea Flour
0-60 min	3090.0 ± 369.9	1322.1 ± 191.8***	2245.9 ± 305.4***	1648.6 ± 189.4***	2719.1 ± 268.7*
0-120 min	9355.4 ± 696.5	3475.1 ± 549.5***	5705.5 ± 858.4**	4355.5 ± 543.3***	5760.3 ± 717.8**
0-180 min	10798.1 ± 1040.7	4027.5 ± 688.9***	7109.1 ± 1280.7	5046.0 ± 755.0**	6660.4 ± 964.7*

¹ All values are means ± standard error of the mean (SEM). ² Mg * min/dL (calculated by the trapezoidal rule); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

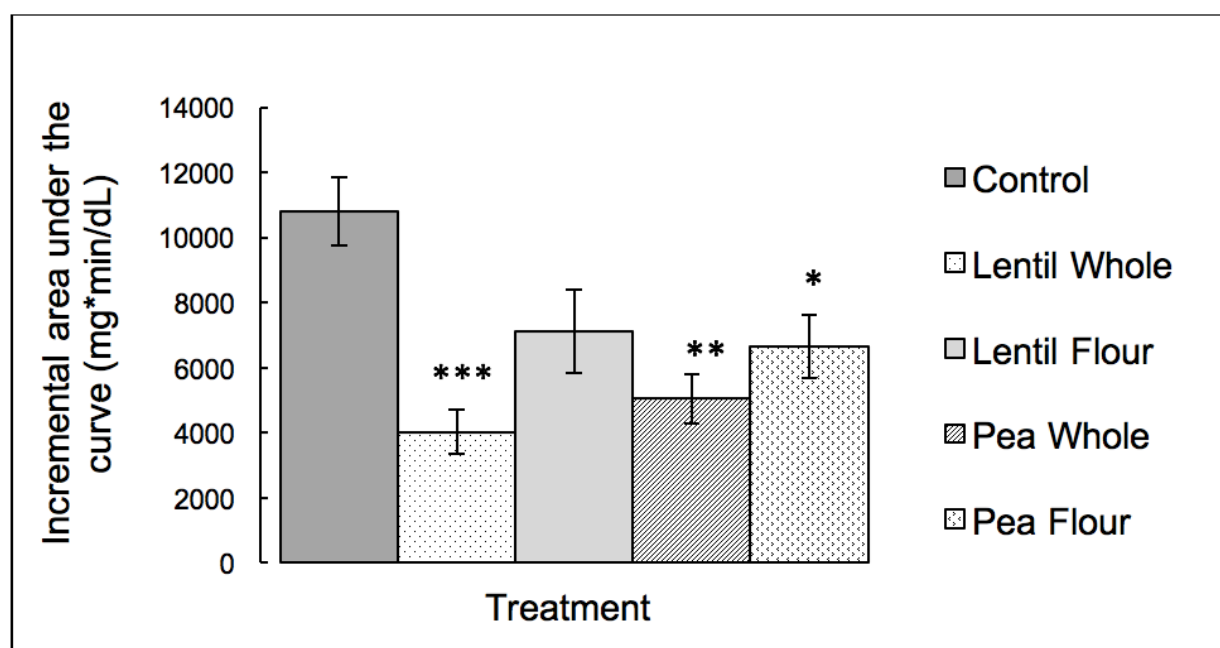


Figure 4.4. Incremental area under the curve differences in net glucose 0-180 minutes postprandial (n = 11)^{1,2}

¹ All values are means ± standard error of the mean (SEM). ² Mg * min/dL (calculated by the trapezoidal rule); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Differences in glycemic response between the pulses may be explained nutritionally, such as type of fiber (soluble or insoluble) or amount of resistant starch present. Differences may also be explained by milling. Milling results in the separation of starch granules from protein bodies, by increasing surface area (Hoover et al., 2010; Vaz Patto et al., 2015). This may increase the availability of starch to digestive enzymes. Further, particle size is reduced by milling. Larger particle sizes are thought to elicit a lower postprandial glycemic response, which was seen in the trends of our whole pulses. The pea flour had a larger particle size than the lentil flour, but generally had a larger glycemic response, contrary to the expected outcome. Thus, there may be something beyond particle size that is influencing the postprandial glycemic response. From all of the observations, the mechanisms behind the glycemic response are multifaceted and cannot be explained based on one factor alone.

Statistical Power

Statistical power indicates the likelihood of “correctly rejecting a false null hypothesis and thus detecting a genuine effect” (Richardson, 2011). Observed power values were computed by SPSS. Our statistical power was between 0.938-1.00, indicating at least a 94% probability of making correct assumptions from our statistical test results. Our power was above 0.80, which is a common goal for clinical trials (Gupta et al., 2016).

Another measure computed by SPSS was partial eta squared values. Partial eta squared statistics are commonly used by researchers to interpret the effect size of clinical trial data (Ferguson, 2009). This measure is used for complex models with more than one factor (Sánchez & Cervantes, 2016) and is commonly reported due to SPSS capabilities (Fritz et al., 2012). A comparison of partial eta squared to Cohen’s 1988 criteria of effect sizes is documented (small = 0.0099; medium = 0.0588; large = 0.1379) (Richardson, 2011). Our partial eta squared values were well above the large cut offs.

Wilks' Lambda was used as a multivariate test with $\alpha = 0.05$ and showed significant effects from time points with a value of 0.063 and an observed power of 1.0. Partial eta squared value for this was 0.937, indicating 94% of variability is attributed to the time points. A test of between-subjects (treatment type) showed a power of 0.938. Likewise, an observed power was 0.983 when looking at time point x treatment meal type with a Wilks' Lambda value of 0.371. The partial eta squared value for this was 0.219.

Further, power of within-subjects (net glucose changes at postprandial time points) effects using Greenhouse-Geisser (adjusts for lack of sphericity) was 1.0. The Greenhouse-Geisser test had partial eta squared values for time points and time point x treatment meal interactions of 0.805 and 0.304, respectively. This indicates that 81% of variation is associated with the time point, and the time point combined with the treatment type accounts for 30% of variation.

CHAPTER 5. CONCLUSIONS & RECOMMENDATIONS FOR FUTURE RESEARCH

Findings from dietary forms and physical activity questionnaires show that the guidelines for physical activity and dietary recommendations were not met by the participants. Both diet and exercise are significant factors that impact glycemic control, for better or worse. Considering the rising prevalence of diabetes, and specifically T2DM, these are urgent concerns that must be addressed. The subpar quality of the American diet can be improved with the incorporation of pulses.

Although pulses are nutritionally dense, this does not mean Americans will consume pulses in the form they have traditionally been presented (i.e., whole pulses). Appeal of products mainly comes from taste, and pulses do not necessarily meet this, with their low fat and high fiber status. With the rise in pulse processing and more specifically pulse flour processing, the question of the integrity of pulses comes into question. It has been documented that whole pulses are beneficial for adults with T2DM, but this question is inconclusive with pulse fractions such as flours. Commercial flours (i.e., split flours) have hulls removed, losing a large portion of the pulse fiber. Information comparing split and whole flours in glycemic response studies was not readily found, suggesting this is a research gap which future work can explore. This study's pulse flours trended lower than the control treatment, but commercial flours should be used to comment on industry practices.

Additionally, overall diet must be taken into consideration. American diets tend to be high in fat, refined grains, and added sugar. As seen in previous research (Winham et al., 2007; Winham et al., 2017), pulses have varying effects when eaten with high glycemic index foods. The glycemic index of our current consumption patterns, as well as the nature of our foods, will influence the effectiveness of implementing pulses in novel ways. Further, the amount of pulse

flour used in this study is considerably higher than what is common for products on the market or likely plausible in terms of functionality. The question of commonly consumed serving sizes will influence the validity of health claims capitalizing on the beneficial glycemic properties of pulses. This is also another research gap, and very relevant in the context of our diets.

