THE EFFECTS OF WHEAT GERM SUPPLEMENTATION ON INFLAMMATION, METABOLIC, AND GUT HEALTH MARKERS IN OVERWEIGHT ADULTS

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2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2021

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ACKNOWLEDGEMENTS

I have always felt unconditional love and support from my family, despite our physical distance. Thank you, Mom and Dad for giving us the gift of education and for instilling in us a love for learning. To my siblings, as your *kuya*, I want you to know how proud I am of how you've excelled in your own lives. I take comfort in knowing that I can always rely on you when I need it. To my Tita Ruth and Tito Mike, I am forever indebted to the support you've always given me. I wish you knew how highly I speak of you to my friends. And to my precious nieces Sophie and Ellie, we love you so dearly and we are so glad that you have joined our family.

Dr. Lucas, I know I caused you to age by a decade just in the last year and a half that we worked together, but I want you to know how grateful I am for your guidance. Thank you for seeing my potential and believing in me even when I didn't. Your pragmatic approach to life taught me to keep my eye on the prize. There truly could not have been any better adviser for me at this point in my life. Drs. Emerson and Smith, thank you for being a part of my committee and for inspiring me to continue exploring and discovering knowledge. I have truly felt that this experience led me to the limits of our knowledge. I can't wait to push past them and see what I might find.

To my countless friends in Stillwater: thank you for the good times. Thank you for holding me up when I felt down. I hate to think that one day we will all be in different places. Know that I cherish the moments we spend together.

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Date of Degree: JULY, 2021

Title of Study: THE EFFECTS OF WHEAT GERM SUPPLEMENTATION ON INFLAMMATION, METABOLIC AND GUT HEALTH MARKERS IN OVERWEIGHT ADULTS

Major Field: NUTRITIONAL SCIENCES

Abstract: **Objectives:** Overweight and obesity is linked to several metabolic disturbances including type 2 diabetes. Functional foods such as wheat germ (WG) have been shown to improve metabolic markers in animals and humans alike. Our study investigated the effects of wheat germ supplementation on inflammation, metabolic, and gut health markers in overweight adults.
Methods: Forty overweight (body mass index = 25.0–30 kg/m^2) adults between the ages of 18–45 years old were recruited to participate in this single-blinded randomized controlled study. After initial screening, participants were asked to consume energy balls containing either cornmeal (control) or 30 g of WG daily for 4 weeks. Participants were asked to otherwise maintain their normal diet and physical activity throughout the supplementation period. Anthropometric and metabolic parameters, as well as dietary (3-day food record), medical history, physical activity (Yale Physical Activity Survey), stool measures (Bristol Stool Chart, BSC and the Cleveland Clinic Constipation Scoring System, CSS), gut integrity markers, and fecal bacterial population were assessed at baseline and at the end of the 4-week supplementation period.
Results: Thirty-nine participants completed the 4-week supplementation (n = 20 and 19 for the WG and control group, respectively). There were no differences in the lipid profile, but glycated hemoglobin ($P = 0.04$), insulin ($P = 0.03$), and homeostatic model assessment of insulin resistance ($P = 0.04$) were significantly decreased in the WG but not the control group. Additionally, the adipokine resistin, which is correlated with insulin resistance, was also significantly reduced ($P = 0.03$) by WG supplementation but not the control. There were no changes in stool characteristics between the two groups before and after supplementation as indicated by the BSC and CSS. The phyla Bacteroidetes ($P = 0.03$) and Proteobacteria ($P = 0.048$) and the genus *Bacteroides* ($P = 0.03$) were significantly decreased in the control group. No significant changes were observed in plasma inflammatory markers, fecal short-chain fatty acid (SCFAs) concentrations, and markers of gut integrity in both supplements.
Conclusion: Four weeks of WG supplementation resulted in improvements in markers of glucose homeostasis and reduction of the pro-inflammatory adipokine, resistin. However, these improvements in markers of glucose homeostasis due to WG consumption is not due to changes within the gut (i.e., bacterial population, gut integrity, and SCFAs production). The mechanism by which WG improve glucose homeostasis is unclear at this time and needs to be investigated in future studies. Our findings indicate that WG may be a safe, effective and economical approach to improve glucose homeostasis.

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CHAPTER I

INTRODUCTION

Overweight and obesity are the cumulative results of long-term positive energy balance and is primarily characterized by excess adiposity. The Centers for Disease Control and Prevention (CDC) clinically defines overweight as a body mass index (BMI) of 25 to 30 kg/m^2, whereas obesity is a BMI above 30 kg/m^2.[1] In the US and around the world, overweight and obesity have been public health concerns for decades. In the US alone, the CDC estimates that from 2015-2016, 39.8% of US adults were reported to be obese, while 31.6% were overweight, accounting for nearly 72% of adults above the normal weight range.[1] The CDC also estimates that from 2017-2018, 42.4% of US adults are obese, which is equivalent to approximately 93.3 million people.[1,2]

Obesity is linked to various chronic diseases such as cardiovascular diseases (CVD), type 2 diabetes (T2D), and several types of cancer.[3] The rise in the prevalence of T2D has paralleled that of obesity. The CDC estimates that 34.2 million people in the US have been diagnosed with T2D, of which 34.1 million are adults.[4] These figures represent nearly 11% of all US adults.[4] In 2019, 463 million people were estimated to have T2D around the world.[5] It is also estimated that in 2045, this number will rise to 700 million.[5] Additionally, obesity is deemed a costly condition. In 2013, obese adults have higher medical costs by $3429, of which $3210 or 93.6% was paid for by insurance companies.[6] Therefore, a reduction in the prevalence of overweight and obesity will not only improve health but also economic outcomes.

On a pathophysiological level, inflammation has been observed to accompany the excess adiposity in obesity. Typically, inflammation is an acute localized response to injury that characterizes the classic immune response. However, the inflammation that occurs in overweight and obesity is a low-grade and chronic type of inflammation that is marked by the presence of cytokines and adipokines. Immune cells such as macrophages and neutrophils that infiltrate adipose tissue are involved in this response by producing inflammatory molecules.[7] In obesity, pro-inflammatory molecules such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and leptin are increased in obesity, whereas the anti-inflammatory hormone adiponectin is decreased.[8-10]

In addition to inflammation, the last few decades have presented compelling evidence regarding the link between gut health, obesity and its accompanying complications. The colon, once thought to be nothing more than a waste repository and the major site of water absorption[11], is known to be the home of the gut microbiota. Composed of at least 1,800 genera and between 15,000-36,000 species of bacteria, the gut microbiota has a genetic diversity that surpasses that of their human hosts.[12] Clusters of these bacteria known as "enterotypes" exert either beneficial or pathologic effects on its host.[12] Evidence suggests that bacteria belonging to the *Firmicutes* and *Bacteroidetes* phyla make up about 90% of the adult gut microbiota and that the imbalance between these two phyla distinguish normal weight subjects from those that are obese.[13] Despite each enterotype's individual functions, the overall composition and diversity of the gut microbiota ultimately determines whether it is protective against disease or not. When large shifts in the ratio in microbial composition occur in the gut microbiome, an imbalance is created that eventually leads to several pathologic conditions.[14] Known as dysbiosis, this imbalance is linked to a host of diseases that includes not only metabolic disorders, but also inflammatory bowel disease and neurological disorders.[14]

Microbial dysbiosis can also compromise gut integrity by affecting tight junction proteins that prevent the entry of unwanted pro-inflammatory substances from the gut lumen into the bloodstream. One marker that indicates a compromised gut barrier is intestinal fatty-acid binding protein. Although involved in the metabolism of fatty acids inside the enterocyte, it is released into the intestinal lumen when the gut barrier is impaired.[15] On the other hand, zonulin is a protein that regulates the tight junctions in the intestine and has been observed in high concentrations in the plasma of obese individuals.[16] When triggered by enteric pathogens or gliadin, it is released into the intestinal lumen where it stimulates an inflammatory response.[17] Another marker of gut integrity is secretory IgA, which works by sequestering pathogenic bacteria to keep them from coming in direct contact with the gut epithelium.[18] The presence of these markers suggests altered gut permeability and inflammation that is typical in obesity.[19]

Another prominent function of the gut microbiota is the production of short-chain fatty acids (SCFAs). SCFAs are produced by the gut microbiota's fermentation of non-digestible carbohydrates. Of the known SCFAs, the most common in the gut are butyrate, acetate, and propionate, all of which exhibit a wide range of functions.[20] For example, butyrate maintains gut barrier integrity by regulating the tight junction proteins that hold the enterocytes together.[20] Evidence has also shown that increased propionate flux through the liver decreases the amount of triglycerides in the liver, which leads to a decrease in risk for non-alcoholic fatty liver disease.[21] Acetate has also been shown to stimulate the release of the satiety hormone leptin in adipocytes.[22]

A healthy eating pattern is widely regarded as an important approach to combat overweight and obesity. Such pattern encourages consuming nutrient-dense foods such as fruits, vegetables, whole grains, and lean meat in place of calorie-dense foods that are common in the Western diet. A healthy eating pattern would likely include the consumption of functional foods, which confer anti-inflammatory and antioxidant effects as well as favorably affecting the gut microbiome and gut health. Functional foods refer to foods that contain bioactive compounds that

confer health benefits beyond their fundamental nutritional value. These benefits improve gut health and therefore constitute a holistic approach in combatting obesity and related chronic diseases.

One such functional food that may improve health outcomes associated with obesity is wheat germ (WG). WG is the embryonic part of the wheat grain that is a by-product of flour milling and is used in animal feed, cosmetics, and in baked foods.[23] WG is rich in bioactive compounds such as fiber, B-vitamins, tocopherols, phytosterols, policosanols, and polyphenols.[23] A study with mice revealed that WG supplementation improves insulin resistance, as marked by a decrease in fasting insulin, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1).[24] Fermented WG extract (Avemar^{TM}) has been shown to improve glucose tolerance, systolic blood pressure, and visceral fat deposition in rats.[25] Similarly, the addition of WG to the diet of overweight male subjects resulted in a significant decrease in fasting blood glucose.[26] Additionally, a recent study by Moreira-Rosario et al. revealed that daily supplementation of 6 g of wheat germ for 2 months resulted in improvements in gastrointestinal function accompanied by elevated levels of beneficial bacteria in healthy adults.[27] Despite these promising results, more research needs to be conducted with humans to examine the relationship between WG consumption, gut health, inflammation and metabolic outcomes that are associated with obesity.

The *objective* of this study is to examine the effects of four weeks of WG supplementation on markers of gut health and metabolic outcomes in overweight individuals. Our *overall hypothesis* is that WG, because of its many bioactive components, will improve markers of gut health and, consequently, metabolic outcomes in overweight individuals. The *specific aim* of the study is to determine the effects of 4 weeks of WG supplementation on:

a. bacterial population and markers of gut integrity,

Working hypothesis: WG, because of its many bioactive compounds such as insoluble fiber, will act as a prebiotic and increase the population of commensal bacteria and increase the production of SCFAs. SCFAs are known to upregulate tight junction proteins and overall improvement in gut integrity.

b. markers of inflammation, and

Working hypothesis: Because of the role of WG in maintaining gut integrity, less inflammatory substances such as the bacterial endotoxin, lipolysaccharides (LPS), will enter the circulation. Moreover, SCFAs are known as signaling molecules that can suppress the production of pro-inflammatory cytokines by immune cells and modulate the expression of adipokines such as leptin and adiponectin.

c. metabolic markers of overweight adults.

Working hypothesis: The increase SCFAs production due to WG consumption will stimulate the release of the gut hormones called incretins such as glucagon-like peptide (GLP-1) that are known to induce the production of insulin by the pancreas and affect glucose homeostasis. Additionally, the reduction of systemic inflammation due to WG consumption will improve markers of insulin resistance.

CHAPTER II

REVIEW OF LITERATURE

Overweight and obesity

Weight status above the normal range is classified as being either overweight or obese, whose clinical definitions are based on body mass index (BMI). BMI only considers an individual's height and weight. Although limited by its exclusion of several health-related factors such as genetics and body fat percentage, BMI is widely used as an assessment tool, especially on a population level. Clinicians, then, must consider other factors to assess accurately an individual's health status.

The differences between overweight ad obesity lie in their BMI numerical range and their level of associated risk. The BMI range for the overweight category is 25-30 kg/m^2, while BMI greater than 30 kg/m^2 is considered to be in the obese category, which is then divided into three subcategories based on increasing BMI: Class I, II, and III.[1] Overweight and obesity correspond to different levels of risk of co-morbidities related to increased adiposity such as diabetes, hypertension, and cancer.[28] While overweight is associated with a mildly increased risk of such diseases, obesity, on the other hand, is associated with a moderate to very severe risk.[28] A British study of 3.6 million adults reported that BMI has a J-shaped association with overall mortality risk.[29] The same study also found that for every 5-point increase in BMI above 25 kg/m^2, the risk for all-cause mortality increases by 21 percent.[29] Additionally, the study determined that life expectancy declines with higher BMI.[29]

The prevalence of obesity around the world is increasing, but the rates in the US are especially high. The burden of obesity was once a unique feature of developed countries such as the United States where food supply is relatively abundant, but the rising trends can now be observed in developing countries in Latin America, Asia, Africa, and the Pacific.[30,31] The World Health Organization (WHO) estimates that obesity rates worldwide have almost tripled since 1975.[31] About 1.9 billion adults age 18 years and older are in the overweight or obese BMI categories, while 650 million of these individuals are classified as obese. Thus, 52% of the world's adult population are either overweight or obese.[31] Additionally, 340 million children and adolescents between the ages of 5 and 19 years old are overweight or obese, representing 18% of this population.[31] While this rate is not as high as that of the adults, it is concerning that in 1975, the overweight and obesity rate among children and adolescents ages 5-19 years old was 4%, nearly a five-fold increase.[31]

Similarly, in the United States, the rates of overweight and obesity have steadily risen to alarming rates over the last few decades.[32] Results from the 2015-2016 National Health and Nutrition Examination Survey (NHANES) indicate that 31.8% of adults are overweight. On the other hand, 42.4% were obese, of whom 9.2% were severely obese.[2] Altogether, these numbers represent nearly 80% of US adults who have excess weight.

Obesity is costly, both directly and indirectly. Direct costs represent the medical expenses to treat the conditions associated with overweight and obesity.[33] Alternatively, indirect costs are lost resources due to complications from overweight and obesity and are typically more difficult to ascertain.[33] No matter the type of costs, various studies indicate that a large fraction of costs for health care systems and for societies around the world can be attributed in part to obesity.[34] In 1986, it was estimated that obesity accounted for 5.5% or $39 billion of the direct and indirect costs with medical conditions like type 2 diabetes and cardiovascular disease (CVD).[35] In 1998, the estimate rose to 6% or $42 billion, and in 2006, it rose even higher to 10% or $86 billion.[36] In

2016, the total cost associated with obesity was $1.27 trillion across chronic diseases.[37] If obesity continues to rise at its current rate, direct costs alone from obesity will amount to between $48 to $66 billion a year in the US.[38] On an individual level, researchers have estimated that people who are obese spend between $1,429 and $2,741 annually more than their non-obese counterparts.[36,39] Given the exorbitant medical costs associated with obesity, effective strategies are needed to prevent obesity and its comorbidities.

The health risks of overweight and obesity have been clearly identified by several research studies.[3] Individuals who have a higher than normal weight are at a higher risk for metabolic diseases such as dyslipidemia and type 2 diabetes, and cardiovascular diseases such as coronary heart disease and congestive heart failure.[3] Unsurprisingly, the rise of obesity rates is also associated with an increase in obesity-related comorbidities, particularly type 2 diabetes. About 463 million people worldwide had diabetes in 2019 and it is projected that in 2045, this number will rise to 700 million.[5] Obesity also increases the risk for certain types of cancer (colon, breast, endometrial, gallbladder), sleep apnea, and stroke.[3] In women, obesity is associated with irregular menstrual cycles, amenorrhea, and polycystic ovarian syndrome.[3] Additionally, individuals who are obese may experience psychosocial effects such as social stigmatization, binge eating disorder, and body image issues.[3] Mortality rates among individuals with obesity have also been found to increase between 50 to 100 percent more than those who are not.[3] This multitude of health risks is incredibly concerning and therefore require immediate attention and effective courses of action.

