The Effects of Corn Flour on

Heart Health and the Gut Microbiome on

Hyperlipidemic Adults

by

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ABSTRACT

Grains are a major dietary staple in many cultures and countries. Corn can be consumed as either a fruit, vegetable, or grain. In the case of the Corn and Heart Health Study (CHS), the effect of corn flour was examined. The study used three varieties of corn flour (whole grain, refined, and a refined plus bran blend), provided by the North American Millers Association (NAMA), to examine the effectiveness of corn flour on lowering low-density lipoprotein (LDL) cholesterol as well as its effects on the diversity of the gut microbiome. The objective of this analysis was to determine the magnitude of change between pre- and post- intervention serum blood samples and the changes in alpha (within-sample) diversity in the gut microbiome. The study utilized a randomized-single blinded, crossover model. The study was 16 weeks long, with three 4-week long treatment periods with two-week washout periods in between. During each treatment period blood samples, stool samples, a diet record, and questionnaires were collected from participants. Two blood samples were collected at the beginning and end of each treatment period to account for potential day to day changes on LDL cholesterol. For the purpose of this study, the results of blood and fecal analysis were used to determine the effectiveness of the intervention. Fecal analysis using the Shannon Index showed that there was no significant difference in the within-sample microbiome diversity by corn flour type ($H=2.86$, $p=0.72$). Pre-treatment plasma LDL levels were subtracted from post-treatment levels and analyzed using a general linear model that controlled for sequence, period and a nested (ID[sequence]) variable to account for the within-person crossover design. This showed that the bran-enriched flour had the highest mean reduction in LDL cholesterol while the refined and whole grain flour resulted in increases in LDL cholesterol. The change in LDL cholesterol for bran-enriched flour was significantly different from the refined flour (Mean Difference of -14.97 mg/dL; P=.041). The results of this study indicate that refined corn flour enriched with bran could be a recommended addition to the diet to prevent cardiovascular disease and reduce LDL cholesterol in individuals who are at low risk.

DEDICATION

This thesis is dedicated to the family and friends who have supported me throughout my educational journey. Without your support, I would never have been able to reach for the stars and achieve my dream.

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TABLE OF CONTENTS

CHAPTER Page

APPENDIX

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Purpose of Study

According to the World Health Organization, cardiovascular disease kills approximately 17.9 million people each year, that's two-and a half times the population of Arizona (1,2). Cardiovascular disease has been the leading cause of death in the United States since 1921(3). This disease is a cause for serious public health concern that has far reaching effects on individuals and the health system. Cardiovascular disease can cause multiple long-term health issues that can require long-term care (4). The cost of care in the United States is also significant, at around 219 billion dollars per year (5).

Elevated cholesterol levels contribute to an elevated risk for cardiovascular disease via the formation of plaques along blood vessels (6). Long-term high cholesterol can lead to atherosclerosis, or hardened plaques on the blood vessels (6). These plaques can block off important arteries leading to cardiovascular events like heart attacks and strokes (6). Although there are many medications that are prescribed to reduce this risk and treat cardiovascular disease, there are gaps in the literature regarding the effectiveness of nutrition-based interventions.

Dietary fiber has been found to reduce the risk of cardiovascular events and death (6). For example, a study found that consuming 10 grams of dietary fiber each day reduced the risk of a cardiovascular event by 14% and death by 27% (6). Choosing whole grains in the diet is an efficient and affordable way to enhance dietary fiber intake. Studies cited in a review by Harris and & Kris-Etherton, cited that studies found evidence of risk reduction ranging from 7% to 40% (8). Corn more specifically, has been shown to have different benefits to cardiovascular health. Some of the positive health effects of whole grains are attributed to its fiber content. Corn in particular has been shown to have many medicinal benefits, with nearly every part of the fruit of a corn plant (9). The cob of the corn plant has been used to cure stomach problems, while the silk has been used to treat problems related to the urinary system (9). The overall nutrient content also makes corn an important part of a nutrient dense diet (9).

Corn, also known as maize, serves important cultural roles in addition to its health benefits. The origin of corn can be traced back 10,000 years from its domestication from the teocintle (10). Although it was first produced in Mesoamerica, it has spread throughout the world to be the third highest crop in production (9). Over time, corn has been modified to withstand different threats to production through genetic modification (10). Corn contains many micronutrients and macronutrients that contribute to its ability to improve cardiovascular health (9). A review cites the B-complex vitamins, essential fatty acids, anthocyanins, phytosterols, and resistant starch play an important role in lowering cardiovascular disease risk (9). The review also cites potential evidence for corn silk to be a method of lowering blood pressure (9).

There are many studies that seek to examine the relationship between corn and cholesterol. However, gaps in the literature fail to address different components of corn, non-cholesterol effects of a corn-based intervention, and the level of experimental validity. Many of the studies of corn use corn oil, instead of corn flour. Many of the published studies on the subject also fail to address how a corn-based intervention affects blood cholesterol and other health effects (in this case the intestinal microbiome).

Another gap in the literature is the method of examining effects as many of the studies utilize animals instead of human participants.

Research Aim and Hypothesis:

The purpose of the Corn and Heart Health Study is to examine the acute effect of corn flour consumption on reducing LDL cholesterol in adults with high cholesterol, and the acute effects of corn flour consumption on the gut microbiome in a 16-week randomized crossover study of participants in Phoenix, Arizona. There are several objectives this study seeks to assess. The first objective was to address the effect of different corn flours on circulating concentrations of cholesterol. The second objective was to determine how the consumption of corn flour affected the gut microbiome composition. In this study refined, whole grain, and a bran and refined corn flour blend were tested. The consumption of the excellent corn flour over a two-week period will cause a greater change (pre-intervention subtracted from post intervention) in circulating LDL cholesterol levels when compared to refined flour and whole grain corn flour treatments. Results will also show an increase in alpha diversity at the genus level due to the increase in fiber in the diet.

Definitions and Terms:

- Cardiovascular disease: A term used to describe several different health conditions affecting the heart and vascular system. These include, but are not limited to stroke, heart failure, and heart attack.
- Cholesterol: A macronutrient that can be created by the body or consumed in the diet. It is described by the CDC as similar to fat, with wax like characteristics. Cholesterol is a significant determinant of cardiovascular disease risk (11).

- LDL Cholesterol: LDL, also known as low density lipoprotein, is a type of fat and protein-based particle that circulates cholesterol in the body. This type of cholesterol is associated with negative health outcomes due to its tendency to collect on blood vessel walls forming plaques. (12)
- HDL Cholesterol: HDL, also known as high-density lipoprotein, is a type of particle made of fat and protein that carries cholesterol through an excretory process via the liver.
- VLDL Cholesterol: VLDL, also known as very low-density lipoprotein, is a type of cholesterol particle that delivers endogenous cholesterol (13). Similar to VLDL, high levels signify risk for health issues.
- NDSR: Also known as the Nutrition Data System for Research. This is software that facilitates dietary analysis of 24-hour recall or diet record data.
- Gut Microbiome: A term used to describe the organisms that live within the digestive tract. The population of these organisms can be affected by an individual's dietary habits and contribute toward the overall health of the host.
- Dietary Fiber: Carbohydrate components in the diet that can not be digested by the human digestive enzymes (14).
- B-vitamins: A family of vitamins that contribute to the process of nutrient metabolism in the body (15).
- Anthocyanins: A water soluble pigment in plants that also plays a role in human health (16). An example of a source of anthocyanin is red cabbage (16).
- Phytosterols: Plant based bioactive compounds (17). Examples of sources of phytosterols include vegetable oils (17).

