Are Weight and Diet Related to the Gut Microbiome in Healthy College Students Living in the Dorms? : A Cross-Sectional Observational Analysis

by

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ABSTRACT

College weight gain and obesity are significant problems impacting our society, leading to a considerable number of comorbidities during and after college. Gut microbiota are increasingly recognized for their role in obesity and weight gain. Currently, research exploring the gut microbiome and its associations with dietary intake and body mass index (BMI) is limited among this population. Therefore, the purpose of this study was to assess associations between the gut microbiome, BMI, and dietary intake in a population of healthy college students living in two dorms at Arizona State University (n=90). Cross-sectional analyses were undertaken including 24-hour dietary recalls and anthropometrics (height, weight and BMI). High throughput Bacterial 16S rRNA gene sequencing of fecal samples was performed to quantify the gut microbiome and analyses were performed at phyla and family levels. Within this population, the mean BMI was 24.4 ± 5.3 kg/m^2 and mean caloric intake was 1684 ± 947 kcals/day. Bacterial community analysis revealed that there were four predominant phyla and 12 predominant families accounting for 99.3% and 97.1% of overall microbial communities, respectively. Results of this study suggested that a significant association occurred between one principal component (impacted most by 22 microbial genera primarily within Firmicutes) and BMI (R^2=0.053, p=0.0301). No significant correlations or group differences were observed when assessing the Firmicutes/Bacteroidetes ratio in relation to BMI or habitual dietary intake. These results provide a basis for gut microbiome research in college populations. Although, findings suggest that groups of microbial genera may be most influential in obesity, further longitudinal research is necessary to more accurately describe these associations over me. Findings from future research may

be used to develop interventions to shift the gut microbiome to help moderate or prevent excess weight gain during this important life stage.

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CHAPTER 1

INTRODUCTION

Obesity is a significant problem that is impacting our society, with 31.8% of youth/teens being classified as overweight or obese, leading to a considerable number of comorbidities, such as hypertension, dyslipidemia, type 2 diabetes, and heart disease.[1-4] Obesity is now viewed as the consequence of complex interactions between genetics, environment, socio-economic status, and diet, rather than simple imbalances in energy consumption.[5] The prevalence of overweight children and childhood obesity has become a growing problem that continues into the college years. The most significant change in weight occurs during the initial transition to college amongst college freshmen and students living in the dorms and can ultimately result in a transition from a healthy weight into overweight or obese categories.[1,6-11]

During the freshman year of college, students are exposed to significant environmental and dietary changes. This transitory period between adolescence and adulthood has been characterized as "emerging adulthood," during which, health behaviors are thought to be driven by increased social development and susceptibility to peer influence.[12,13] The impact that this period has on adult health makes the transition to college a unique time to study interrelationships between general health and weight outcomes; however, this time period is infrequently studied in obesity research.[14]

Gut microbiota have become increasingly recognized for their crucial role in metabolic functioning and subsequent states of health and disease.[2] Studying the dynamic relationship between weight and intestinal microbiota is important for

understanding the college weight gain phenomenon. The current research connecting obesity and the gut microbiome demonstrates that, in obese populations, microbial diversity decreases as compared to that of lean individuals.[15,16] The connection between the gut microbiome and obesity has been further strengthened by animal studies in which feces of diet-induced obese (DIO) mice were transplanted into germ-free lean mice.[16,17] The results of these studies demonstrated that mice receiving transplants from DIO mice experienced weight gain whereas those receiving transplants from lean donors did not.[16,17] These relationships connecting the gut microbiome and obesity have been most predominantly characterized using two main phyla classifications that seem to be most evident in the gut: Firmicutes and Bacteroidetes.[2,5,18]

The majority of research suggests that there is an increased Firmicutes/Bacteroidetes (F/B) ratio in obese patients as compared to healthy, lean individuals.[2,5,18] Further obesity research demonstrates that, by placing obese adult patients on weight loss diets such as low-fat or low-carbohydrate diets, the F/B ratio can be returned to that of healthy, lean persons.[18] Therefore, while the gut microbiota can help promote obesity through increases in Firmicutes, an individual may be able to alter this ratio to a healthier composition with lifestyle changes that increase Bacteroidetes abundance.[2,5,18] Although the majority of research supports the findings of an increased F/B ratio in obese patients, there are studies of similar populations that report an increase in the opposite direction[19] or find no correlation between weight and phyla proportions.[20,21] As a whole, research in this area is still in its early stages and more work is needed to fully understand the connections between the microbiome and weight status.

Another factor that is shown to play a role in determining the gut microbial make-up and its shifts during weight change is diet. One study looked at the association between the gut microbiome and diet by looking at children in Europe that consumed a Western diet compared to children in Burkina Faso (a rural city in Africa) that consumed a Neolithic diet.[22] The differences between the two diets were that the Western diet was high in sugar, animal fat, and calorie-dense foods whereas the Neolithic diet was low in fat and animal protein and rich in starch, fiber, and plant polysaccharides (predominantly vegetarian).[22] This study found that the European children had lower overall microbial diversity and a high F/B ratio as compared to that of the African children who had a rich microbial diversity and a low F/B ratio. African children also had a higher presence of short chain fatty acid (SCFA) producing microbes, which is believed to provide a protection against enteric pathogens.[22] Other studies have aimed to assess how dietary changes influence the gut microbiome. In a cross-sectional study of healthy, lean and obese individuals, gut microbiome data were taken at multiple time points with all the subjects consuming a controlled baseline diet, a 2400 kcal, and a 3400 kcal balanced diet (24% protein, 16% fat, and 60% carbohydrates).[23] This incremental increase in total calories resulted in a greater F/B ratio and greater energy harvesting demonstrated by decreased energy loss in feces.[23] In another study ten healthy adult male and female subjects were fed either a low fat/high fiber diet or a high fat/low fiber diet and the gut microbiome was assessed. Similar to the study by De Filippo et.al., consuming a low fat/high fiber diet resulted in a low F/B ratio while the high fat/low fiber diet resulted in a gut profile with greater F/B ratio.[24] Both studies demonstrated that changes in diet can

rapidly begin to alter the gut microbiome, although it is not clear whether these changes shift back with discontinuation of the diet.[23,24]

While diet has a large impact on the composition of the gut microbiome, microbiota of obese individuals have also been associated with greater energy harvesting from the diet which may contribute to increased fat/energy stores in the body.[2,5] In the animal studies that transplanted feces from diet-induced obese (DIO) mice into germ-free, lean mice, the transplant recipients also had greater fat deposition than those receiving transplants from lean donors.[16,17] The greater fat deposition in this study was determined to be associated with the increased F/B ratio, thereby supporting the hypothesis that an obese microbiome is more efficient at energy harvesting. Similar findings from human studies also support the hypothesis that Firmicutes is the key phyla responsible for increasing obesity through energy harvesting.[2,5,18]

While the current research has demonstrated some correlation between diet, weight status and the gut microbiome, healthy college freshmen have not yet been utilized as a specified population to explore these correlations further. The present study will explore the connections between the gut microbiome, weight status and diet in health healthy college students living in the dorms. Utilizing this population is crucial to understanding these relationships further as college freshmen and other students living in the dorms generally experience significant changes to their diet and weight status.[14] Lastly, results from this research will provide a basis for future longitudinal studies and interventions to understand the relationships between diet, weight status, and the gut microbiome in order to decrease the prevalence of college weight gain and impede the increasing obesity rate in young adults into adulthood.

Study Purpose

The purpose of this cross-sectional observational analysis was to characterize the gut microbiome and identify relationships between body mass index (BMI), dietary intake, and the gut microbiome of healthy college students living in the dorms.

Aims

- Aim 1: To characterize the gut microbiome profiles of healthy college students living in the dorms.
    - Purpose: To determine the relative abundance of microbial taxa in feces from a diverse group of college students living in the dorms.
- Aim 2: To investigate the association of BMI with the fecal microbiome composition in healthy college students living in the dorms.
    - Purpose: To compare the Firmicutes to Bacteroidetes (F/B) ratio of underweight, normal weight, overweight, and obese college students living in the dorms and assess associations between fecal microbiome communities at the genus level and BMI after using principal components analysis (PCA) to reduce the data.
- Aim 3: To investigate the association of dietary intake with the fecal microbiome composition in healthy college students living in the dorms.
    - Purpose: To identify the dietary factors that correlate with the F/B ratio in healthy college students living in the dorms.

Definitions

- Gut Microbiome (plural of gut microbiota) - The community of bacteria inhabiting the gut that work symbiotically with the body.
- Gut Microbiota- The different bacteria inhabiting the gut that work symbiotically with the body.
- Fecal Microbiota- The bacteria present in the feces that are considered representative of the gut microbiota.
- Underweight- BMI<18.5 kg/m^2
- Normal weight- 18.5≤ BMI<25 kg/m^2
- Overweight- 25 $kg/m^2 \leq$ BMI $\leq$ 29.9 kg/m^2
- Obese- BMI $\geq$ 30 kg/m^2
- Prebiotic- Specialized plant fibers, non-digestible for humans, that are used to nourish good bacteria in the gut.
- Probiotics- Live bacteria often found in fermented dairy products that may improve digestive health.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

Obesity is a significant problem that is impacting society, leading to a considerable number of comorbidities such as hypertension, dyslipidemia, type 2 diabetes, heart disease, stroke, osteoarthritis, sleep apnea, and some cancers.[1,2] According to the Centers for Disease Control and Prevention, the prevalence of overweight and obesity among all demographic groups has continued to increase during the past three to four decades in the United States.[9,25-28] This increase is best described as the result of complex interactions between genetics, environment, socio-economic status, and diet, rather than simple imbalances in energy consumption and expenditure.[5]

Recent data suggest that the prevalence of obesity in 2011-2012 was 16.9% in youth (2-19 years old) and 34.9% in adults (20 years and older). These percentages are not significantly different from the NHANES 2003-2004 data, thereby demonstrating that the increase in obesity has leveled off.[4] In addition, NHANES data from 2011-2012 suggests that the prevalence of overweight individuals between the age of 12-19 years was 14.9%. This percentage is also not significantly different from the NHANES 2003-2004 data, thereby demonstrating that the increased percentage of overweight individuals has leveled off.[3,4] This age range includes the important transition from high school to college, a period that has been associated with weight gain.[10,11]

College Students

The transition from adolescence to adulthood (high school to college), is a time period that is filled with major behavioral, social, and lifestyle changes.[14] As these individuals move away from home and become more independent they often engage in unhealthy eating, reduced physical activity, and inadequate sleep habits.[1,7,8] In addition, college students face a large number of health problems such as the common cold, sexually transmitted diseases, development of chronic diseases, and alcohol abuse as they are exposed to new environmental and social pressures during this transition period.[9,29,30] These influential factors can have long term effects on weight and overall health such as remaining overweight/obese through adulthood, developing hypertension, Type II diabetes, heart disease, and other weight related comorbidities.[9] Therefore, identifying determinants of weight gain and dietary choices in this population are crucial for informing health programs in college for the prevention of obesity later in life.[1,6-8]

College Freshmen and Weight Issues

Many students entering college are exposed to the idea of the "freshmen 15" or the tendency for students to gain 15 pounds during their first year of college.[1,7-11] While this myth is still presented by the popular press, a vast amount of research refutes this finding, reporting that weight gain during freshmen year accounts for only 2.5-6 pounds on average and that this excess weight is the result of increased fat mass.[1,6-10] Findings from a study of 478 college students found that the greatest increase in weight occurred during freshman year, but that students continued to gain across the college years; men gained an average of 9 pounds while women gained an average of 7 pounds over a four

year college period.[11] Ultimately, these changes in weight status can result in a transition from a healthy weight into overweight or obese categories. In fact, it has been shown that the greatest increase in overweight and obesity is seen between the ages of 18-29 years, especially among those who reported having a college education.[6,25] While the weight gain issue in college students is not quite as drastic as that presented by the "freshmen 15" myth, there is still a pattern that has emerged suggesting that weight increases during freshmen year continue throughout college and may lead to overweight or obesity in adulthood. This weight gain pattern makes this a crucial time period to identify contributing factors and reduce the risk for obesity and related comorbidities.

College Students' Dietary Intakes

One of the primary factors that may contribute to weight gain in college freshmen is the change in dietary intake as students begin to make their own food-related decisions.[31,32] Such poor dietary choices may result in inadequate intakes that fail to meet dietary recommendations.[32,33] Previous research has reported that college students consumed diets with fewer nutrients and greater fat, sugar, and sodium intakes.[32,34,35] A number of studies have found that college diets are severely lacking in fruits, vegetables, dairy products, fiber, and healthy grains.[31,32,36-38]

Despite having increases in calorically dense snack foods, multiple studies have found that total energy consumption actually decreases after students begin college.[32,36,39-42] This decrease in total caloric intake also tends to correspond with a reduction in the variety of foods consumed and overall diet quality.[32,36,39-42] In a study of 54 female college freshmen followed during the first five months of college, an increase

in BMI was observed despite reductions in caloric intake; decreases in fruit, vegetable, meat, carbohydrate and dairy consumption; and increases in the proportion of calories from fat and alcohol.[39] These results demonstrate that despite a decreased caloric intake, having a diet with less variety and a greater than recommended intake of calories from fat, weight gain remains prevalent in this high-risk population. Mechanisms for this observed weight gain are not well defined and further research is needed to understand this phenomenon in a college setting.