Pathophysiology of Overweight and Obesity

Excess adipose tissue may be located in either the subcutaneous (under the skin) or visceral (abdominal cavity) depots. The difference between these two storage areas lies in the risk associated with them, although anatomical, cellular, molecular, physiological, clinical and

prognostic distinctions can be made.[40] Scientific literature largely supports that visceral adipose tissue poses greater cardiometabolic risks as compared to its subcutaneous counterpart.[41]

Adipocytes respond to an energy surplus by hyperplasia and hypertrophy. Excess lipids are first directed to SAT where hyperplasia takes place. Over an extended period as is the case in overweight and obesity, they are then deposited in ectopic sites such as skeletal muscle, the liver, and VAT when the expansive capabilities of SAT are diminished.[42,43] Eventually, blood flow to the adipocytes decreases (i.e., hypoxia) and they lose the ability to increase through hyperplasia. Adipose tissue expansion or hypertrophy then occurs. Hypertrophic adipose tissue is what is associated with metabolic disturbances.[42] Hypoxia also induces the recruitment of immune cells such as macrophages which then produce the pro-inflammatory markers tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6).[44] Further evidence also indicates that impaired cell differentiation via the suppression of the Wnt1 signaling pathway and that the protein SWELL1 may promote adipocyte enlargement instead of expansion.[45] This cascade of physiological events characterizes the chronic inflammation that occurs in overweight and obesity.

Several studies support this idea about the difference in the relationships between the different storage sites for adipose tissue and health outcomes. In a study conducted by Foster et al., diet-induced obese mice transplanted with subcutaneous adipose tissue (SAT) demonstrated improved insulin sensitivity and insulin and leptin concentrations. However, excising SAT resulted in the aggravation of metabolic dysregulation by way of increased insulin and leptin.[46] In a study involving Chinese subjects with pre-diabetes, researchers found that visceral adipose tissue (VAT) represented a stronger association with insulin resistance than SAT.[47] Another Chinese study found that VAT was associated with a diagnosis of diabetes, whereas SAT seemed to be associated with a lower risk among Chinese women, but not men.[48] Lastly, in a CVD-free sample with subjects who either had diabetes, pre-diabetes, or a healthy weight, it was found that the volume of VAT and the ratio of VAT/SAT volumes are positively associated with impaired

glucose metabolism.[49] Altogether, these studies indicate the danger of VAT accumulation and therefore present a potential therapeutic target in the treatment of obesity and its comorbidities.

A five-year study conducted in Canada observed the cross-sectional and longitudinal effects of SAT and VAT on the risk of developing metabolic syndrome (MetS). The researchers found significant relationships between VAT and MetS and metabolic risk factors. They determined that for every 10 cm^2 increase in VAT, the odds of MetS, high-risk fasting glucose levels, and high-risk HDL-C levels increased by 16%, 11%, and 7%, respectively. Similarly, they found that with the same increase in VAT, the odds of MetS and high-risk triglyceride levels also significantly increased by 23% and 30%, respectively. In contrast, no such significant relationships were identified between changes in SAT and MetS and metabolic risk factors.[50] Therefore, in obesity, VAT is more concerning than SAT because of its metabolically active properties which lead to an increased risk of chronic diseases.

Inflammation in Overweight and Obesity

In response to any kind of damage, the body activates the body's immune system to produce an inflammatory response. This involves the release of immune cells that fight off any intrusion and consequently heal the injury. A double-edged sword, inflammation can also exhibit harmful effects. Acute inflammation constitutes a robust response that takes place over the course of a few days. Immune cells such as neutrophils, macrophages, lymphocytes, and B cells regulate this type of innate inflammatory response.[51] In overweight and obesity, however, inflammation is both low-grade and chronic: low-grade because it exhibits lower levels of pro-inflammatory cytokines which permits it to persist over a period of time.[51]

In the case of obesity-induced inflammation, macrophages are thought to be some of the key regulators in this process. Classified as either M1 (classically activated) or M2 (alternatively activated), they develop from monocytes that differentiate in response to certain physiological

conditions.[52] M1 activation happens in response to molecules by bacteria such as LPS. In contrast, M2 activation respond to parasites and their related cytokines, IL-4 and IL-13. Interestingly, the two types of macrophages have opposite functions: M1 macrophages are highly inflammatory and promote insulin resistance, whereas M2 macrophages promote insulin sensitivity and inhibit M1 macrophages.[52] Multiple studies have shown that adipose tissue from lean mice were found to have more M2 macrophages, whereas those of obese mice had more M1 macrophages.[7,53,54] The M1 macrophages from obese mice were also found to secrete pro-inflammatory molecules.[53] M1 and M2 also have opposite effects on insulin control with M1 cells promoting insulin resistance by secreting pro-inflammatory cytokines (e.g., resistin, IL-6, and TNF-α), while M2 cells promoting insulin sensitivity by secreting IL-10, an anti-inflammatory cytokine.[52]

Another prominent player in obesity-induced inflammation is interleukin-6 (IL-6), a cytokine that is produced not only by macrophages, but also by adipocytes, skeletal muscle, fibroblasts, and endothelial cells.[55,56] The association between IL-6 and increased adiposity is undeniable. For example, higher concentrations of IL-6 were observed in overweight and obese individuals and are considered a risk factor for type 2 diabetes.[7] Additionally, a strong link between IL-6 levels and BMI was found in a study of morbidly obese patients who were about to undergo bariatric surgery.[57] Twelve months after the procedure, their plasma IL-6 concentrations were significantly decreased.[57] In another study of lean, overweight, and obese adults ages 24-71 years old, the expression of IL-6 receptor (IL-6R) and IL-6 was found to be enhanced in obesity.[58] Furthermore, IL-6 has also been recognized as the main stimulus for the production of C-reactive protein (CRP), an acute phase protein.[59] Supporting this notion is a separate study of morbidly obese patients who underwent bariatric surgery that reported that IL-6 concentration was moderately correlated to systemic CRP concentrations.[60]

TNF-α, another well-known marker of inflammation, is also known to contribute to the development of insulin resistance. The transcription of insulin receptor substrate 1 (IRS-1) and glucose transporter type 4 (GLUT4) and the phosphorylation of insulin receptor substrates (IRSs) has been shown to be inhibited by TNF-α.[51] Earlier studies on TNF-α demonstrated that it directly affects pancreatic beta cells so as to decrease insulin secretion in the presence of glucose.[61] A later study on pancreatic beta cells revealed TNF-α works by suppressing the transcription of insulin.[62] In contrast, deletion of TNF-α and its receptors in obese mice significantly improved insulin resistance.[63]

Adipocytes also produce inflammatory molecules such as the hormone leptin. A key player in metabolic regulation, leptin has also been reported to increase the production of TNF-α.[64] In a British study of non-diabetic men aged 60-79 years old, leptin was positively associated with markers of insulin resistance, triglycerides, inflammatory markers such as IL-6 and CRP, and waist circumference, and negatively with level of physical activity and HDL-c.[65] In another study of type 2 diabetic individuals, leptin was also positively associated with triglycerides, lipoprotein A, Apo-A1, glucose, blood pressure, BMI, and insulin resistance, and negatively with HDL levels.[66]

Another hormone with metabolic regulatory effects is ghrelin. Although known as the "hunger hormone," its function extends beyond it orexigenic effects. It has been shown to influence thermogenesis, lipogenesis, gastric and intestinal motility, cardiac output.[67] Notably, ghrelin directly targets the pancreas to stimulate the secretion of glucagon while consecutively inhibiting insulin.[68] However, studies that investigated the effects of ghrelin on glucose metabolism revealed its negative effects. In a randomized controlled trial conducted by Tong et al., 13 subjects were infused with ghrelin on three separate occasions. The authors found that doing so decreased insulin sensitivity while also impairing pancreatic beta cell function. This is

even despite an observed increase in glucagon-like peptide 1 (GLP-1), an incretin that stimulates that release of insulin. [69]

Gut Microbiome in Overweight and Obesity

The gut microbiome is an ecosystem of microorganisms that exist in a symbiotic relationship with its human host whose diversity and complexity is thought to be greater than that of the brain.[9] Among all humans, an estimated 1,800 genera and 15,000-36,000 bacteria reside in the gut.[12] It has been dubbed a "virtual endocrine organ" because it acts in a way similar to other endocrine organs such as the pancreas.[9,70] The gut microbiota produce several metabolites that are released into the bloodstream to exert different effects in different parts of the body.[9,70] Short-chain fatty acids (SCFAs), neurotransmitters, and deconjugated bile acids are some of the metabolites that originate from the gut microbiome.[9]

It is estimated that the gut microbiome contains trillions of microorganisms that comprise about 50% of the cells in the human body.[71] Known as "enterotypes," classes of bacteria that inhabit the gut may either be commensal (beneficial) or pathogenic (harmful). Of these enterotypes, the most prominent are bacteria from the phyla *Bacteroidetes* and *Firmicutes*.[72] Bacteria from the *Actinobacteria* and *Proteobacteria* phyla have also been found along the gut.[73] The microbial composition of the gut microbiome, which depends on several factors such as age, genetics, environment, diet, and gut barrier integrity, ultimately determines its beneficence.[74] In a condition called dysbiosis, a shift in the ratio of commensal and pathogenic bacteria creates an imbalance that has been linked to several diseases such as T2D and inflammatory bowel diseases.[75]

The differences in the composition of the gut microbiome between overweight and obese individuals compared to that of lean individuals have been noted in several studies.[13,76] Bacteria belonging to the phyla *Bacteroidetes* and *Firmicutes* are specially sensitive as weight status

shifts.[13] A study on *ob*/ob mice revealed that in obese mice, *Bacteroidetes* is significantly reduced, whereas the opposite is true for *Firmicutes*.[76] Similar observations have also been made in several human studies.[77-79] A study by Armougom et al. reported that compared to normal weight individuals, obese individuals had lower levels of *Bacteroidetes,* higher levels of *Lactobacillus* species, and similar levels of *Firmicutes*.[77] Furthermore, a recent study on Ukrainian adults demonstrated that obese individuals had higher *Firmicutes* levels and lower *Bacteroidetes* levels compared to those who had normal or lean weights.[78]

However, a study by Schwiertz et al. found the opposite to be true: *Bacteroidetes* was elevated in overweight and obese subjects when compared to lean subjects.[79] The authors suspected that this might have been due to some compounding factors such as diet and overall fitness that were not considered.[79]An increase in *Firmicutes* phylum seemed to counterbalance a corresponding reduction in *Bacteroidetes,* but decreased levels were observed when the subjects lost weight through a weight-loss program.[80]

As briefly mentioned above, diet is a modulator of the gut microbiome. Several studies mentioned in an extensive review conducted by Telle-Hansen et al. indicate the undeniable effect that diet has on the gut microbiome.[81] Of the many nutrients that humans consume, dietary fiber has received much attention in gut microbiome research.[81] Unfortunately, most Americans do not meet the daily recommendations for dietary fiber.[82] Fibers are complex carbohydrates that are abundant in fruits, vegetables, and whole grains. They are typically not digested in the small intestine because of a lack of human enzymes that permit such action, so they become substrates for gut bacteria in the large intestine which then produce metabolites such as SCFAs, CO_2, and H_2.[83] In a study of individuals consuming an omnivore, vegetarian, or vegan diet showed that the plant-based Mediterranean diet, which is rich in fiber, has been shown to increase the population of genera of beneficial bacteria like *Roseburia, Lachnospira,* and *Prevotella*.[84] In contrast, a study

on mice by Desai et al. reported that deprivation of dietary fiber leads to the degradation of the gut mucosal barrier, allowing access to the pathogenic *Citrobacter rodentum*.[85]

Gut Integrity

In addition to the composition of the gut microbiome, the integrity of the epithelial lining of the intestines that house these microorganisms indicate a healthy gut. The tight junction proteins on the apical surface of the enterocytes regulate the entry of molecules in between them.[86] A compromised barrier has been associated with a host of diseases that range from metabolic disturbances such as type 2 diabetes to neurodegenerative disorders such as Alzheimer's disease.[87] Various markers of gut integrity are therefore useful indicators of a potentially serious health condition. The SCFA butyrate has been shown to play an essential role in maintaining gut integrity by upregulating the tight junction proteins claudin-1 and zonula occludens-1 between enterocytes.[88-90] Butyrate has also been found to be a player in mucin production that separates luminal bacterial and epithelial cells, conferring therefore added protection from bacterial invasion.[91]

Found mainly in the duodenum and the jejunum, intestinal fatty acid binding protein (I-FABP) is used in fatty acid metabolism and in the maintenance of cellular components. In a healthy status, enterocytes undergo programmed apoptosis without liberating their contents, including I-FABP. When the gut barrier is compromised, however, I-FABP is released into the bloodstream, allowing it to become an indicator of decreased gut integrity.[15]

Another protector of gut integrity is zonulin, a protein that, in turn, upregulates the tight junction proteins that keep the intestinal barrier intact.[17,92] When enterocytes come in direct contact with bacteria or gliadin, a subcomponent of gluten in wheat, they release zonulin to bind to receptors on the apical surface and begin disintegrating tight junction proteins.[17] Zonulin is then deactivated and released into the intestinal lumen.[17] Increased concentration of fecal zonulin

has been shown in multiple studies to be elevated in overweight and obese subjects, in combination with other inflammation markers such as TNF-α and IL-6.[16,93,94]

Secretory IgA (SIgA) also serves as a marker that indicates gut integrity. SIgA is a polymeric form of IgA with a secretory component in its structure and is found on mucosal surfaces such as that of the gut.[18] SIgA itself promotes the expression of tight junction proteins while attached to commensal bacteria and induce the targeting of dendritic cells by intestinal M cells.[18] Its functions also include what is known as "immune exclusion" where SIgA bind to pathogenic bacteria and become coated in mucus, and then excreted in the feces.[18]

Short-chain Fatty Acids

Short-chain fatty acids (SCFAs) are the most studied metabolites produced by the gut microbiota by digesting the dietary fibers that the human hosts cannot digest themselves. Of the SCFAs of varying lengths, the most prominent are acetate, propionate, and butyrate, which all together comprise 95% of the SCFAs produced by the gut microbiota. The production of these three SCFAs also differ in location: acetate and propionate are mostly produced in the small and large intestines while butyrate is produced in the colon and the cecum.[20] It is also estimated that SCFAs provide around 10% of the daily energy requirement of humans.[95]

Propionate and butyrate have been shown to activate intestinal gluconeogenesis (IGN). Propionate acts as a ligand to free fatty acid receptor 3 (FFAR3) that is located along the peripheral nervous system.[96] Butyrate, on the other hand, activates IGN more directly via a mechanism dependent on cyclic adenosine monophosphate (cAMP) by activating the expression of glucose-6-phosphatase-α (*G6PC*) and phosphoenolpyruvate carboxykinase 1 (*PCK1*) genes.[96] Another study found that serum acetate levels are negatively correlated to VAT and fasting insulin levels.[97] The relationship between serum acetate and adiposity may be explained by the SCFAs binding to G-protein coupled receptor-41 and 43 (GPR41, GPR43). These receptors,

which are expressed in several sites including adipocytes and colonic epithelial cells, regulate leptin and GLP-1 which, in turn, regulate adiposity.[97,98] This negative correlation between acetate and VAT also explains the relationship between acetate and fasting insulin, as these two variables demonstrate a strong positive correlation in this study ($r = 0.66$, $P < 0.003$).[97]

The role of SCFAs in inflammation has also been documented. A study found that butyrate treatment of patients with ulcerative colitis (UC) for 4-8 weeks inhibits the activation of nuclear factor kappa B (NF-κB), a transcription factor that regulates the expression of pro-inflammatory genes.[99] This decreased activity of NF-κB also correlated with a decrease in the severity of UC.[99] In another study, butyrate attenuated hepatic injury by inhibiting neutrophil activity and the expression of TNF-α and IL-6.[100] Furthermore, SCFAs may also serve as histone deactylase (HDAC) inhibitors. HDAC is a class of enzymes that make DNA less accessible to transcription factors by removing the acetyl groups found on the histone proteins attached to DNA. Butyrate and propionate are the most efficient HDAC inhibitors, while most studies indicate no such effect from acetate.[101] HDAC inhibition by butyrate and propionate allows the acetylation of histones in the promoter and conserved non-coding sequence regions of the Foxp3 locus, which leads to an increase in the production of peripheral regulatory T-cells. This, in turn, may stem the development of ulcerative colitis.[102]

The list goes on for the roles that SCFAs play in human physiology. In addition to their roles in the maintenance of gut integrity, carbohydrate metabolism, and immunity, other functions reported for SCFAs include their participation in lipid metabolism, appetite regulation, prevention of atherosclerosis, and carcinogenesis.[20,103,104] For example, propionate has been shown to inhibit lipid synthesis in rat hepatocytes.[105] Another study reported that GPR43 activation by acetate inhibits insulin signaling in adipocytes, thereby hampering lipid accumulation.[106] With respect to appetite regulation, SCFAs are associated with increased levels of circulating GLP-1 and peptide YY (PYY), which are both anorectic hormones.[107] Enteroendocrine L cells, which are found

mostly in the distal ileum and colon, release these hormones via the binding of SCFAs to the FFA2 and FFA3 receptors.[107] This wide array functions of the SCFA emphasize their importance in the human health.