- Essential Fatty Acids: Fatty acids that can not be produced by the body but are needed for different bodily processes (18).
- Resistant Starch: Starches that are not easily digested by the human body and digestive processes (19).

CHAPTER 2

REVIEW OF LITERATURE

Cardiovascular Disease

Cardiovascular disease is a serious condition that poses a major health concern for the population. According to the World Health Organization, 17.9 million people died in 2019 as a result of cardiovascular disease (20). Cardiovascular disease affects individuals of all races and ethnicities (21). According to the CDC, the top three race/ethnic groups that die due to cardiovascular disease are white (non-Hispanic), native Hawaiian and pacific islanders, and black (non-Hispanic) individuals (21). Cardiovascular disease is an overarching term used to describe several different medical conditions which can lead to potential damage to the blood vessels or the heart (20).

There are several different types of health issues that fall under the category of cardiovascular disease. These different subtypes represent what parts of the body they affect. The main types are coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolisms (20). Coronary heart disease affects blood vessels that play a role with the heart (20). Cerebrovascular disease involves the blood vessels that involve the bran (20). Peripheral heart disease involves the blood vessels that supply the arms and legs (20). Rheumatic heart disease is caused by bacterial infections affecting heart function (20). Congenital heart disease is caused by defects in the heart that have been present since birth (20). Finally, there are pulmonary embolisms and deep vein thrombosis which involve clots in the legs (20). These clots can break off and travel which can have catastrophic effects (20).

Costs of Cardiovascular Disease

The health care cost of cardiovascular disease is stark. Individuals with cardiovascular disease can incur many costs due to treatment needs. According to data collected by the American Heart Association the price of care for cardiovascular disease has increased over time (22). From 1996 to 2016 the price for cardiovascular care in the US increased from $212 billion to $320 billion dollars (22). Of this spending, a majority came from public insurance, followed by private insurance, and finally out-of-pocket costs (22). A majority of this spending was used for inpatient care, ambulatory care, nursing home care, and pharmaceutical treatment (23). A study by the American Journal of Managed Care found that the average cost per patient per year was $18,953, the cost increased with further hospitalizations (23). This cost places a large burden on the American population who are at risk or who have been diagnosed with cardiovascular disease.

Signs and Symptoms of Cardiovascular Disease

Initial signs and symptoms of cardiovascular disease may not be obvious but will progress to major events without intervention. Signs of cardiovascular disease related events include (but are not limited to) shortness of breath, fatigue, pain in chest or limbs, or confusion (20). However, there are more serious and potentially deadly events that can occur as a result of cardiovascular disease. There are five main types of cardiac events that signal cardiovascular disease. These are angina, heart attack, heart failure, stroke, and transient ischemic attack (24). Angina indicates the ability to oxygenate the blood, the symptoms of this are pain or discomfort in the area of the heart (25). Heart attacks are a common symptom of cardiovascular disease, approximately 800,000 people a year experience heart attack (26). Heart attacks occur due to a loss of access to oxygenated blood in the heart which can result in the death of heart muscle (26). This is a dangerous condition that can lead to cardiac arrest and cause long-term effects (26). There are several symptoms that indicate a heart attack is occurring include chest pain, feeling faint, shortness of breath, pain in neck, shoulders, jaw, or arms (27). Heart failure is another part of cardiovascular disease, it involves a reduction in blood flow. This reduction requires the body to compensate by reducing blood flow to organs (28). This condition is not curable and may be connected to other heart conditions (28). Strokes are another condition that can occur as a result of bad cardiovascular health. A stroke typically occurs when there is a blockage or rupture in a blood vessel in the brain (29). This can cause long-term cognitive and physical effects (29). Transient ischemic attack (TIA), also known as a “mini stroke”, is a short-term blockage of a blood vessel in the brain (29).

High cholesterol is often a sign of increased risk for atherosclerosis and cardiovascular disease (30). The most common treatment for high cholesterol is to administer statin drugs (29). When administered this drug class targets the liver to prevent the output of endogenous cholesterol (30). The elimination of endogenous cholesterol production results in receptor changes in the liver (30). The receptors put in place, which were triggered by statin use, remove LDL cholesterol from the bloodstream and reduce the amount available to stick to arterial walls (30). There are several types of cholesterol that medical professionals monitor that indicate cardiovascular health these are: LDL cholesterol, HDL cholesterol, total cholesterol, and triglycerides (31). When assessing blood lipid levels for cardiovascular health, the goal is to see LDL and total cholesterol reduced, while HDL increases (31).

Diagnosis of Cardiovascular Disease

Cardiovascular disease can be diagnosed using a variety of tests. One method of testing that can be used to suggest increased risk or diagnose cardiovascular disease is blood testing. When looking at the blood profile, the main measures of concern are total cholesterol, lipoprotein profiles, triglycerides, C-reactive proteins, and homocysteine (32). Imaging can also be used to diagnose heart issues that may signal a cardiac event. Examples of imaging tests include electrocardiogram, ultrasound, the nuclear stress test, and echocardiogram (32). There are also more invasive tests that can be conducted to diagnose heart conditions are angiography, cardiac catheterization, or an electrophysiology study (32).

Diagnosis of cardiovascular disease can often be inconsistent between men and women. A meta-analysis by Keteepe-Arachi and Sharma found that several factors are attributed to the discrepancies in how cardiovascular disease is diagnosed (33). The article cites one of the reasons for underdiagnosis and misdiagnosis is a lack of research involving female participants (33). These studies, which helped inform medical professionals on the diagnosing of cardiovascular disease, contributed to this issue (33). Another issue that women face in medical care is the belief that women are less likely to be affected by cardiovascular disease which attributes to underdiagnosis or misidentification (33). This study highlights the importance of gender diversity in studies and how these studies can affect the public (33).

Cholesterol

Cholesterol is a vital macronutrient that plays important roles in the body. There are several different types of cholesterol within the body: low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high density lipoprotein (HDL) (34). The main cholesterol types of medical professionals focus on when accessing health are HDL, LDL cholesterol, and total cholesterol (31). The process starts with chylomicrons carrying lipids (34). During the lipid metabolism process, this is broken down into remnants which are incorporated into VLDL in the liver (34). The metabolism of lipids leads VLDL to become IDL (intermediate density lipoprotein) which is then metabolized to LDL (34). LDL cholesterol is made up of low-density lipoprotein and is considered to be the "bad cholesterol" due to its negative effects on health (31). While HDL, serves the opposite effect of LDL, as it works to remove excess cholesterol in the body (34). The brief summary of the cholesterol cycle summarized above is a metabolic process that is constantly occurring and changing as it reflects the dietary habits of each individual.