This study found that meals containing pulses yielded glycemic response-lowering properties compared to a reference food item in adults with type 2 diabetes. Likening the Glucola 50 g control beverage to other carbohydrate-heavy and calorie dense foods, such as refined grain products in the American diet, pulses play an evident role in our health. It is important to note that every pulse treatment was significantly different from the control treatment at some point. A lower glycemic response is likely attributed to the nutrient dense profile of pulses, with their fiber and protein contents. This finding highlights the nutritional benefits pulses provide over carbohydrate dense foods.

The main effects of the pulse flours were not significantly different from the control treatment or the whole pulses, but the main effects of the whole pulses were different from the control treatment. This illustrates the complexity of the glycemic response and the multiple factors influencing it. Though main effects of whole pulses were similar to the pulse flours, there were some differences at the time point level. These differences become critical when considering short-term and long-term glucose control, in particular when concerned about high blood sugar peaks. This also shows that some attributes influencing the glycemic response are time dependent, such as the absorption and digestion of starches. Overall trends showed lower postprandial glucose values for the whole pulses compared to the pulse flours. Regardless of statistical significance, the findings show that health benefits of pulses are maximized when they are consumed whole.

While there is substantial information presented in this thesis, there is still a level of uncertainty with some relevant factors. For example, there are some missing details on fiber (i.e., insoluble or soluble fiber and SDS, RDS, or RS). Future research should include an in-depth nutrient analysis to determine type of fiber present in pulses. Going further, a complete picture of starch analysis is worth looking into. This study did not have information available on the RS for the cooked pulses, or other starch types such as SDS and RDS.

An investigation into the cell wall structure and digestibility is also warranted. Previous literature illustrates the importance of processing on the accessibility of digestive enzymes, as well as other factors such as dietary fiber (including RS) and antinutrient components. Digestion is interconnected with several things: starch, polyphenols, particle size, among several other factors. The importance of digestion cannot be downplayed. While our study shows that whole pulses produce a lower glycemic response, further work is required on processing to effectively evaluate the significance and implications of pulse flour processing on the postprandial glycemic response.

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APPENDIX A. INSTITUTIONAL REVIEW BOARD APPROVAL

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Institutional Review Board
Office for Responsible Research
Vice President for Research
2420 Lincoln Way, Suite 202
Ames, Iowa 50014
515 294-4566

Date: 05/22/2019
To: Donna Winham, DrPH, MA
From: Office for Responsible Research
Title: **Metabolic effects of pulse consumption on biomarkers in adults**
IRB ID: 17-191
Submission Type: Continuing Review & Modification **Review Type:** Full Committee
Approval Date: 05/21/2019 **Approval Expiration Date:** 05/21/2020

The project referenced above has received approval from the Institutional Review Board (IRB) at Iowa State University according to the dates shown above. Please refer to the IRB ID number shown above in all correspondence regarding this study.

To ensure compliance with federal regulations (45 CFR 46 & 21 CFR 56), please be sure to:

- Use only the approved study materials in your research, including the recruitment materials and informed consent documents that have the IRB approval stamp.
- [Retain signed informed consent documents](#) for 3 years after the close of the study, when documented consent is required.
- Obtain IRB approval prior to implementing any changes to the study or study materials.
- Promptly inform the IRB of any addition of or change in federal funding for this study. Approval of the protocol referenced above applies only to funding sources that are specifically identified in the corresponding IRB application.
- Inform the IRB if the Principal Investigator and/or Supervising Investigator end their role or involvement with the project with sufficient time to allow an alternate PI/Supervising Investigator to assume oversight responsibility. Projects must have an [eligible PI](#) to remain open.
- Immediately inform the IRB of (1) all serious and/or unexpected [adverse experiences](#) involving risks to subjects or others; and (2) any other [unanticipated problems](#) involving risks to subjects or others.
- IRB approval means that you have met the requirements of federal regulations and ISU policies governing human subjects research. Approval from other entities may also be needed. For example, access to data from private records (e.g., student, medical, or employment records, etc.) that are protected by FERPA, HIPAA, or other confidentiality policies requires permission from the holders of

IRB 01/2019

those records. Similarly, for research conducted in institutions other than ISU (e.g., schools, other colleges or universities, medical facilities, companies, etc.), investigators must obtain permission from the institution(s) as required by their policies. **IRB approval in no way implies or guarantees that permission from these other entities will be granted.**

- Your research study may be subject to [post-approval monitoring](#) by Iowa State University's Office for Responsible Research. In some cases, it may also be subject to formal audit or inspection by federal agencies and study sponsors.
- Upon completion of the project, transfer of IRB oversight to another IRB, or departure of the PI and/or Supervising Investigator, please initiate a Project Closure to officially close the project. For information on instances when a study may be closed, please refer to the [IRB Study Closure Policy](#).

If your study requires continuing review, indicated by a specific Approval Expiration Date above, you should:

- **Stop all human subjects research activity if IRB approval lapses**, unless continuation is necessary to prevent harm to research participants. Human subjects research activity can resume once IRB approval is re-established.
- **Submit an application for Continuing Review** at least three to four weeks prior to the **Approval Expiration Date** as noted above to provide sufficient time for the IRB to review and approve continuation of the study. We will send a courtesy reminder as this date approaches.

Please don't hesitate to contact us if you have questions or concerns at 515-294-4566 or IRB@iastate.edu.

IRB 01/2019

APPENDIX B. STUDY PROCEDURES AND PAYMENT SCHEDULE

Study procedures and payment schedule for an individual participant – T2DM

Study Step	Activities or instruments to complete
1. Screening eligibility interview Nonfasting. Scheduled at any time. Payment amount = \$0 15-30 minutes	Telephone or in-person eligibility interview Receive instruction for fasting appointment including map and directions. Review consent form. Provide written study protocol summary
2. Screening Appointment Non-Fasting or Fasting Payment amount = \$0 20-40 minutes	Forms to complete: Informed consent, medical history Fasting blood draw (if applicable): glucose, HbA1c, lipids Height, weight, waist circumference, and blood pressure Explanation of how to complete 24-hour food records, satiety, physical activity, and gastrointestinal (GI) symptom forms Highlight study protocol: drink plain water after evening meal, no consumption of legumes on the day before testing, no caffeinated beverages or foods, no alcohol, no exercise, adequate sleep Pick out frozen meal choice for pre-test evening meal Schedule Test Day #1 study start appointment
3. Test Day #1 – Test meal Fasting - scheduled between 7 to 8 am Payment amount = \$0 Plan on 4.0 hours	Review 24-hour food records, physical activity, satiety, GI symptoms questionnaires; fasting status; medications not taken that morning; standard meal eaten; no caffeine, no alcohol; adequate sleep. Weight and blood pressure; waist circumference and height if not previously completed Fasting blood draw (if applicable): HbA1c, lipid panel Consume food intervention product Blood draws every 30 minutes after meal Schedule Treatment 2 appointment Provide frozen meal and additional forms for test day #2 of 5.
4. Test Day #2 – Control Fasting - scheduled between 7 to 8 am Payment amount = \$80 Plan on 4.0 hours	Review 24-hour food records, physical activity, satiety, GI symptoms questionnaires; fasting status; medications not taken that morning; standard meal eaten; no caffeine, no alcohol. Weight and blood pressure Fasting blood draw: TSH, CBC Consume food intervention product Schedule Treatment 3 appointment Provide frozen meal and additional forms for test day #3
5-7. Test Days #3, 4, and 5 Payment amount = \$80 (Days 3 & 4) Payment amount = \$160 (Test Day 5)	Repeat steps in 4 above Vitamin D on test day #3 Comprehensive Metabolic Panel on test day #4
8. Post-testing (not in person)	Ensure last Post-test GI Tract Activity form is completed and submitted to study coordinators Receive blood test results (HbA1c, Lipid Panel, TSH, CBC, Vitamin D, and CMP)