The Gut Microbiome

The human body is home to microbial communities consisting of a total number of microorganisms that significantly outnumbers the total number of human cells by ten-fold.[43-45] The majority of microbes in the human body reside in the gut and play a large role in nutrient utilization, immune / inflammatory responses, and overall intestinal health.[43-47] The gut microbiome develops throughout life based on genetics, dietary intake and environmental exposures.[48-50] Prior to birth, it was originally thought that the gut was completely sterile,[48,51] but new research shows there is a placental microbiome that initiates gastrointestinal colonization prior to birth.[52] Despite these findings, colonization of the gut in utero is minimal in comparison to microbial exposures that occur during and after birth.[48-50]

The first stage of life is most crucial in the development of a healthy, diverse microbiome as it is the time when the microbiome is most fragile.[51] Differing exposures throughout this period help determine how the gut is colonized such as mode of delivery, location of birth (home vs. hospital), and whether infants were formula or breast-fed

received antibiotics.[46,48,51] As infants age throughout childhood and into adulthood, the gut microbiome reaches a state of greatest diversity and stability; however, established microbe communities during adulthood can be influenced by transient changes related to diet, weight status, and long-term antibiotic usage.[46,51,53] These exposures throughout life play a significant role in the development of the gut microbiome and, in turn, determine its diversity and how it functions in relation to both health and disease.[46,48,49,51,53,54]

The Gut Microbiome's Role in Health and Disease

The gut microbiome has emerged as having an important, formerly underappreciated role, in human gut physiology.[54,55] Functions of the gut microbiome include nutrient utilization, host immunity, and intestinal health which, depending on the prevalence of specific species, can impact health and influence disease risk.[44,46,47,50,51,53,56-58]

Nutrient utilization. The gut microbiome plays a key role in nutrient utilization by hydrolyzing and fermenting nutrients, such as complex polysaccharides/fiber, that cannot otherwise be broken down by human enzymes.[50,51,59-61] The breakdown of these nutrients by gut microbes increases the body's ability to harvest and utilize these otherwise unavailable sources of energy from the diet.[2,5,16,17,50,51,62-65] The wide variety of usable roducts that are produced from hydrolysis and fermentation of nutrients are not fully understood. However, specific byproducts have been associated with benefits including improved intestinal health, as well as detriments, such as increased fat mass and obesity.[16,50,63,65-68]

Hydrolysis of glycosides and glucuronides. In the human body, many polysaccharides cannot be fully digested by intestinal enzymes. A couple of these polysaccharides are glycosides (organic substances predominantly found in plants that consist of one sugar molecule and one non-sugar molecule) and glucuronides (a specific type of glycoside derived from glucuronic acid that can combine with toxic organic compounds leading to excretion).[69] Gut microbes can aid this process by hydrolyzing polysaccharides that contain glycosidic bonds resulting in the production of fermentable products and monosaccharides that can be absorbed by the body.[50,51,59,61] Intestinal microbes can also scavenge compounds that are excreted from the intestinal mucosa to produce a usable energy source when dietary intake does not provide adequate energy.[51,61] In both cases, the microbes in the gut are able to use glycoside hydrolysis in order to help the host (the human) produce and use energy more efficiently.

In addition to providing usable substrate to microbes and human hosts for energy utilization, the ability of these microbes to hydrolyze plant glycosides and glucuronides has been a focus of modern medicine to formulate medications for timed release at specific intestinal locations.[59,60,70] For instance, in a study performed in rabbits evaluating the laxatives senna and cascara, the glucose moiety of the aglycone component protected the product from becoming activated until it reaches the colon where microbes hydrolyzed the glycoside bond.[59,70] Similarly, the process of glucuronide hydrolysis is important in enterohepatic circulation and was therefore taken into account when creating drugs such as morphine, chloramphenicol, and phenylphthalein.[59,60] These specific medications require extensive enterohaptic circulation so they are retained in the body for an extended period of time, resulting in a prolonged therapeutic effect.[59,60]

Microbial hydrolysis of glycosides and glucuronides is only one way in which hosts benefit from these symbiotic relationships; the gut microbiota can also utilize other nondigestible compounds to produce usable products for the host.

Dietary fiber and production of short chain fatty acids (SCFAs). Complex carbohydrates, especially dietary fiber, have nondigestible components that are fermented by the gut microbiota in order to produce SCFAs, (fatty acids consisting of less than six carbons).[44,50,62-64,66-68] While there are many SCFAs that are useful to the body, the most prevalent are butyrate, proprionate, and acetate.[50,63,65-68] Butyrate is the preferred nutrient for colonocyte (epithelial cell in the colon) metabolism and development in order to promote healthy intestinal tissue. Proprionate is transported to the liver to be used in gluconeogenesis and regulate cholesterol synthesis. Acetate is an important energy source for peripheral tissues, such as the liver, where it is used for lipogenesis, cholesterol synthesis and is also the primary circulating SCFA in the blood.[50,66,67] SCFAs absorbed in the colon provide up to 10% of the energy used by humans and are used more effectively in those who consume high levels of dietary fiber.[50,71,72] While SCFAs are thought to provide an important source of energy, it has also been determined that excessive production of certain SCFAs may play a role in obesity.[50,64,65,68]

Although the majority of the current literature agrees that excessive production of some SCFAs can play a role in obesity, the literature is contradictory as to which SCFAs and microbial genera are most influential.[16,50,62,64,65,68] For instance, in an animal model where microbiota from lean and obese mice were transplanted into germ free mice, butyrate and acetate production was higher in the mice given the obese microbiota as

compared to the lean microbe recipients.[16] Similarly, in a study of obese and normal weight children, butyrate and proprionate were produced in higher amounts in obese subjects and were associated with gut dysbiosis/decreased microbial diversity when compared to their normal weight counterparts.[50,68] Another human study performed in obese and normal weight adults, determined that there was a greater concentration of SCFAs, specifically in proprionate, in the obese adults compared to their lean counterparts.[65] These results are in line with the child study regarding proprionate, but the adult study did not report significant differences in butyrate production.[50,65,68] Conversely, a third human study in which lean microbiota was transplanted into obese patients demonstrated an increased microbial diversity with an overall increase in butyrate production provided by the lean microbiota.[62] These results differ from other presented findings that demonstrated that butyrate production was associated with obesity and decreased microbial diversity.[62,64,65,68] This discrepancy is suggestive of the complex interactions that occur in the gut in relation to SCFA production, microbial diversity, and obesity and bring to light the need to account for differences in subject populations as well as methodological approaches. For instance, one study was carried out in children and did not involve any intervention, whereas the contrary findings were demonstrated in an adult population who underwent microbiome transplants.[62,68] The fragility of the microbiome in children and the complexities surrounding a microbiome transplant in adults who previously had a stable microbiome demonstrate the difficulty in truly comparing these studies.[16,46,51,53,62,68] In order to properly consider the role of SCFAs in obesity, further research is merited.

Energy harvesting. Another key function performed by the gut microbiota is energy harvesting, in which the microbiota utilize undigested carbohydrates (plant polyssachrides) and some proteins to produce energy that can be stored and used by the host body.[16,44,50] While the previous sections demonstrate similar concepts, this specific process takes nutrient utilization mechanisms one step further and focuses specifically on storing the energy produced by the gut microbiome. The microbiota can accomplish this by collectively expressing larger numbers of glycogen hydrolases than human gut cells, which allows them to digest larger quantities of glycogen with greater efficiency.[44,50,61] While energy harvesting is a very useful process in helping humans extract the most energy from dietary sources, certain microbial profiles have been associated with overly efficient energy harvesting abilities, which are thought to be one of many contributors in obesity.[2,5,15-17,44,50,61]

Inflammation. As the functions of the gut microbiota continue to be explored, it has been determined that microbial diversity can also play a large role in preventing or promoting low-grade inflammation in obesity, Type 2 Diabetes, atherosclerosis, and other metabolic disorders.[50,56,57,73-78] Chronic, systemic, low-grade inflammation seen in these disease states is possibly related to a condition known as metabolic endotoxemia.[50,74,75,77,78] Metabolic endotoxemia is a condition in which there is an increase in the endotoxin, lipopolyssachride (LPS) (a pro-inflammatory antigen derived from the cell walls of gram negative bacteria), circulating in the blood as compared to the low levels of LPS found in the circulation of healthy dividuals.[50,74,75,77,78]

There have been multiple studies carried out manipulating diet as well as the gut microbiome composition in order to determine the association between the gut microbiota and the low-grade inflammation seen in these metabolic disorders.[73-78] An animal study carried out in mice with a control group fed normal chow, a high fat (HF) fed group, and a HF-fed group plus prebiotic oligofructose (OFS) aimed to study the impact of LPS on inflammation and prevalence of fecal *Bifidobacterium* species.[75] The HF fed group had a significant decrease in many commensal gut microbiota, including Bifidobacteria, and had prevalent endotoxemia, increased intestinal permeability, and inflammation as compared to the control group that maintained a healthy gut microbiome.[75] The addition of OFS restored quantities of Bifidobacteria and resulted in lower endotoxin and inflammation levels equivalent to that of the control diet.[75] Studies carried out in humans strengthen and further demonstrate the association between the gut microbiome and the promotion and prevention of metabolic endotoxemia,[56,73,77,78] which plays a large role in the development of obesity and other metabolic disorders characterized by chronic, systemic, low-grade inflammation.[50,56,57,73-78] For instance, in a study carried out in a population of 12 healthy males that were provided with high fat meals while measuring plasma endotoxin levels, it was determined that low-grade endotoxemia may contribute to post-parandial inflammation and could be another contributor to the development of atherosclerosis.[77]

Immune response to gut pathogens. Intestinal health is largely determined by the composition of the gut microbiome which assists in protecting against enteric pathogen colonization, mediating immune system responses, and allowing the growth and

colonization of beneficial bacteria.[47,79-81] The adaptive (specific attack of foreign invaders following previous exposure) immune system responds to enteric pathogens by releasing immunoglobulin A (Sig A). The commensal gut microbiome has the ability to remain fairly stable despite microbial exposures from dietary intake and the environment. Exposure to pathogenic bacteria can alter the composition of gut microbiota, thereby influencing immune responses.[80-87] Innate immunity components (immunity present at birth that reacts quickly but lacks specificity) such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generally employed during infrequent inflammatory responses. When the body senses a serious assault these components promote oxidative stress[47,88,89] and antimicrobial peptides (AMP) are released. AMP help eliminate infections by enteric pathogens, but can also harm the commensal gut microbiota.[47,88-91] For individuals that have inflammatory bowel disease (IBD), this immune response is greatly elevated, which plays a role in damaging the intestine.[47,88,92] This demonstrates that, while immune responses are crucial in helping to prevent the colonization of unwanted pathogens, broad spectrum immune responses can be especially detrimental to the commensal bacteria.

Antibiotics. Antibiotics are used extensively in both clinical and agricultural settings to help treat and prevent infections. The overexposure to antibiotics has led to pathogen antibiotic resistance as well as pathogen resistance to immune responses.[85,93-96] One example of this resistance is demonstrated by pathogenic *E. coli* strains that have developed a specific SigA binding antigen that interferes with the immune response, thereby allowing this pathogen to populate space that was originally competitively

occupied by the commensal bacteria.[47,82,97] Overall, antibiotics, while useful in treating infections, have a significant effect on the composition of commensal gut microbiota which may persist long-term[47,87]; therefore, external efforts to restore the commensal gut microbiota may be extremely beneficial when targeting enteric pathogens.

Probiotics and prebiotics. Probiotics and prebiotics have been discussed as ways to help restore a healthy, commensal intestinal composition after antibiotic use and in relation to GI diseases such as IBD.[47,96,98,99] Probiotics, which have shown promise as therapeutic treatments for IBD, have had mixed effects on health due to the difficulty in adhering to the intestinal lumen alongside commensal microbes.[47,98,100] In fact, a recent study demonstrated that probiotic administration in healthy adults was undetectable in the feces in over half the population after two weeks and undetectable in essentially all individuals after 48 weeks.[47,100] Prebiotics such as oligofructose may help provide nutrients to help the commensal bacteria recolonize after antibiotic use. They can also be used alongside probiotics as a means to promote the colonization of the probiotic microbes.[47,96,99] The combination of prebiotics and probiotics, known as synbiotics, may also be a promising way to restore commensal gut microbiota and maintain intestinal health.[47,96,99,100]

The roles that immunity, antibiotics, probiotics, and prebiotics play in the prevention of enteric pathogen colonization and the restoration and maintenance of the commensal gut microbiota prove to be complex. Broad immune responses to infections or improper immune functioning can lead to diseases such as IBD. Similarly, excessive use of antibiotics can cause significant compositional changes in the gut microbiota from

which, the long term effects are less understood. Further research is needed in order to determine how to maximize the positive effects and minimize the detrimental effects of these processes on the commensal gut microbiome and subsequent intestinal health.