Blood Cholesterol Testing

Blood cholesterol testing is a common medical test conducted throughout many individuals' lifespan to determine risk for cardiovascular disease or the severity of the condition. The main measures medical professionals look at are total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (31). When looking at blood cholesterol levels to determine CVD risk, there are set parameters that signal cardiovascular health. The American Heart Association has set the guidelines on these laboratory values (CDC) in the format of milligrams per deciliter (mg/dl) (31). Total cholesterol, LDL, and triglycerides are the same regardless of biological sex (31). HDL cholesterol is the only measure that varies based on biological sex, with the optimal levels set at greater than 40 mg/dl for females and 50 mg/dl for males (31). The optimal level for LDL cholesterol is 100 mg/dL (31). The optimal level for triglycerides is less than 150 mg/dL. Total cholesterol should be about 150 md/dl as well (31).

When an individual is at risk for cardiovascular disease or has been diagnosed with cardiovascular disease it is important to get consistent blood cholesterol testing done to determine risk. When individuals are being tested, individuals need to fast for nine to twelve hours before the test to ensure accuracy (35). During the appointment individuals are seen by a phlebotomist, who is trained in specimen collections (36). During the appointment, the phlebotomist will find a vein in the arm and hand and insert a needle that dispenses the blood into tubes (36). These tubes are then analyzed using specialized machines that have been calibrated for accuracy.

Cardiovascular Disease Treatment

Treatment of cardiovascular disease depends on the severity. When cholesterol rises above the optimal levels, statins and lifestyle changes should be recommended (37). One of the first steps that may be taken when an individual is clinically diagnosed with high cholesterol are statins (38). However, there are other pharmacological interventions like aspirin that can be administered to protect heart health (38). As mentioned above, lifestyle habits play a role in cardiovascular disease. Making changes to these habits can play a significant role in treatment (39). The three main lifestyle changes are increased physical activity, dietary changes, and reducing or eliminating smoking habits (39). If the administration of medicine and lifestyle changes do not work, surgery may be an option to improve cardiovascular health and prevent cardiovascular events (39). Some of the surgeries may be non-invasive, while others require a long-term recovery. Examples of this include: coronary angioplasty, coronary artery bypass grafts, or atherectomy (39).

Cardiovascular Disease Risk

There are several modifiable and non-modifiable factors that contribute to the risk for cardiovascular disease. Non-modifiable risk factors contribute to an individual's risk for cardiovascular disease but can not be changed. Examples of this type of risk include age, genetics, and ethnicity or family history (40). In terms of age, increasing age is associated with increasing risk for cardiovascular disease (41). This is due to the damage that occurs over time to blood vessels and the heart (41). Genetics is also a non-modifiable risk factor that plays a role in cardiovascular disease risk (40). These genetic differences may affect how the body responds to different items that are consumed (42). Ethnicity is another risk factor for cardiovascular disease. Scientists have found that certain ethnic groups have varying genetic risk due to different hereditary predispositions (42). An example of this can be found in a study by the American Heart Association that found that there was a genetic relationship between salt sensitivity and hypertension in black Americans (42).

Modifiable risk factors contribute to the likelihood of cardiovascular disease but can be changed through various interventions. Examples of modifiable risk factors include dietary habits, physical activity habits, environment, and smoking (43). There are several dietary habits that put individuals at higher risk for cardiovascular disease, such as high intake of salt, high cholesterol intake, high sugar intake, and high intake of fats (44). Physical activity habits also play a role in the risk of cardiovascular disease.

Generally, 30 minutes each day over 5 days of the week has shown to be related to lowering risk of cardiovascular disease (45). Environment also contributes to the risk of cardiovascular disease in many ways. One example is the level of population surrounding the environment of an individual. If someone lives in an area with high pollution levels, they may not be able to participate in activities that take place outdoors (46). The pollution could not only affect the breathability of the air, but the risk for oxidative stress (46). Another example of environmental impacts on cardiovascular disease risk is in the walkability of the environment around (47). An area with a lack of sidewalks, which reduces safety when walking, may reduce a resident's desire or ability to exercise in the area. Smoking is an activity that puts individuals at high risk for many health issues, one of which is cardiovascular disease. According to Johns Hopkins Medicine, of the five individuals whose death is related to smoking, one is from cardiovascular disease (48). Smoking does not only put the person actively using at risk, but those around them (48). Yearly, about 34,000 people die of cardiovascular disease who were exposed to secondhand smoke (48). This is why reduction or cessation of smoking is important in addressing cardiovascular disease.

Lifestyle Changes

One of the major recommendations for individuals with high risk or who have been diagnosed with cardiovascular disease is to quit smoking. Taking this step at the point of prevention or treatment is important in improving health outlook. It is also necessary to point out that non-traditional forms of smoking also carry the risk of affecting cardiovascular health. Like cigarettes, cigars and e-cigarettes all come with similar risks (49). This is an important topic to emphasize when discussing smoking cessation, as recommendations do not only include cigarettes.

Another major recommendation in the literature is dietary changes. Like smoking, this topic can be viewed from both the scope of prevention and treatment. The main focuses of which should be fat, cholesterol, and salt consumption (50). When discussing fat consumption, individuals should be conscious of their consumption of saturated and trans fats as these pose significant health risks (50). Salt consumption is also an important dietary consideration that should be made (50). Salt plays an important role in the regulation of blood pressure, with overconsumption leading to elevated blood pressure (41). Implementation of a Mediterranean diet or implementation of the Diet Approaches to Stop Hypertension (DASH) diet are recommended (50,51). These diets have shown positive effects on heart health because their guidelines lead to reduction of sodium, saturated fats, and trans fats (50,51). Over consumption of these can lead to adverse effects on the cardiovascular system that may require treatment or surgical intervention (50). An example of how long-term overconsumption leads to health issues is atherosclerosis. Atherosclerosis is caused by buildup of a fat substance in the blood vessels in the body (52). Over time, this builds up will harden and impact blood flow (52). Without proper care, this can lead to more serious health issues such as heart attack, stroke, or blood clots depending on the location (52).

The third recommendation to the public to prevent or manage cardiovascular disease is to participate in regular physical activity (50). The minimum recommendation for physical activity is thirty minutes a day, at least five days per week (50). Physical activity does not have to be vigorous, adding walking into a daily routine can also have positive effects (50). It is important to make these habit changes maintainable. Taking this step to prevent or manage cardiovascular disease can also help improve overall health through weight loss and reducing the risk of other diseases (50).

A fourth, lesser known, way to prevent cardiovascular disease, is to promote improving sleep quality and length (53). The recommendations to work on sleep were introduced in 2010 in the Presidential Advisory from the American Heart Association (53). The American Heart Association determined that getting a healthy amount of sleep each night, with the current recommendation being 7 to 8 hours, is necessary to mitigate adverse health outcomes (53). Improving sleep is not only positively connected with improved cardiovascular health, it is also connected to improved overall mortality risk (53).