APPENDIX C. PARTICIPANT INSTRUCTIONS

PARTICIPANT INSTRUCTIONS

Metabolic response to peas and lentils as part of a meal

1. Contact us at isubeanstudy@gmail.com if you have any questions. Please contact us as soon as possible if any issues or concerns arise.
2. On the day prior to each testing day:
 - a. Do not drink beverages, eat food products, or consume medications with caffeine. Examples include painkillers with caffeine, coffee, tea, herbal tea, soda, energy drinks, energy bars, chocolate, or coffee/mocha-flavored foods.
 - b. Do not consume alcoholic beverages, including beer, wine or liquor.
 - c. Do not consume legumes, such as chickpeas, beans (includes butter beans, navy beans, cannellini beans, red kidney beans, adzuki beans, black-eyed beans and soybeans), peas, or lentils.
 - d. Refrain from moderate or heavy physical activity.
 - i. Examples of activities to avoid:
 1. Walking for exercise (walking the dog, walking to and from your car or between classes is OK)
 2. Jogging or running
 3. Workouts at the gym
 4. Sports such as basketball, baseball, soccer, golf, etc. that require running, walking or jumping for extended periods of time
 5. Hiking
 6. Biking (even if it is just biking to work)
 - ii. Examples of activities that are OK
 1. Walking the dog
 2. Walking to get your mail
 3. Walking to and from your car
 4. Walking around at home (as part of your normal activities)
 - iii. If you have a question about an activity, please ask Mariel
3. Please complete these forms on the day before your testing day.
 - a. Food Log
 - b. Four (4) Satiety forms about your lunch meal (Pre-eating, 1 hour after eating, 2 hours after eating, and 3 hours after eating)
 - i. Do not eat additional food during these 3 hours
 - c. Pre-Test Day GI Tract Activity form (completed in the evening)
4. Please follow these instructions for the Food Log: record what you eat and drink for the entire day prior to coming to the testing site. This will start when you wake up and end with the prepared frozen meal you have selected.
 - a. Forms for recording your dietary intake will be provided.
 - b. Measure your food consumption to the best of your ability (i.e. if you are having a cup of dry cereal, use a measuring cup to serve this amount).
 - c. Measure all beverages you consume, including water.

- d. Record the time in which you eat and drink all foods and beverages to the nearest minute.
 - e. Include the amounts and brand names of all foods eaten, including seasonings, condiments and garnishes.
 - f. Include the volumes and brand names of all beverages consumed.
 - g. Record all dietary supplements, herbs and other non-food items you consume throughout the day.
 - h. Record all medications consumed throughout the day.
5. The evening before each test day,
- a. Please consume the entire meal (and cookie, if applicable) provided to you by the researchers. Do not leave any unfinished food.
 - i. **Save packaging to bring on test day.**
 - ii. This meal will consist of a frozen prepared meal and a cookie (unless you opted out).
 - iii. You will need to consume this meal approximately 12 hours prior to your scheduled appointment on the testing day.
 - iv. For this meal, consume only the food items that you have selected before. Do not add any other foods to the meal. Each evening meal must be the same before the 4 test mornings.
 - b. **Please get an adequate amount of sleep.** Recommended amount is 7-9 hours.
6. You will then fast for 12 hours prior to your scheduled appointment on each testing day.
- a. This means that you will not consume any food or beverage with the exception of water on the night prior to a testing day. **DO drink plain or unflavored water!**
 - b. If your appointment on the testing day is scheduled for 7 a.m., you will need to start fasting at 7 p.m.
7. On the morning of your testing appointment, continue to fast.
- a. This means that you will not consume any food or beverage other than water.
 - b. Consume at least 2 cups of plain water prior to leaving for the testing site.
 - c. Please do NOT consume coffee, tea (hot or cold), decaffeinated coffee, decaffeinated tea, colas or any beverage OTHER than water.
 - d. Do not drink any alcoholic beverages including beer, wine, or liquor.
 - e. Do not take any medications, unless pre-approved by the study coordinators.
 - f. If you do accidentally consume food or a beverage other than water, please inform the investigators. Your results may not be valid and we may need to reschedule you for another test date.
8. On the morning of your testing appointment, report to the testing site – the Nutrition and Wellness Research Center (NWRC, 2325 North Loop Drive, Building #6, Ames, Iowa 50010).
- a. If you are running late, or cannot make your appointment, for any reason, please call us as soon as possible. This is very important!
9. In the early evening after your test date, please complete the Post-Test GI Tract Activity form. You can complete this online, or email it back to us. It must be completed the evening of your test day. The link will be emailed out to you.

EXAMPLE OF SCHEDULE PRIOR TO A TESTING DAY

One day before testing day

1. Refrain from moderate or heavy physical activity.
2. Do not drink beverages, eat food products, or consume medications with caffeine. Examples include painkillers with caffeine, coffee, tea, soda, energy drinks, energy bars, chocolate, or coffee/mocha-flavored foods.
3. Do not drink herbal tea.
4. Do not drink alcoholic beverages, including beer, wine, or liquor.
5. Do not consume legumes, such as chickpeas, beans (includes butter beans, haricot/navy beans, cannellini beans, red kidney beans, adzuki beans, black-eyed beans and soybeans), peas, or lentils.
6. Complete your study forms: Food Log, four (4) Satiety forms, and Pre-Test Day GI Tract Activity form.

Approximately 12 hours before testing appointment

1. Consume evening meal provided in its entirety.
2. Continue to refrain from moderate or heavy physical activity.

12 hours before testing appointment

1. Begin fast.
2. Can drink only plain water in the evening.
3. Continue to fast and to refrain from moderate or heavy physical activity.
4. Be well rested, get enough sleep. 7-9 hours is recommended.

Morning of testing appointment

1. Continue fast, but drink some plain water. No other beverages besides plain water.
2. Continue to refrain from moderate or heavy physical activity and drinking caffeine.
3. Report to testing site. Bring all completed forms. Bring outside packages of frozen meal and cookie.
4. Bring any medications that you will need to take after completion of the test day (~11am).

Evening after testing appointment

1. Complete the Post-Test GI Tract Activity form. Bring this on your next test date, or if you have completed all your test dates then email or mail it to us. Or complete online (link emailed to you).

APPENDIX D. RECRUITMENT HALF PAGE FLYER

ISU IRB: 17-191-00
Approved Date: 05/21/2019
Expiration Date: 05/21/2020



Has a doctor told you that you have high blood sugar or 'prediabetes', high blood pressure, high triglycerides, or metabolic syndrome? **Or** type 2 diabetes?

If, yes, we invite you to participate in a research study on the effects of eating foods made with beans, peas or lentils on blood sugar. The study involves a screening interview, 1 pre-study meeting and 5 morning visits for fasting blood draws at the Nutrition Wellness Research Center in Ames, IA (near the Gateway Hotel). On the 5 different study test mornings, each participant will have blood drawn before consuming a test meal, and then every 30 minutes for 3 hours afterwards. Four of these meals will contain beans, peas or lentils. If you successfully complete all 5 test days, you may receive up to \$400.

We are recruiting 24-75-year-old men and women who have 'prediabetes', high blood pressure, high triglycerides, low HDL, and large waist size, **OR** have been diagnosed with type 2 diabetes. Your BMI must be between 22-39.9. Participants who are allergic to beans, peanuts, soy, peas, lentils, gluten, eggs, dairy, wheat, or tomatoes are not eligible. Other eligibility criteria will be reviewed at the screening interview.

If you are interested or would like more information please contact: Mariel Camacho-Arriola, Graduate Research Associate.

- Email: isubeanstudy@gmail.com
- Phone: (515) 294-5040
- *Recruitment ends October 15, 2019*

Thank you!

Donna M. Winham, DrPH, RD, LD
Department of Food Science and Human Nutrition
Iowa State University, Ames, IA 50011, dwinham@iastate.edu
The study, "Metabolic effects of pulse consumption on biomarkers in adults" in the Department of Food Science and Human Nutrition, is funded by the USA Dry Pea & Lentil Council, and the Pulse Crop Health Initiative.



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Thank you!