Wheat Germ

Wheat germ (WG) is the embryonic part of a wheat grain that comprises between 2.5-3.8% of the seed's weight.[23] It is a by-product of flour milling and is used in animal feed, cosmetics, and in baked foods. WG is particularly rich in bioactive compounds such as fiber, B vitamins, tocopherols, phytosterols, policosanols, and polyphenols.[23] Although wheat germ by itself is not widely consumed in the US, its nutrient density makes it a viable candidate as a healthy and cost-effective supplement that bestows several health benefits.[23]

The research on the effects of wheat germ on metabolic and gut health is scant, but the results from both animal and human studies are promising. A study on mice revealed that WG improves insulin resistance, as marked by a decrease in fasting insulin, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1, all of which are metabolic markers.[24] Another study on mice concluded that WG produced induced a 4-fold increase in *Lactobacillaceae*, a commensal genus of bacteria.[108] In the same study, WG also increased the expression of the *Il10* gene expression in the ileum and reduced serum pro-inflammatory cytokines, IL-1B, IL-6, interferon-γ, and TNF-α.[108] Furthermore, Avemar™, a nutraceutical product made from fermented WG extract, has been shown to improve glucose tolerance, systolic blood pressure, and visceral fat deposition in rats.[25]

In humans, the daily addition of 15 g of WG in bread rolls for 8 weeks as opposed to inulin and refined grain resulted in a significant decrease in fasting blood glucose in overweight male subjects who were at a higher risk of CVD. However, no significant differences in glucose control, lipid status, and 24-h ambulatory blood pressure were observed compared to the control

group.[26] In another study that recruited healthy adults, daily consumption of 6 g of wheat germ for 8 weeks increased the population of the beneficial *Bacteroides* and *Bifidobacterium* species in the gut microbial population.[27] The study also found that WG did not improve gastrointestinal discomfort.[27] The same intervention did not produce any significant changes in serum cholesterol and triglyceride, postprandial glucose response, and insulin sensitivity, possibly due to a lower dosage.[109,110]

Additional studies involving wheat germ investigated its effects on blood lipids. One study investigated the effects phytosterols in WG on cholesterol absorption. Ten subjects randomly consumed three different muffins at three different times that were two weeks apart from each other. The muffin either had 80 g wheat germ with 328 mg of phytosterols, phytosterol-depleted wheat germ, or extracted wheat germ reconstituted with purified phytosterols. Plasma cholesterol was then monitored 4-5 days later using the cholesterol tracer incorporated in all the muffins. While no difference was detected between the plasma cholesterol after consumption of the original wheat germ and reconstituted wheat germ muffins, plasma cholesterol was found to be 42.8% higher after eating the phytosterol-depleted muffin.[111] Additionally, in an Iranian study of 15 hyperlipidemic patients with an average age of 45.7 years, the consumption of 30 g of raw wheat germ for 4 weeks significantly lowered total cholesterol, triglycerides, and VLDL, without any changes to HDL.[112] In contrast, a study by Lin et al. provided chocolate pellets with or without 20 mg of WG policosanols to 58 adults with normal to mildly elevated plasma cholesterol concentrations. At the end of their 4-week study, the authors detected no significant changes in the total cholesterol, LDL, HDL, and triglycerides in each group. No differences between the blood lipids were also observed between the two groups at the end of the intervention.[113]

The limited research on WG discussed above demonstrate that in animal models, WG showed significant improvements in health outcomes while human studies produced less than

stellar results. One explanation might be a difference in dosage. While the dosages in the animal models might have been supraphysiological, their equivalent dosages in humans may have not been achieved. There are many other unanswered questions about the health potential of WG in humans including is its effects on markers of gut health, inflammation, and in metabolic outcomes. This study sought to understand the effects of daily wheat germ supplementation on metabolic markers of overweight adults.

CHAPTER III

METHODS

Study design and inclusion and exclusion criteria

The study utilized a randomized, single-blinded study design. The protocol was approved by the Institutional Review Board at Oklahoma State University. Individuals 18-45 years of age with a BMI between 25-30 kg/m^2 but otherwise healthy regardless of gender were included in the study. The exclusion criteria were: having a medical condition that affects gut health and metabolic outcomes such as diabetes and hypertension, antibiotic use within the previous 3 months, heavy use of dietary supplements, smoking, known allergy to the ingredients of both the wheat germ and control supplements (wheat, corn meal, milk, honey, and peanut), pregnant or lactating status, and major surgery occurring within the last 6 months. One hundred thirty participants were interested and screened to determine whether they meet the inclusion and exclusion criteria. Of the individuals that were screened, 44 individuals qualified for the study and were consequently randomized into either the experimental or control group. The participants consumed their respective supplements for four weeks and visited the study site four times during this period for the administration of the study.

Wheat germ and control supplements

The control and wheat germ supplements were prepared as energy balls made by the research team. The recipe included 30 g of wheat germ or corn meal (control), 10 g of peanut butter, 10 g of honey, and 5 g of powdered milk. These ingredients were mixed and then rolled

into 2 balls of the same weight and packaged into small opaque bags. Supplements were stored in the refrigerator until distributed to the participant. Supplements were analyzed for macronutrient composition (Table 1) by NP Analytical Laboratories (St. Louis, MO).

Table 1: Study supplement composition

Analyte	Control	Wheat germ
	Amount in two energy balls (g)	
Moisture	5.2	6.4
Protein	5.9	12.1
Fat	6.3	7.6
Total dietary fiber	1.45	4.4
Soluble fiber	0.2	0.4
Insoluble fiber	1.3	4.0
Ash	0.9	1.8
Carbohydrate	36.6	27.1
Calories (kcal)	227.0	225.0

Questionnaires

Participants completed medical history, physical activity, stool characteristics, and food intake questionnaires. A standard medical history form assessed information about past and present medical conditions, such as history of disease and surgery, allergies, smoking status, and alcohol intake. Additionally, a standard 3-day food record was also given to the participants to provide a detailed list of their food intake for 2 weekdays and 1 weekend day during the week prior to their appointments. Food items from the 3-day food records were entered and analyzed using the ESHA Food Processor (ESHA, Salem, OR) software to determine caloric and nutrient intake at baseline and at the end of the study.

To measure their energy expenditure, participants filled out the Yale Physical Activity Survey (YPAS)[114] at baseline and at the end of the study. This questionnaire assessed the participants' duration and frequency of physical activities during the previous week and month. From these responses were calculated energy expenditure in the week prior to their visit and a monthly summary index that represents their level of physical activity within the past month. The weekly energy expenditure was obtained by multiplying the duration of the activity in minutes with an intensity code that corresponds to the activity which then generates the amount of kilocalories spent. Monthly summary indices assessed the intensity and duration of different categories of activities: vigorous activity, leisurely walking, moving about on their feet, standing, and sitting. A corresponding weight factor was multiplied to each response and the resulting values from each category were combined to form the monthly summary index.

Lastly, stool characteristics were measured using the Bristol Stool Chart (BSC) and the Cleveland Clinic Constipation Scoring System (CSS) at baseline and at the end of the study.[115,116] The BSC asked the participants to identify the consistency of their most recent stool on a scale of 1 to 7, with 1 being separate hard lumps and 7 being completely watery. In this scale, a lower score indicates constipation and higher score signifies loose bowel movement. The CSS asked the participants to rate aspects of their constipation (frequency of bowel movements, painful evacuation effort, incomplete evacuation, abdominal pain, length of time to defecate unsuccessful attempts at evacuation per 24 hours, and duration of constipation) on a scale of 0-4 while use of assistance such as enema or laxatives is on a scale of 0-2. The scores for each portion of the CSS are then added together to form a final constipation score (maximum of 30). Unlike the BSC, a lower CSS score indicates less constipation.

Study visits

The participants visited the study site four times during the study. After a screening interview via phone or email, the participant arrived at the study site for the first visit. During visit 1, the exclusion and inclusion criteria were re-assessed to make sure the participants qualify for the study. The research personnel then explained the study to the participant and answered any questions. Those who agreed to participate in the study provided written consent.

After giving consent, height was measured using a Seca stadiometer CE 9123 (Seca, Hamburg, Germany). Waist and hip circumferences were also measured using a tape measure around the belly button and the top of the iliac crest of the pelvis, respectively. Blood pressure was determined using an automatic Omron blood pressure monitor (Omron Healthcare, Inc., Lake Forest, IL). Weight and body composition were assessed using Seca medical body composition analyzer 514 (Seca, Hamburg, Germany). Participants also filled out the YPAS and a medical history questionnaire. Participants then received instructions on filling out a 3-day food record, collecting their fecal sample, and preparing for the blood draw on the second visit. For the fecal collection, participants were given a Fe-Col® fecal sample collection kit (Alpha Laboratories, England, United Kingdom), an ice pack, and an insulated Styrofoam box. Participants were asked to collect their fecal sample within 24 hours of visit 2 and to store their fecal samples in the Styrofoam box with the ice pack.

For visit 2, participants came to the study site after fasting for 12 hours and brought their 3-day food record and fecal samples. Blood was drawn by a trained phlebotomist to collect plasma and serum samples using standard venipuncture techniques. A small amount of whole blood was used for lipid panel and glucose analysis using Alere Cholestech LDX® Analyzer (Abbott, Abbott Park, IL) and for glycated hemoglobin using DCA Vantage Analyzer (Siemens, Malvern, PA). Lastly, the participants filled out the BSC and CSS. The participants then

randomly drew their treatment group and were given a 2-week supply of the corresponding treatment regimen, a calendar to keep track of their supplement intake, and another 3-day food record. The participants were instructed to keep the supplement in the refrigerator until ready for consumption and to keep the empty supplement bags to assess compliance.

The third visit occurred approximately two weeks after visit 2. Here, participants brought their empty supplement bags and calendar to assess compliance as well as their 3-day food record. Body weight, waist and hip circumferences, and blood pressure were measured again. Participants also filled out the YPAS, BSC, and the CSS questionnaires and were asked about any concerns about the study. They were given another 2-week supply of their treatment regimen, a new calendar, a new fecal sample collection kit, and another 3-day food record. Data collected in this visit, however, is not reported here.

On the fourth and final visit, participants came to the study site after fasting for 12 hours. The 3-day food record, the empty supplement bags, and calendar were collected to assess compliance. The same anthropometric measures and questionnaires that were assessed during the first two visits were repeated during the final visit. Blood and fecal samples were also collected and processed as described below.

Processing of plasma and fecal samples

Whole blood was collected in separate serum and plasma tubes. Serum tubes were left for 20 minutes at room temperature to allow clotting. Serum and plasma tubes were centrifuged for 20 minutes at 4,000 rpm at 4°C and were aliquoted and then stored at –80 °C for later analyses. The fecal samples were aliquoted into microcentrifuge tubes and were stored at –80 °C until analyses.

Lipid profile analysis

Blood lipids were analyzed in whole blood samples using Alere Cholestech LDX® Analyzer (Abbott, Abbott Park, IL). Triglycerides are broken down by lipase to form glycerol and free fatty acids. Glycerol is then phosphorylated with ATP into glycerol-3-phosphate through the action of glycerol kinase. Glycerol-3-phosphate and oxygen are catalyzed by glycerol phosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced by the reaction combines with 4-aminoantipyrine and N-ethyl-N-sulfohydroxypropyl-m-toluidine, sodium salt (TOOS), to form a purple quinoneimine dye that absorbs at 490 nm. The absorbance of the quinoneimine dye is directly proportional to the concentration of triglycerides in the sample.[117]

Total cholesterol and HDL cholesterol analyses begin by cholesterol esterase converting cholesterol esters into free cholesterol. These reactions take place in separate pads in the cassette that contains analytes specific to the targeted cholesterols. Free cholesterol is then oxidized by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide in the presence of oxygen. The hydrogen peroxide produced from this reaction reacts with TOOS, forming a purple quinoneimine dye that is proportional to the concentration of total and HDL cholesterol in the sample.[117] Lastly, LDL cholesterol and non-HDL cholesterol are estimated using the Friedewald equation based on the values determined for total and HDL cholesterol.[118]

Serum non-esterified fatty acids (NEFA) using a commercial kit from Fujifilm Medical Systems (Lexington, KY) were analyzed using a Biolis 24i Clinical Chemistry Analyzer (Carolina Liquid Chemistries Corp., Greensboro, NC). A reagent acylates coenzyme A from NEFAs and eventually results in a purple dye that is read by the analyzer. As with the other lipid analyses, the intensity of the dye is correlated to the concentration of NEFAs in the sample.

Markers of glucose homeostasis

Blood glucose in whole blood samples was also measured by Alere Cholestech LDX® Analyzer (Abbott, Abbott Park, IL). With the action of glucose oxidase, glucose and oxygen combine to form o-D-gluconolactone and hydrogen peroxide. Like the lipid profile analysis, the process ends with the conversion of the hydrogen peroxide to the purple quinoneimine dye via the action of peroxidase. The intensity of the quinoneimine dye is proportional to the concentration of glucose in the sample[117]

Plasma insulin, C-peptide, and GLP-1 were measured with the Bio-Plex Pro™ Diabetes Assays Kit (Bio-Rad, Hercules, CA). In this method, beads are internally colored with red and infrared fluorescent dyes. Different ratios of these dyes are found in 100 different regions on each bead, with each region corresponding to a specific analyte. A biotinylated detection antibody then binds to the bead, followed streptavidin-conjugated phycoerythrin. The Bio-Plex® MAGPIX™ multiplex reader (Bio-Rad, Hercules, CA) quantifies the concentrations of the target analytes based on the fluorescent signals emitted by the beads.[119]

The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as a marker of insulin resistance using the following formula:[24]

$$\frac{[\,fasting\ insulin\ (\mu U/mL]\ x\ [fasting\ glucose\ (mg/dL)]}{405}$$

Markers of inflammation and gut integrity

To detect markers of inflammation, IL-6 and TNF-α, and were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, Carlsbad, CA). The instructions from the manufacturer were strictly followed. ELISA uses a bed of microplate wells that are coated with antibodies specific to a target antigen. Antigen bind to these antibodies, followed by enzyme-tagged antibodies. A coloring agent is added to the wells to

determine the concentration of the target analyte. A microplate reader then determines the concentrations of the analyte in each well using the absorbance of the solution.[120] C-reactive protein (hs-CRP) was also analyzed using the clinical chemistry analyzer as described in the lipid profile analysis section. In the quantification of hs-CRP, however, CRP antibodies are added to the sample, which then releases light proportional to the CRP levels within and is measured by the analyzer. The other markers of inflammation (ghrelin, leptin, resistin, plasminogen activator inhibitor-1 [PAI-1]) were measured using Bio-plex Multiplex Immunoassay System, as described in the previous paragraph. The measures of gut integrity (serum zonulin and I-FABP, fecal SIgA) were also measured using ELISA kits (I-FABP: R&D Systems, Minneapolis, MN; zonulin and SIgA: ALPCO, Salem, NH).

Fecal short chain fatty acid analyses

Fecal SCFAs were measured using gas chromatography (GC) as previously described.[121] To prepare for GC analysis, fecal samples were freeze-dried overnight using a VirTis freeze-dryer (SP Scientific, Warminster, PA). Approximately 0.25 g of freeze-dried fecal samples were added to 1.125 mL ice-cold Millipore water and 0.125 mL of an internal standard (10 mM of 2-ethylbutyric acid in 12% formic acid). The resulting solution was then vortexed for 3 minutes. Thereafter, the pH of the solution was adjusted to fall between 2-3 pH using hydrochloric acid. Once the proper pH was achieved, the volume of the final solution was recorded. The fecal samples were then incubated at room temperature for 10 minutes before being centrifuged for 45 minutes at 4000 rpm. Lastly, the supernatant was filtered through a 45 µm syringe filter into a microcentrifuge tube.