It is important when making recommendations to utilize the principles of nutrition counseling and consider the circumstances surrounding each individual. The social determinants of health are an important tool to use when addressing issues that are widespread that disparities may contribute to. When addressing cardiovascular disease, looking at the environment is important. Differences in the environment affects the ability of individuals to try to adhere to the lifestyle recommendations set forth (54). Examples of how the environment affects social determinants of health include lack of sidewalks preventing physical activity (54). Studies also found a connection between vegetation in the environment and heart health (54). The food environment is also an important determinant that should be considered when making nutrition recommendations. Lack of access to grocery stores, also known as food deserts, may affect individuals' ability to purchase and maintain a healthy, balanced diet (54). This study also looked at how segregation within an environment could impact cardiovascular disease risk (54). They specifically looked at how individuals were affected when the segregation occurs at an ethnic and/or racial level (54). A link between the occurrence of cardiovascular disease was found in longitudinal studies (54). Psychosocial determinants also play a role in cardiovascular disease risk (54). One example of a psychosocial determinant that may affect individuals' risk of cardiovascular disease is psychological stress (54). Psychological stress is associated with increases in increased cardiovascular disease mortality, increased incidences of stroke, and increased incidences of ischemic heart disease (54). Depression and social isolation were also psychosocial determinants that affect cardiovascular disease risk (54). Longitudinal studies found that depression specifically affected the likelihood of individuals experiencing heart attacks or death due to heart disease (54). Social isolation was only associated with increased occurrence of cardiovascular disease, and not related to death or cardiovascular events (54). Many of the social determinants of health play a role in the development of chronic inflammation leading to cardiovascular disease (54). The article cited the neuro-hematopoietic axis, the SAM axis, the HPA axis, epigenetic changes, and glucocorticoid and catecholamine signaling caused by psychosocial and environmental factors contributing to cardiovascular disease (54). A review by Lang et al. also supports the importance of social determinants of health in the development of cardiovascular disease (55). The study expanded on the effect of work on cardiovascular disease risk (55). Specifically, the study further expands on the ways that chronic stress and work environment contribute to cardiovascular disease risks long-term (55). These pieces of literature support the need for individualized approaches when making recommendations to prevent or improve cardiovascular disease.

Grains and Dietary Fiber

Corn, specifically, is a staple in the American diet, where it is utilized in many different forms (56). Corn can be used to produce oils, animal feed, cereals, and many other items (57). Due to its versatility, it is one of the largest crops produced in the United States (56). Corn can be found in many cultures under the name maize (58).

Corn is composed of three different parts, each with different uses and nutritional values. The outer layer of the corn kernel is called the bran, it contains the dietary fiber of the corn kernel (59). The endosperm is another nutrient containing, portion of the corn kernel. The endosperm is 70% starch and 8 to 10% percent protein and does not contribute significantly to fat intake (60). The final, inner portion of the corn kernel is called the germ. The germ contains the highest amount of amino acids of the corn kernel (61). In corn production, the kernels are processed into several by-products. These by-products are used for a variety of products from cereal, oil, or animal feed (61). The germ layer of the corn kernel gets separated from the kernel and is used in oil and animal feeds (61). The endosperm is generally separated off to be used in human foods (61).

Corn Effects on Overall Health

There is evidence that different parts of the corn plant provide health benefits. A study by Maki et al. showed that the corn oil was more effective at reducing blood cholesterol in comparison to coconut oil (62). Corn silk has also been shown to reduce low density lipoprotein cholesterol (63). The researchers found that corn silk played a role in HMG-CoA reductase inhibition which is an important mechanism that reduces low density lipoprotein and very low-density lipoprotein cholesterol (63). HMG-CoA reductase is a rate controlling enzyme, it serves the purpose of bonding to an HMG-CoA substrate to form a product that will be used in the cholesterol synthesis (63). Another example of corn oil's benefits for human consumption, showed that phytosterols in the corn oil played an important role in how much cholesterol was being absorbed (64). Phytosterols are plant sterols and stanols, these components are similar in structure to cholesterol (65). There are several reasons why the phytosterols in the corn oil reduce cholesterol absorption. Some of the possible reasons for this reaction were competition against the cholesterol molecules and trans intestinal cholesterol excretion (65). This competition reduces the amount of circulating LDL cholesterol in the blood (65).

Gut Microbiome

The human intestine is home to a variety of microorganisms that serve an important role in our health and react to changes in our nutrition habits (66). There are thousands of microorganisms in the intestinal tract that can vary depending on genetics, diet, environment (food availability), age, and pregnancy status (67). There are several different subtypes of microorganisms in the gut microbiome such as: bacteria, viruses, prokaryotic microorganisms, and eukaryotic microorganisms (68). The balance between these microorganisms is exceedingly important for it to properly function. The microbial population serves several functions in the gut. One important role is protection (66). A balanced microbiome prevents the overgrowth of harmful microorganisms and communicates with the immune system via IgA and Toll-like cell receptors (66). Another important function of the gut microbiome is related to its connection to the nervous system (66). The nervous system and gut microbiome are connected through its involvement in neurotransmitters and neurotrophic factors, involvement with the sensory barrier, regulation of intestinal mucosa, control of intestinal sensory input, and production of bacterial metabolites (66). The third known function of the gut microbiome is its role in the metabolism. This metabolism is dependent on the microbiota. A study by Martin et al. found that the gut microbiome plays a role in metabolic activities like insulin sensitivity, glucose regulation, fat storage (69).

Stool consistency can be a signal of changes in diet and the gut microbiome. The Bristol Stool Scale can be used to categorize stool samples into seven different categories (70). The categories are labeled numerically one through seven. One describes samples that resemble pebbles, while samples categorized at seven are completely liquid (70). The scale can also be broken down into three sections (70). The first section includes categories one and two, and can be summarized as constipation (70). The second section includes categories three and four and are considered normal stool consistency (70). The third section includes categories five through seven and are considered to be within the diarrhea category (70).

Stool is affected by the health of the microbiome. A study by Vandeputte et al. compared analysis of the gut microbiome from stool samples to self-reported data on stool-consistency (71). Stool consistency can be attributed to many factors, one of which is transit time (71). The study found that there was a relationship between consistency and microbial population (71). Specifically, as reported firmness increased, the microbiome sample was negatively affected (71). The study also found that the enterotype that each person did possess, was important to consider when viewing the results of fecal analysis (71).

Microbiome Balance

The balance of the microbiotic environment in the intestinal tract is important to our health. Previous studies and literature suggest that imbalances in the microbiome can be related to metabolic disorders like diabetes (66). Studies have also found that imbalances in the microbiome are linked with irritable bowel disease, crohn's disease, and colorectal cancer (72). Another significant finding regarding microbiome balance was the effect of E. Coli's presence in the intestinal microbiome (72). Studies of the microbiome found that E. Coli overpopulation in the microbiome corresponded with the occurrence of irritable bowel disease and colorectal cancer (72).