Donna M. Winham, DrPH, RD, LD
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The study, "Metabolic effects of pulse consumption on biomarkers in adults" in the Department of Food Science and Human Nutrition, is funded by the USA Dry Pea & Lentil Council, and the Pulse Crop Health Initiative.

APPENDIX E. RECRUITMENT FULL PAGE FLYER

Research study \$400 'prediabetes' or type 2 diabetes

ISU IRB: 17-191-00
Approved Date: 05/21/2019
Expiration Date: 05/21/2020

Has your doctor told you that you have
high blood sugar or 'prediabetes'? **OR**
Have you been diagnosed with **type 2 diabetes**?

Who is potentially eligible?

- We are specifically recruiting 24-75-year-old non-smoking men and women who have 'prediabetes' or **high blood sugar** and any 2 of the following 4 conditions?:
 - large waist circumference
 - low HDL
 - high blood pressure
 - high triglycerides
- OR**
- Have been diagnosed with **type 2 diabetes** and control it with diet/exercise, metformin or weekly injections of Trulicity, Bydureon or Ozempic.

What is this study about?

- Nutrition researchers are looking at the effects of test foods on blood glucose and insulin values
- Study participants will come to ISU on 5 different mornings for a 4 hour period
- After eating a test meal, blood samples will be drawn every 30 minutes for 3 hours.
- Participants will also complete surveys on food intakes and satiety

• **Recruitment ends Oct 15, 2019**

Your BMI must be between 22-39.9. Participants who are allergic to beans, peanuts, soy, peas, lentils, gluten, eggs, dairy, wheat, or tomatoes are not eligible. Other eligibility criteria will be reviewed with you at the screening interview.



If you are **interested** or would like **more information**

Please contact

Mariel Camacho-Arriola, Research Associate

Email: isubeanstudy@gmail.com

Phone: (515) 294-5040

APPENDIX F. MEDICAL HISTORY QUESTIONNAIRE

MEDICAL HISTORY QUESTIONNAIRE

Participant ID# _____

Date: _____ Age: _____

Gender: Male Female

Height _____ ft. _____ in.

Weight: _____ lbs.

1. Are you taking any medications regularly? (including insulin, aspirin, etc.) Y N
 If yes, what medications and how often? _____

2. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Y N
 If yes, what supplements and how often? _____

3. Has a doctor ever told you that you have any of the following conditions:

Kidney disease?	Y	N
Liver disease?	Y	N
Thyroid problems?	Y	N
Cancer?	Y	N
Gastrointestinal disease or disorders?	Y	N

4. Are you allergic to any foods? Y N
 If yes, please specify _____

5. Do you have a tendency to faint when a blood sample is taken? Y N

6. Do you have an allergy to tape or latex (e.g., latex gloves)? Y N

7. Please describe any other medical conditions that may affect your participation below or on the backside of this form.

APPENDIX G. FOOD FREQUENCY QUESTIONNAIRE

TODAY'S DATE: _____ **AGE:** _____ **GENDER:** **MALE** **FEMALE**

Dietary Assessment Screener

Think about your eating habits over the past month or so. About how often do you eat each of the following foods either at home or in restaurants? Mark an "X" in ONE box for each food.

<i>How often do you eat ...</i>	Do Not Eat	Once per MONTH or less	2-3 times per MONTH	1-2 times per WEEK	3-4 times per WEEK	5 or more per WEEK
Hamburgers, ground beef, meat burritos, tacos						
Beef or pork, such as steaks, roasts, ribs, or in sandwiches						
Fried chicken						
Hot dogs, or Polish or Italian sausage						
Cold cuts, lunch meats, ham (not low-fat)						
Bacon or breakfast sausage						
Salad Dressings (not low-fat)						
Margarine, butter or mayonnaise on bread or potatoes						
Margarine, butter or oil <i>in cooking</i>						
Eggs (not Egg Beaters or just egg whites)						
	Do Not Eat	Once per MONTH or less	2-3 times per MONTH	1-2 times per WEEK	3-4 times per WEEK	5 or more per WEEK
Pizza						
Cheese, cheese spread (not low-fat)						
Whole milk (not low-fat or skim)						
French fries, fried potatoes						
Corn chips, potato chips, popcorn, crackers						
Doughnuts, pastries, cake, cookies (not low-fat)						
Ice Cream (not sherbet or low-fat)						

NOTE: These questions ask about what you eat each WEEK or by DAY, not by the month as in the previous questions.

Think about your eating habits over the past month or so. About how often do you eat each of the following foods either at home or in restaurants? Mark an "X" in ONE box for each food.

How often do you eat ...	Do not eat	Less than once per WEEK	About 1 time per WEEK	2-3 times per WEEK	4-6 times per WEEK	Once per DAY	2 or more times per DAY
Fruit juice, like orange, apple, grape, fresh, frozen or canned (Not sodas or other drinks)							
How often do you eat any fruit, fresh or canned? (Not counting juice)							
Vegetable juice, like tomato juice, V-8, carrot							
Green salad (like lettuce or spinach salad)							
Potatoes, any kind, including baked, mashed or french fried							
Vegetable soup, or stew with vegetables							
Any other vegetables, including string beans, peas, corn, broccoli, or any other kind							
Fiber cereals like Raisin Bran, Shredded Wheat or Fruit-n-Fiber							
Beans such as baked beans, pinto, kidney, peas, chickpeas, or lentils (not green or yellow beans)							
Dark bread such as whole wheat or rye							

These questions ask about your education, marital status, household size, race/ethnic identification, income, and some health questions.

1. Please check the box below that best describes the highest degree or level of school that you have **completed**.

- | | |
|---|----|
| Did not graduate from high school or complete GED | 1 |
| High school graduate, diploma or GED..... | 2 |
| Some college credit, but less than 1 year..... | 3 |
| 1 or more years of college, no degree..... | 4 |
| Associate degree (ex: AA or AS)..... | 5 |
| Bachelor's degree (BA, BS, AB)..... | 6 |
| Master's degree (MS, MA, MEd, MBA)..... | 7 |
| Professional degree (MD, DDS, DVM)..... | 8 |
| Doctorate degree (PhD, DrPH, ScD)..... | 9 |
| Other:..... | 10 |

[SPECIFY] : _____

2. What is your current marital status?

- | | |
|-------------------------------------|---|
| Single | 1 |
| Married..... | 2 |
| Living w/partner, not married | 3 |
| Divorced/Separated/Widowed | 4 |

3. How many total people live in your household including yourself? _____

4. Have you ever met with a Registered Dietitian to discuss type 2 diabetes?

- | | |
|----------------------------|---|
| Yes | 1 |
| No..... | 2 |
| Don't know/ not sure | 7 |

5. Have you participated in any diabetes education programs?

- | | |
|---|---|
| No, I have not participated in such a program | 1 |
| No, I have not participated in these programs, but have heard of them ... | 2 |
| Yes, I have participated in a program | 3 |

Name of program(s): _____

6. Are you Spanish or Hispanic or Latino?

- No, not Spanish/Hispanic/Latino..... 1
 Yes, Mexican, Mexican-Am., Chicano.... 2
 Yes, Puerto Rican 3
 Yes, Cuban 4
 Yes, other Spanish/Hispanic/Latino— ... 5
 [SPECIFY] : _____

7. Which one or more of the following would you say is your race or ethnicity?

(CHECK ALL THAT APPLY)

- African American or Black 1
 Afro-Caribbean 2
 African (continent) 3
 White 4
 Asian 5
 Native Hawaiian or other Pacific Islander 6
 American Indian or Alaskan Native 7
 Other: 8
 [SPECIFY] : _____

9. Would you say that in general your health is?:

- Excellent 1
 Very Good..... 2
 Good 3
 Fair 4
 Poor 5

APPENDIX H. SAMPLE SIZE FORMULA

$$\delta = (t_{\Gamma\alpha, df} - t_{\Gamma\beta, df}) * \frac{\sqrt{2}}{n} * sd$$

δ = difference between treatment means

sd = standard deviation

$\alpha = 0.05$, $1 - \beta = 80\%$ conventional values for clinical studies (Hickey, Grant, Dunning, & Siepe, 2018).