Factors that Influence the Gut Microbiome

Non-modifiable factors. Recently, research has begun to explore different factors that are associated with the gut microbiome. The primary non-modifiable factors that have been a focus of recent research include genetics, intestinal diseases, ethnicity/race, and age. The effect of host genetics on the gut microbiome has been studied in both humans (primarily twins)[15,101-105] and mice[103,106-110] and has demonstrated that there is at least some effect of the host genome on gut microbe communities. Additionally, there are studies that have related single host genes to differing gut microbiome profiles.[104,105,109,110] Further research must still be carried out in order to determine what the specific profile differences are, ensure whether the differences are based on the expression of the studied gene, or to determine whether there are secondary disease state factors that are altering the microbiome instead.[104,105,109,110] When focusing on intestinal diseases that are promoted by specific, non-modifiable genetic profiles, such as Crohn's Disease (CD),[111-113] Ulcerative Colitis (UC),[112,114] Irritable Bowel Syndrome (IBS),[115,116] and colon cancers,[114,117-119] the research is fairly new, but suggests that microbial diversity is decreased with slight differences between these diseases. Despite these findings, specific relationships have been inconsistent and require further research to determine the specific microbiome profiles related to each intestinal disease and the

roles they may play in disease-related symptoms to continue exploring whether the specific gene expressions or secondary disease factors are altering the microbiome.[111-119]

Ethnicity/race and age are two non-modifiable factors that had a smaller focus in the research thus far. For instance, studies focusing on race/ethnicity in relation to the gut microbiome have ranged from comparing the gut microbiome of smaller ethnic groups within China[120]; Belgians with Japanese[121]; and both Hispanic and non-Hispanic African Americans, Native Americans, and Caucasians in the United States.[122] These and other ethnic populations that have been explored suggest only minor racial and ethnic differences, although the characterizations of each ethnicity/race have only been defined in a single study or are inconsistent between studies.[120,120-125] In order to verify and strengthen the findings of these studies, further studies need to be carried out between ethnicities/races that have already been explored and confounders such as lifestyle factors that can be tightly connected with race/ethnicity should also be considered in order to determine whether ethnicity/race is truly the cause of the differences. The relationship between age and the microbiome has demonstrated a variety of inconsistent findings in relation to which microbial communities are most abundant in different age groups.[124,126,127,127-133] Although differences in microbial composition are inconsistent, the majority of research suggests that microbial diversity and function increase rapidly throughout infancy, with more subtle shifts in abundance between childhood/adolescence and adulthood, and decreased diversity in the elderly.[124,126,127,127-133] In order to verify and strengthen the findings of these studies, further studies need to be carried out between ethnicities/races that have already been explored while considering confounders, such as lifestyle factors, in order to determine whether ethnicity/race is truly the cause of

the differences in microbial profiles. Additionally, based on the contradictions and minimal amount of research focused on age and the gut microbiome, further research must be carried out within and between age groups, especially the least studied age range of adolescences, in order to determine the general microbial profiles related to age groups so that health interventions can take these general profile abundances into considerations for optimum results.

Modifiable factors. There are also modifiable factors that have become a recent focus of gut microbiome research and are of special importance as they can actively shift or promote certain gut microbiome profiles. Modifiable factors that have been a focus of gut microbiome research include mode of birth, exercise, and diet. Mode of birth provides the first microbial exposure other than the minimal exposure in utero.[48-50,52] Studies comparing vaginal and cesarean deliveries show conflicting results with some demonstrating no differences in bacterial richness and diversity and others demonstrating significantly lower diversity in infants delivered by cesarean section.[134-140] Based on these findings, more research needs to be carried out to determine how such differences may influence health in later life.

Exercise and the gut microbiome has become an increased area of focus in recent years and is another modifiable factor. Current research in mice with a range of exercise intensities has consistently demonstrated that exercise may alter the microbiome by increasing microbial diversity.[141-145] Additionally, a recent human study has been carried out exploring this association further.[146] This study recruited 40 male elite rugby players who performed intense physical activity and 46 healthy, male controls of similar size

(BMI). The study demonstrated that the elite athletes had significantly more diverse gut microbiota compared to the controls, similar to the results seen in the previous mice studies.[146] Further research is needed to study the moderating effects of diet and exercise, as well as the type of exercise, on gut microbial communities.

Diet and the gut microbiome. The link between dietary intake and obesity has prompted the study of possible connections with the microbiome.[15,147,148] Results from studies focusing on the association between diet and the gut microbiome vary widely, but data suggest that certain dietary interventions can be associated with shifts in microbial proportions present in the colon.[22-24,147-156] There are three studies that demonstrated some of the most significant findings, especially in relation to how the diet-gut microbiome association plays a role in obesity, through the use of dietary comparisons and dietary interventions.[22-24]

The first study compared the gut microbiome of children in Europe (Eu) on a Western diet high in sugar, animal fat, and calorically-dense foods) and children in Burkina Faso (BF) on a Neolithic diet (low in fat and animal protein; rich in starch, fiber, and plant Polysaccharides).[22] In order to compare changes in the gut microbiome, this study focused primarily on the ratio of Firmicutes to Bacteroidetes, the two most predominant microbial phyla found in the gut.[2,5,18,20-22] The results of the study demonstrated that Eu children had lower overall microbial diversity and a high Firmicutes/Bacteroidetes ratio, whereas the BF children had a rich microbial diversity, lower Firmicutes/Bacteroidetes ratio, and higher prevalence of SCFA-producing microbes.[22] Similar to findings that a Western diet has been associated with obesity,[157]

this study demonstrated that such a diet may promote a less healthy microbiome compared to diets of the BF children.

Dietary interventions have been successfully implemented to alter the gut microbiome.[23,24] One study of lean and obese healthy adults evaluated the influence of increased caloric consumption on gut microbial changes by incrementally increasing total calories, with consistent macronutrient distributions, until consumption reached 2400 and 3400 kcals.[23] The results of this study demonstrated that greater caloric consumption increased the Firmicutes/Bacteroidetes ratio as well as energy harvesting.[23] Another study evaluating the effects of low fat-high fiber and high fat-low fiber diets on microbiota proportions found that the Firmicutes/Bacteroidetes ratio was low in subjects consuming a low fat-high fiber diet and high in subjects consuming a high fat-low fiber diet.[24]

These three studies demonstrated that less healthy dietary components, e.g. high fat, low fiber, and very high caloric intake appear to be negatively associated with a healthy gut microbiome.[22-24] There are additional studies that have been carried out focusing on other dietary patterns and components such as low carbohydrate diets, high protein diets, vegan diets, carnivorous diets, and diets including artificial sweeteners.[147,148,151,153,155] These studies have demonstrated a wide variety of shifts in the gut microbiome, which strengthen the concept of diet playing a large role in the composition of the gut microbiome. Although they have strengthened the general association between diet and the gut microbiome, the wide range of dietary components that have been researched and have resulted in varied shifts to the gut microbiome make it difficult to specify or draw conclusions on the health effects associated with these

microbiome changes.[147,148,151,153,155] Therefore, further research needs to be carried out to reinforce the specific interactions between these additional dietary components and the gut microbiome and how the effected microbiomes are related to other health outcomes such as obesity. Furthermore, the majority of the research in this area has not focused on the long term changes of the gut microbiome in relation to acute dietary changes and the role that this plays on obesity and weight status.[22-24,147-156] Therefore these concepts need to be explored further to strengthen the ties between diet, the gut microbiome, and obesity or weight change.

Obesity and the Gut Microbiome

Obesity was previously viewed as the result of simple imbalances in energy consumption, but more recently it has been considered as the consequence of complex interactions between genetics, environment, socio-economic status, and diet.[2,5,65] In addition, gut microbiota have become increasingly recognized for their crucial role in metabolic functioning and subsequent states of health and disease.[2] Microbiome data suggest that obese individuals have significantly different gut microbiomes than their lean counterparts.[15-18,20-22,65] Data also suggest that gut microbial proportions may shift with weight changes.[18,20,21]

Currently, it remains difficult to elucidate the full association between dietary intake and the gut microbiota. For instance, it has been found that obese individuals have an overall less diverse microbiome than their lean counterparts.[15,16,50,62,64,65,68] This was demonstrated in studies where the microbiome of diet induced obese (DIO) mice, was transplanted into germ-free lean mice, which resulted in weight gain as compared to those

who received transplants from lean mice.[16,17] When analyzing the differences in the composition of the microbiome, decreased microbial diversity was a primary characteristic in the mice that received transplants from DIO mice.[16,17] These results have also been seen indirectly in human studies focusing on diet and the gut microbiome, such as the Burkina Faso study, in which individuals consuming diets associated with obesity, such as a Western diet, had a significantly lower microbial diversity than those consuming Neolithic diets.[22]

In addition to microbial diversity, obesity has also been associated with an altered composition of two major microbial phyla. The majority of current research demonstrates that obese individuals have an increase in the Firmicutes/Bacteroidetes ratio as compared to healthy, lean counterparts.[2,5,18] For example, in a study comparing 12 obese individuals to lean controls, the obese individuals had a higher overall Firmicutes/Bacteroidetes ratio. Furthermore, when the obese individuals were placed on a long term restricted, weight loss diet, their microbiome began to shift toward that of their lean counterparts as they lost weight.[18] Some studies have contradicted this finding, demonstrating no association between the Firmicutes/Bacteroidetes ratio and weight[20,21] or observing a decrease in the Firmicutes/Bacteroidetes ratio.[65] Other studies have identified differences at the genus and species level. In one study, *Bacteroides and Prevotella* (two genus level classifications within the phyla Bacteroidetes) were lower in obese individuals, whereas *Lactobacillus*, *Leuconostoc*, and *Pediococcus* (three genus level classifications within the phyla Firmicutes) were higher in obese individuals prior to gastric bypass-induced weight loss.[21] In another study, *Roseburia* (genus within the Firmicutes phyla) and *Eubacterium rectale* (species within the Firmicutes phyla)

increased with weight gain and decreased with weight loss.[20] Based on these findings, it appears that, even if the microbiome is not influenced at the phylum level (Firmicutes/Bacteroidetes ratio), changes at specific genus levels still follow a similar pattern of increasing bacteria within the Firmicutes phyla and decreasing bacteria within the Bacteroidetes phyla. Given these findings, the Firmicutes phyla should be a specific area of focus as the results most consistently suggest that increases in Firmicutes or specific bacteria within this phylum are associated with obesity or weight gain.[2,5,18,20-22]

Recently studies have explored potential mechanisms for higher levels of Firmicutes with obesity/weight gain.[2,5,16-18] While research on the gut microbiota and the previously discussed mechanism of energy harvesting in relation to obesity is fairly new, the current research is fairly unanimous in stating that increased energy harvesting is associated with greater fat stores in obese subjects as compared to lean individuals.[2,5,16,17,50] An example of this is demonstrated in animal studies in which intestinal microbiota from diet-induced obese mice were transplanted into lean germ-free mice.[16,17] The results of this study demonstrated that there was greater total body fat deposition in the germ-free mice receiving transplants from the obese mice.[16,17] These data suggested that the increased fat deposition was potentially associated with increases in Firmicutes.[16,17] Human studies have also demonstrated that the proportion of Firmicutes is elevated in obese subjects, which may be associated with increased fat deposition.[2,5,18] The current research strongly points toward an increase in Firmicutes in the gut microbiome being a primary cause of increased fat deposition and, in turn, playing a significant role in obesity. More research must be done on a more specific genus level in order to identify specific bacteria within the phyla that are most

responsible for promoting increased fat deposition and to understand the discrepancies in the literature.

Summary

Obesity is an ever growing problem that has significant impacts on the health of our society. This disease not only reduces the quality of life but also leads to a wide range of comorbidities including hypertension, dyslipidemia, type 2 diabetes, heart disease, stroke, osteoarthritis, sleep apnea, and some cancers.[1,2,9,25-28] The transition between adolescence and adulthood that occurs during college is a crucial time period during which children are influenced by a wide range of behavioral, environmental, and social factors. Poor health behaviors acquired during this time lead to weight gain and are often carried into adulthood.[1,7-9,14,29,30] Therefore, this important period in life represents a crucial time for interventions to reduce the risk for obesity later in life. The gut microbiome has emerged as a key player in health functions including, nutrient utilization, immunity, and inflammation.[44,46,47,50,51,53,56-58] While current evidence suggests the importance of the gut microbiome in health, its role in relation to dietary intake and obesity has yet to be evaluated in adolescents as they transition to college.

CHAPTER 3

METHODS

Participants/Study Design

Subject Selection and Recruitment

Subjects were considered eligible for this study if they were healthy college students living in the selected dorms, spoke English, and were at least 18 years old. The study cohort was recruited from a larger study aiming to understand the impact of social networks on dietary intake, physical activity, and weight gain among Arizona State University students living in two representative dormitories. Subjects were excluded from the study if they had any history of eating disorders, malabsorption diseases, HIV infection, high blood pressure, diabetes, or were taking probiotics, antibiotics, or antifungals within the last two to three months.

A sample size of 90 subjects participated in this study. Prior studies showing significant correlations between variables such as fiber, obesity, and F/B phyla ratios have had varying sample sizes. For instance, a previous cross-sectional analysis of 30 children found significant correlations between F/B phyla ratios and dietary fiber intake.[22] Using a slightly larger sample size of 35, significant correlations between weight and the F/B phyla ratios (measured using the ratio of median $\log_{10}$cells in each phyla/g feces) were observed in obese patients.[65] The proposed sample size was chosen based on the findings above and the expectation that, when exploring a new population, a

larger cohort would be needed to evaluate significant differences in dietary intake and BMI in relation to the gut microbiome.

Study Design

Data were collected at the time of recruitment and at up to two time points during a one week period. Each subject was either provided with a fecal sample collection kit (Commode Specimen Collection Kit, Fisher Scientific, Anthem, AZ) for stool collection in the dormitory or directions to the School of Nutrition and Health Promotion clinical facility for on-site collection. Subjects provided one fecal sample during the Fall 2014 or Spring 2015 semester. Participants that preferred dorm ollections were asked to return their sample to the clinical lab or request that study staff pick up their sample. All samples were delivered or picked up within 24 hours. This study was approved by the Arizona State University Institutional Review Board (See Appendix A). **Figure 1** summarizes the flow of the study.