Macronutrients in the Microbiome

The macronutrients we consume also have important reactions with the gut microbiome. Protein is an important part of the human diet that contributes to body composition (73). Studies have shown that how the gut microbe interacts with protein is dependent on its characteristics (74). The main characteristics of concern when considering the relationship between protein and the gut microbiome are: how much protein is in the meal, the other macronutrients contained in the meal, glycation of protein, how it was processed, the level of oxidation (74). The source the protein is coming from is also an important factor to consider: plant proteins, animal proteins, and synthetic proteins can have different effects on the body (74). Bioavailability also plays an important role in interactions with the gut (74). Protein is also metabolized by bacteria in the gut microbiome into short chain fatty acids (SCFA) (74). It is important to note that other by-products are created by protein metabolizing in the microbiome, metabolites that are helpful or harmful to health can be produced (74).

Carbohydrates breakdown in the gut microbiome also takes place in the gut. Microbes in the gut help with the breakdown of carbohydrates into more useful forms (75). Essentially it takes the carbohydrates from a polysaccharide structure to monosaccharide structure (75). This occurs because of the enzymes that many of the microbes in the gut possess (75). An example of this process is the breakdown of starch into glucose by a microbe like Bifidobacterium spp. (75).

Fiber is another important nutrient that plays an important role in health and has important interactions with the gut microbiome. There are two types of fiber present in the diet: insoluble fiber and soluble fiber (76). It is important to note that insoluble fibers can not be utilized by the gut microbiome (76). Dietary fiber that is accessible to the gut microbiome is put through the process of anaerobic fermentation, with the product being short-chain fatty acids (SCFA) (76). Fiber consumption also benefits the microbiome, consumption of a variety of fibers can affect the populations in the intestine (76). Assessments of the microbiome populations can indicate to scientists that fiber content in the diet is different, this is especially prevalent in different rural and urban communities (76).

Previous studies have explored the connection between the gut microbiome and the consumption of whole grains and cereal grains which can contribute a significant amount of fiber to the diet. A meta-analysis by Jefferson and Adolphus found that based on thirty-nine studies, there is evidence that supports the conclusion that grain-based fiber added to the diet causes significant differences in the microbiome abundance (77). A study by Carvalho-Wells et al. looked at how the consumption of a corn-based breakfast cereal affected the microbiome over the course of 21 days (78). The study found that fecal bifidobacteria increased significantly while the cereal was being consumed when compared to a control group (78).

CHAPTER 3

METHODS

Participant Recruitment

Participants were recruited for this study via flyers distributed by the research team in the Phoenix metropolitan area. The inclusion criteria for individuals participating in this study were: individuals between the ages of 18 and 70 years with circulating LDL cholesterol >110 mg/dL. Participants with LDL cholesterol >190 mg/dL were permitted to participate pending physician approval. There were several reasons why a participant may be excluded from the study this included: abnormal or significant weight changes, use of statins or other drugs that could impact study results, women could not be pregnant or breastfeeding during the duration of the study, following restrictive diet habits, supplement use, food allergies (egg, dairy, gluten), pre-existing conditions known to impact cholesterol, and being active in other studies. Physical activity >30 min/d on more than 5 days per week.

Study Design

This study used a single-blind, randomized, cross-over design, with each participant receiving three different varieties of corn flour. The timeline for this study was 16-weeks, with each of the interventions lasting four weeks. Between each intervention there was a two-week wash-out period for blood lipid levels and the microbiome to return to normal. The wash-out period was extended for some of the patients if their diet was going to be affected by dietary changes, specifically around significant meal-related holidays (e.g., Thanksgiving, Christmas, New Years).

Study Protocol

Prior to speaking directly with study coordinators, potential participants were directed to fill out a survey to determine eligibility. It focused on collecting information on age, sex, location, contact information, as well as questions regarding their health. The questions regarding health helped to determine if the potential participant met the criteria necessary for inclusion. After completion of the survey, potential participants were contacted, and an initial visit was scheduled. During this visit, initial blood tests were run to confirm high LDL blood cholesterol levels. A sample of blood was sent to Sonora Quest labs where a standard lipid panel was run including total cholesterol, HDL, LDL, VLDL, and triglycerides. The research team also explained the study and addressed concerns prior to having initial consent forms signed. Once the blood test confirmed eligibility, a general health and demographic questionnaire was provided to participants for completion. Another consent form was signed at this point. At the second visit of the study, another blood draw was collected, and more questionnaires were provided regarding each participant's gastrointestinal health and potential issues. A diet record was also provided at this time. Participants were directed to fill it out with two weekdays and one weekend to reflect their diet during different parts of the week. Additionally, anthropometric data was collected during this visit. Height, weight, waist circumference, and blood pressure measurements were taken. Each was collected three times to ensure accuracy and averaged for final data analyses. The third visit occurred the day after visit number two. Another blood sample was collected at this time. This will let researchers know about day-to-day variation. A baseline fecal sample was also collected during this visit. Additional questionnaires were provided at this time specifically focusing on physical activity and socio demographic questions. At this time participants were provided with corn-flour based baked goods to be consumed for the first 4-week intervention.

This study intervention involves the distribution of food items to participants. Participants were given one week of food supplies of each item to be consumed twice per day in the fourth, fifth, and sixth visits. These visits involve additional food distributions. On week six another fecal sample collection kit and diet record to be collected at the post treatment visits (seven and eight) were provided to each participant. During the 7th visit, post-intervention testing was completed which involved a blood sample collection, collection of anthropometric measurements, collection of compliance paperwork and uneaten corn-flour food items. Questionnaires covering gastrointestinal symptoms, acceptability of food and physical activity were also provided. The following day, visit eight occurred. During this appointment an additional blood sample was collected, questionnaires covering gastrointestinal symptom and food acceptability and the diet record from the previous visit was collected. Finally, a fecal sample kit will be distributed to establish a baseline for the 2nd intervention after the two-week washout period. Visits two through eight were repeated twice for the other two interventions. By the end of the study a total of about 22 meetings occurred.

Production of Baked Goods

The baked goods being provided to participants were in the form of either pita bread or corn bread muffins. The baking occurred in a controlled and sanitary environment in the ASU Downtown metabolic kitchen spaced in the Arizona Biomedical Collaborative building and the Wexford Phoenix Biomedical Campus building.

A standardized recipe was used to create the muffins and pita bread during the course of the study. Each ingredient in the recipe was measured by weight to prevent differences between batches. The only changes made to the recipe was the flour to create goods for each intervention period. The different flour types used in the study were whole grain corn flour, refined corn flour, and excellent corn flour. The whole grain corn flour and refined corn flour were distributed by North America Millers Association-Corn Division. The excellent corn flour is a mixture of bran and refined flour which were received separately and mixed together in a 75 gram:165 gram ratio.

The pita recipe involved the use of the corn flour variation needed for the batch (refined, whole grain, or excellent flour), wheat gluten, dry yeast, water, granulated sugar, salt, ICS 56, softase 4040, baking powder, shortening, and lecithin/topsithin UB. The total caloric yield for the excellent fiber pita was 146 calories with 10 grams of dietary fiber. The total caloric yield for the whole grain fiber pita was 164 calories with 1.99 grams of dietary fiber. The total caloric yield for the refined fiber pita was 167 calories with 0.68 grams of dietary fiber.