df = degrees of freedom

APPENDIX I. DRY WEIGHT BASIS EQUIVALENCY CALCULATIONS

Lentils:

One cup of cooked lentils were determined to be 160 g through multiple trials, which means that ½ cup was 80 g. This is the pulse amount used for the whole lentil treatment. *To determine an equivalent ½ cup dry weight basis amount for lentil flour, the following calculations were made based on moisture content values from Eurofins analysis. The moisture content for the samples were 54.9% for the boiled lentils and 10.1% for the lentil flour:*

Amount of dry solids in 100 g cooked lentils = $100 \text{ g} - 54.9 \text{ g} = 45.1 \text{ g}$ dry solids per 100 g cooked lentils

Amount of dry solids in 160 g cooked lentils per cup = $160 \text{ g cooked lentils/cup} * 45.1 \text{ g dry solids/100 g cooked} = 72.16$ dry cooked lentil solids per cup

Amount of dry solids in 100 g lentil flour = $100 \text{ g} - 10.1 \text{ g} = 89.9 \text{ g}$ dry solids per 100 g lentil flour

Flour equivalent amount to cooked lentils = $72.16 \text{ g dry cooked lentils solids per 1 cup} / (89.9 \text{ g dry solids/100 g lentil flour}) = 80.27 \text{ g dry lentil flour solids per 1 cup}$

Thus, 40.13 g of lentil flour was used for an equivalent ½ cup dry weight basis to cooked lentils.

Peas:

One cup of cooked peas were determined to be 152 g, meaning that ½ cup was 76 g. This is the pulse amount used for the whole pea treatment. *To determine an equivalent ½ cup dry weight basis amount for pea flour, the following calculations were made based on moisture*

content values from Eurofins analysis. The moisture content for the samples were 60.8% for the boiled lentils and 9.2% for the pea flour:

Amount of dry solids in 100 g cooked peas = $100 \text{ g} - 60.8 \text{ g} = 39.2 \text{ g}$ dry solids per 100 g cooked peas

Amount of dry solids in 152 g cooked peas per cup = $152 \text{ g cooked peas/cup} * 39.2 \text{ g dry solids/100 g cooked} = 59.58 \text{ g dry cooked pea solids per cup}$

Amount of dry solids in 100 g pea flour = $100 \text{ g} - 9.2 \text{ g} = 90.8 \text{ g}$ dry solids per 100 g pea flour

Flour equivalent amount to cooked peas = $59.58 \text{ g dry cooked pea solids per 1 cup} / (90.8 \text{ g dry solids/100 g pea flour}) = 65.62 \text{ g dry pea flour solids per 1 cup}$

Thus, 32.81 g of pea flour was used for an equivalent $\frac{1}{2}$ cup dry weight serving to cooked peas.

APPENDIX J. FOOD LOG

Food Intervention Study
Participant Food Log
Study Entry Form

Thank you for participating in this ISU Food Science and Human Nutrition Department Research Study on the blood glucose responses to pulse consumption. Please complete the following food record as best as you can for one day starting when you wake up. Bring the forms with you when you come in for your testing day. The nutritionist will go over the forms with you and answer questions you may have. Handwritten forms are ok.

On the forms provided, please record ALL food and beverages that you consume. Water should be included.

Try to provide as much detail as possible about the foods or beverage that you consumed. For example, instead of listing "ham and cheese" sandwich, write out the specific ingredients that went into the sandwich on the lines below the item. See the examples below:

How not to do it:

Time of Meal	Food Item	Brand or Source	Ingredients and/or Type of Preparation	Amount
Noon	Ham and cheese sandwich		ham, cheese, bread, mustard, lettuce, tomato	one

How we would like you to record the foods:

Time of Meal	Food Item	Brand or Source	Ingredients and/or Type of Preparation	Amount
Noon	Ham and cheese sandwich	Fareway deli	ham, deli	two slices (1 ounce each)
		Fareway deli	cheese, Swiss	two slices (1 ounce each)
		Hy-Vee Bakery	wheat bread	two slices
		Kraft	mustard, Dijon	1 teaspoon
		Kraft	mayonnaise	1 teaspoon
			lettuce, red leaf	1 leaf
			tomato	2 slices

Where possible, please bring in the labels from convenience and/or packaged foods. You can attach these to your forms. Remember to include beverages (including water) and snacks that are consumed between meals or afterwards.

Be sure to write down any supplements or medications you might be taking. Please attach a label for the supplement or write the information from the label on the record form.

Time: Note the time you ate or drank an item and indicate if am or pm for clarity.

Food Item: Write what the food or drink item was, e.g. crackers, casserole, shrimp, Coca-Cola

Brand or Source: In this space, please write down what the brand name is for the food item or where it came from. Examples: Nabisco Wheat Thins, Burger King, church potluck supper, or homemade.

Type of Preparation: Write down how a food was prepared if not obvious. Some common ways of preparing foods include baking, boiling, frying, micro waving, steaming or roasting. If you use oil or other sauces in preparing the food item, list them on a separate line.

Amount: Record how much of an item you ate or drank. Example: orange juice, 6 ounces. We would like you to weigh or measure your foods as much as you can during the day. Record as much detail as you can.

You can also take pictures of the food and drink to supplement your written details, or if you are unable to weigh or measure the amount.

Food Code: For office use only. Please leave blank.

Here is another example:

Time (am/pm)	Food Item	Brand or Source	Ingredients or Type of Preparation	Amount
<i>Example: 6:45 am</i>	<i>Raisin Bran</i>	<i>Post</i>		<i>½ cup</i>
<i>6:45 am</i>	<i>skim milk</i>	<i>Good Day</i>		<i>½ cup</i>
<i>6:45 am</i>	<i>egg</i>	<i>don't know</i>	<i>scrambled, used Pam nonstick spray</i>	<i>1 large</i>

If you have questions or difficulties before we meet next week, please feel free to email us at isubeanstudy@gmail.com. We look forward to your participation in this project!

APPENDIX K. INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

PGR Study
International Physical Activity Questionnaire

ID # _____

Date _____

Circle:

Male Female

The questions will ask you about the time you spent being physically active in the last 7 days.

Please answer each question even if you do not consider yourself to be an active person.

To describe the intensity of the physical activity, two terms (Moderate and Vigorous) are used:

Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

Examples include:

- a. Walking for Exercise (walking a golf course, walking around a lake, etc)
- b. Hiking
- c. Biking (even just biking to work)
- d. Gardening
- e. Cleaning

Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.

Examples Include:

- a. Jogging or running
- b. Workouts at the gym
- c. Sports such as basketball, baseball, soccer that require running, walking or jumping for extended periods of time
- d. Heavy gardening or construction work
- e. Chopping wood

1. The first question is about the time you spent sitting during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the last 7 days, how much time did you spend sitting during a day?

_____ Hours _____ Minutes

2. Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

During the 7 last days, on how many days did you walk for at least 10 minutes at a time?

_____ Days →

How much time did you usually spend walking on one of those days?

_____ hours _____ minutes

3. During the last 7 days, on how many days did you do moderate physical activities like gardening, cleaning, bicycling, at a regular pace, swimming or other fitness activities?

Think *only* about those physical activities that you did for at least 10 minutes at a time. Do not include walking.

_____ Days →

How much time did you usually spend on one of those days?

_____ hours _____ minutes

4. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, heavier garden or construction work, chopping wood, aerobics, jogging/running or fast bicycling?

Think *only* about those physical activities that you did for at least 10 minutes at a time.

_____ Days →

How much time did you usually spend on one of those days?

_____ hours _____ minutes

APPENDIX L. ANTHROPOMETRIC METHODS

Anthropometric Methods Instructions

Note: Always explain to the participants what you are going to do before you do it. Explain all of your procedures/decisions.