GC analysis was carried out using an Agilent 6890N GC system with a flame ionization detector (FID), N10149 automatic liquid sampler (Agilent Technologies, Santa Clara, CA), and using an Agilent J&W DB-FFAP column with an Agilent FS as the pre-column. Hydrogen was

the carrier gas set at a flow rate of 14.4 mL/min. The inlet temperature was set to 200ºC and the detector temperature at 240ºC. Calibration was also conducted using standard solutions containing acetic, propionic, n-butyric, i-butyric, valeric, i-valeric, caproic, and heptanoic acids.

Fecal microbiome analysis

Fecal samples were sent to the University of Arkansas (Fayetteville, AR) for gut microbiota analysis. Samples were initially frozen to -80 ºC and were shipped overnight with dry ice to ensure their integrity. DNA was extracted from frozen stool samples by the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Germantown, MD, USA) following the manufacturer's protocol. DNA concentration was measured by a NanoDrop One (Thermo Fisher Scientific, Madison, WI, USA) and diluted to 10 ng/μL. The V4 region of 16S rRNA from each sample was amplified using the forward primer (5-GTGCCAGCMGCCGCGGTAA-3) and reverse primer (5'-GGACTACHVGGGTWTCTAAT-3') with attaching Illumina sequencing primer and barcode sequence. The PCR amplicons were pooled together in equimolar concentrations using the SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA, USA). Library concentration was determined by qPCR using the Kappa Library Quantification Kit (Roche, Indianapolis, IN, USA) with primers specific to the Illumina adapters. The quality of the library was determined by an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). The pooled library was then sequenced on an Illumina MiSeq sequencer with paired end (2 × 250 bp, MiSeq Reagent Kit v2, 500 cycles (Illumina, San Diego, CA, USA). A commercial community DNA was included as a positive control (ZymoBIOMICS™ Microbial Community Standard, Zymo Research, Irvine, CA, USA). Negative controls from DNA extraction and PCR amplification were also sequenced for quality controls.

Statistical Analyses

Data were checked for normality and equal variance, and datasets deviating from these standards were subjected to nonparametric analysis. Statistical analyses were performed using SAS 9.4 software package (SAS Institute, Cary, NC). An independent t-test was used to detect the differences between the two groups at each timepoint, while a paired t-test was used to detect the changes within each group from baseline to final. Chi-squares test was used to analyze the categorical variables gender and ethnicity.

For the gut microbiome data, sequencing reads were analyzed using mothur v1.39.1 following the MiSeq SOP, including steps for quality-filtering, alignment against a 16S reference database (SILVA v132), and clustering into operational taxonomic units (OTUs) with a 97% identity threshold. The OTUs were then classified against the RDP (Ribosomal Database Project) database. The gut microbial diversity within each subgroup and the distances between subjects were evaluated by alpha-diversity (Shannon index, Chao index, observed OTUs) and beta-diversity (Bray–Curtis, Jaccard) measures, respectively. ANOSIM (analysis of similarity) was performed to evaluate the dissimilarity between groups (or subgroups) by using mothur v1.39.1. Because the microbiome data was not normally distributed, the Kruskal-Wallis test was also used to detect any changes within each group and differences between the two groups at both timepoints.

CHAPTER IV

RESULTS

Participant characteristics

A total of 130 individuals were screened with a target enrollment of n = 40 study participants (n = 20 individuals per group). Forty-four individuals started the study but only thirty-nine participants completed the four-week intervention. Four participants who dropped out were assigned to groups (1 in WG and 3 in control) while one participant dropped out before being assigned to a group. Participants discontinued their involvement with the study for different reasons: loss of interest (3 participants), unexpected out-of-state move (1 participant), and sudden onset of illness unrelated to the study (1 participant).

Table 2 shows the demographic information of the study participants. The participants consisted of 19 men (49%) and 20 women (51%). Twenty participants were in the wheat germ group (WG) with 9 (23%) females and 11 (28%) males. The control group, in contrast, consisted of 19 participants, 11 (28%) of whom were male and 8 (21%) were female. There was no statistical difference ($P = 0.855$) on the number of individuals by gender between the two treatment groups. More than half of the study participants were non-Hispanic Whites. In the WG group, 10 participants were non-Hispanic White (26%), 4 were Black (10%), 5 were Asian (5%), and 1 was Hispanic (3%). The control group, on the other hand, had 10 participants who were non-Hispanic White (26%), 5 were Black (13%), 2 were Asian (5%), 1 was Hispanic (3%), and 1

was Middle Eastern (3%). As with the gender, there was also no statistical difference ($P = 0.67$) in the number of individuals by ethnicity between the two treatment groups. The average age of the participants for the WG and control groups were 28.1 and 24.9 years, respectively which is statistically similar ($P= 0.67$).

Nutrient intake

Three-day food recall was used to assess nutrient intakes of the study participants. Comparisons were made before and after supplementation for each trial as well as comparing between the control and WG treatment at baseline and after supplementation (Table 3). There were no significant differences in nutrient intake before and after supplementation in the WG group. Despite having no differences in total caloric intake, the control group had significant decrease in total calories ($P = 0.04$), carbohydrates ($P = 0.02$), thiamin ($P = 0.03$), biotin ($P = 0.03$), vitamin C ($P = 0.03$), vitamin D ($P = 0.02$), folate ($P = 0.03$), pantothenic acid ($P = 0.03$), and molybdenum ($P = 0.03$). Lastly, when comparing the differences in the intake of both groups in each time point, total sugars were significantly higher ($P = 0.03$) at baseline while selenium was significantly lower ($P = 0.04$) after supplementation in the control group.

Physical activity

The Yale Physical Activity Survey was used to assess the exercise habits of the study participants (Table 4). Participants were asked to report their physical activity within the most recent week and month relative to taking the survey. Before the start of supplementation, the control group had significantly lower total amount of time spent on physical activity ($P = 0.02$), work calorie expenditure ($P = 0.03$) and total calorie expenditure ($P = 0.01$) than the WG group. Interestingly, individuals on the WG group demonstrated a significant reduction in time spent on weekly exercise during the 4-week study duration ($P < 0.001$), and hence, energy expenditure due to exercise ($P < 0.01$). Although the total time spent on weekly exercise was not different in

the control group before and after supplementation, the energy expenditure due to exercise was also significantly reduced (*P = 0.04*) at the end of the 4-week intervention compared to baseline.

In terms of the monthly activity indices, there was no change in the WG and the control groups after supplementation compared to baseline. The groups had similar activity index at baseline and after supplementation except for the leisurely walking index which is lower in the control group than the WG group (*P = 0.03*).

Anthropometrics and stool measures

Table 5 shows the results for the anthropometric parameters and stool characteristics after 4 weeks of WG supplementation compared to control. There were no significant changes in weight, BMI, fat mass, % body fat, visceral fat, waist and hip circumferences, waist-to-hip ratio, and systolic and diastolic blood pressure at baseline and after one month consumption of either the WG or control regimen. However, skeletal mass was significantly reduced in the WG group (*P = 0.01*) after supplementation which was not observed in participants that received the control energy ball. There were also no differences in the aforementioned parameters when comparing between the two groups at baseline and after the supplementation period.

As for the stool measures, constipation score tended to improve after four-week consumption of both the WG (*P = 0.06)* and the control (*P = 0.09)* energy balls. There was no difference in constipation score between the two groups at baseline and after supplementation. There were no changes in stool consistency as indicated by the responses to the Bristol chart questionnaire before and after one month of consumption of both types of energy balls. Additionally, there were also no difference when comparing the baseline and final values of the Bristol stool chart of the wheat germ and control group.

Lipid profile and markers of glucose homeostasis

There were no significant changes in lipid panel (i.e. total cholesterol, HDL-, LDL-, non-HDL- cholesterol and LDL/HDL ratio), triglycerides, and NEFA after four-week consumption of both types of energy balls (Table 6). There were also no significant differences on these lipid parameters when comparing the two groups before and after supplementation.

In contrast to the lipid parameters, four week consumption of WG significantly improved markers of glucose homeostasis including HbA1c ($P = 0.04$), insulin ($P = 0.03$), and HOMA-IR ($P = 0.04$), which was not observed in the control group (Table 6). In support of the improved HOMA-IR, the adipokine, resistin, which is correlated with insulin resistance, was also significantly reduced ($P = 0.03$) by WG supplementation but not the control. The changes in glucose homeostasis markers with WG supplementation was not reflected by changes in fasting blood glucose. Likewise, consumption of WG and control supplements had no effects on C-peptide, ghrelin and the incretin, GLP-1. When comparing these glucose homeostasis markers between the two supplements at baseline and after supplementation, no differences were observed between the two groups.

Markers of inflammation and gut integrity

Markers of inflammation and gut health were also assessed and shown on Table 7. There were no significant differences in the plasma concentrations of the inflammatory markers, TNF-α, IL-6, leptin, hs-CRP, and PAI-1 at baseline and after supplementation with both WG and control supplement. There were also no differences in these markers between the WG and control group at baseline and after four weeks of supplementation.

Plasma concentrations of intestinal fatty acid binding protein (I-FABP), a marker of gut integrity, were not significant for both groups at baseline and final, as were differences between the two groups at each timepoint. As for the tight junction protein zonulin, 4-week supplementation of WG and control had no effect on this marker of gut integrity. Plasma

concentrations of zonulin tended (*P = 0.09*) to be lower at baseline in the WG group compared to the control. Fecal concentrations of secretory IgA, the immunoglobulin abundant in mucosal areas that is important in promoting gut health, was also not affected by treatment.

Gut microbial diversity

Fecal bacterial populations were analyzed before and after supplementation and Figures 1 and 2 demonstrates gut bacterial diversity. Observed operational taxonomic units (OTUs), and Shannon and Chao1 indexes were used as markers of alpha diversity, the variation of bacterial species in a sample. Figure 1A showed no significant change in counts of unique OTUs in each sample after supplementation with both the WG and control supplements. Shannon index, which take into account both the abundance and evenness of bacterial species, was unaffected by both the WG and control supplements (Figure 1B). Chao1 index, which measures species richness tended to be lower (*P = 0.065*) after the control supplementation but unaffected by the WG supplements (Figure 1C).

On the other hand, beta diversity measures, which indicate variation of microbial communities between samples, are presented in Figures 2A-J. Bray-Curtis, which measures differences in microbial abundances between two samples, and Jaccard, which assesses the presence or absence of species and does not take into account the abundance, were used as markers of beta diversity. Figures 2A & 2B show no significant differences in the Bray-Curtis and Jaccard indexes of the two treatments at baseline and after supplementation. Comparison of the Bray-Curtis and Jaccard indexes for the baseline values of the two supplements (Figures 2C & 2G, respectively), change at baseline and after supplementation with the control supplement (Figures 2D & 2H, respectively), final values of the two supplements (Figures 2E & 2I, respectively), and change at baseline and after supplementation with the WG supplement (Figures 2F & 2J, respectively) indicate no significant differences.

Gut Microbiota Composition

We analyzed the gut microbial composition of the fecal samples at the phylum and genus levels using 16s RNA sequencing. Figure 3A compares the average relative abundances of each phylum before and after supplementation of both the WG and control supplements and Table 8 is the quantification of the relative abundances of these bacterial phyla. Figure 3B represents the relative abundances of bacterial phyla before and after supplementation for each study participants on the WG and control group. Of the phyla detected, the three most abundant were *Firmicutes, Actinobacteria,* and *Bacteroides* (Figure 3A and Table 8). WG supplementation has no effect on the relative abundance of these bacterial phyla while a significant decrease in the relative abundances of the phyla *Bacteroidetes* (*P = 0.03*) and *Proteobacteria* (*P = 0.048*) were observed after supplementation with the control energy ball (Table 8). The Firmicutes/ Bacteroidetes ratio, a marker of obesity, yielded no changes after supplementation of either wheat germ or control treatment. There were also no differences in the relative abundance of the bacterial phyla before and after supplementation when comparing the two supplements.

We also analyzed the fecal relative abundance of the bacterial population at the genus level (Figure 4 and Table 9). Figure 4A compares the average relative abundances of each genera before and after supplementation for both the WG and control supplements and Table 9 is the quantification of the relative abundance of these bacterial genera. Figure 4B represents the relative abundances of bacterial genus before and after supplementation for each study participants on the WG and control group. The most abundant genus is *Blautia* and other abundant genera include *Bifidobacterium, Lachnospiraceae,* and *Roseburia* (Table 9). Supplementation with the control energy ball tended to reduce the genus *Faecalibacterium* (*P =0.07*) and significantly reduced *Bacteroides* (*P = 0.03*). Like the bacterial phyla, there were no significant differences in the relative abundance of bacterial genera after WG supplementation compared to baseline. In comparing the WG and control groups, there were no significant differences in the relative abundance of the bacterial genera at baseline but *Lachnospiriceae* was

significantly lower (*P = 0.03*) and *Faecalibacterium* higher (*P = 0.007*) at the final time point with WG supplementation.

Fecal short-chain fatty acid concentrations

In addition to assessing gut bacterial population and markers of gut integrity, we also determined the effects of WG supplementation on short-chain fatty acids (SCFAs) production (Figure 5 and Table 10). Four-week supplementation of both WG and control supplements has no significant effects on fecal SCFAs. Similarly, there were no significant differences in the fecal SCFAs concentration between the two groups at both baseline and final.

Table 2. Demographic Information of Study Participants

	Wheat Germ (n=20)	**Control (n=19)**	**P-value[1]**
Gender & Age			
Male (n, %)	11 (28%)	11 (28%)	0.86
Female (n, %)	9 (23%)	8 (21%)	
Age (y)	28.1 ± 7.4	24.9 ± 6.0	0.16
Ethnicity			
White, non-Hispanic (n, %)	10 (26%)	10 (26%)	0.67
Black (n, %)	4 (10%)	5 (13%)	
Hispanic (n, %)	1 (3%)	1 (3%)	
Asian (n, %)	5 (13%)	2 (5%)	
Middle Eastern (n, %)	0 (0%)	1 (3%)	

[1]Age is presented as mean ± SD. Chi-square test was used for gender and ethnicity to determine goodness of fit while t-test was used for age.