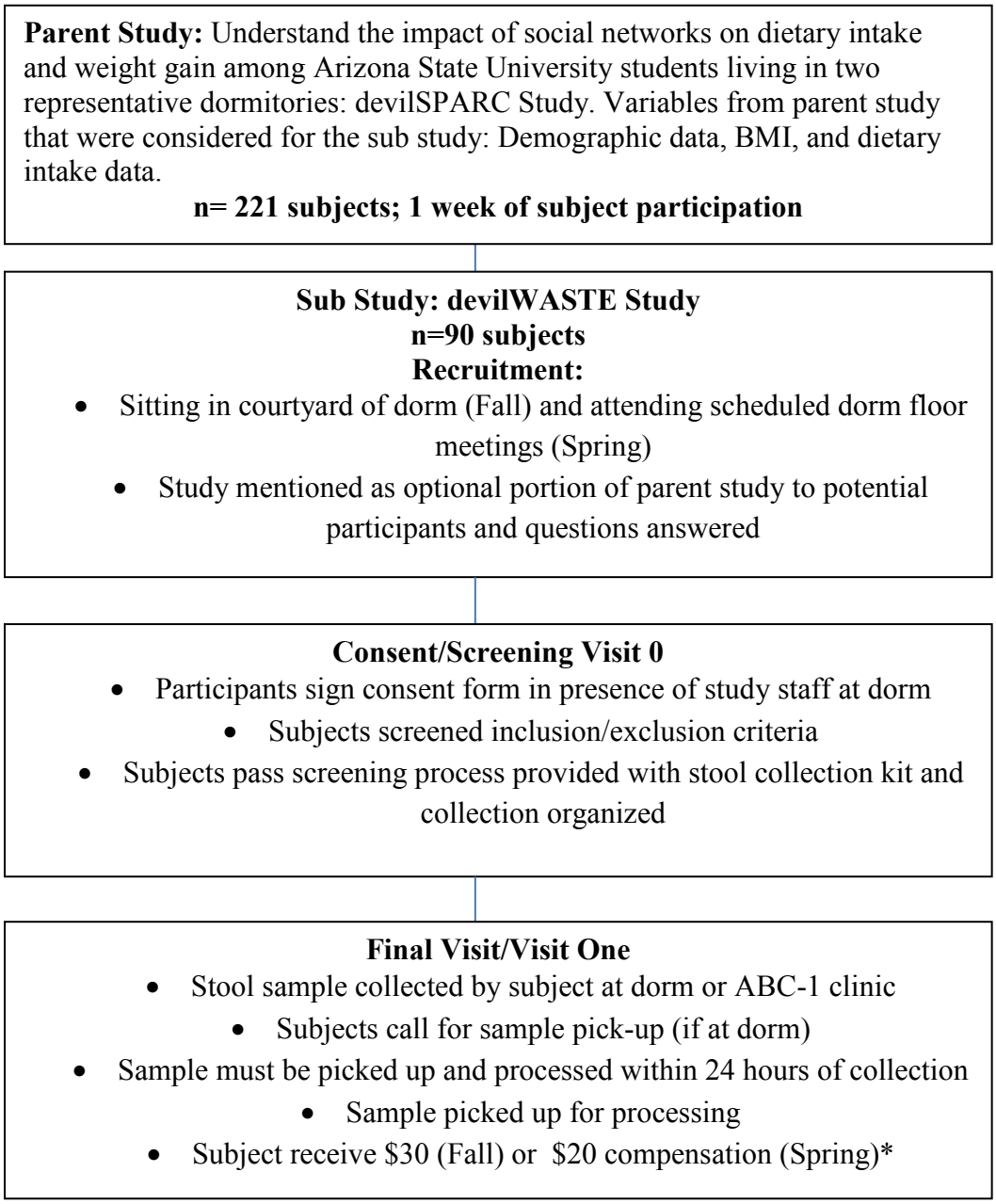

Figure 1: *Study design flow chart. *Compensation varied by semester to explore participants' willingness to participate based on compensation for future studies.*

Measures

Demographic Data

Demographic data such as gender, age, and race/ethnicity were collected at entry into the parent study through a self-report, computer-based survey. This information was used to determine the diversity of the participants.

Body Mass Index

Height and weight were collected at entry into the parent study by trained personnel and BMI was calculated as (weight in kg)/ (height in m)^2. Height and weight were measured up to three times in order to obtain two values within 0.5 cm and 0.5 kg, respectively, which were then averaged. Each subject was asked to remove their shoes and jackets, empty their pockets and ensure that their hair did not interfere with the measurement equipment. Weight was assessed with a Seca scale and height was measured using a Stadiometer in order to assure consistent collection of anthropometric measures. BMI values were separated into four categories based on the CDC cutoffs: BMI values <18.5 were considered underweight, BMI values greater than or equal to 18.5 and less than 25 kg/m^2 were considered normal weight, BMI values greater than or equal to 25 kg/m^2 and less than or equal to 29.9 kg/m^2 were considered overweight, and BMI values ≥30 kg/m^2 were considered obese.[158]

Dietary Intake

Participants completed up to three ASA24 24-hour dietary recalls (including one weekend and/or one or more week days). The mean daily nutrient intakes were calculated from a range of one to three dietary recalls per participant that fell within the caloric cutoffs (with a maximum of one weekend day and a range of zero to three week days) and were energy adjusted based on the mean caloric intake of the study population.[159] This method adjusts for variation in intake and reduces confounding related to total energy intake. The 24-hour dietary recalls were completed using the National Cancer Institute's ASA24 24-hour automated dietary recall software.[160] Mean intake for all major macronutrients and micronutrients were provided and compared to DRIs provided from the Institute of Medicine DRI Summary Table[161] and DRI Estimated Average Requirement Table.[162] The analyses involving dietary intake utilized total energy intake in kilocalories (kcals) and carbohydrate, protein, total fat, saturated fat, total fiber, and added sugar intake measured in grams (g).

The Gut Microbiome- Fecal Samples

The Firmicutes/Bacteroidetes gut microbiome phyla ratio, as well as the overall gut microbiome characterization at the phyla, family, and genus levels were the primary dependent variables that were collected and utilized specifically for this study. Fecal samples were collected and processed per the protocols outlined by the National Institutes of Health Human Microbiome Project (HMP) (commonfund.nih.gov/hmp/). The specific instructions for both stool collection and the lab processing procedures are outlined in the Stool Specimen Collection Study Protocol Procedures for ASU's School of Nutrition and

Health Promotion (SNHP) research facility (See Appendix D). The supplies required for the patient stool sample collection were as follows: one Commode Specimen Collection System that included a sealable plastic collection container with tightly fitting, snap-on (leak resistant) lid and collection frame, one Ziploc bag, one small bag liner for collection bowl, one pair of latex or nitrile gloves, instant ice pack(s) and one small cooler for transport. All samples were delivered to the clinical facility within 24 hours of collection.

Fecal microbial communities were assessed at the Biodesign Institute at Arizona State University in Tempe, AZ. Microbial genomic DNA was extracted from fecal samples using the PowerSoil DNA isolation kit as described by the manufacturer (MoBio Laboratories Ltd, Carlsbad, CA). The samples were homogenized using a beadbeater (BioSpec, Bartlesville, OK). Bacterial 16S rRNA gene sequences from each sample were amplified in triplicate PCRs and performed in 96-well plates. Barcoded universal primers 515F and 806R; http://www.earthmicrobiome.org/emp-standard-protocols/16s/,[163] containing Illumina adapter sequences which target the highly conserved V4 region were used to amplify the microbial sequences from individual samples. PCR reactions, amplicon cleaning and quantification were performed as outlined.[163] Equimolar ratios of amplicons from individual samples were pooled together before sequencing on the Illumina platform at Arizona State University's DNASU Core Facility (Illumina MiSeq instrument, Illumina, Inc., San Diego, CA).

Statistical Analysis

JMP and SPSS statistical software[164,165] packages were used to carry out all analyses. Prior to running analyses, all data were organized and cleaned by removing outliers (>3 SD from the mean) and participants who failed the screening process or dropped out prior to fecal sample collection. All data were tested for normality using the Kolmogorov–Smirnov test (in lieu of a population size >50) and $p>0.05$ was considered normally distributed. All data were considered significant at $p<0.05$. Additional exclusions for data analyses included subjects not meeting the minimum number of diet recalls (at least one recall), and any subjects with recalls that were >5000 or <500 kcals/day (cutoffs to minimize under and over reporting) were excluded from analyses, as previously described.[166-171] QIIME software [172] was used to clean raw Illumina microbial data by removing short and long sequences (truncated sequence should be > 75 bases long), sequences with primer mismatches, uncorrectable barcodes and ambiguous bases. A closed reference Greengenes database was used to pick operational taxonomic units (OTUs) and assign taxonomy.

The fecal microbial data were expressed as mean $\pm$ SD or median (interquartile range) of microbiota frequencies/proportions at the phyla level based on the normality on the data. The BMI data were expressed both continuously and categorically (underweight, normal weight, overweight, and obese). The diet data included total caloric intake (expressed as kcals), macronutrient intakes (carbohydrates, fat, saturated fat, and protein, sugar, dietary fiber), vitamin intakes (vitamins A, B6, B12, C, D, E, and K) and macromineral intakes (calcium, magnesium, phosphorus, potassium, and sodium).

Principal components analyses (PCA) were used to reduce the dimensionality of the microbial data for regression analyses. Assumptions required to run PCA included: 1) normality of the variable data (the data was transformed if necessary to fit the normality assumption), 2) incomplete/missing data removed, and 3) outliers excluded from the final data set. Once all assumptions were met, the PCA model was run and the data sets were reduced into components equal to the number of variables tested. Components explaining the variation among microbial data components were then organized from most to least variance explained. PCA was performed with the genus level microbial data (part of Aim 2; run once with the genus level microbial data from all subjects and once with outliers removed).

Multivariate and linear regression analyses were completed using eigenvectors for the PCA components. Components that explained the greatest variation in microbial proportions were retained for these models. Four a priori assumptions were required to run this model: 1) variables were normally distributed, 2) there was a linear relationship between independent and dependent variable(s), 3) error variance was the same across all levels of the independent variable, and 4) the independent variables were uncorrelated (collinearity). Independent variables were transformed as necessary to ensure that model residuals were normally distributed.

The Wilcoxon-Kruskal Wallis analysis was carried out with the F/B ratio and categorical BMI data (Aim 2). Two a priori assumptions were required to run this non-parametric model: 1) the samples were independent of one another and 2) there were two or more categorical groups. This non-parametric test was used as the data were not

normally distributed and there were four BMI category groups, from which the F/B ratios were compared to identify group differences.

Spearman's correlation analyses were carried out with the F/B ratio data and the continuous dietary intake data for energy intake (kcals), and the continuous energy adjusted data for carbohydrate (g), protein (g), total fat (g), saturated fat (g), dietary fiber (g), and added sugar (g) (aim 3). Two a priori assumptions were required to run this non-parametric model: 1) the samples were independent of one another and 2) the variables were continuous. This non-parametric test was used as the data were not normally distributed and both variables were continuous.

CHAPTER 4

DATA AND RESULTS

Descriptive Characteristics

One hundred and twelve college students living in two Arizona State University dorms provided informed consent for this study. After screening for eligibility criteria, 10 participants were disqualified for not meeting one or more of the exclusion criteria, resulting in a sample size of 102 at enrollment. Of these 102 enrolled participants, 12 participants dropped out of the study, leaving 90 total participants who provided fecal samples and 75 participants who also provided eligible diet data. A recruitment diagram is provided in Figure 2.

Of the 90 college students who participated, 35.6% (n=32) of the students were recruited in the fall of 2014 from one dorm. The remaining 64.4% (n=58) of participating students were from dorm two and recruited in the spring of 2015. Subject characteristics are included in Table 1. The participants were racially and ethnically diverse with the majority of participants self-identifying as either Hispanic/Latino/Spanish (31.1%, n=28), non-Hispanic, White (41.1%, n=37), non-Hispanic, Black (7.8%, n=7) and non-Hispanic, Asian (10%, n=9).

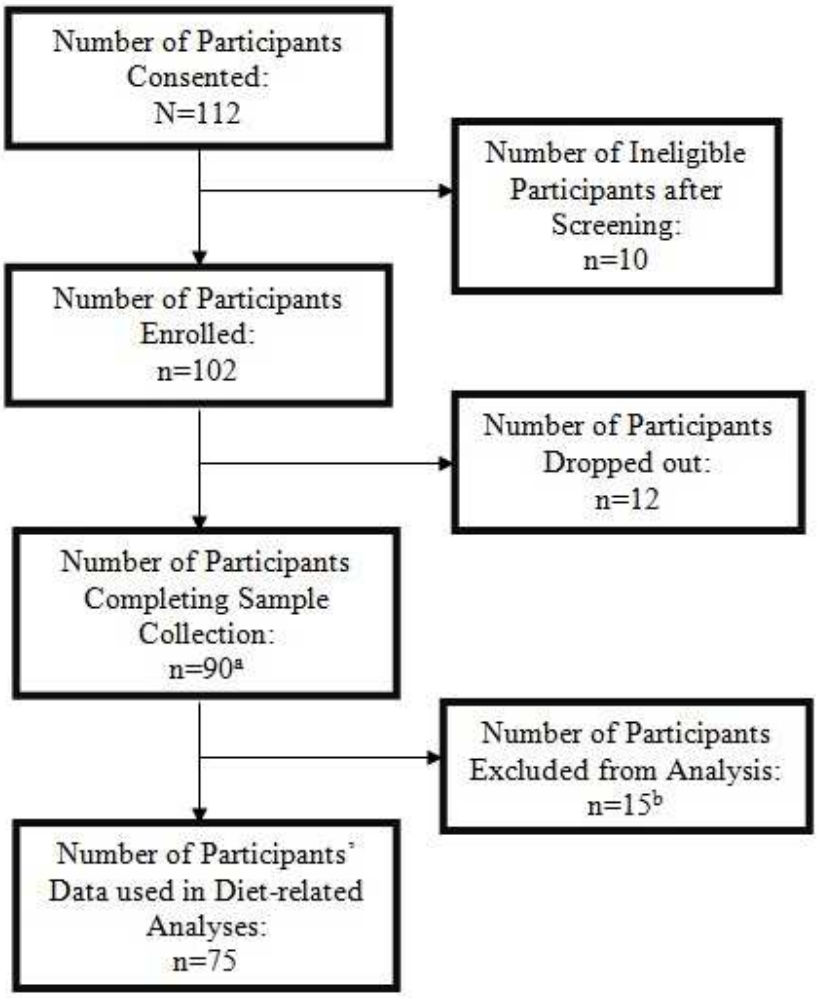

Figure 2. *Participant recruitment diagram.* [a]*All subjects completing the fecal sample collection were included in subject characteristic data and analyses that did not involve dietary intake data.* [b]*Rationale for excluding participant data from analyses: 15 participants did not have a minimum of one diet recall that fell within the 500-5000 kcal cutoffs.*

Table 1. Characteristics of study participants (n=90)	
Characteristic	*Mean±s.d.*[a]
Gender, % (n)	
Male	44.4 (40)
Female	54.4 (49)
Transgender	1.1 (1)
Age, y	18.57 ± 0.77
Race/Ethnicity, % (n)	
Hispanic, Latino, or Spanish origin	31.1 (28)
White (Non-Hispanic)	41.1 (37)
Black/African American (Non-Hispanic)	7.8 (7)
American Indian/Alaskan Native	1.1 (1)
Asian	10 (9)
Mixed	8.9 (8)
BMI, kg/m^2	24.37 ± 5.31
Underweight, % (n)	5.6 (5)
Normal Weight, % (n)	57.8 (52)
Overweight, % (n)	23.3 (21)
Obese, % (n)	13.3 (12)

Abbreviations: BMI, body mass index. [a]Mean± standard deviation (s.d.) or as otherwise specified.