Weight: adapted from NHANES Anthropometry Procedures Manual (2017) and Fitness & Metabolism Unit, NWRC

1. The participant will take their shoes off. Light clothing may be worn, but no shoes, sweaters, or jackets.
2. The researcher will clean the scale before/after the participant gets on the scale. Ensure the scale is zeroed before telling the participant to get on the scale.
3. The participant will stand still with their weight evenly distributed over the center of the scale platform. Their arms will be at their sides, and they will look straight ahead.
4. Record their weight in kilograms. Repeat steps for a total of 2 times. You may show the participant their weight in pounds if they are curious.

Blood Pressure:

1. The participant should be sitting for a few minutes (set a 5 minute timer), then with both feet flat on the floor you will wrap the blood pressure cuff around their non-dominant arm at the brachioradial artery. The cuff will have an arrow, align this arrow with the inside of their arm.
2. The researcher will turn the machine on and press start, then record the number. Repeat as many times as necessary (2-3 times; 3 times at screening visit and if their 2nd value is unusually high on test days). Do not talk with the participant while the machine is reading their values. The settings of the machine should not be changed. Arm cuffs may be changed if a different size is required.
3. Do not let the participant see their values while their measurement is being taken. You may share their values after their last measurement is taken.

Blood Pressure Guidelines (from American Heart Association):

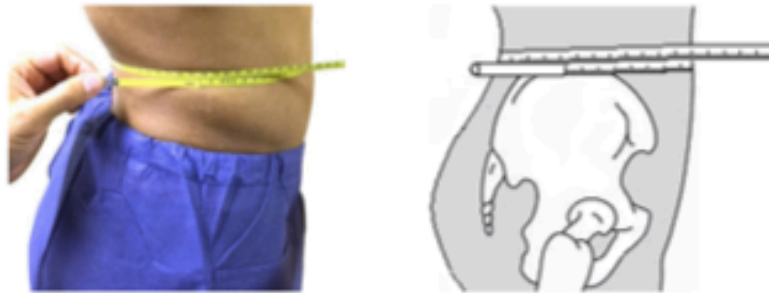
Blood Pressure Category	Systolic mm Hg (upper #)		Diastolic mm Hg (lower #)
Normal	Less than 120	AND	Less than 80
Elevated	120-129	AND	Less than 80
High Blood Pressure (Hypertension) Stage 1	130-139	OR	80-89
High Blood Pressure (Hypertension) Stage 2	140 or higher	OR	90 or higher
Hypertensive Crisis/possible stroke (*requires immediate action)	Higher than 180	AND/OR	Higher than 120

*Hypertensive Crisis:

- If participant is experiencing signs of possible organ damage, such as chest pain, shortness of breath, back pain, numbness/weakness, change in vision or difficulty speaking, **call 911 immediately**.
- If participant is not experiencing any of those symptoms, wait five minutes and then test blood pressure again. If values are still unusually high, ask participant to contact their doctor immediately. They may not require hospitalization, but should contact their doctor.

Waist Circumference: adapted from NHANES Anthropometry Procedures Manual (2017)

1. Wash your hands prior to taking participant's waist circumference. Two researchers should take the waist circumference: one staff will hold the tape measure and the other will help visually determine if the tape is aligned correctly.
2. The participant's shirt will be lifted to see the iliac crest (the start of their hip bone), as the measurement will be taken with direct skin contact. Ask the participant to show you where their iliac crest (hip bone) is, then confirm by finding it with your hands. If necessary, lower their waistband to expose the iliac crest. *As always, tell the participant what you will be doing before you do it.*
3. Extend the measuring tape around the waist. Position the tape in a horizontal plane at the iliac crest (see picture below). **Stand on the right side** of the participant, not facing them. Ask 2nd staff member to ensure the horizontal alignment of the tape. Always position the zero end of the tape below the section containing the measurement value.

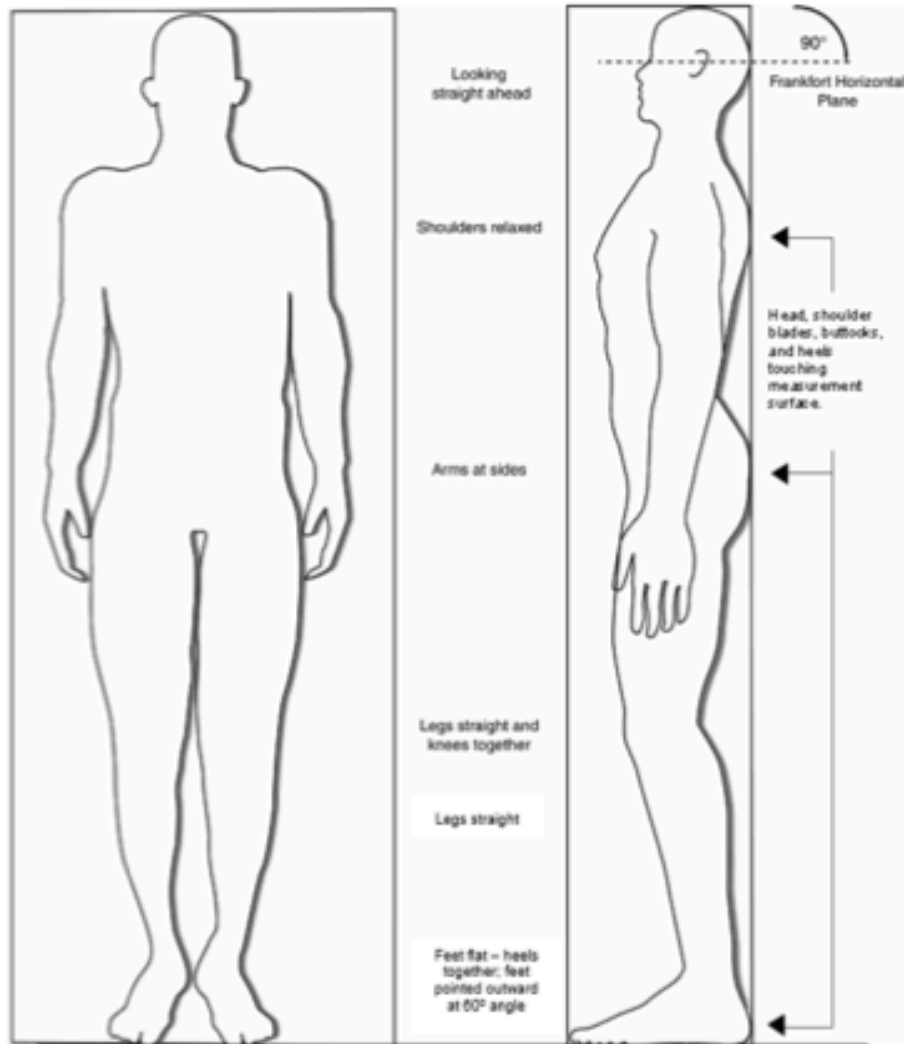


4. The participant will cross their arms at the elbows (like giving themselves a hug), and you will instruct them to breathe in and then out. You will read the tape measure when they breathe out.
5. The researcher should read the measurement with their line of sight directly in front of the value rather than at an angle or slightly off to the side.

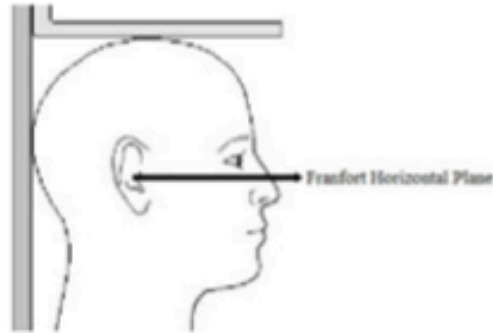
Height: adapted from NHANES Anthropometry Procedures Manual (2017) and Fitness & Metabolism Unit, NWRC

1. Ask participants to remove anything from the top of their heads (i.e. buns, braids, hat, etc.). They should remove shoes as well.