Table 3. Effects of Four Weeks of Wheat Germ Supplementation on Nutrient Intake of Overweight Individuals

Nutrient	Wheat Germ (n= 20)		P-value (WG baseline vs final)	Control (n=19)		P-value (CT baseline vs final)	P-value (WG vs CT baseline)	P-value (WG vs CT final)
	Baseline	Final		Baseline	Final			
Calories (kcal)	1797.1 ± 494.8	1812.4 ± 552.4	0.93	1940.7 ± 726.4	1681.0 ± 631.2	**0.04**	0.47	0.49
Calories from Fat (kcal)	661.0 ± 322.2	629.2 ± 304.1	0.74	687.5 ± 333.4	618.2 ± 249.7	0.18	0.80	0.90
Calories from Saturated Fat (kcal)	203.8 ± 89.3	197.9 ± 93.1	0.82	222.7 ± 109.0	192.2 ± 85.8	0.14	0.56	0.84
Protein (g)	75.3 ± 23.4	76.0 ± 21.7	0.92	87.3 ± 48.9	80.0 ± 39.4	0.38	0.34	0.70
Carbohydrates (g)	206.8 ± 53.3	216.2 ± 76.8	0.64	225.7 ± 67.9	189.0 ± 70.6	**0.02**	0.34	0.26
% kcal from carbohydrates (%)	47.1 ± 10.3	48.4 ± 11.9	0.63	47.8 ± 7.2	46.5 ± 10.1	0.54	0.82	0.50
% kcal from fat (%)	35.8 ± 9.1	33.4 ± 9.0	0.31	34.8 ± 7.3	36.2 ± 8.1	0.42	0.69	0.31
% kcal from protein (%)	16.8 ± 3.7	17.2 ± 3.3	0.70	17.6 ± 3.9	18.6 ± 4.8	0.38	0.55	0.28
Total Dietary Fiber (g)	19.6 ± 10.8	17.3 ± 9.4	0.39	18.1 ± 8.0	15.4 ± 7.1	0.10	0.62	0.50
Total Soluble Fiber (g)	0.77 ± 0.77	1.1 ± 1.0	0.19	0.80 ± 0.88	0.72 ± 0.69	0.70	0.93	0.19
Total Sugars (g)	56.2 ± 23.0	68.5 ± 43.9	0.31	77.0 ± 38.8	63.3 ± 39.5	0.06	**0.05**	0.70
Added Sugar (g)	7.6 ± 9.7	23.7 ± 43.2	0.14	15.6 ± 22.9	13.5 ± 15.1	0.69	0.17	0.33
Monosaccharides (g)	6.5 ± 7.6	17.8 ± 41.7	0.20	11.2 ± 10.8	8.7 ± 9.6	0.18	0.12	0.36
Disaccharides (g)	9.6 ± 9.7	6.6 ± 5.2	0.23	8.1 ± 8.8	7.1 ± 10.1	0.42	0.61	0.86
Other Carbs (g)	124.2 ± 41.2	122.3 ± 57.4	0.88	124.1 ± 46.1	105.4 ± 42.0	0.07	0.99	0.30
Fat (g)	73.5 ± 35.8	70.2 ± 33.8	0.75	76.5 ± 37.1	68.7 ± 27.8	0.18	0.80	0.89
Saturated Fat (g)	22.6 ± 9.9	22.0 ± 10.3	0.82	24.8 ± 12.1	21.3 ± 9.5	0.14	0.56	0.84
Monounsaturated Fat (g)	18.1 ± 12.8	15.6 ± 9.9	0.45	17.1 ± 13.1	12.5 ± 7.1	0.13	0.81	0.27
Polyunsaturated Fat (g)	13.5 ± 15.2	10.6 ± 6.4	0.43	9.5 ± 8.2	8.2 ± 3.8	0.44	0.35	0.18

Trans Fatty Acid (g)	0.62 ± 0.60	0.41 ± 0.56	0.27	0.58 ± 0.50	0.46 ± 0.51	0.38	0.82	0.76
Cholesterol (mg)	308.8 ± 211.1	323.4 ± 191.3	0.83	340.6 ± 189.0	286.4 ± 183.4	0.26	0.62	0.54
Vitamin A - RAE (mcg)	493.4 ± 382.0	421.0 ± 360.5	0.32	622.7 ± 681.5	375.5 ± 248.4	0.09	0.47	0.65
Carotenoid RE (mcg)	334.4 ± 482.6	239.5 ± 398.2	0.35	423.5 ± 795.3	263.0 ± 323.5	0.33	0.68	0.84
Retinol RE (mcg)	239.1 ± 184.4	193.5 ± 103.4	0.39	286.0 ± 311.4	187.5 ± 125.4	0.17	0.57	0.87
Beta-Carotene (mcg)	2509.6 ± 3179.7	2023.2 ± 3417.7	0.47	3585.5 ± 6013.6	1487.5 ± 1789.2	0.12	0.49	0.54
Vitamin B1 - Thiamin (mg)	1.0 ± 0.46	1.0 ± 0.65	0.79	1.1 ± 0.69	0.78 ± 0.41	**0.03**	0.70	0.17
Vitamin B2 - Riboflavin (mg)	1.4 ± 0.74	1.3 ± 0.58	0.58	1.6 ± 1.3	1.23 ± 0.82	0.20	0.60	0.85
Vitamin B3 - Niacin (mg)	16.7 ± 10.7	18.2 ± 10.2	0.60	21.0 ± 17.2	16.5 ± 9.5	0.18	0.35	0.60
Vitamin B6 (mg)	1.4 ± 1.0	1.6 ± 0.99	0.64	2.1 ± 1.9	1.8 ± 1.8	0.46	0.16	0.59
Vitamin B12 (mcg)	3.2 ± 2.5	2.7 ± 1.6	0.34	4.4 ± 4.7	3.5 ± 3.9	0.31	0.34	0.40
Biotin (mcg)	14.9 ± 11.9	10.6 ± 8.2	0.21	24.6 ± 29.1	11.2 ± 13.8	**0.03**	0.20	0.87
Vitamin C (mg)	61.8 ± 28.4	66.1 ± 59.8	0.76	65.1 ± 46.6	38.6 ± 40.6	**0.03**	0.79	0.10
Vitamin D (mcg)	3.6 ± 3.4	3.2 ± 138.4	0.59	4.5 ± 5.0	2.3 ± 3.1	**0.02**	0.48	0.43
Vitamin E - Alpha-Tocopherol (mg)	6.2 ± 4.9	5.0 ± 3.5	0.25	6.4 ± 5.4	4.5 ± 2.4	0.12	0.93	0.56
Folate (mcg)	264.2 ± 141.3	262.9 ± 184.4	0.97	302.6 ± 221.4	223.8 ± 189.1	**0.03**	0.52	0.52
Vitamin K (mcg)	106.1 ± 117.7	104.0 ± 212.3	0.95	52.4 ± 53.7	69.1 ± 98.9	0.49	0.08	0.51
Pantothenic Acid (mg)	2.2 ± 1.3	2.3 ± 1.2	0.79	3.0 ± 1.9	1.9 ± 1.5	**0.03**	0.14	0.42
Calcium (mg)	589.0 ± 266.5	660.4 ± 292.0	0.43	718.6 ± 438.8	638.4 ± 389.3	0.15	0.27	0.84
Chromium (mcg)	1.6 ± 1.3	2.3 ± 2.2	0.26	10.5 ± 20.0	6.0 ± 16.3	0.49	0.09	0.36
Copper (mg)	0.71 ± 0.41	0.69 ± 0.42	0.87	0.72 ± 0.52	0.57 ± 0.27	0.17	0.94	0.29
Fluoride (mg)	0.46 ± 0.58	0.27 ± 0.34	0.15	0.28 ± 0.56	0.37 ± 0.52	0.86	0.33	0.49

Iodine (mcg)	43.0 ± 41.2	33.4 ± 27.7	0.42	45.6 ± 46.1	35.8 ± 44.9	0.08	0.86	0.85
Iron (mg)	12.2 ± 4.0	33.4 ± 27.7	0.83	13.1 ± 5.5	11.0 ± 6.4	0.13	0.56	0.63
Magnesium (mg)	172.7 ± 87.2	195.9 ± 125.6	0.35	185.5 ± 137.1	150.9 ± 81.8	0.14	0.73	0.20
Manganese (mg)	1.7 ± 2.2	1.7 ± 1.2	0.91	1.4 ± 0.88	1.5 ± 1.5	0.89	0.49	0.60
Molybdenum (mcg)	16.9 ± 14.5	17.4 ± 18.4	0.81	17.7 ± 13.3	9.8 ± 11.1	**0.03**	0.86	0.18
Phosphorus (mg)	821.6 ± 440.2	881.6 ± 421.6	0.60	832.6 ± 642.7	736.0 ± 428.9	0.46	0.95	0.94
Potassium (mg)	1756.9 ± 682.6	1551.8 ±724.2	0.20	1793.7 ± 1401.7	1342.9 ± 762.8	0.09	0.92	0.39
Selenium (mcg)	79.1 ± 55.4	90.8 ± 57.0	0.48	79.2 ± 58.2	58.5 ± 35.1	0.10	1.00	**0.04**
Sodium (mg)	3131.9 ± 1171.5	3149.8 ± 1428.6	0.95	3081.5 ± 1395.5	2691.7 ± 1210.3	0.18	0.90	0.29
Zinc (mg)	6.2 ± 4.2	6.3 ± 2.8	0.96	7.7 ± 5.8	6.9 ± 6.5	0.62	0.36	0.72
Omega 3 Fatty Acid (g)	1.3 ± 1.6	0.89 ± 0.53	0.31	0.79 ± 0.67	0.63 ± 0.35	0.24	0.22	0.07
Omega 6 Fatty Acid (g)	11.1 ± 13.0	8.5 ± 5.1	0.40	6.5 ± 5.1	6.5 ± 3.7	0.98	0.16	0.18
Alcohol (g)	4.0 ± 7.6	4.7 ± 9.6	0.78	1.4 ± 5.0	2.0 ± 8.5	0.52	0.22	0.36
Caffeine (mg)	70.0 ± 96.9	44.8 ± 63.4	0.05	48.0 ± 64.5	34.2 ± 59.5	0.26	0.41	0.60
Choline (mg)	262.2 ± 172.8	232.0 ± 151.2	0.54	254.1 ± 182.9	200.6 ± 178.5	0.29	0.89	0.56

Values are presented as mean ± SD.

Table 4. Effects of Four Weeks of Wheat Germ (WG) Supplementation on Physical Activity of Overweight Individuals

	Wheat Germ (n=20)		P-value (WG baseline vs final)	Control (CT) (n=19)		P-value (CT baseline vs final)	P-value (WG vs CT baseline)	P-value (WG vs CT final)
	Baseline	**Final**		**Baseline**	**Final**			
Weekly Energy Expenditure from Physical Activity (kcal)								
Work	2672.8 ± 2072.0	3258.0 ± 7789.9	0.75	1420.7 ± 1309.0	1224.8 ± 923.9	0.42	**0.03**	0.26
Yard Work	366.0 ± 682.6	228.5 ± 548.6	0.45	86.1 ± 312.2	149.2 ± 410.1	0.20	0.11	0.62
Caretaking	84.0 ± 183.0	150.0 ± 323.1	0.19	50.5 ± 220.2	0 ± 0	0.33	0.61	0.05
Exercise	2868.8 ± 1921.4	1649.3 ± 1254.4	**0.002**	1814.9 ± 1411.6	1263.2 ± 1193.5	**0.04**	0.06	0.33
Recreation	654.3 ± 445.0	597.5 ± 512.1	0.66	738.0 ± 831.4	602.4 ± 664.0	0.59	0.70	0.98
Total Minutes (min)	1535.8 ± 833.8	995.2 ± 503.0	**0.0002**	981.5 ± 597.4	799.4 ± 496.3	0.16	**0.02**	0.23
Total kcal	6645.8 ± 3755.3	5883.2 ± 8584.4	0.69	4110.2 ± 2081.8	3239.6 ± 1932.9	0.08	**0.01**	0.19
Monthly Summary Index								
Vigorous Activity Index	29.8 ± 17.5	26.3 ± 17.8	0.29	35.5 ± 18.9	25.5 ± 18.0	0.05	0.33	0.90
Leisurely Walking Index	20.8 ± 11.6	20.0 ± 9.0	0.81	15.6 ± 11.9	13.1 ± 9.9	0.24	0.17	**0.03**
Moving Index	8.1 ± 3.1	7.1 ± 2.6	0.15	8.4 ± 3.4	8.7 ± 3.3	0.69	0.80	0.09

Standing Index	5.0 ± 2.3	5.1 ± 1.9	0.77	6.2 ± 2.5	5.5 ± 2.4	0.11	0.12	0.59
Sitting Index	2.3 ± 0.85	2.5 ± 1.4	0.52	2.5 ± 0.9	2.4 ± 0.9	0.72	0.79	0.94
Total Summary Index	65.9 ± 25.4	60.9 ± 23.0	0.30	68.2 ± 24.8	55.2 ± 24.5	0.05	0.78	0.46

Values are presented as mean ± SD. The work category includes chores like grocery shopping, doing laundry, housework, food preparation, and home repair. The yard work category includes gardening, lawn mowing, and cleaning driveways. Caretaking accounts for any activity where one tends to another person, be it older, disabled, or children. Exercise refers to planned physical activities like brisk walking, pool exercises, aerobics, and cycling. Recreation involves hobby-like activities like slow walking, needlework, bowling, golf, and billiards. Participants wrote down the duration for each activity they performed, which was then multiplied to an intensity code. This generated the energy spent doing each activity. Monthly summary indexes, by contrast, assign a number to the participants' activity based on their intensity and duration without regard to specific activities. The survey asks the participants to indicate how frequently they participated in vigorous activities, leisurely walking, moving about on their feet, standing, and sitting. Their responses corresponded to a score which was then multiplied by a weight factor depending on the classification of activity. Total summary indexes range between 0-137, and up to 605 if the participant does not know or refused to respond to the questions. A higher score signifies higher energy expenditure during the month prior to completing the survey.

Table 5. Effects of Four Weeks of Wheat Germ Supplementation on Anthropometric Parameters and Stool Characteristics of Overweight Individuals

Measures	Wheat Germ (n= 20) (mean ± SD)		P-value (WG baseline vs final)	Control (n=19) (mean ± SD)		P-value (CT baseline vs final)	P-value (WG vs CT baseline)	P-value (WG vs CT final)
	Baseline	Final		Baseline	Final			
Anthropometric Measurements								
Height (cm)	172.3 ± 11.9		-	170.2 ± 9.2		-	-	-
Weight (kg)	80.8 ± 12.9	80.3 ± 27.2	0.17	81.0 ± 11.2	80.8 ± 11.2	0.62	0.98	0.90
BMI (kg/m^2)	26.9 ± 1.7	27.0 ± 1.9	0.98	27.7 ± 1.7	27.8 ± 2.0	0.63	0.14	0.17
Body fat (%)	18.5 ± 13.5	17.3 ± 13.8	0.79	20.1 ± 12.6	19.3 ± 12.7	0.95	0.63	0.41
Fat Mass (kg)	23.2 ± 6.8	23.7 ± 6.5	0.12	22.2 ± 6.5	22.0 ± 6.5	0.73	0.65	0.41
Skeletal Mass (kg)	26.5 ± 7.2	26.1 ± 7.1	**0.02**	28.6 ± 6.7	29.0 ± 6.8	0.45	0.73	0.46
Visceral Fat (kg)	1.20 ± 0.85	1.22 ± 0.83	0.75	1.20 ± 0.82	1.09 ± 0.84	0.40	0.99	0.70
Waist circumference (cm)	81.4 ± 10.0	82.1 ± 12.3	0.67	79.3 ± 9.0	78.5 ± 9.1	0.41	0.49	0.31
Hip circumference (cm)	88.2 ± 9.6	87.1 ± 14.7	0.77	83.8 ± 8.3	83.7 ± 9.2	0.94	0.13	0.39
Waist-to-Hip Ratio	0.92 ± 0.06	0.99 ± 0.4	0.47	0.95 ± 0.06	0.94 ± 0.07	0.56	0.19	0.61
Systolic blood pressure (mmHg)	106.6 ± 12.3	108.5 ± 10.9	0.34	109.7 ± 9.4	106.8 ± 11.3	0.25	0.37	0.65

Diastolic blood pressure (mmHg)	71.6 ± 9.0	71.8 ± 9.7	0.91	71.3 ± 7.0	70.4 ± 8.9	0.57	0.91	0.65
Stool characteristics								
Constipation score[1]	4.4 ± 1.8	3.6 ± 2.03	0.06	4.3 ± 2.1	3.3 ± 2.3	*0.09*	0.89	0.74
Bristol stool chart[2]	3.8 ± 1.2	3.6 ± 1.1	0.51	4.0 ± 1.3	3.8 ± 1.1	0.74	0.71	0.39

Values are mean ± SD. [1]Using the Cleveland Clinic Constipation Scoring System (CSS) with 8 questions and answers are in the scale of 0-4 and then added for a total score with a range of 0-30. A higher CSS score indicates more constipation.[2]Using the Bristol stool chart questionnaire and answers are in the scale of 1-7 with lower number indicating harder stools.