Dietary Intake

All nutrient intake values were energy-adjusted based on the mean caloric intake (1684 ± 947 kcals). Mean daily dietary intake is provided in Table 2. The average dietary intake of this group of students was fairly well balanced on a macronutrient level and fell within the DRI ranges for protein (20% of kcals) and carbohydrates (50% of kcals), although the average fat intake was much higher than the recommended range (40% of kcals). Average dietary vitamin intake did not meet the DRI levels for vitamins A, D, E and C, but did meet the recommended intake for vitamins B6, B12, and K. Lastly, the average dietary mineral intake for these students did not meet the DRI for many minerals (including potassium, calcium, and magnesium), although they greatly exceeded the DRIs for both sodium and phosphorous.

Table 2.	Dietary nutrient intake (n=75)		
Nutrients	*Mean±S.D. or Median (IQR)*	*DRI*[b]*	*Range*
Energy Intake, kcals	1519 (947)	N/A[c]	639-3720
Macronutrients[a]			
Protein, g	76.6 (0.8)	10-35%[d]	74.6-80.8
Carbohydrate, g	188.0 (1.1)	45-65%[d]	184.5-191.4
Added Sugar, g	78.6 (0.9)	Limited[c]	76.1-83.1
Total Fat, g	68.9±1.0	20-35%[d]	66.1-71.7
Saturated Fat, g	23.2 (1.2)	Limited[c]	21.1-26.9
Fiber, g	12.4 (1.3)	38; 25[e]	10.8-15.7
Vitamins[a]			
Vitamin C, mg	68.4(0.5)	90; 75[e]	67.6-76.6
Vitamin B6, mg	2.3 (0.3)	1.3	1.4-10.7
Vitamin B12, mcg	4.7 (0.9)	2.4	3.3-10.5
Vitamin K, mcg	101.0 (1.0)	120; 90[e]	100.4-105.7
Vitamin D, mcg	3.3 (1.2)	5	1.6-6.6
Vitamin A, mcg	325.8 (1.2)	900; 700[e]	323.5-330.1
Vitamin E, mg	7.9 (0.2)	15	7.1-16.4
Minerals[a]			
Calcium, mg	799.3±1.0	1000	796.4-802.2
Magnesium, mg	221.6 (1.1)	400; 310[e]	219.8-225.1
Phosphorous, mg	1130.8±1.0	700	1128.2-1134.0
Potassium, mg	1882.7±1.0	4700	1879.5-1885.0
Sodium, mg	2831.4 (1.2)	1500	2829.0-2834.7

[a]Adjusted for energy intake.
[b]Dietary Reference Intakes reported as intake value per day.
[c] General population DRI values have not been provided; kcals require individual calculations where sugar and saturated fat recommendations are to limit as much as possible with added sugars not exceeding 25% of total dietary intake
[d]Values represented as Acceptable Macronutrient Distribution Ranges (AMDR)
[e]Values represented as the requirements for adult males; requirements for adult females
*DRIs provided from the Institute of Medicine DRI Summary Table[161] and DRI Estimated Average Requirement Table.[162]

Fecal Microbiome

A diverse range of fecal microbes were observed with sequences from 20 phyla (Figure 3). Phyla proportions varied by participant with the four most abundant being Bacteroidetes (57.2 ± 11.4%), Firmicutes (24.3 ± 9.9%), Proteobacteria (6.0± 3.9%), and microbes that have not yet been assigned a phyla classification ("unassigned," 11.8 ± 5.4%). At the family level, 134 unique groups were identified with 12 families accounting for the greatest proportion (97.1%) of the community (Figure 4). Almost half of these prominent families were from the phylum Firmicutes (Erysipelotrichaceae, Ruminococcaceae, Lachnospiraceae, Clostridiaceae and "unassigned" with proportions of 2.0 ± 2.7%, 12.4 ± 8.2%, 6.9 ± 4.4%, 0.7 ± 0.6% and 1.2 ± 0.8%, respectively). A total of four families were from the phylum Bacteroidetes (Rikenellaceae, Prevotellaceae, Porphyromonadaceae and Bacteroidaceae with proportions of 17.0 ± 11.6%, 1.2 ± 1.9%, 10.5 ± 5.9% and 27.5 ± 13.4%, respectively). Pasteurellaceae (0.7 ± 2.3%) and Alcaligenaceae (5.2 ± 3.1%) were from the phlyum Proteobacteria. An additional 11.8 ± 5.4% of the variation at the family level was explained by "unassigned" microbial groups.

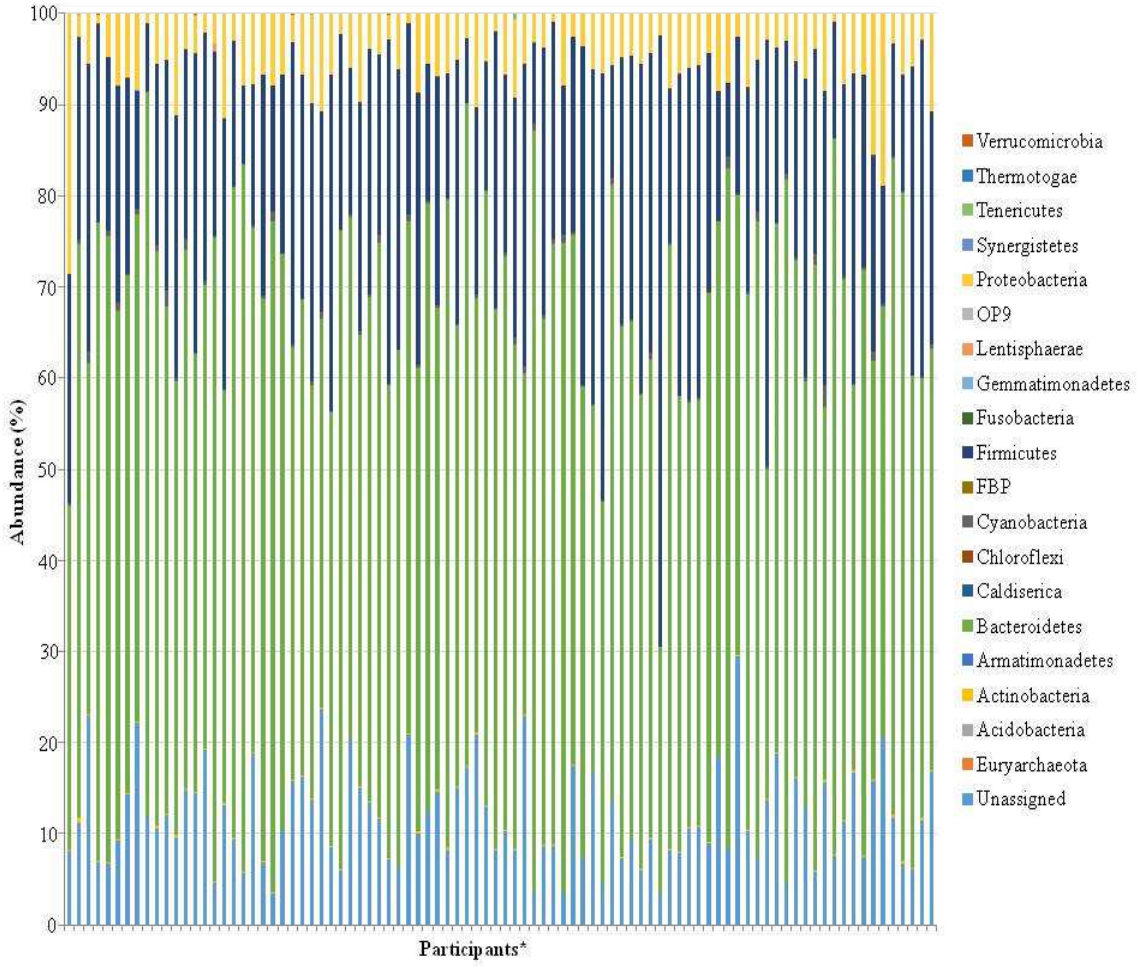

Figure 3. *Phyla level classification of the gut microbiome of college students living in the dorms. *Each bar represents one participant (N=90).*

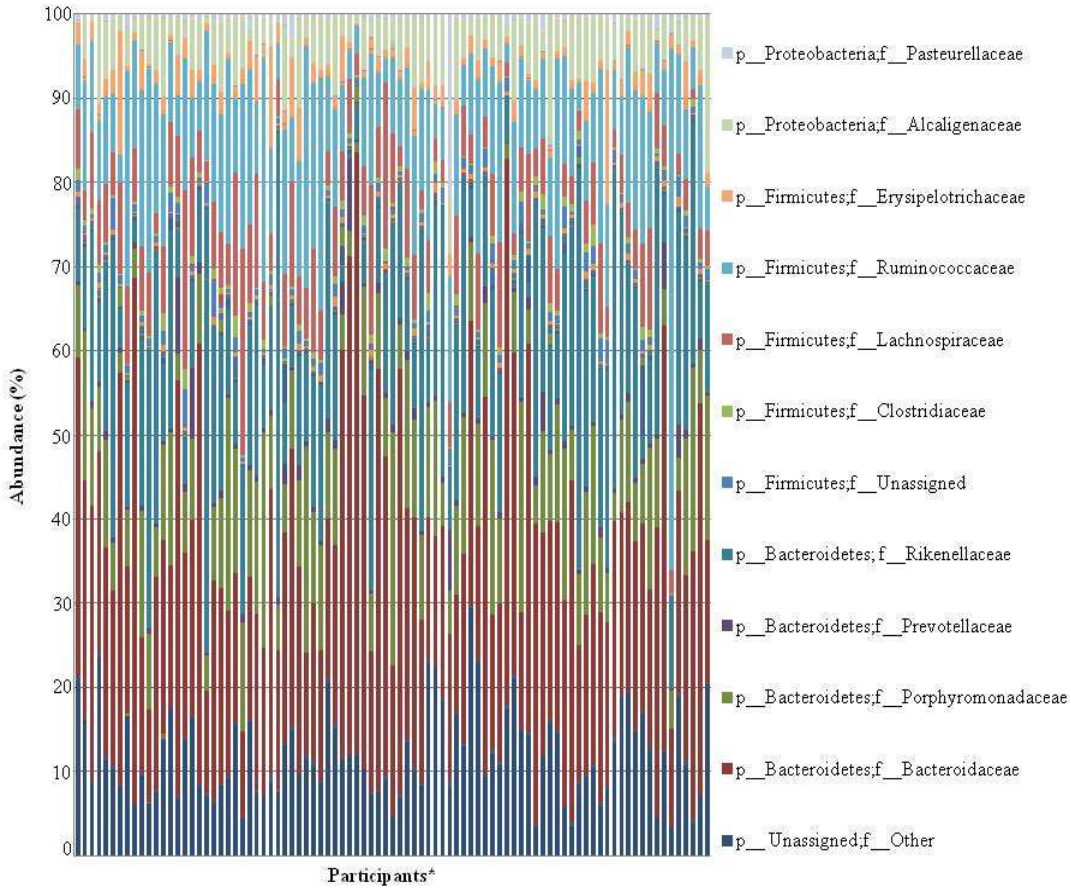

Figure 4. *Family level classification of the gut microbiome of college students living in the dorms. *Each bar represents one participant (N=90); the most abundant family classifications are presented in the legend with p__ representing phylum and f__ representing family level classifications. Only the most abundant families are represented in the legend (97.1% of the total community).*

BMI and the Gut Microbiome

Phyla level classifications by participant BMI category are visualized in Figure 5. The mean and standard deviations of the F/B ratio for each BMI category are represented in Figure 6 (Underweight: 0.4 ± 0.2, Normal: 0.5 ± 0.4, Overweight: 0.5 ± 0.2, and Obese: 0.4 ± 0.1). No significant differences were observed in the F/B ratios when comparing BMI groups using the Wilcoxon-Kruskal Wallis test (mean BMI values by group; Underweight: 17.8 ± 0.6 kg/m^2, Normal: 21.7 ± 1.7 kg/m^2, Overweight: 26.7 ± 1.5 kg/m^2, and Obese: 34.6 ± 5.7 kg/m^2; $p=0.445$).