2. They will stand up straight against the backboard with the body weight evenly distributed and both feet flat on the platform. Instruct them to stand with the **heels together and toes apart** (see picture below). The toes should point slightly outward at approximately a 60° angle. Check that the back of the **head, shoulder blades, buttocks, and heels make contact with the backboard** (see picture below). Subjects who are unable to do this due to their size and shape, should be instructed to place the **buttocks and heels** or their head in contact with the board.



3. Align the participant's head in the Frankfort horizontal plane (see picture below). This is when the upper part of the ear canal and the lower part of the eye are parallel with the floor.



4. Instruct the participant to take a deep breath and hold their position. This will help straighten the spine.
5. The researcher will then bring down the stadiometer head piece, so that it rests firmly on top of the participant's head, with enough pressure to compress their hair.
6. Instruct the participant to crouch down and out from under the stadiometer, so that they do not push up the head piece.
7. Record the height in centimeters.
8. Repeat the steps one more time, for a 2nd reading.

APPENDIX M. STANDARD OPERATING PROCEDURE FOR OBTAINING AND WORKING WITH HUMAN BLOOD SAMPLES

SOP for Obtaining and Working with human blood samples

At Nutrition Wellness Research Center 2017 by Jeanne Wempe Stewart

Protection when handling human blood samples.

These human blood samples may or may not have blood borne pathogens (BBP) in them. We treat all human blood samples as if they do contain BBP and use universal precautions and PPE at all times when working with the samples.

Universal Precautions:

- keep biohazard waste containers near work area
- minimize splashing of blood
- use extreme caution when working with sharps
- use needles that have safety shields
- wash hands often-even after wearing gloves
- label all containers with biohazard symbol and color coding tape
- use leak-proof, unbreakable containers
- use sealed secondary containers for transport
- do not eat, drink or smoke while working with human blood samples
- do not apply cosmetics or handle contact lenses when working with human blood samples
- do not store food with human blood samples
- do not rub eyes, nose or mouth with hands, gloves, or lab coat sleeve when working with human blood samples

Personal Protective Equipment (PPE) The quantity of BBP depends upon the amount of splashing.

Minimal Protection

- gloves
- eye protection
- lab coat or disposable coveralls

Additional protection

- head and foot coverings
- face shield or face mask

Protection when handling human urine or fecal sample samples.

Urine and fecal samples are **not** considered potentially infectious unless they are contaminated with blood. Please be sure to give your participants containers a small, insulated lunch bag and bags that are tight so that they will not spill urine or feces.

SOP for drawing a blood sample to test for glucose in a glucose meter via a lancet

ReliOn Confirm/micro test strips only need 0.3 uL of blood to analyze the glucose. The strip draws blood in the sample application tip by capillary action. The blood glucose reacts with the enzyme on the test strip producing a current proportional to the blood glucose level. The meter detects the current and converts it into a blood glucose reading. These glucose monitors are checked by reading calibrators just like samples at the beginning of the day.

ReliOn Glucose meters are available from Walmart.

Lancets are available from many suppliers. I generally purchase lancets from MarketLab (ML5418 1.8 mm and ML5419 2.2 mm)

Procedure for testing glucose

1. Calibrate each glucose meter every day that blood samples are drawn and keep record of the results. Standards are good for 3 months once the bottle is opened.
2. Have participants wash their hands.
3. Once participant is sitting in chair in front of you, put on your fresh gloves.
4. Wipe location on side of finger with alcohol wipe.
5. While alcohol is drying, let arm hang down for 15 seconds. You may massage the wrist, palm and finger to obtain a blood drop.
6. Choose a site on the side of a fingertip to minimize pain of hitting a nerve.
7. Press the lancet against the side of the finger to obtain a blood sample.
8. Lower the hand to help the blood flow.
9. Get out a strip and slide it into the glucose meter with the contact strip inside the meter and the sample application tip is available to draw up blood. Check that the Code number on the screen agrees with the code number on the test strip bottle.
10. Once you can see the Apply Blood symbol, obtain a blood drop by touching the sample application tip to a blood drop. Capillary action will pull the blood sample into the test strip. When the test strip check window is full of blood, a beep sounds and you can remove the test strip from the drop of blood.
11. The meter will count down, showing a flashing dash. After 7 seconds, the glucose results will show on the meter window. Record result on the participant's data sheet in blue ink.
12. Remove the test strip and dispose it in the autoclavable, BBP sharps container.
13. Put lancet in the autoclavable, BBP sharps container.
14. Put bandaid on site of blood stick.
15. Test a different site each time you test on one day. I would recommend that you start on the non-dominant hand index finger first one side and then the other. Avoid taking blood from the little finger and thumb by using both sides of the three fingers on the left and right hand; the little finger tends to hurt more and the thumb has thicker skin.
16. Practice on each other before actually taking blood from your participants.

NWRC SOP for drawing an antecubital blood sample

NWRC will hire phlebotomists that will have at least 120 hours of supervised experience of drawing blood before they are hired to draw participant blood samples for NWRC here at ISU. Generally, the phlebotomists work/have worked at McFarland Clinic, Mary Greeley Hospital or Thielen Student Health Center here in Ames. Advance Services Inc (ASI) is a firm in Ames that does allow us to hire their hourly phlebotomists but I still insist that they have the 120 hours of supervised experience as indicated on their CV. I do require that the phlebotomists must be able to stand to draw the blood samples and be ambidextrous. We have three locations under the NWRC umbrella: NWRC in Research Park, Human Nutritional Sciences Building, and the Forker Building. The phlebotomists are expected to drive themselves to the appropriate location and they will only be at one location each day. They will be provided with parking passes if the blood draw is on campus. The phlebotomists are expected to wear their own scrubs or wear the laboratory coat that we provide. We do provide nitrile gloves and safety glasses/goggles/face shield for the phlebotomist's protection. The phlebotomists have been trained to let each participant see them put on fresh nitrile gloves for their blood draws and to remove the gloves when finished with the participant. All phlebotomists will be required to take yearly ISU Blood Borne pathogen training.

NOTES: Occasionally, as the students are filling out the Quest test requisitions for each participant's blood samples, they forget to indicate which tests the PI wants analyzed for each participant so no tests are analyzed. Have also had problems where the tube and Quest requisition do not have exactly the same information or the handwriting is very sloppy and the Quest Lab can't read the info. Sometimes I receive phone calls to straighten the problem out and other times they do not call me and that necessitates that the participant comes in for another blood draw. **The IRB has agreed that scheduling the participant to come in for an extra blood draw due to the labeling issue is not an adverse event!**

Another note: Occasionally a participant will come in fasted to the point they are dehydrated and the phlebotomist can't obtain a blood sample. NWRC does not want a phlebotomist to make more than 3 sticks to get a blood sample. The Project manager should make the choice between having the participant come back another day and be sure to stress that they drink plenty of water while fasting or transport the participant to Mary Greeley or McFarland and having the lab personnel draw the blood sample. We have account numbers that we have to use for these needs so the Project manager needs to contact Jeanne Stewart for that info before they leave to have the blood drawn. In reality, it is less stressful for the participant to have them fast again and come in another day.

A fasted blood sample is preferred for a majority of the tests performed on serum, plasma, or whole blood. Fasting is defined as no consumption of food or beverages except water for 10-12 hours before the blood draw.

Proper identification of specimens needs identical two patient identifiers on **both** the requisition and the tube:

1. Patient name: Study name instead of their last name and ID number for their first name. An example would be HMBVITD2 (last), HMB133JS (first). It is advisable to use the ID number and the person's initials because sometimes it is hard for Quest to read some people's hand writing and the initials are the difference between having the sample identifiable or losing the sample.
2. Participant requisition number sticker placed lengthwise on the tube.
3. Date of birth because some tests have different high and low points depending upon the age of the person.

Serum, plasma or whole blood collection collected by a single stick:

Quest recommends that participants should be instructed to not clench their fists just prior to or during the phlebotomy procedure as this may alter some of the patient's laboratory results, such as the concentration of potassium in serum. Participant's posture (sitting or supine) can depend upon their past experience with blood draws. If participant says that it is common for them to faint upon having blood drawn or that they have never had blood drawn before and they are scared of the procedure, please have them lie down on the phlebotomy bed before drawing blood. The phlebotomy chairs do recline and can be rapidly reclined by squeezing the release handle and pushing the chair back to elevate the participant's feet above their head (Trendelenberg Position) if they should faint. We also have boxes of ammonia inhalants on the phlebotomy cart in each Phlebotomy Room if the participant doesn't come to on their own.