Table 6. Effects of Four Weeks of Wheat Germ Supplementation on Lipid Profile and Markers of Glucose Homeostasis of Overweight Individuals

Parameters	Wheat Germ (n=20)		P-value (WG baseline vs final)	Control (n=19)		P-value (CT baseline vs final)	P-value (WG vs CT baseline)	P-value (WG vs CT final)
	Baseline	Final		Baseline	Final			
Lipid profile								
Total cholesterol (mg/dL)	176.0 ± 35.4	173.8 ± 34.4	0.56	183.4 ± 30.8	187.4 ± 48.1	0.55	0.49	0.32
HDL-C (mg/dL)	59.1 ± 16.5	56.2 ± 17.3	0.89	56.9 ± 20.3	56.6 ± 22.7	0.19	0.71	0.95
Triglycerides (mg/dL)	83.3 ± 58.6	93.0 ± 73.4	0.52	89.7 ± 42.9	85.7 ± 40.5	0.26	0.70	0.71
LDL-C (mg/dL)	100.2 ± 25.0	98.8 ± 24.6	0.33	108.6 ± 30.1	113.8 ± 37.8	0.73	0.35	0.15
Non-HDL-C (mg/dL)	116.7 ± 33.8	117.4 ± 31.5	0.43	126.5 ± 33.8	131.0 ± 42.4	0.83	0.37	0.27
LDL-C/HDL-C ratio	1.87 ± 0.8	2.0 ± 0.9	0.35	2.3 ± 1.3	2.4 ± 1.2	0.47	0.26	0.25
NEFA (mEq/L)	0.46 ± 0.25	0.45 ± 0.21	0.87	0.52 ± 0.22	0.46 ± 0.20	0.32	0.46	0.94
Glucose homeostasis markers								
HbA1c (%)	5.3 ± 0.3	5.2 ± 0.3	**0.04**	5.3 ± 0.4	5.3 ± 0.3	0.64	0.88	0.63
Insulin (pg/mL)	273.4 ± 253.9	201.6 ± 162.0	**0.03**	178.0 ± 137.6	216.5 ± 177.5	0.15	0.16	0.79
Glucose (mg/dL)	88.4 ± 8.5	86.8 ± 6.9	0.60	87.5 ± 6.2	88.5 ± 7.6	0.39	0.69	0.48
HOMA-IR	1.7 ± 1.7	1.3 ± 1.0	**0.04**	1.2 ± 0.94	1.3 ± 1.0	0.31	0.19	0.91
C-peptide (pg/mL)	36.8 ± 39.1	27.4 ± 33.2	0.32	38.5 ± 35.2	36.0 ± 35.6	0.64	0.89	0.45

GLP-1 (pg/mL)	4530.6 ± 2952.5	4493.3 ± 3325.1	0.86	5639.92 ± 4363.0	5326.43 ± 4385.7	0,13	0.36	0.51
Ghrelin (pg/mL)	150.5 ± 68.6	161.6 ± 90.1	0.40	137.2 ± 52.2	146.4 ± 81.0	0.51	0.64	0.70
Resistin (pg/mL)	788.3 ± 240.4	728.8 ± 233.7	**0.03**	804.6 ± 332.6	818.5 ± 353.6	0.60	0.86	0.36

Values are mean ± SD. HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; NEFA, non-esterified fatty acids; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; GLP, glucagon-like peptide-1

Table 7. Effects of Four Weeks of Wheat Germ Supplementation on Markers of Inflammation and Gut Health of Overweight Individuals

Parameters	Wheat Germ (n= 20)		P-value (WG baseline vs final)	Control (n=19)		P-value (CT baseline vs final)	P-value (WG vs CT baseline)	P-value (WG vs CT final)
	Baseline	Final		Baseline	Final			
Serum or plasma inflammation markers								
Leptin (pg/mL)	1012.2 ± 1007.2	1025.8 ± 1098.3	0.80	985.2 ± 1005.1	1036.6 ± 1065.9	0.57	0.94	0.98
PAI-1 (pg/mL)	1067.3 ± 278.6	988.8 ± 302.4	0.10	967.6 ± 265.9	1064.8 ± 268.9	0.09	0.27	0.42
IL-6 (pg/mL)	1.05 ± 0.68	1.02 ± 0.81	0.88	0.91 ± 0.70	1.00 ± 0.61	0.45	0.52	0.91
TNF-α (pg/mL)	0.39 ± 0.24	0.38 ± 0.23	0.49	0.44 ± 0.22	0.41 ± 0.24	0.11	0.54	0.68
hs-CRP (mg/L)	1.6 ± 1.9	1.8 ± 3.4	0.79	1.7 ± 2.0	2.0 ± 2.2	0.29	0.84	0.85
Gut integrity markers								
Serum I-FABP (pg/mL)	722.2 ± 320.7	636.0 ± 239.2	0.12	658.9 ± 344.0	629.0 ± 207.8	0.73	0.56	0.92
Serum zonulin (ng/mL)	42.3 ± 6.5	44.8 ± 12.3	0.50	46.5 ± 7.7	43.4 ± 6.0	0.31	0.08	0.67
Fecal sIgA (μg/mL)	1211.3 ± 1662.4	1585.2 ± 1592.8	0.54	1829.7 ± 1698.6	1270.3 ± 1511.7	0.80	0.27	0.54

Values are mean ± SD. PAI-1, plasminogen activator inhibitor-I; IL-6, interleukin-6; TNF-α, tumor necrosis factor- α; hs-CRP, high sensitivity C-reactive protein; I-FABP, intestinal fatty acid binding protein; sIgA, secretory immunoglobulin A

Figure 1. Effects of Four Weeks of Wheat Germ Supplementation on Fecal Microbial Alpha Diversity Measures of Overweight Individuals
A = control; B = wheat germ

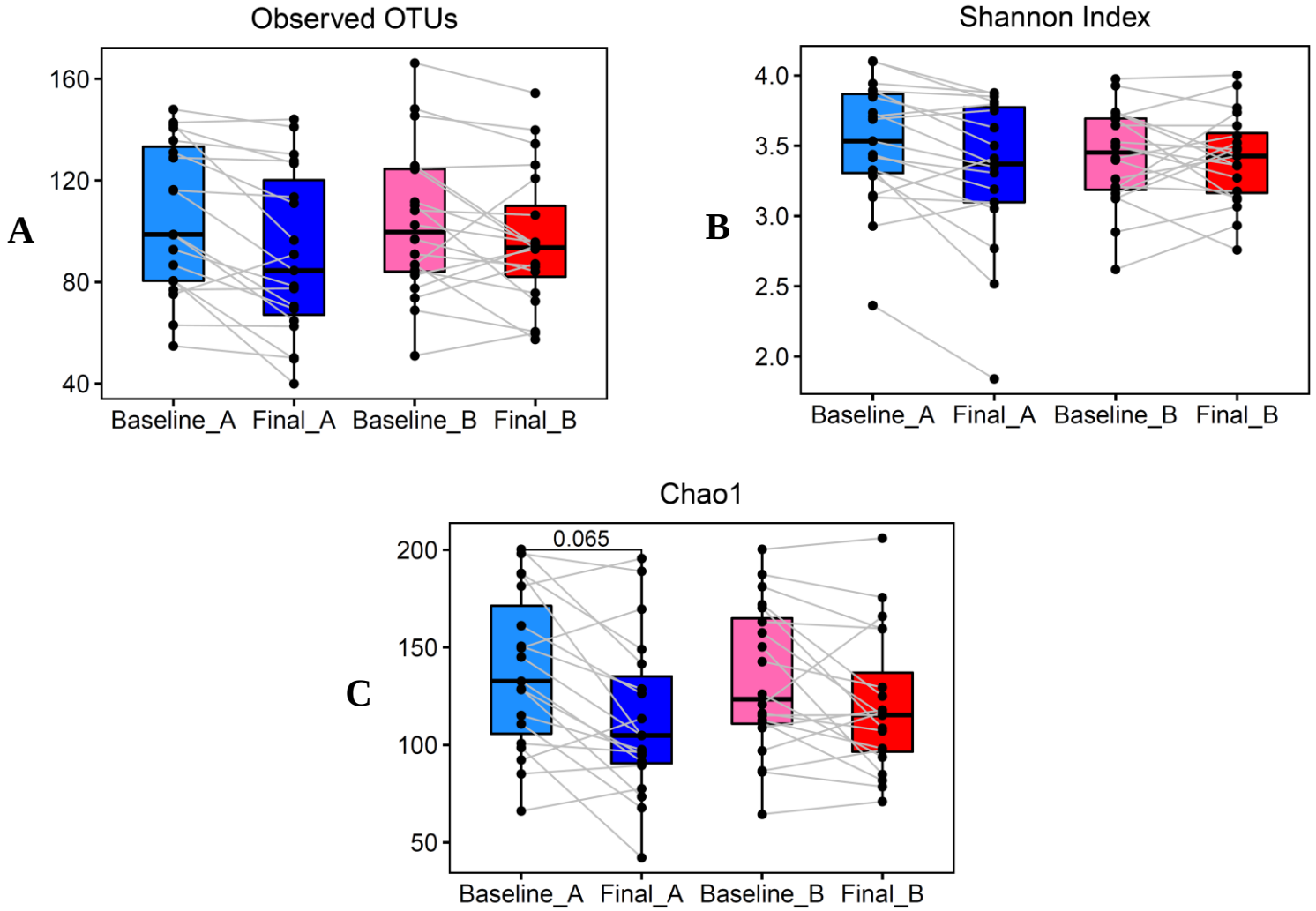
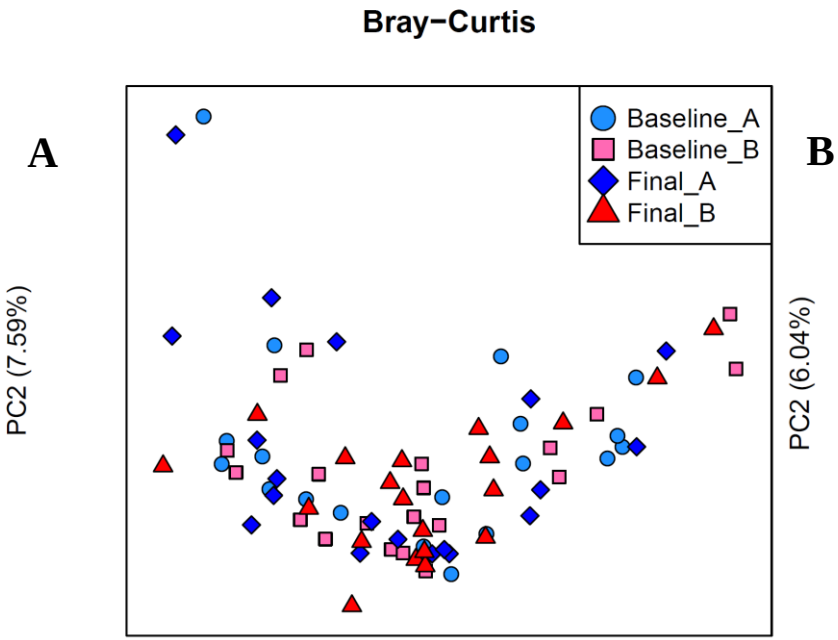
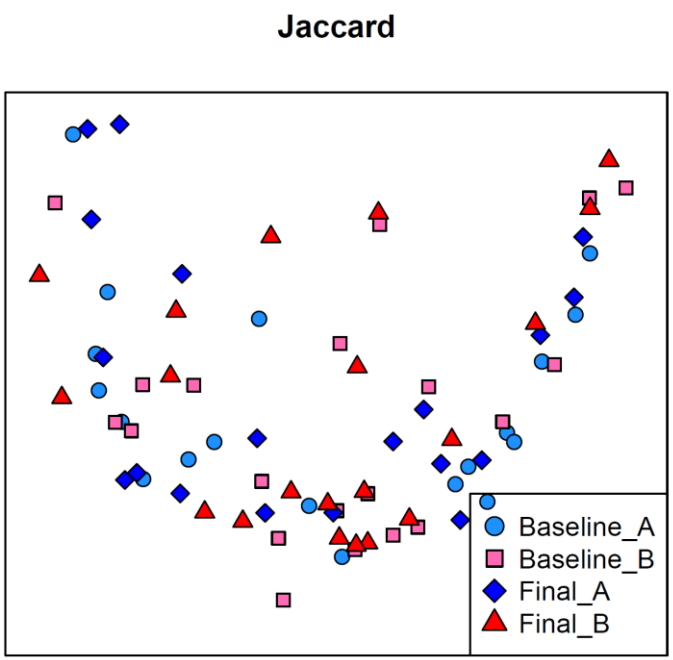


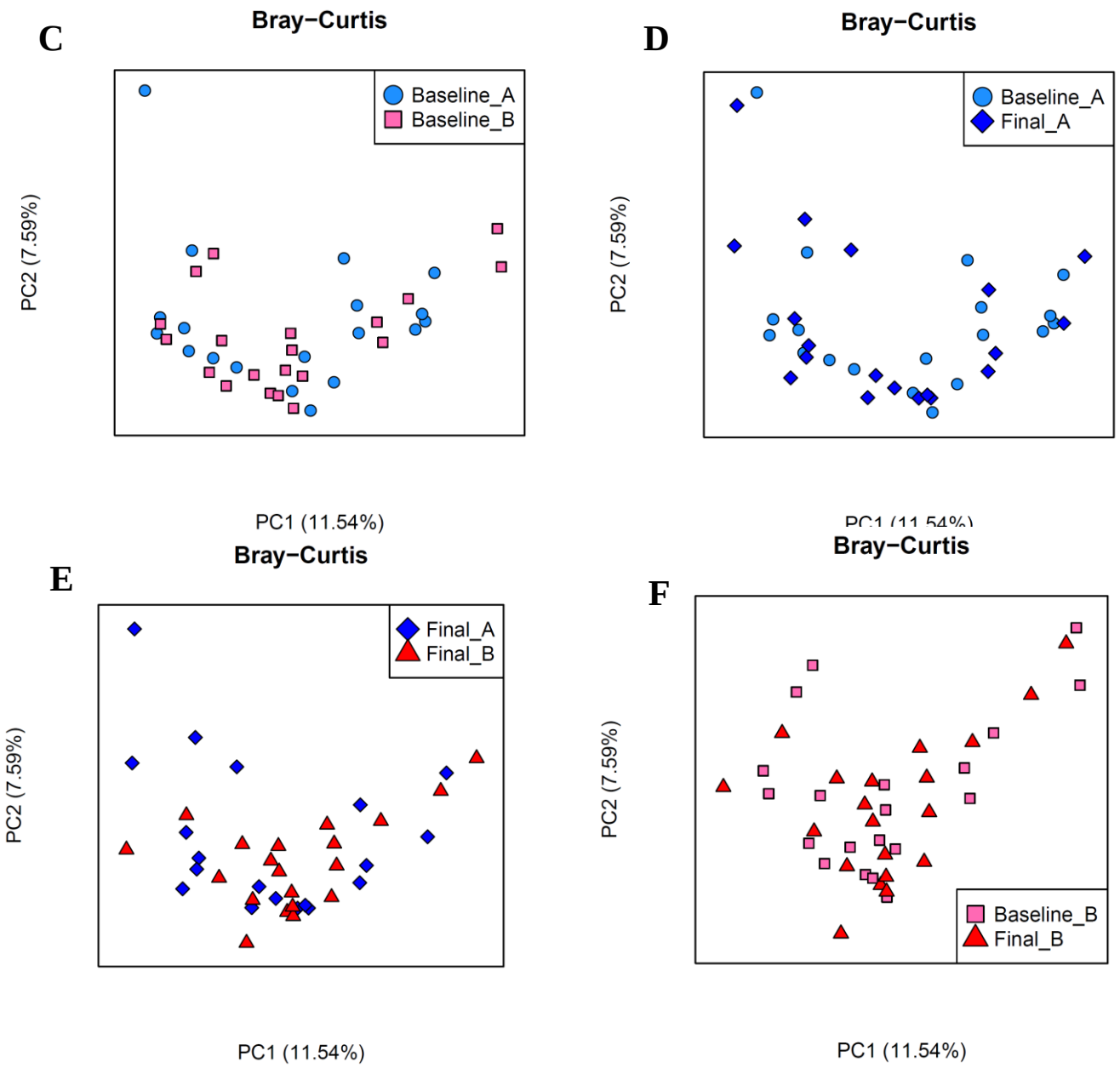
Figure 2. Effects of Four Weeks of Wheat Germ Supplementation on Fecal Microbial Beta Diversity Measures of Overweight Individuals
A = control; B = wheat germ



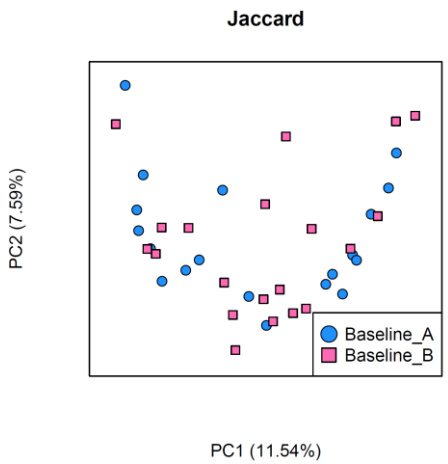
comparison	R-value	P-value
Baseline_A-Baseline_B-Final_A-Final_B	0.007527	0.2949



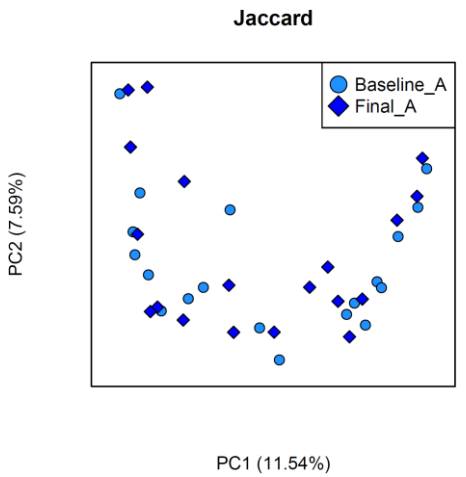
comparison	R-value	P-value
Baseline_A-Baseline_B-Final_A-Final_B	-0.01163	0.721