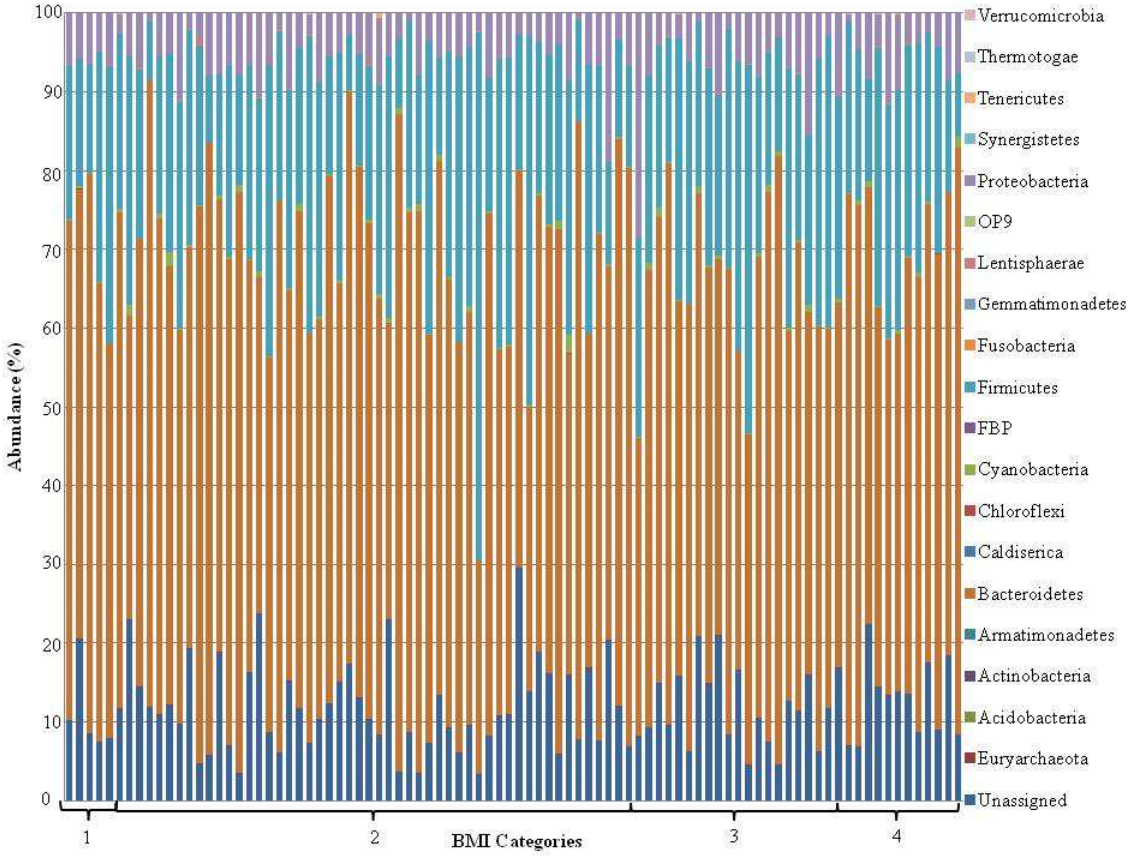

Figure 5. *Phyla level classification of the gut microbiome of healthy college students living in the dorms by BMI categories. Each bar represents one participant (N=90). Bars in the bracket section labeled 1 represent underweight BMI participants (BMI<18.5 kg/m^2; n=5), 2 is normal weight BMI participants (18.5≤BMI<25 kg/m^2; n=52), 3 is overweight BMI participants (25 kg/m^2 ≤ BMI ≤ 29.9 kg/m^2; n=21), and 4 is obese BMI participants (BMI ≥ 30 kg/m^2; n=12).*

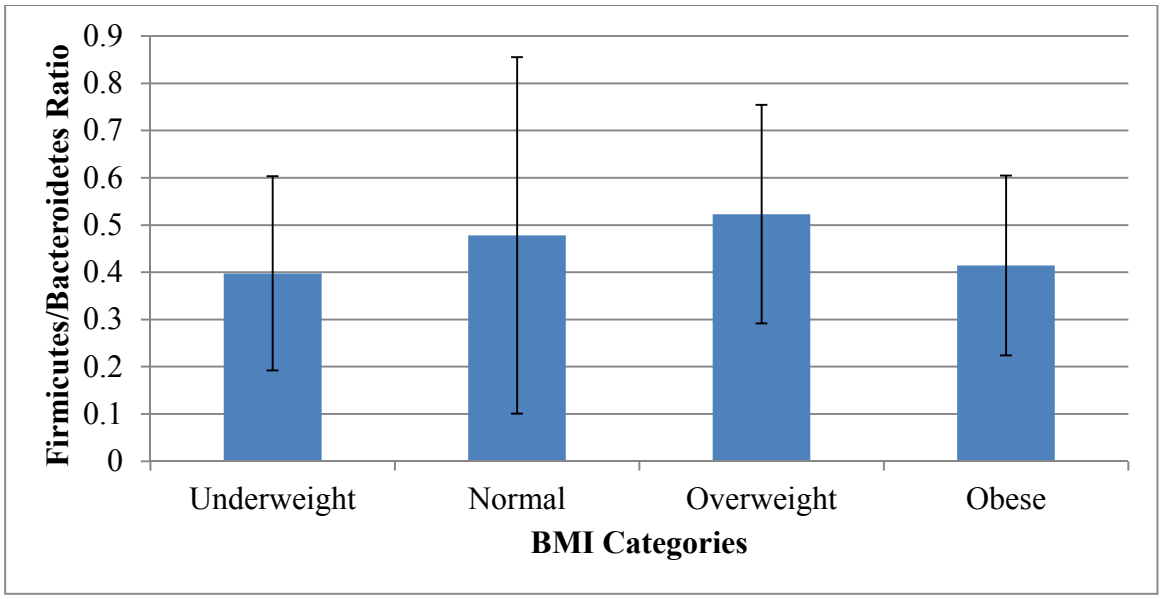

Figure 6. *Mean±s.d. of Firmicutes/Bacteroidetes ratio by BMI category (N=90). The number of participants contributing to each BMI category included: underweight BMI participants (BMI<18.5 kg/m^2; n=5), normal weight BMI participants (18.5≤BMI<25 kg/m^2; n=52), overweight BMI participants (25 kg/m^2 ≤ BMI ≤ 29.9 kg/m^2; n=21), and obese BMI participants (BMI ≥ 30 kg/m^2; n=12).*

At the genus level, 275 unique microbial groups were sequenced and identified. In a preliminary iteration of PCA, data from all 90 participants were used but as seen in Figure 7a, an outlier was identified. Removal of this participant from the PCA resulted in the identification of 12 principle components (PC) that explained approximately 30% of the variation in microbial community structure (Figure 7b). Multivariate linear regression was used to screen for potential relationships between these 12 components and participant BMI. Model results from this analysis were not significant (R^2=0.12, p=0.5891), but PC12 appeared to have an association with BMI (p=0.0357). Regression analysis of this component independently suggested that PC12 was significantly associated with BMI (R^2=0.053, p=0.0301; Figure 8).

A total of 261 microbial genera contributed to PC12 with 23 genera from 7 different phyla having the greatest influence on this component (Table 3). Negative

eigenvectors from these 23 genera were suggestive of microbial groups that may contribute to a higher BMI while positive eigenvectors were associated with microbial genera that may contribute to a lower BMI (Table 3).

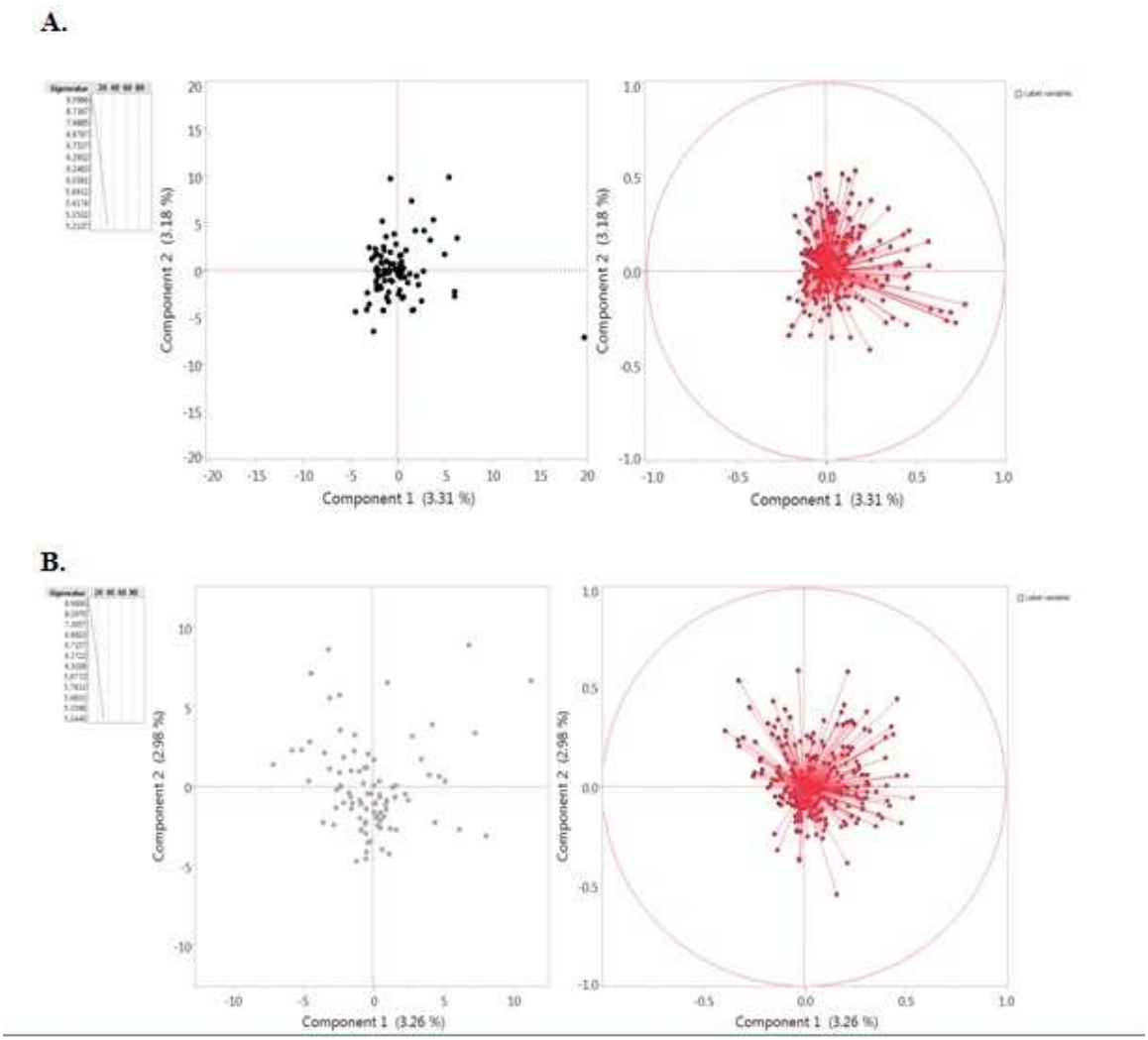

Figure 7. *Principal Components Analysis (PCA) of community composition by abundance of microbial genera. Panel A includes PCA of all 90 subjects and Panel B includes PCA results after removing one outlier (n=89). Charts on the left represent the dimensionality of study subjects and charts on the right represent the genera distributions. In both panels, data are presented with axes for principal components one and two.*

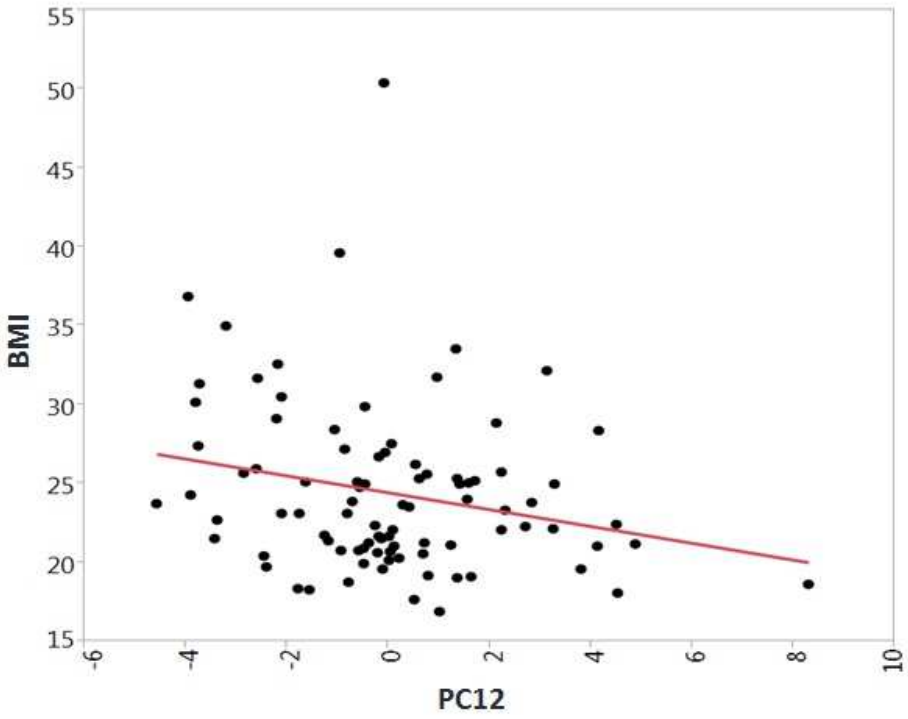

Figure 8. *Principal component 12 (PC12) had a significant negative relationship with college student BMI (R^2=0.053, p=0.0301).*