The muffin recipe involved the use of corn flour variation needed for the participant (whole grain, refined, or excellent corn flour). The recipe involved the use of shortening, granulated sugar, salt, instant starch, native starch, maltodextrin, non-fat dry milk whole eggs, honey, vanilla extract, wheat gluten, baking powder, enzyme softase 4040, and water. The total caloric yield for the excellent flour muffin was 286 calories with 9.92 grams of dietary fiber. The total caloric yield for the whole grain muffin was 301 calories with 1.95 grams of dietary fiber. The total caloric yield for the refined fiber muffin was 304 calories with .65 grams of dietary fiber.

Microbiome and Blood Analysis

Blood and fecal samples were collected over the course of this study. Blood samples were collected by a trained phlebotomist, who collected around 7mL of blood during each collection. After collection, these samples were frozen at -80 degrees Celsius until they could be analyzed. Blood analysis was completed using the Beckman Coulter AU480 following manufacturer protocols. In brief, total cholesterol, HDL cholesterol, and triglycerides were measured, and LDL cholesterol was calculated using the Friedewald formula equation.

While blood samples only need to be thawed to be analyzed, fecal samples require processing prior to analysis. During this processing, fecal weight, pH, and Bristol Stool Scale observations were recorded. A smaller sample from the larger sample was collected, along with three extra samples. Microbial genomic DNA was extracted from fecal samples using DNeasy PowerSoil Pro DNA isolation kits (Qiagen) and a vortex-based beadbeater. The hypervariable V4 region of the 16S rRNA gene was amplified from stool samples using barcoded 806R and 515F primers (79) and 5 Prime Hot MasterMix (5 Prime, Germany) in triplicate. Quality of the amplicons and potential contamination was checked on an agarose gel. Amplicons were quantified using Picogreen (Invitrogen), according to the manufacturer's protocol. A total of 200 ng of amplified DNA from each sample was pooled and cleaned using UltraClean PCR Clean-Up Kit, and then diluted, denatured (0.2 N NaOH), and sequenced on the MiSeq platform (Illumina), as previously described (79). Due to the limited sequence diversity among 16S rRNA gene amplicons, 10% of the PhiX control library (Illumina) made from phiX174 was added to the run. A 7 pM aliquot of the pooled 16S rRNA gene library was subjected to paired-end sequencing using 2×250 bp MiSeq Reagent Kit V2 (Illumina). Sequencing was performed by the Genomics Core at ASU using the Illumina MiSeq and MiSeq Control Software. Pooled sequences were de-multiplexed and quality filtered using the QIIME2 software package (80). Sequences were assigned to operational taxonomic units (OTU) with a 99% similarity threshold using QIIME's uclust-based open-reference OTU picking protocol against the most recent SILVA reference database. Sequences that did not match the reference database were clustered de novo; thus, all sequences were included in the analysis. Core diversity analyses were performed on the OTU tables, from which the alpha diversity metric, Shannon Index, was used for statistical analyses.

Statistics and Data Analysis

In this study the independent variable was the consumption of corn-flours being used as the intervention in the study. Each of the three corn flours being tested aimed to elicit a response that could be measured after four weeks. The dependent variables in this study were magnitude of change in plasma LDL levels and gut microbiome alpha diversity.

The sample size suggested for this study was 45 individuals, this estimation was created based on the number of people needed to collect significant data using power calculations. The recruitment of 45 individuals, accounts for a predicted 20% dropout rate and allows for a sample size that would produce significant results.

Data analysis was completed using several different software programs. Dietary analysis was completed using the nutrition data system for research (NDSR) from the University of Minnesota. This software collects information on the day, time, location, and contents of meals. Redcap was another software program used for data management over the course of the study. Statistical analysis was completed using SPSS, this software was used to analyze data for significant changes, analyze the strength of relationships, and to create graphs representing the data. For this study a linear model will be utilized to analyze data. This model will account for the different treatments utilized in the study as well as the sequence and period assigned to each participant. Participant's data will be nested because the study was a crossover and multiple sets of data were collected on each participant. The linear equation for LDL will be LDL change ~ ID(sequence) + sequence + period + treatment. Post-hoc pairwise comparisons will be used to further analyze the difference in results between each corn flour treatment on blood cholesterol. For analysis of the gut microbiome's alpha diversity the same method of linear model will be used (change in alpha diversity ~ ID(sequence) + sequence + period + treatment).

CHAPTER 4

RESULTS

Participation Characteristics

A total of 52 participants were screened for the study. This population consisted of 18 males and 22 females, an average age of 39.4 (±14.2) years old. Of the participants that completed at least one period (n=30), 23 individuals completed all three periods.

Table 1: Participant Characteristic Breakdown (N=30)

Variable	Subcategories	Mean +/- SD, Percentage of Participants
Age		39.3± 14.9
Biological Sex		
	Male	13(43.3%)
	Female	17 (56.6%)
Race		
	American Indian/Alaskan Native	0 (0%)
	Asian	4 (13.3%)
	Native Hawaiian or other Pacific Islander	0 (0%)
	African American	0 (0%)
	Caucasian	23 (76.6%)
	Individuals identifying with multiple races	2 (6.7%)
	Unknown	1 (3.3%)
Body Mass Index (BMI)		37.53±45.7

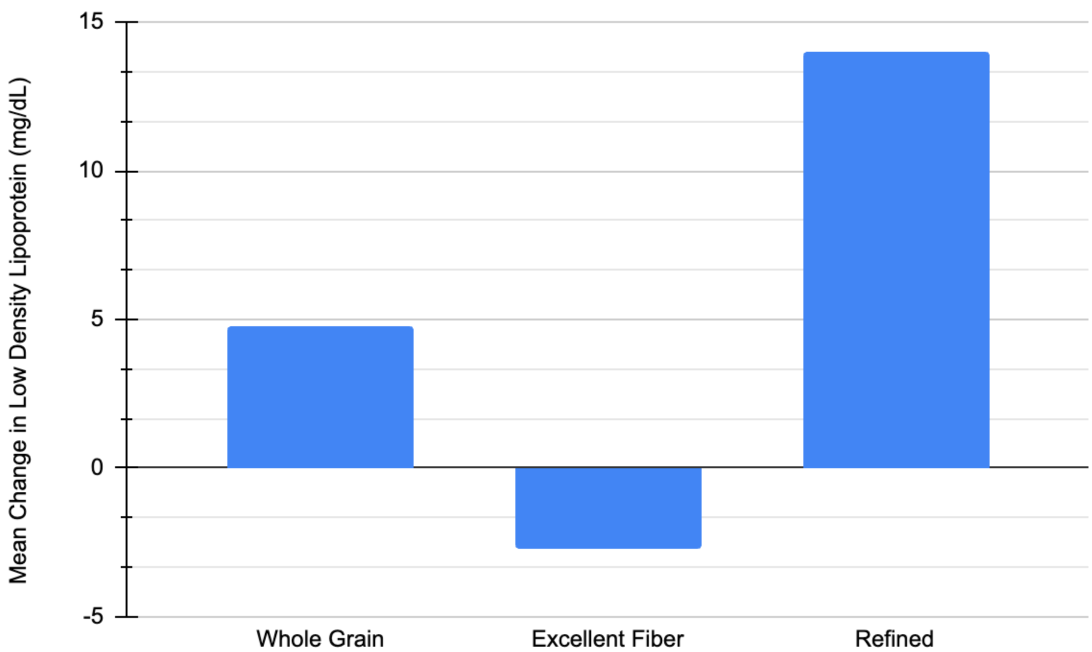
Serum Baseline Data		
	Cholesterol (mg/dL)	272.12±40.2
	High Density Lipoprotein (HDL-c) (mg/dL)	58.95±11.3
	Triglycerides (mg/dL)	170.98±70.32
	Low Density Lipoprotein (LDL) (mg/dL)	178.98±39.74