C
Bray–Curtis
Baseline_A
Baseline_B
PC2 (7.59%)
PC1 (11.54%)
D
Bray–Curtis
Baseline_A
Final_A
PC2 (7.59%)
E
Bray–Curtis
Final_A
Final_B
PC2 (7.59%)
PC1 (11.54%)
F
Bray–Curtis
Baseline_B
Final_B
PC2 (7.59%)
PC1 (11.54%)

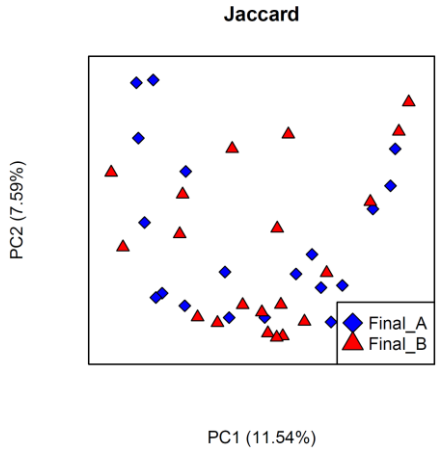
G


Jaccard
PC2 (7.59%)
PC1 (11.54%)
Baseline_A
Baseline_B

H


Jaccard
PC2 (7.59%)
PC1 (11.54%)
Baseline_A
Final_A

I


Jaccard
PC2 (7.59%)
PC1 (11.54%)
Final_A
Final_B

J

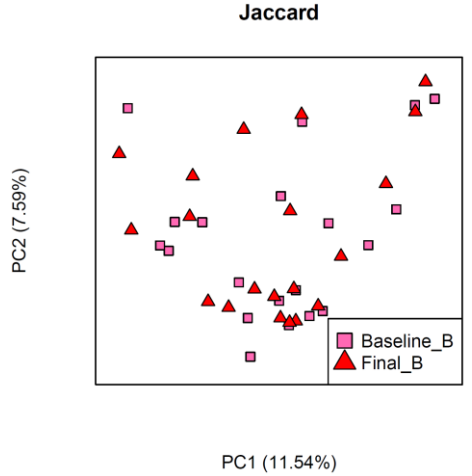
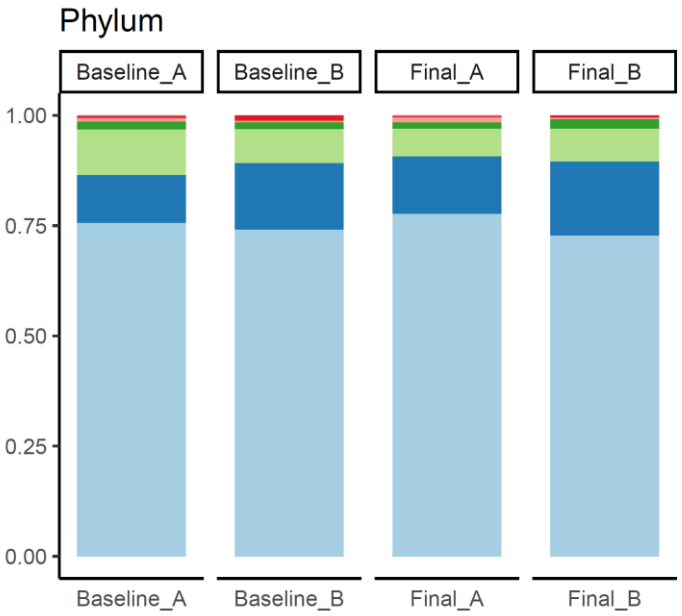
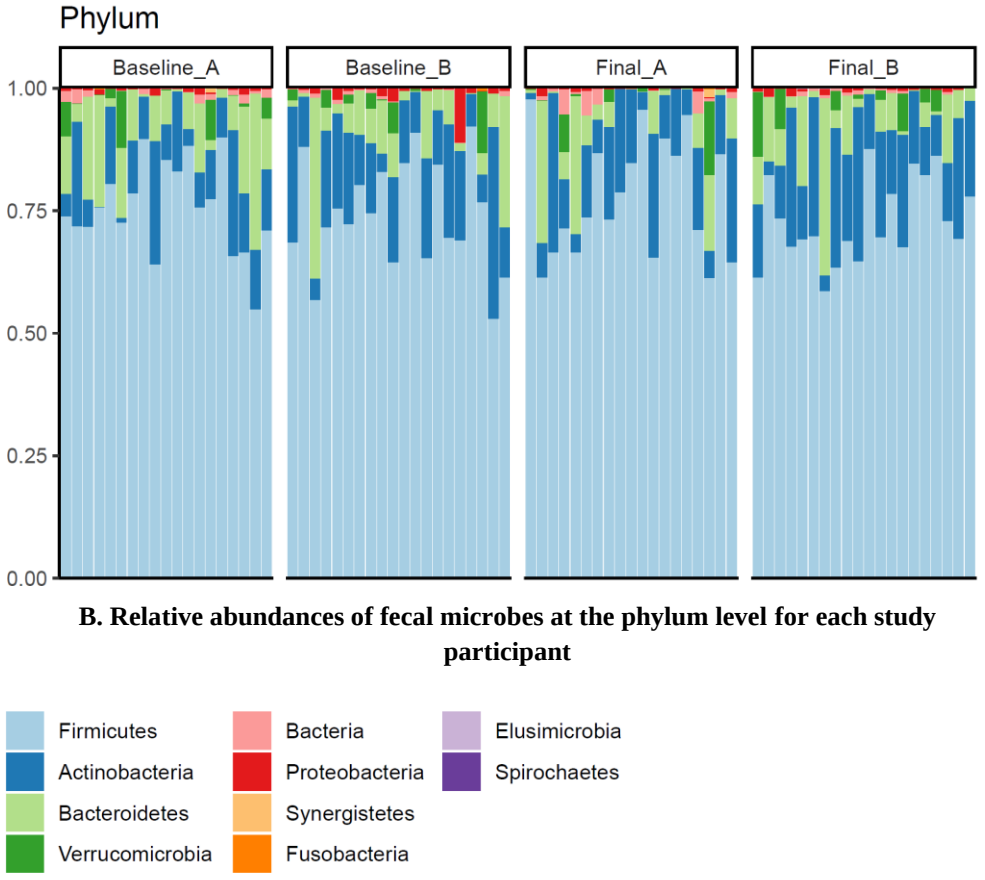

Jaccard
PC2 (7.59%)
PC1 (11.54%)
Baseline_B
Final_B

Figure 3. Effects of Four Weeks of Wheat Germ Supplementation on Fecal Microbial Composition at the Phylum Level in Overweight Individuals
A = control; B = wheat germ



A. Average relative abundances of fecal microbes at the phylum level



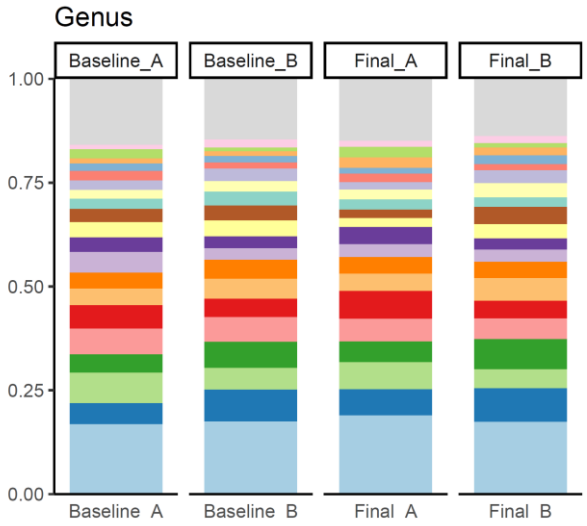
B. Relative abundances of fecal microbes at the phylum level for each study participant

Table 8. Effects of Four Weeks of Wheat Germ Supplementation on Relative Abundance (%) of Fecal Microbial Population at the Phylum Level in Overweight Individuals

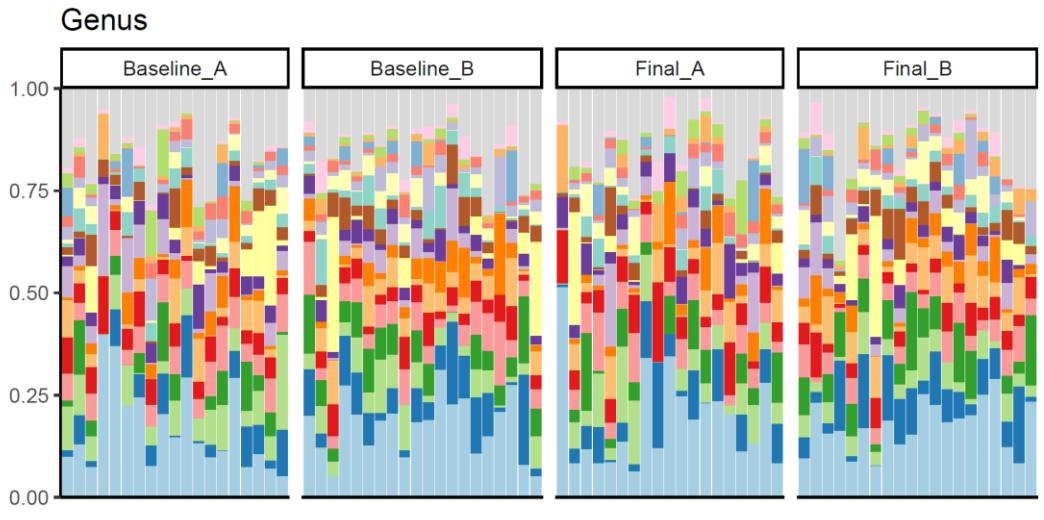
Bacterial Phylum	Wheat Germ (n = 20)		P-value (WG baseline vs final)	Control (n = 19)		P -value (CT baseline v final)	P -value (WG v CT baseline)	P -value (WG v CT final)
	Baseline	Final		Baseline	Final			
Firmicutes	74.1 ± 11.0	72.8 ± 8.5	0.63	75.6 ± 9.3	77.7 ± 12.2	0.78	0.57	0.22
Actinobacteria	15.1 ± 8.7	16.7 ± 8.6	0.40	10.9 ± 7.3	13.0 ± 8.6	0.57	0.13	0.20
Bacteroidetes	7.7 ± 9.2	7.5 ± 8.5	0.96	10.3 ± 8.5	6.34 ± 8.9	**0.03**	0.14	0.21
Verrucomicrobia	1.5 ± 3.1	2.2 ± 3.7	0.61	1.8 ± 3.45	1.4 ± 3.7	0.72	0.60	0.71
Bacteria	0.34 ± 0.37	0.33 ± 0.33	0.98	0.82 ± 0.92	1.1 ± 1.8	0.65	0.15	0.78
Proteobacteria	1.2 ± 2.4	0.47 ± 0.52	0.32	0.48 ± 0.45	0.29 ± 0.39	**0.049**	0.45	0.43
Firmicutes/Bacteroidetes ratio	64.1 ± 132.0	43.1 ± 71.8	0.91	30.7 ± 52.4	224.6 ± 383.4	0.11	0.21	0.48

Values are mean % ± SD.

Figure 4. Effects of Four Weeks of Wheat Germ Supplementation on Fecal Microbial Composition at the Genus Level of Overweight Individuals
A = control; B = wheat germ



A. Average relative abundances of fecal microbes at the genus level



B. Relative abundances of fecal microbes at the genus level in each study participant

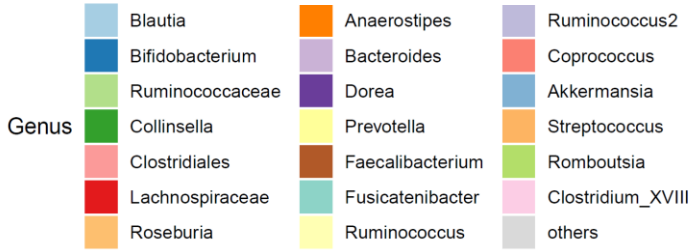


Table 9. Effects of Four Weeks of Wheat Germ Supplementation on % Relative Abundance of Fecal Microbial Population at the Genus Level of Overweight Individuals

Bacterial Genera	Wheat Germ (n = 20)		P-value (WG baseline v final)	Control (n = 19)		P-value (CT baseline v final)	P-value (WG v CT baseline)	P-value (WG v CT final)
	Baseline	Final		Baseline	Final			
Blautia	17.5 ± 7.4	17.5 ± 6.2	0.94	16.9 ± 10.6	19.0 ± 11.8	0.49	0.56	0.91
Bifidobacterium	7.7 ± 6.3	8.1 ± 6.0	0.77	5.1 ± 4.6	6.3 ± 5.8	0.70	0.15	0.33
Lachnospiriceae	4.4 ± 2.2	4.2 ± 2.6	0.67	5.6 ± 2.8	6.7 ± 4.5	0.74	0.12	**0.03**
Roseburia	4.8 ± 3.2	5.5 ± 3.9	0.68	4.0 ± 3.8	4.1 ± 4.1	0.80	0.29	0.24
Bacteroides	2.8 ± 3.2	3.0 ± 3.3	0.85	5.0 ± 5.7	3.1 ± 5.6	**0.03**	0.11	0.25
Prevotella	3.8 ± 8.7	3.5 ± 7.3	0.37	3.7 ± 6.7	2.2 ± 6.4	0.26	0.50	0.21
Faecalibacterium	3.6 ± 2.7	4.2 ± 3.0	0.63	3.1 ± 2.5	2.1 ± 2.8	0.07	0.74	**0.007**
Ruminococcus	2.5 ± 2.2	3.4 ± 3.1	0.55	2.1 ± 2.1	2.4 ±2.9	0.98	0.44	0.30
Akkermansia	1.5 ± 3.1	2.2 ± 3.7	0.61	1.8 ± 3.4	1.4 ± 3.7	0.72	0.60	0.71
Streptococcus	1.1 ± 1.3	1.8 ± 2.2	0.77	1.2 ± 2.6	2.5 ± 4.2	0.26	0.11	0.89

Values are mean % ± SD.