The trained Phlebotomist will choose the appropriate spot to perform venipuncture to withdraw a blood sample and proceed to withdraw the blood in prelabeled tubes (**Study group labels tubes and fills out the Quest Test Requisition ahead of time**). We generally use the antecubital spot in the arm to draw blood. The tubes will be inverted slowly 10 times by the phlebotomist and placed in the secondary containment on the clean cart to be moved to the lab for centrifugation by the study team. The time the blood draw is completed should be written on the tape on the test tube rack by the phlebotomist. The serum tubes (SST tiger top) are allowed to clot in the upright position at room temperature for longer than 30 minutes but less than 60 minutes. Whole blood for CBCs should be collected in lavender-topped tubes. These tubes should be filled to capacity since the anticoagulant could cause distortion in the test results due to osmolality of the anticoagulant. The lavender tubes should be inverted 8 times to mix the blood thoroughly with the anticoagulants. There are many different tests that can be analyzed so it is imperative that the study group determine what tubes each test requires and

what order of draw should be performed and if the tube needs to be kept on ice until the tube is centrifuged. The preferred order is light blue, tiger top, red, green or tan, lavender or tan, royal blue, gray and lastly yellow tubes. Tubes are placed in the transport bags along with the requisition and put in the laboratory refrigerator in secondary containment to await Quest Pickup. If Quest samples are drawn on Saturday morning, we have to transfer the samples to remove the serum from the RBCs and study personnel must meet the Quest driver for sample pickup as the outside doors are locked.

When filling out a Quest Test Requisition please be sure to fill out the following: Bill to MY account. Patient name, Date of Birth, Sex, Date collected, Time collected, Fasting or non fasted state, Physician (PI of the study), and Test codes wanted for analyzing each sample.

NWRC Standard Operating Procedure for working in the Phlebotomy Room and Laboratory at NWRC.

Before the study starts for the day, lab personnel will push the **Clean Cart** (labeled in the lab) with the secondary containment pan from the lab down the hall to the Phlebotomy Room without gloves or lab coat. Never touch the **Clean Cart** with gloved hands to avoid contaminating the cart and therefore, ungloved hands.

All blood samples will be withdrawn from the human participant by a trained phlebotomist (single sticks) or nurse (indwelling venous catheter) in the Phlebotomy Room. Samples will be placed in either ice or in a test tube rack and the time noted on tape. The samples will be placed in secondary containment on the **Clean Cart** by the Phlebotomist or Nurse who is wearing PPE (nitrile gloves, safety glasses and scrubs). The lab personnel will then push the **Clean Cart** back to the lab and then put on their PPE (lab coat, nitrile gloves, and safety glasses). Some methods need to have the enzymes in the blood deactivated. Aprotinin is a competitive serine protease inhibitor which forms stable complexes with and blocks the active sites of enzymes. If it is necessary to add aprotinin, the blood tubes should be placed in the BSC and lids removed, aprotinin added, tubes recapped, inverted slowly 10 times and left to sit to allow coagulation (> 30 min but < 60 minutes) before centrifugation. If samples do not need aprotinin, they can either stay in the Phlebotomy Room or on the cart until it is time to centrifuge the samples. Centrifugation should occur at 1450 to 1600 relative centrifuge force (RCF) for at least 15 minutes; this equates to 3450 to 3380 revolutions per minute (RPM). Quest samples should be handled appropriately and placed in the refrigerator in the lab. Lab Personnel will not wear lab coats or nitrile gloves out in the hallway. We are working with blood which may have blood borne pathogens in the samples so we need to limit potential accidental exposure to participants, staff, and neighboring personnel passing through the halls to/from dining rooms, phlebotomy room, lab and kitchen. The **Clean Cart** will then be pushed back to the Phlebotomy Room by lab personnel who are not wearing lab coats or nitrile gloves to wait for the next blood samples. When the samples are properly aliquoted into microcentrifuge tubes, they will be placed in secondary containment and placed on the **Clean Cart** and rolled down to the ultralow freezers by lab personnel who are not wearing lab coats or nitrile gloves. When you get to the ultralow freezers, open the freezer and using the blue ultra cold gloves, pull out the boxes you need and close the door. You may use nitrile gloves to put your samples in the appropriate box but then you need to take them off to touch the ultralow freezer door and to take the **Clean Cart** back to the lab or phlebotomy room. At the end of the blood draw session and you are done using the **Clean Cart**, please spray it with 409 to clean any possible contamination from the cart.

NWRC SOP for inserting a Peripheral Indwelling Venous Catheter

Indwelling venous catheters allow venous access for a period of hours, allowing withdrawal of multiple venous samples; this is easier on the vein than doing multiple sticks. The indwelling catheter is inserted into the antecubital fossa. Catheters may be made of polyurethane or silicone that is firmly secured with tape. Based on

the guidelines that have been established at McFarland Clinic, we only hire nurses to insert an indwelling catheter because the catheters must be flushed with saline every time blood is withdrawn. This is considered administering a medication (even saline) that is why it is considered a nursing procedure.

Flushing

To prevent occlusion and thrombosis, such devices require regular flushing, usually with a heparinised saline solution.

Hygiene

- The venous access port and entry site should be kept scrupulously clean and the latter regularly checked for any signs of infection.
- Alcohol-impregnated wipes may be used to clean the exterior surfaces of the devices.
- **Prevention of air entry into line**
- Non-valved lines must be kept clamped closed when not in use, and care taken not to unclamp them until the port entry is sealed.

NWRC SOP for inserting Indwelling Venous Catheters

Nurses will wear appropriate Personal Protective Equipment (PPE) while drawing blood samples. The PPE will consist of gloves, eye protection (safety glasses or goggles), and either a lab coat or scrubs. Please take off gloves as soon as you are done using them on each participant but do not put on a fresh pair until the next participant is sitting in front of you. That way the participant can see that the gloves are new.

1. Ask participant for ID number to be sure that ID number matches with tube numbers.
2. Ask participant if you should use foam tape or paper tape to secure indwelling catheter to their arm or hand.
3. Visualize antecubital site (1st choice), any place on arm or hand (2nd choice) for site to insert indwelling venous catheter.
4. Sanitize selected site with isopropanol.
5. Insert BD Insyte Autoguard indwelling venous catheter-22 GA 1.00 in (Ref # 381423).
6. Attach BD Q-Syte (Ref # 385100) to indwelling catheter.
7. Sanitize Q-Syte with isopropanol every time before use.
8. If no blood sample will be taken immediately depending upon approved IRB study protocol, flush indwelling catheter with 3 mL of sterile 0.9% sodium chloride (normal saline).
9. If blood sample will be taken immediately depending upon approved IRB study protocol, attach the BD Luer-Lok access Device (Ref # 364902) to the Q-Syte and then insert the vacutainer blood tube(s) to collect the needed blood samples.
10. Remove the vacutainer tube and Luer-Lok access device and then flush indwelling catheter with 3 mL of sterile 0.9% sodium chloride (normal saline).
11. If there is a problem and you cannot obtain a sample using the above steps, please use a sterile Luer-Loc syringe and withdraw a blood sample from the Q-Syte. PPE for using the Luer-Lok syringe to obtain the blood sample will include gloves, lab coat, and face mask. Attach the Luer-Lok syringe to a BD Blood Transfer device (Ref # 364880) and then insert a vacutainer tube inside the transfer device to safely transfer the blood sample to the vacutainer tube.

We will discontinue the use of the Specimen Collection Assembly with blunt plastic cannula (Ref # 303380) immediately as it causes irritation to the vein, can damage the Q-Syte, and run a risk of exposing the Nurse to BBP.