Low Density Lipoprotein Cholesterol Analysis and Statistical Modeling

Low density lipoprotein cholesterol was analyzed using a generalized linear model to analyze differences in LDL cholesterol between treatments. The generalized linear model output contained an omnibus test with a likelihood-hood ratio of chi-squares was 54.06 with a significance level of p equals .035. This indicates that the model assesses treatment, period, sequence, and participant within sequence. The results indicate we should reject the null hypothesis, meaning one population of the models was different.

Post hoc analysis using Tukey HSD showed a significant mean difference between the excellent fiber flour and refined flour with a mean difference of 14.92 and a significance of p equals .041. The graph below shows the difference in marginal means for each treatment. The whole corn flour had a marginal mean change of 4.78 mg/dL with a standard error of 3.33 mg/dL. This indicates there was a slight increase between the beginning and end of the 30-day treatment period. Excellent flour had a mean marginal change of -2.69 mg/dL with a standard error of 3.44 mg/dL. This indicates that on average, participants experienced a decrease in LDL cholesterol during the treatment period. The refined flour had the largest average increase in LDL cholesterol. During the 30-day treatment period participants experienced an average 13.99 mg/dL increase in LDL cholesterol, with a standard error of 3.50 mg/dL.

Figure 1: Bar Plot of Mean Change in LDL Cholesterol for Three Corn Flour Treatments Over a Four Week Treatment Period (N=30)



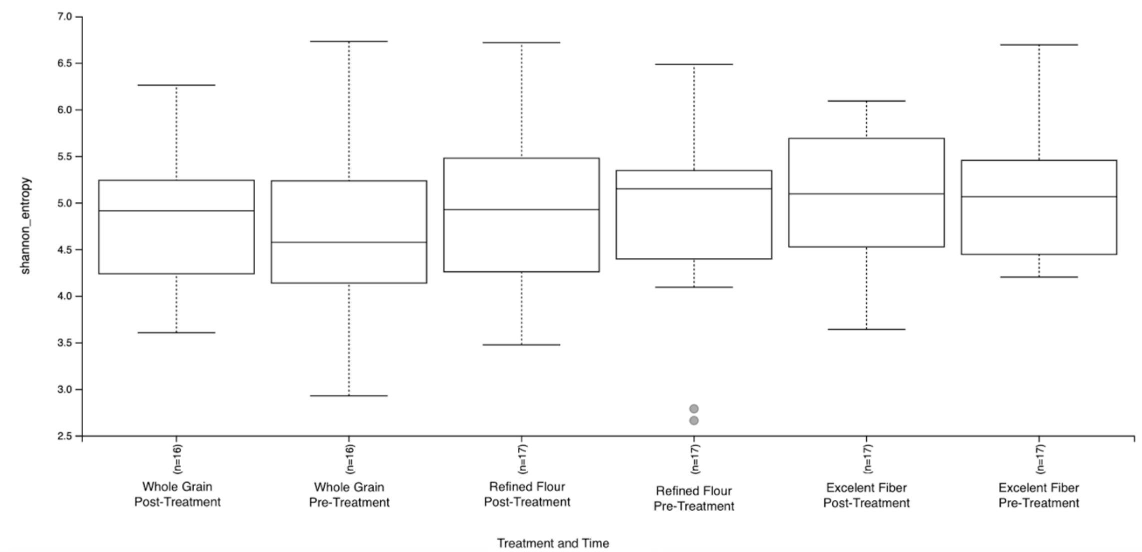
Statistical analysis was also performed to determine if age significantly impacted LDL. Due to the limited sample size, the current data did not contain enough degrees of freedom to power the model.

Gut Microbiome Analysis and Statistical Modeling

Figure two illustrates the microbiome data collected from participants of the study. Samples were collected before (pre) and after (post) each treatment. Data was analyzed specifically using the Shannon Index to illustrate the within sample diversity.

Shannon diversity did not differ by flour group (H=2.86, p=0.72). This suggests that fiber content does not significantly affect the richness of the microbial community at the genus level.

Figure 2: Box and Whisker Plot of Shannon Index Data to Compare Pre and Post Within Sample Diversity Between Three Corn Flour Treatments Over Four Weeks (N=21)



CHAPTER 5

DISCUSSION

The results of this study indicate that the addition of approximately six grams of fiber from corn bran added to refined corn meal had beneficial effects on heart health. The refined corn flour enriched with bran produced a significant average decrease in LDL cholesterol of -2.69 mg/dL. In contrast, the whole grain corn flour did not result in improvements in LDL cholesterol when consumed over the same period of time. The results also indicated that the consumption of corn flour enriched with a fibrous bran had no significant effect on the alpha diversity of the gut microbial community. The expectation prior to analysis was that the fiber content of the different flours would contribute to a difference in bacterial diversity within the gut microbiome. However, analysis using the Shannon Index yielded insignificant results (H=2.86, p=0.72).

According to a review by Soran et al. the risk of heart disease is reduced by 10% when a reduction of 1 mmol/L (39 mg/dL) of LDL cholesterol is achieved (81). This indicates that the change in LDL cholesterol seen with the excellent fiber treatment would produce a much smaller and less clinically relevant effect on LDL cholesterol. Due to the limited reduction in LDL cholesterol, this would likely not be recommended to patients as a way to reduce LDL cholesterol when they are at higher-than-average risk for heart disease. However, it can be used as a better alternative to maintain current LDL levels when compared to refined or whole grain corn flour products in the diet.

The study found that refined corn flour enriched with corn bran decreased LDL cholesterol in men and women with mild-to-moderately high LDL cholesterol not taking statin or other blood lipid-lowering drugs. Previous studies have established the relationship between other bran-enriched flours and cholesterol levels, but corn bran has not been well studied in humans for several decades. However, studies exist analyzing the effectiveness of corn bran in different populations. A study by Shane and Walker studied the addition of corn bran to a low-fat controlled diet in men with hypercholesterolemia (82). The study found that the addition of corn bran to a controlled diet resulted in a significant reduction in VLDL cholesterol (1.01±.052 to 0.88±0.39), triglycerides (2.24±1.12 to 1.94±0.87), and total cholesterol (6.03±0.83 to 5.72±0.86) (82). A study by Berg et al. explored the effect of oat bran on participants with high risk for heart disease (83). This study utilized a controlled randomized trial, where participants were randomly assigned a diet with or without bran (83). The study found that the oat bran treatment resulted in a significant reduction in LDL cholesterol of 56.3 mg/dL with a standard deviation of 35.1 mg/dL (83). A study on the effect of wheat bran by Osfor et al. found that the mean LDL cholesterol was significantly reduced (149.67±39.62 to 121.89±44.38) when consumed over a month-long period (84). The study also found that the consumption of wheat bran resulted in a non-significant increase in HDL cholesterol (from 42.45±12.47 to 43.22±13.69) (84). Taken together, the effects of bran fiber from grain products do not seem to be the same for all grains. Studies of rice bran show that the fibrous portion of rice bran is not effective in changing LDL cholesterol (85). However, the study found that rice bran oil caused a change in LDL cholesterol showing a difference in the mechanism of effect (85).