Figure 5. Effects of Four Weeks of Wheat Germ Supplementation on Fecal Short-Chain Fatty Acid Concentrations of Overweight Individuals

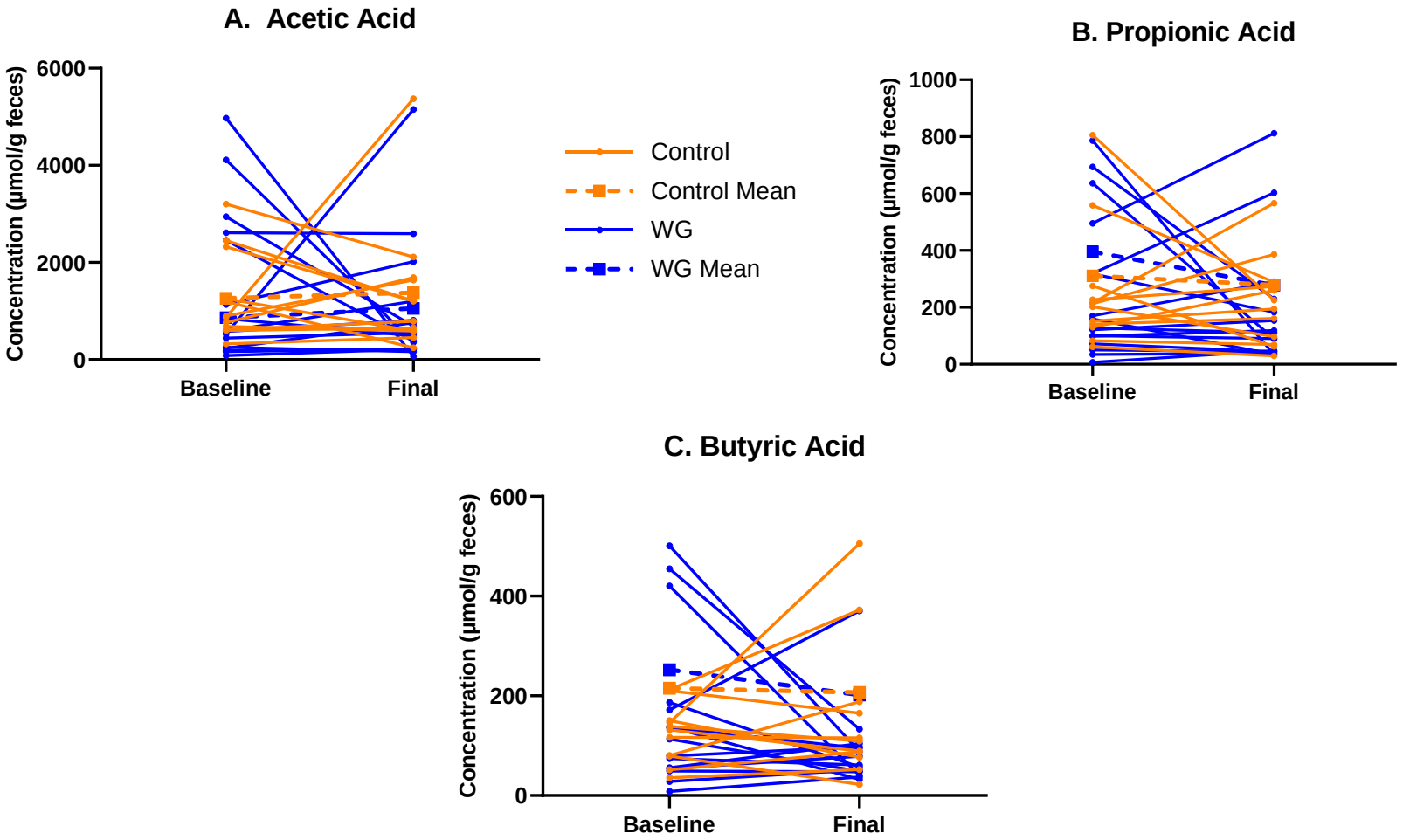


Table 10. Effects of Four Weeks of Wheat Germ Supplementation on Fecal Short-Chain Fatty Acid Concentrations of Overweight Individuals

Short-chain fatty acid (µmol/g feces)	Wheat Germ (n= 20) (n = 20)		P-value (WG baseline vs final)	Control (n=19) (n = 19)		P-value (CT baseline vs final)	P-value (WG vs CT baseline)	P-value (WG vs CT final)
	Baseline	Final		Baseline	Final			
Acetic acid	1382.0 ± 1547.1	991.9 ± 1309.7	0.48	1264.1 ± 900.5	1377.6 ± 1382.0	0.81	0.82	0.83
Propionic acid	262.1 ± 254.1	182.9 ± 221.4	0.28	255.4 ± 215.1	218.0 ± 153.7	0.60	0.78	0.52
Isobutyric acid	106.5 ± 203.1	357.3 ± 886.9	0.29	51.2 ± 75.9	69.5 ± 187.3	0.63	0.34	0.24
Butyric acid	190.3 ± 186.2	127.6 ± 171.9	0.17	122.9 ± 58.0	162.1 ± 147.4	0.33	0.95	0.87
Isovaleric acid	51.3 ± 54.4	81.4 ± 144.9	0.44	51.7 ± 81.0	26.3 ± 19.6	0.27	0.99	0.17
Valeric acid	54.8 ± 59.3	42.6 ± 67.9	0.55	46.8 ± 68.7	41.6 ± 51.8	0.69	0.73	0.96
Caproic acid	9.7 ± 17.5	3.9 ± 4.1	0.17	10.5 ± 27.0	3.0 ± 3.2	0.29	0.93	0.52
Heptanoic acid	3.3 ± 5.4	8.1 ± 23.2	0.45	5.1 ± 14.0	0.95 ± 1.78	0.24	0.64	0.25

Values are mean ± SD.

CHAPTER V

DISCUSSION

The rise in the prevalence of obesity and the complications associated therewith present a daunting challenge that often seems insurmountable. With the advent of gut microbiome research, our knowledge of the pathophysiology of obesity has increased as we discover the powerful influence of microscopic organisms that call our gut home. However, with the gut microbiome in play as a virtual endocrine organ[9], many questions regarding obesity and related conditions remain unanswered. For one, the effects of functional foods on the gut microbiome and metabolic outcome is an area of active research. One functional food that contains many bioactive components including fiber, B-vitamins, tocopherols, phytosterols, policosanols, and polyphenols is wheat germ (WG).[23] Limited animal[24,25,108,122] and human[26,111-113] studies demonstrate the potential of WG in improving metabolic outcomes. However, the effects of WG on the gut microbiome and its relationship with gut integrity and metabolic outcomes have not been extensively investigated. In this study, we sought to determine whether daily WG supplementation for four weeks affected gut bacterial population and gut health, inflammation and metabolic markers in overweight adults.

Using alpha and beta diversity measures, our study demonstrated that four weeks of WG supplementation had no effects on the bacterial diversity in overweight adults. We determined alpha diversity using the number of observed OTUs, the Shannon and the Chao1 indexes while beta diversity was determined using the Bray-Curtis and Jaccard indexes. There were no changes

in these measures of bacterial diversity before and after 4-week supplementation of both WG and control supplement except for a tendency for a reduction in Chao1 index with the control supplement. In addition to the effects on microbial diversity, we also observed minimal changes in the various bacterial phyla and genera with WG supplementation. The only changes that we observed were significant reductions in the phyla Bacteroidetes and Proteobacteria as well as the genus *Bacteroides* with the control supplement while WG maintained these bacterial populations. Because of the alterations in bacterial population due to the control supplement, the WG group have higher relative abundance of the genus *Faecalibacterium* and lower *Lachnospiriceae* compared to the control group at the final time point. *Faecalibacterium*, whose only known species is *F. prausnitzii*, has been found to be positively associated with various markers of health.[123] In a study of 30 obese individuals who went through Roux-en-Y gastric bypass surgery, *F. prausnitzii* was found to be higher in those who have type 2 diabetes and was also consistently associated with decreased levels of hs-CRP and IL-6 during the multiple follow-up visits.[124] *F. prausnitzii* has also been shown to discriminate those who have type 2 diabetes from those who do not in a study of European women.[125] *Lachnospiriceae* on the other hand, has been found to be associated with metabolic disorders such as type 2 diabetes in a study of 20 individuals ages 58 to 71 years old.[126] Another study further observed *Lachnospiriceae* levels to be higher in those who are obese and have metabolic syndrome.[127] In germ-free *ob/ob* mice, colonization of *Lachnospiriceae* from hyperglycemic mice resulted in impaired glucose metabolism.[128] The significance of WG in maintaining these bacterial populations compared to the control is not clear at this time.

Gut microbial diversity is important because changes in composition due to different factors such as diet, stress, and antibiotic use induce a state called dysbiosis that is associated with several diseases.[14] Although dysbiosis has no definite clinical criteria and is therefore not a diagnostic tool, it is widely regarded that an increase in gut microbial diversity is linked to a

healthier status.[14] Our findings indicate no significant changes in microbial composition with four weeks of WG supplementation despite evidence that the gut microbial composition could shift within 24 hours in response to changes in dietary intake.[129] This is in contrast with the few studies examining the effects of WG on gut bacterial population. Moreira-Rosario and colleagues found an increase in the *Bacteroides* and *Bifidobacterium* species after four-week supplementation of 6 g of WG in the form of bread in healthy adults. [27] Similarly, Ojo and colleagues found that 12-week WG supplementation in mice that are fed a high-fat, high-sucrose diet, resulted in an increase in the bacterial family Lactobacillaceae, which includes the phylum Firmicutes.[108] Several factors may have contributed to the differences in our findings compared to that of Moreira-Rosario and Ojo.[24,27,108,109] The dose, the matrix by which the WG is mixed with, compliance, study population, and supplementation duration are just the few factors to consider in future studies.

This result is in agreement to the study by Moreira-Rosario et al. where 55 healthy adults who consumed 6 g of wheat germ for four weeks reported a significant decrease in gastrointestinal discomfort-related quality of life compared to the control group that consumed refined white bread.[27] The significant change in the study by Moreira-Rosario and colleagues might be due to the participants' worries and concerns regarding their bowel movement, which our study did not measure. In the beginning, our participants' scores from both BSC and CSS already reflected normal stool consistency and low levels of constipation, so the intervention only served to maintain or slightly improve their stool measures.

We also investigated the effect of wheat germ on glucose measures. Among the parameters we examined, HbA1c, fasting insulin, and HOMA-IR were improved by WG supplementation. Similarly, Ojo et al. found improved HOMA-IR and insulin in mice who were fed a high-fat, high-sucrose diet after 12 weeks of WG supplementation.[24] However, the human study by Moreira-Rosario et al. reported no changes in these parameters.[109] This may be due to

the lower WG dose used in their study. Similarly, Tripkovic et al. found no significant differences for HOMA-IR and insulin after 4-week supplementation in overweight men given 15 g of wheat germ added to dinner rolls.[26] The positive effects of WG on HbA1c, fasting insulin, and HOMA-IR we observed in our study compared to the no effects on these parameters observed by Moreira-Rosario and colleagues[109] as well as Tripkovic et al.[26] may be explained by the difference in dosage administered in the studies. Our 30 g dose of WG may represent a value within a range of WG doses that elicits a favorable response in humans. However, more clinical studies are still needed to determine the factors that contributes to the positive effects of WG on glucose homeostasis.

The significantly lower levels of resistin in the WG group might partly explain the improvement in HOMA-IR that we have observed. Resistin, a small protein produced by peripheral blood mononuclear cells (PBMC) and macrophages in humans, has been shown to play a role in metabolic disorders, such as type 2 diabetes, although the mechanisms by which it exerts its effects are poorly understood.[130,131] Resistin, however, has been demonstrated to promote inflammation that leads to decreased insulin sensitivity. Resistin stimulates the activation of pro-inflammatory genes and cytokines via the action of the transcription factor NF-κB. Resistin has also been shown to bind to toll-like receptor 4 (TLR4), the same receptor to which LPS binds to induce an inflammatory response.[130] However, the reduction in resistin due to WG that we have observed is not associated with the modulation of inflammatory markers as we did not observe significant changes in the inflammatory markers TNF-α, IL-6 and C-reactive protein. Supplementation with WG might improve glucose homeostasis through improved insulin signaling or glucose uptake. However, this is speculative and needs to be investigated in future studies.

We also observed no effects of WG supplementation on lipid profile. These results echo that of the study by Moreira-Rosario et al. where participants consumed 6 g of WG daily for 4

weeks and experienced no changes in cholesterol and triglyceride concentrations.[109] Similarly, a study by Tripkovic et al. examined triglycerides, total cholesterol, and HDL in overweight males. After 4 weeks of daily consumption of 15 g of WG, no significant differences were found in the lipid measures.[26] The lipid profile results for our participants were already within normal limits, indicating a homeostatic state that needed no correction.[132] One component of WG that has been reported earlier to lower cholesterol is policosanol.[133,134] However, a study by Lin et al. show that WG policosanol has also no effect on lipid profile. These studies[26,109,113] and our findings indicate that WG is not involved in modulating lipids.

Despite our findings about the positive effects of WG on glucose homeostasis, our studies have several limitations. One of these limitations is the self-reporting of dietary intake, physical activity, and compliance by the participants. Self-reporting, by nature, is highly prone to inaccuracies. Although participants were given instructions to keep their dietary intake and physical activity constant throughout the study, they were not given specifications as to how to achieve this. Therefore, the amounts reported in the questionnaires were potential sources of bias as they were based on the participants' estimations. Participants were also asked to record their supplement intake in a calendar and to turn in the bags that contained their supplement. While only a few participants reported missing a few days resulting therefore in a near-perfect compliance, the fact still stands that this was based on their self-reports without our verification. Another limitation of the study is that we used 16s RNA sequencing for fecal microbial analysis. Although it is a common approach utilized in gut microbiome research, 16s RNA only identifies to the genus level and not the species level of the bacteria. This limited the full characterization of the gut microbiome of our participants. We also observed a high variability in our fecal SCFAs concentrations. Whether this variability is due to the supplement or other factors needs to be addressed in future studies. Our WG dose of 30 g per day might have also been insufficient to

produce changes outside the markers of glucose homeostasis. Future studies will need to determine an optimal daily WG dose that will elicit the desired effects.

We suggest that future studies examining WG and its effects on metabolic and gut health markers include subjects from the obese category because the chronic disease risks associated with increased adiposity such as T2D and CVD are greatly elevated in this category. Another challenge with our study is that many participants verbally expressed during their visits about their difficulty in consuming the study supplements (e.g., undesirable flavor, dry and crumbly texture, excessive amount). Future studies should incorporate a variety of food products that contain WG, such as including the wheat germ in baked products such as bread, muffins, cookies, etc. However, cooking may affect the bioactive component in WG. Recruitment of the participants should also include criteria based on body fat percentage in addition to BMI, and ability to give blood and fecal samples. Another challenge of our study is that two and four participants had difficulty providing blood and fecal samples, respectively. One participant was completely unable to have blood drawn and those who had trouble with the fecal samples provided so little fecal matter for our data to be complete. In future studies, recruitment will need to ask about the ability to give blood while fecal sampling instructions will need to request as much fecal matter as can be provided by the participant. We also suggest the use of shotgun metagenomic sequencing to fully characterize and identify the species of the gut bacteria in the fecal samples.

In conclusion, our study demonstrated that 4-week WG supplementation improved several markers of glucose homeostasis: HbA1c, insulin, and HOMA-IR. These positive effects on glucose markers is not due to changes within the gut as we did not see any effects of WG on gut bacterial population, markers of gut integrity, SCFAs concentrations as well as the gut hormone GLP-1 that can stimulate insulin secretion. Additionally, because HbA1c is a measure of long-term glucose control, WG's effect on HbA1c might even be amplified had the study been

extended to at least 8 weeks. The positive effect of WG on glucose homeostasis may be partly attributed to a decrease in the pro-inflammatory adipokine resistin which can improve insulin signaling or glucose uptake. Based on our findings, these improvements in glucose homeostasis markers by WG supplementation provides a rationale to use WG as an effective and economical option for individuals who needs to improve glucose control. Future studies will need to explore the effects of WG in other populations such as those in the obese BMI category and those who are insulin resistant or diagnosed with type 2 diabetes. The elucidation of the mechanisms by which WG exerts its effects on metabolism is also warranted.

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APPENDICES

Oklahoma State University Institutional Review Board

Date:	01/15/2019
Application Number:	HS-18-88
Proposal Title:	Wheat germ supplementation will improve markers of gut health, inflammation, and insulin resistance in overweight individuals
Principal Investigator:	Edralin Lucas
Co-Investigator(s): Sam Emerson	
Faculty Adviser:	
Project Coordinator:	
Research Assistant(s):	
Processed as:	Expedited

Status Recommended by Reviewer(s):
Approved Approval Date:

0

1/09/2019

Expiration Date: **01/08/2020**

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

The final versions of any recruitment, consent and assent documents bearing the IRB approval stamp are available for download from IRB Manager. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be approved by the IRB. Protocol modifications requiring approval may include changes to the title, PI, adviser, other research personnel, funding status or sponsor, subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.
2. Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3. Report any unanticipated and/or adverse events to the IRB Office promptly.
4. Notify the IRB office when your research project is complete or when you are no longer affiliated with Oklahoma State University.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact the IRB Office at 223 Scott Hall (phone: 405-744-3377, irb@okstate.edu).

Dawnett Watkins

Sincerely,
Dawnett Watkins, CIP
IRB Manager
Oklahoma State University IRB

VITA

Levin Matthaeu Gencianeo Dotimas

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF WHEAT GERM SUPPLEMENTATION ON MARKERS OF INFLAMMATION AND GUT HEALTH IN OVERWEIGHT ADULTS

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in July, 2021.

Completed the requirements for the Bachelor of Science in Dietetics at Brigham Young University, Provo, Utah in 2017.

Experience:

Graduate Research and Teaching Assistant, Department of Nutritional Sciences, Oklahoma State University, August 2018-present

Production Supervisor, Vie de France-Yamazaki, Rockville, MD
March 2018 – July 2018

Dining Room Supervisor, Sodexo, Silver Spring, MD
August 2017 – April 2018

Dietary Cook, Provo Rehabilitation and Nursing, Provo, UT
April 2016 – June 2017

Professional Memberships:

Academy of Nutrition and Dietetics (2015-present)
American Society of Nutrition (2020-present)