Table 3. Most influential microbial contributors to PC12 (n=89)

Taxa[a]	*Eigenvector*[b]
p_Firmicutes;c_Clostridia;o_Clostridiales;f_[Tissierellaceae];g_WAL_1855D	0.181
p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Micrococcaceae;g_Micrococcus	0.170
p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae;g_Brevundimonas	0.170
p_OP9;c_JS1;o_BA021;f_;g_Unassigned	0.154
p_Firmicutes;c_Clostridia;o_Clostridiales;f_[Tissierellaceae];g_Finegoldia	0.146
p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Collinsella	0.134
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Lactococcus	0.128
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptostreptococcaceae;Other	0.128
p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Comamonadaceae;g_Unassigned	0.122
p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;Other	0.119
p_Firmicutes;c_Bacilli;o_Bacillales;f_Bacillaceae;g_Bacillus	0.119
p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales];f_Chitinophagaceae;Other	0.113
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Megamonas	0.113
p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Adlercreutzia	0.109
p_Firmicutes;c_Bacilli;Other;Other;Other	0.108
p_Gemmatimonadetes;c_Gemm-3;o_;f__;g_Unassigned	0.102
p_Proteobacteria;c_Betaproteobacteria;o_Rhodocyclales;f_Rhodocyclaceae;g_Dechloromonas	-0.100
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;g_Unassigned	-0.106
p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;Other;Other	-0.109
p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae;g_Unassigned	-0.110
p_Bacteroidetes;c_Cytophagia;o_Cytophagales;f_Cytophagaceae;g_Unassigned	-0.114
p_Firmicutes;c_Clostridia;o_Clostridiales;f_[Tissierellaceae];g_Parvimonas	-0.134
p_Verrucomicrobia;c_Opitutae;o_[Cerasicoccales];f_[Cerasicoccaceae];g_Unassigned	-0.145

Abbreviations: PC12, principal component 12.

[a]The taxa for the microbes of the 23 most prevalent genera from 7 phyla are represented; within each taxon, p_ represents the phylum, c_ represents the class, o_ represents the order, f_ represents the family, and g_ represents the genus.

[b]Coefficients of the eigenvectors for each microbial genera; the most influential genera contributors were determined through PCA as coefficient values ≥ 0.1 or ≤ -0.1.

Diet and the Gut Microbiome

In order to determine if there was an association between dietary intake and the gut microbiome, correlation analyses were performed between the F/B phyla ratio and kcals, protein, carbohydrates, total fat, added sugar, and dietary fiber. There were no significant correlations between the F/B phyla ratio and the intake of energy or macronutrients ($p>0.05$; Table 4).

Table 4. Spearman's correlations between nutrient intake and F/B ratio (n=75)

[a]*Nutrient*	*Correlation coefficient*	[b]*P-value*
Energy Intake, kcals	0.122	0.296
Carbohydrates, g	0.076	0.519
Protein, g	-0.078	0.506
Total Fat, g	0.015	0.898
Saturated Fat, g	-0.07	0.548
Added Sugar, g	0.036	0.767
Dietary Fiber, g	0.017	0.883

[a]All nutrients were energy-adjusted based on the average kcal intake of the subject population.
[b]P-values were significant if $p<0.05$.

CHAPTER 5

DISCUSSION

The purpose of this cross-sectional analysis was to characterize the gut microbiome and identify relationships between BMI, diet, and the gut microbiome of healthy college students living in the dorms. To our knowledge, the current study was the first to characterize the gut microbiome of college students living in the dorms. Overall, this cohort of college students were normal weight (n=52), followed by overweight (n= 21), obese (n=12), and underweight (n=5). At the phyla level using the F/B ratio, no associations were identified between BMI categories and the gut microbiome. Further analyses of BMI were performed at the genus level following data reduction by PCA. Predominant microbial groups that were identified were from four phyla and 12 families. Analyses of gut microbiome data suggested that one group (principal component) of microbes was significantly associated with BMI at the genus level. For example, *Dechloromonas* from the Proteobacteria phyla and *Parvimonas* from the Firmicutes phyla are examples of genus level microbes that may be associated with obesity from the identified principal component. No associations were observed between dietary intake and the gut microbiota at the phyla level. These findings provide insights into the metabolic importance of gut microbiota in weight-related outcomes among college students living in the dorms and establish a basis for future research to longitudinally explore relations between gut microbiota and BMI in this dynamic population.

Characterization of the Gut Microbiome of College Students

A diverse range of microbes from 20 phyla and 134 families were identified within this population. Of this diverse range, four phyla and twelve families were identified as the most abundant for college students living in the dorm. Although the gut microbiome of this population had not yet been characterized and the percent abundance varied by individual, Firmicutes and Bacteroidetes were consistently the most abundant phyla. These findings of a diverse microbiome and identifying Firmicutes and Bacteroidetes as the most abundant phyla were consistent with the literature characterizing the gut microbiome of adults in a range of studies focusing on diet, weight, and age.[2,5,18,2022,65,126,132] Due to these similarities in findings in the characterization of the gut microbiome in both populations, it is possible that certain findings in adult populations may be applicable to college students.

Body Mass and Intestinal Microbe Genera of College Students

PCA and regression analyses identified one principal component (PC12) of microbial genera that may be associated with BMI. A total of 261 microbial genera contributed to PC12, but 23 genera from 7 different phyla were the most influential contributors to this group of microbes. Of the 23 most influential genera, there were very few for which the functions have been identified. *Lactococcus*, *Parvimonas*, and order level Clostridiales were also identified as important groups which have functions that may explain the association between PC12 and BMI.

The genus *Lactococcocus* is known to ferment glucose into lactic acid.[173,174] The positive eigenvalue associated with this microbe suggests that it may be more common

among students with lower BMIs. The function of *Lactococcus* may help explain the associations with a lower BMI because, by performing glucose fermentation, it may decrease the amount of energy-yielding substrate available to the host through its utilization of anaerobic respiration producing lactic acid over aerobic respiration through the citric acid cycle.[175]

The genus *Parvimonas* is known to have a pathogenic effect, especially in the GI tract as it has been associated with gingivitis and colon cancer.[176,177] This genus had a negative eigenvalue and therefore may be associated with higher BMIs. The pathogenic effects of *Parvimonas* may help explain the association with higher BMI because previous findings suggest that a greater presence of pathogenic microbes is associated with inflammation and higher BMI values.[50,74,75,77,78]

Lastly, genera belonging to the order Clostridiales contributed both positively and negatively to PC12. Clostridiales have been shown to have multiple functions at more specific classification levels including converting dietary components into toxic or carcinogenic compounds, providing a protective effect against the pathogen *Escherichia coli*, and fermenting indigestible polysaccharides, such as dietary fiber, as an energy source.[178-180] This protective effect would explain the association with lower BMI values because chronic inflammation is often associated with obesity and, therefore, individuals with a lower BMI would likely have less inflammation.[50,56,57,73-78] This variety of functions could explain why this order is present with both positive and negative eigenvalues as it is possible that the negative eigenvalue groups fall into the sub-category of Clostridiales that convert dietary components into toxic or carcinogenic components as well as the subcategory of Clostridiales that breakdown non-digestible fiber for energy

utilization. While exploring the functions of these microbes begins to explain the association between the gut microbiome on a genus level, the majority of literature explores the association between BMI and the gut microbiome at the phyla level.

College Student Body Mass and Gut Microbiota at the Phylum Level

No significant association between the F/B ratio and BMI categories was observed. This finding is similar to a few previous investigations in adults that also demonstrated no significant association between the F/B ratio and BMI categories.[20,21] Despite these few findings, the majority of the research in both adults and mice points to a significant association between the F/B ratio and BMI, specifically, demonstrating an increase in the F/B ratio with an increased BMI,[2,5,18] or, as one study demonstrated, a decrease in the F/B ratio with increased BMI.[65]

There are multiple differences in design between the current study and the previous research that have demonstrated phyla levels associations, which could explain why the current study did not result in an association between the F/B ratio and BMI seen in the majority of the literature. The difference between this analysis and previous studies which observed a difference in F/B ratios could be explained by the cross-sectional design of the current study. One study that found a difference compared 12 obese individuals to lean controls and followed them for one year. When the obese individuals were placed on a long-term restricted, weight loss diet, their microbiome began to shift toward that of their lean counterparts with an increased abundance of the Bacteroidetes phyla and a decreased abundance of the Firmicutes phyla as they lost weight.[18] Also, based on the large inter-individual variation in this cohort, it may have

been more difficult to assess changes in the F/B ratio over time. Lastly, larger differences in the gut microbiome are required in order to see significant differences at the phyla level as compared to the genus level, where smaller changes may be more visible.[20,21] This could be an explanation as to why the only association observed was at the genus level and not at the phyla level.

Habitual Dietary Intake and Gut Microbiota at the Phylum Level during College

Analyses of dietary components suggested that no associations were present between energy, carbohydrate, protein, fat, dietary fiber, added sugar intakes and the F/B ratio. The majority of research has demonstrated a significant association between the F/B ratio and dietary intake, but the majority of these studies had either compared the gut microbiome of two groups with significantly different diets[22] or carried out interventions within a population where the diet was altered and changes were measured over time.[23,24,147-149,151-156] For example, one study compared the gut microbiome of children in Europe (Eu) on a Western diet (high in sugar, animal fat, and calorically-dense foods) and children in Burkina Faso (BF) on a Neolithic diet (low in fat and animal protein; rich in starch, fiber, and plant polysaccharides). The results of this study demonstrated that Eu children had lower overall microbial diversity and a high F/B ratio, whereas the BF children had a rich microbial diversity and a lower F/B ratio.[22]

Similar to the Burkina Faso study, participants collected only one sample in the present study, while the majority of other studies in the literature that demonstrated an association between the F/B ratio and dietary intake were interventions and collected multiple samples. One reason this research may not have demonstrated an association,

while the Burkina Faso study that used a similar design did, is because the participants were part of a unique population that were in the transition from adolescence to adulthood (high school to college), which is a time period that is filled with major behavioral, social, and lifestyle changes.[14] As these participants are moving away from home and becoming more independent, one of the primary changes seen is their dietary intake.[1,7,8] Therefore, unlike the Burkina Faso study, there was likely more noise among the college-student data in this study which limited the ability to see microbial differences. Collection of a single sample during this transition to adulthood could also explain why an association was not observed between dietary intake and the F/B ratio. Therefore, collecting multiple fecal samples and repeating dietary recalls throughout freshmen year/time in the dorms could potentially elucidate an association between the F/B ratio and dietary changes.

Strengths and Limitations

Strengths of this study include that this was a novel area of research and, therefore, collecting cross-sectional data was useful in forming a basis of knowledge in regard to the gut microbiome and its association with weight and diet in this unique population. In addition, this study suggests the feasibility of collecting samples in this population and suggests that longitudinal dietary interventions may be possible. Limitations of this study include that a convenience sample of college students were recruited from only two dorms on the Arizona State University Tempe campus. This may limit the generalizability of the data to students at other dorms or campuses. Furthermore, the fecal microbiome, diet, and weight data were cross-sectional, thereby limiting our ability

to assess relationships or changes over time. In addition, the use of 24-h dietary recalls for collecting consumption data, while being the gold standard for self-reported dietary intake data, may have introduced inaccuracies due to reporting bias. The potential for reporting bias was made evident by the consistently low reported average caloric intakes by the participants completing the 24-h dietary recalls. Lastly, each of the recruitment and sample collections occurred after fall and winter breaks so the participants' recent exposure to familial dietary behaviors may have impacted the gut microbiome communities.

Summary

This was the first study to characterize the gut microbiome and explore potential associations between BMI, dietary intake, and the gut microbiome in college students living in the dorms. Findings demonstrated that there was a significant association at the genus level between BMI and the gut microbiome, but no significant associations at the phyla level between the gut microbiome and BMI or dietary intake. Continuing to expand the knowledge of these associations could play a part in understanding the role that gut microbiota play in the weight gain patterns commonly seen in this population.[6,11,25] Exploring these associations could eventually be used to develop a means of moderating the weight gain pattern seen in this population of college students living in the dorms, which makes this a crucial time period to reduce the risk for obesity and related comorbidities later in life.

CHAPTER 6

CONCLUSION

Gut microbiota have become increasingly recognized for their crucial role in metabolic functioning and subsequent states of health and disease.[2] Studying the dynamic relations between weight, diet, and the gut microbiome are important for understanding the high prevalence of college weight gain. This study characterized the gut microbiome and assessed relationships between BMI, dietary intake, and the gut microbiome of healthy college students living in the dorms. From this work, 12 microbial families were most abundant in this population and a significant association was observed between BMI and 23 microbial genera. However, contrary to the literature amongst other populations, the associations between the gut microbiome at the phyla level (F/B ratio) and both BMI and dietary intake were not significant.

More research is necessary in order to further explore potential associations between the gut microbiome, BMI, and diet in college students living in the dorms. For instance, because this study was cross-sectional, we were unable to capture changes in BMI, dietary intake, and gut microbiota that may have occurred as college students transitioned from home to dorm environments. A longitudinal study design with a significantly larger population would be necessary in order to more accurately assess longitudinal changes in these outcome measures throughout this transitional life stage. In addition to understanding longitudinal changes in the gut microbiome, pre- and probiotic interventions could be implemented as a possible means to moderate or prevent the shifts toward overweight/obesity that often begin during this important period of development.