In this study, the physiological mechanism that caused the impact on cholesterol levels was the fibrous component of the corn bran flour. According to a study by Anderson and Clydesdale corn bran contains 50.12% total dietary fiber (86). The makeup of this dietary fiber is largely hemicellulose and cellulose (86). The consumption of fiber reduced cholesterol levels through several mechanisms (87). One method involves reduced absorption of cholesterol through the intestinal lumen (87). Another mechanism could occur as a result of increased absorption of short chain fatty acids through the gut which thereby reduces cholesterol synthesis by the liver (87). A third mechanism is that fiber can lower cholesterol through increased bile acid synthesis, also occurring in the liver (87). A fourth mechanism of action is that it reduces gastric transit time, resulting in less time for nutrient absorption by the intestines (87).

The lack of significant reductions in LDL cholesterol following whole grain corn flour consumption is contradictory to evidence for other whole grains. There are many studies that have been conducted assessing the effect of whole grains on cholesterol showing a cholesterol lowering effect. A meta-analysis by Holloender et al. looked at 24 studies assessing the effect of whole grain consumption on LDL cholesterol (88). The analysis included analysis of studies looking at oats, wheat, rice, rye, barley, or mixed whole grain (products with multiple grain sources) (88). The overall consensus based on these studies was that whole grain consumption resulted in lower LDL cholesterol and triglycerides, however it did not have a significant effect on HDL cholesterol (88). Whole grain corn flour, however, elicited the opposite effect resulting in a slightly elevated mean cholesterol change. One potential reason this may have occurred is due to a difference in nutrient differences, specifically dietary fiber quantities and types, between corn and other grains, where the content may be different in comparison to other whole grain sources. Another potential reason for the difference in effect may be due to genetic variations in the study population (89). A study by Tucker et al found that genetic polymorphisms on the APOE E3/E3 gene caused study participants to have increased LDL cholesterol when eating whole grain sourdough bread (89).

The present study did not observe a significant difference in within sample gut microbiome diversity by corn flour treatment. However, studies conducted in the past assessing the effects of corn flour on the gut microbiome found that the consumption of whole grain flour resulted in an increase in bifidobacteria (78). Studies have also examined the effect of whole wheat and refined wheat on the gut microbiome. A meta-analysis by Cooper et al. found that consumption of refined wheat grain products resulted in a decrease in *bacteroides* (90). However, the consumption of whole grains resulted in an increase in *Bifidobacterium* and *lactobacillus* (90).

The gut microbiome plays a role in the metabolism of cholesterol (91). There are several mechanisms that allow the gut microbiome to affect LDL cholesterol within the body. Well known mechanisms include its role in short chain fatty acid formation and the production of bile acids (91). The bacteria in the gut microbiome also plays a role by reducing the amount of cholesterol absorbed by the intestine (91). Enzymes can also play a role in reducing the amount of cholesterol being absorbed (91). The study concluded that IsmA-encoding bacteria in the gut play a role in cholesterol metabolism (91). However more studies are needed to fully understand the clinical implications of this information.

This study had several strengths that helped strengthen the data. The length of each treatment period was a strength because it allowed for better assessment of the effects of the intervention over an extended period of time. Another strength of this study was the randomized aspect, as it prevented potential unintentional bias. There are study limitations that may have affected these results. One way that the results may have been affected is by the limited sample size. The data included in this thesis analysis were from a limited sample of participants due to the fact that the overall study and sample analysis was ongoing. Another potential limitation to the study is that the intervention only added one item to an individual's daily diet. There was no way to control for each participant's habitual diet during each treatment period and washout periods. However, three-day diet records were collected from participants during treatment periods and washouts to provide a view of the daily diet of each individual.

The findings of this study do have public health and clinical implications. This study supports the findings of previous studies that have found that consuming the bran portion of grains can be helpful for reducing LDL cholesterol levels. This can be used to support an evidence-based recommendation by nutrition professionals to add bran enriched corn flour to the diet to reduce LDL cholesterol as a preventative measure for cardiovascular disease in individuals who currently have a low risk for cardiovascular disease. Future research should be conducted to further explore the mechanisms that impact cholesterol. This is necessary because not all grains have the same effect on cholesterol and the gut microbiome. This research can also be continued with a larger more diverse population to strengthen results.

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APPENDIX A

ASU IRB APPROVAL FOR USE OF HUMAN SUBJECTS

APPROVAL: EXPEDITED REVIEW

Corrie Whisner

SNHP: Nutrition

602/827-2261

Corrie.Whisner@asu.edu

Dear Corrie Whisner:

On 1/6/2018 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Evaluating the effects of corn flour consumption on cardio-metabolic outcomes and the gut microbiome in adults with high cholesterol.
Investigator:	Corrie Whisner
IRB ID:	STUDY00007518
Category of review:	(2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (7)(a) Behavioral research
Funding:	Name: North American Millers' Association (NAMA)
Grant Title:	
Grant ID:	

Documents Reviewed:	• Full Study Consent Form_Track Changes, Category: Consent Form; • FoodAcceptablityAndSatisfaction_NAMA.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • IRB Protocol Track Changes, Category: IRB Protocol; • Demographics_NAMACornStudy.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • 3-day food record form_NAMAcorn.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • NAMA corn Study Recrutment Flyer_1.8.18.pdf, Category: Recruitment Materials; • Screening Verification Consent Form, Category: Consent Form; • PhysicalActivityQuestionnaire_NAMAcorn.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Email and Social Media Recruitment_12.27.17.pdf, Category: Recruitment Materials;

	• Visit2DataStart_NAMACornStudy.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Corn Proposal 3.5.2017 Final Revised Submission.docx, Category: Sponsor Attachment; • HealthHistoryScreener_NAMACorn.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Visit1BaselineDataLDLScreening.pdf, Category: Screening forms; • NAMA_Corn_Study_Online_Screener.1.8.18.v3.pdf, Category: Screening forms; • Full Study Consent Form, Category: Consent Form; • IRB Protocol, Category: IRB Protocol; • Gastrointestinal_Symptoms_NAMACornS.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);

The IRB approved the protocol from 1/6/2018 to 1/5/2019 inclusive. Three weeks before 1/5/2019 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 1/5/2019 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the “Documents” tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

CARMEN ORTEGA SANTOS

Sonia Vega-Lopez