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APPENDIX A

INTITUTIONAL REVIEW BOARD FALL AND SPRING APPROVAL LETTERS

ASU Knowledge Enterprise Development

APPROVAL: MODIFICATION

Meredith Bruening
SNHP - Nutrition
602/827-2266
Meg.Bruening@asu.edu

Dear Meredith Bruening:

On 9/22/2014 the ASU IRB reviewed the following protocol:

Type of Review:	Modification
Title:	The Role of Friendship Networks on BMI and Behaviors among College Freshmen
Investigator:	Meredith Bruening
IRB ID:	1309009596
Funding:	Name: NIH: National Institutes of Health; Funding Source ID: HHS-NIH-National Institutes of Health,
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• consent without track changes , Category: Consent Form; • Microbiome pilot consent form, Category: Consent Form; • ValidationConsentForm--Revised, Category: Consent Form; • ApprovedIRBProtocol, Category: IRB Protocol; • irb_microbiome-mod_091714 (1).docx, Category: IRB Protocol; • Response to 9/23 modification request, Dr.docx, Category: IRB Protocol; • Web-based survey, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Response to 9/23 modification request, Dr.pdf, Category: Other (to reflect anything not captured above); • Microbiome pilot flyer, Category: Recruitment

	Materials; • ValidationFlyer, Category: Recruitment Materials; • ValidationEmail, Category: Recruitment Materials;

The IRB approved the modification.

When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

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

Sincerely,

IRB Administrator

cc:

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Meredith Bruening
SNHP - Nutrition
602/827-2266
Meg.Bruening@asu.edu

Dear Meredith Bruening:

On 1/5/2015 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	devilSPARC 2015 Spring Survey testing
Investigator:	Meredith Bruening
IRB ID:	STUDY00002019
Category of review:	(5) Data, documents, records, or specimens, (7)(a) Behavioral research
Funding:	Name: NIH: National Institutes of Health, Funding Source ID: 1DP5OD017910-01
Grant Title:	
Grant ID:	
Documents Reviewed:	• Microbiome Social Pilot Screening Form 12 15 14.pdf, Category: Screening forms; • participant_cards_back.pub, Category: Participant materials (specific directions for them); • Parents_Questionnaire_100814.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Parents_Questionnaire_Spanish_version.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Microbe Pilot Recruitment Flyer 12 15 14.pdf, Category: Recruitment Materials; • Student Survey, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Student height weight form, Category: Measures

	(Survey questions/Interview questions /interview guides/focus group questions); • devilWaste_Female Participant Data Collection Sheet.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Participant_cards_front.pub, Category: Participant materials (specific directions for them); • Parent English Consent, Category: Consent Form; • Student devilSPARC and devilWaste consent, Category: Consent Form; • Email_ASA24_reminder_1524.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • IRB_jan_study_10514.docx, Category: IRB Protocol; • Email_follow_up_reminder.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • SubmittedDP5.pdf, Category: Sponsor Attachment; • IRB_form_translator.pdf, Category: Translations; • IRB_form_backtranslator.pdf, Category: Translations; • Parent English Email, Category: Recruitment Materials; • incentive_followup_monday.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Parent Spanish Email, Category: Recruitment Materials; • Parent Spanish Consent, Category: Consent Form;

The IRB approved the protocol from 1/5/2015 to 1/4/2016 inclusive. Three weeks before 1/4/2016 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 1/4/2016 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

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

Sincerely,

IRB Administrator

cc:

Kayla Shenfeld
Helen Kim
Cassandra Kettenhoven
Demi Keezer
Ariel Lankton
Chelsi Chaira
Lisa Rodriguez
Rebecca Bender
Corrie Whisner
Kara Burnett
Ricci-Lee Hotz
Brett Gruberg
Sheila Selvera
Stephanie Nelson
Megan Ellis
Caroline Dysick
Jessica Vega

APPENDIX B

RECRUITMENT FLYER AND FALL/SPRING CONSENT FORMS

RESEARCH STUDY

JOIN THE DEVIL WASTE STUDY

Researchers from Arizona State University are looking for dorm students to participate in research to study how your diet, fitness and friend groups impact the bacteria that normally live in your gut

YOU CAN PARTICIPATE IN THIS RESEARCH STUDY IF:

- You are enrolled in the devilSPARC study
- You are a healthy, male or female and 18 years or older

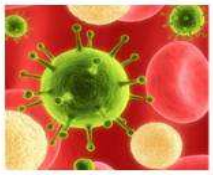
For participating, you will receive:

- $20 if you complete all of the study procedures

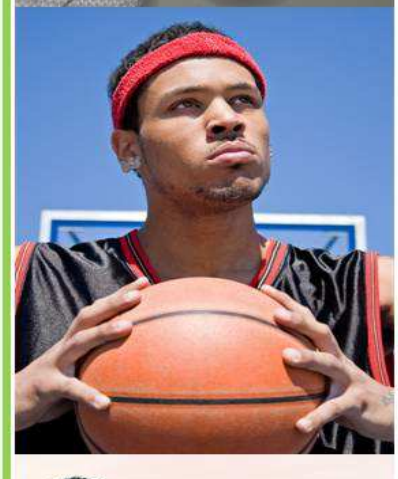
To complete the study, you will be asked to:

- Collect one stool sample

FOR MORE INFORMATION OR TO ENROLL IN THE STUDY:



Email: cwhisner@asu.edu
or
Call: (602) 827-2261



CONSENT FORM

Study Title: Intestinal bacteria profiling in relation to health behaviors and social connections among college-aged freshman males (devilWASTE Study)

Principal Investigators: Corrie Whisner, PhD, Rosa Krajmalnik-Brown, PhD, Meredith Bruening, PhD

Introduction

You are being asked to take part in this study because you are a college-aged freshman male enrolled in the devilSPARC study.

Purpose of Study:

The purpose of the study is to learn more about intestinal bacteria and how your health behaviors during freshman year of college impact these bacteria. Bacteria occur naturally within and on your body. Most of these bacteria are not harmful to you and may play an important role in how you digest the food you eat. Bacteria in your intestine may be impacted by things other than diet, including your level of physical activity and social groups / friends.

Description of Study Procedures:

It is up to you to decide whether or not to participate in this study. If you decide to take part in this study, you will be asked to collect one fecal / stool sample while enrolled in the devilSPARC study. A stool collection kit that contains everything you will need to collect the sample will be provided to you by the study staff. You will have the option of collecting your sample in your dormitory bathroom or at the Nutrition and Health Promotion Clinical Research Lab on ASU's downtown campus, ABC-1 building (425 North 5th Street, Phoenix, AZ 85004-0698). This sample must be kept cold so you will be provided with a cooler and ice packs which must be returned with the sample within 24 hours of collection. If you collect your sample in the dorms, you can drop the sample off at ABC-1 or call the research staff to pick it up from your residence hall. Samples collected in the clinical research facility will not require transport.

Information about the bacterial that live in your gut will be compared to demographic, dietary, physical activity and social data collected in the devilSPARC study.

Duration of the Study:

Your active participation in the study will last 1 day (24 hours), during which you collect your sample and assure that it is received by study staff.

Number of Subjects:

Approximately 60 subjects will take part in this study.

Risks of Participation:
There are no major risks associated with collecting stool samples; however, you may come into contact with the stool during collection. To reduce the risk of exposure to bacteria and other microbes that occur naturally in stool, you will be provided with special collection containers and gloves which will minimize your chance of direct contact with your sample. Additionally, you may experience some social or psychological discomfort as a result of collecting stool samples in your dormitory. To avoid any negative feelings related to collecting samples in the presence of your friends, you may collect your sample in the clinical research facility in ABC-1 (ASU downtown campus).

Benefits of Participation:
You might not benefit from being in this research study. A potential benefit to you from being in this study might be receiving a printout of results from this study. In order to receive results from the overall study, when they are available, you must notify the study staff of your interest.

Costs:
There will be no cost to you to participate in this study. However, if you travel to the ASU downtown campus, your gas, parking or transit costs will not be covered by the study.

Payments:
You will receive $30 in cash for participating in this study.

Confidentiality of Records and Authorization to Use and Disclose Information for Research Purposes
Arizona State University makes every effort to keep the information collected on you private. In order to do so, we will keep your health information and measurements in locked cabinets that will only be available to study personnel. To protect confidentiality, you will be given an identification number and data will be recorded in electronic format, on encrypted networks, accessible only on password protected computers that are kept in locked offices.

If the results of this study are made public, information that identifies you will not be used.

Contact Persons:
For more information concerning this research or if you feel that your participation has resulted in any research related injury, emotional or physical discomfort please contact: Corrie Whisner in the School of Nutrition and Health Promotion at (602) 827-2261 or cwhisner@asu.edu.

This research has been reviewed and approved by the Arizona State University Bioscience IRB. You may talk to them at (480) 965-6788 or research.integrity@asu.edu if:

- You wish to talk to someone other than the research staff about your rights as a research subject;
- To voice concerns about the research;
- To provide input concerning the research process;
- In the event the study staff could not be reached.

Voluntary Participation

Taking part in this study is voluntary. You are free not to take part or to withdraw at any time, for whatever reason. No matter what decision you make, there will be no penalty or loss of benefit to which you are entitled. In the event that you do withdraw from this study, the information you have already provided will be kept in a confidential manner.

****

Subject Consent

I have read (or have had it read to me) the contents of this consent form and have been encouraged to ask questions. I have received answers to my questions. I agree to participate in this study. I have received (or will receive) a signed copy of this form for my records and future reference.

Signature of participant	Date

Printed name of participant

CONSENT FORM: devilSPARC

INTRODUCTION: The purpose of this form is to provide you information about our study that may affect your decision to participate in this research, and to record the consent of those who agree to be involved in the study.

RESEARCHERS: A graduate student under the direction of Professors Meg Bruening, PhD, MPH, RD and Corrie Whisner, PhD from the School of Nutrition and Health Promotion at Arizona State University has invited your participation in a research study.

STUDY PURPOSE: The purpose of this study is to assess healthy eating and physical activity among college students.

DESCRIPTION OF RESEARCH STUDY: If you decide to be a part of this study we will measure your height and weight. You will be asked to take a web-based survey and complete three 24 hour dietary recalls. Participation is voluntary and you can choose to skip any survey question at any time. We will ask you to provide contact information for a parent or guardian as we would like to send a separate survey on their eating and physical activity behaviors. We will link student and parent data through deidentified ID numbers. Additionally, the research team will have limited access to view your SunCard activity during the study week, including entrance and/or exit of ASU facilities. All data collected is confidential. The results are compiled as a group, not individually. Your decision to participate and your responses, should you choose to participate, will not affect your status at Arizona State University in any way. If you agree to participate, your time spent participating will total around 2 hours.

RISKS: You may feel uncomfortable providing personal information about yourself in the study questionnaires. However, as in any research, there is some possibility that you may be subject to risks that have not yet been identified.

BENEFITS: There are no direct benefits to participation. However, indirect benefits of your participation will include helping the researcher to understand ways to promote nutrition and physical activity. These data will also add to the general scientific knowledge about college students contextual factors related to nutrition and physical activity behaviors among friendship networks over time.

CONFIDENTIALITY: All information obtained in this study is strictly confidential. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. In order to maintain confidentiality of your records, Dr. Bruening will assure that your name will only appear on this consent form and will not be associated with any other information provided today. Study data will not be transmitted via the internet. Study data will be stored on a password protected server. To these extents, confidentiality is not absolute.

WITHDRAWAL PRIVILEGE: Participation in this study is completely voluntary. It is ok for you to say no at any time. Even if you say yes now, you are free to say no later, and withdraw from the study at any time.

COSTS AND PAYMENTS: You will receive a $30 incentive for completing this study including:

1. web-based survey _____*(participant's initials indicating understanding)*

2. height and weight by staff _____*(participants initials indicating understanding)*
3. three 24 hour dietary recalls _____*(participants initials indicating understanding)*

All of the above items must be completed to receive the incentive. You can receive a t-shirt if you and you bring a suitemates/roommate or fellow dorm resident from your dorm to participate in the study. In addition, if 50% of the students under the direction of your Community Mentor complete the study your floor will receive support for a pizza party or an ice cream social.

VOLUNTARY CONSENT/ASSENT: Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by the researchers, Dr. Meg Bruening or Dr. Corrie Whisner at devilSPARC@asu.edu or 602.827.2266.
If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board through the ASU Office of Research Integrity and Assurance, at 480.965.6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

_____ _____ _____

Subject's Signature Printed Name Date

You may be eligible for an addition section of the study! If interested, study staff can explain the below information.

Purpose of Section: The purpose of this section of the study, the *devilWASTE* section, is to learn more about how your diet, friends and fitness level during freshman year impact the microbes that live in your intestine. Microbes occur naturally within and on your body. Most microbes are not harmful to you and may play an important role in how you digest the food you eat. You must be 18 to participate in this portion of the study.

Description of Procedures: It is up to you to decide whether or not to participate in this part of the study. If you decide to take part in this section, you will be asked to collect one fecal / stool sample. A stool collection kit that contains everything you will need to collect the sample will be provided to you by the study staff. This sample must be kept cold and returned within 24 hours of collection. Information about the microbes that live in your gut will be compared to demographic, dietary, physical activity and social data collected in this study. Hormone changes can influence intestinal microbes; therefore, female participants will be asked to provide the first date of their last menstrual cycle at enrollment.

Risks and Benefits of Participation: There are no major risks associated with collecting stool samples; however, you may come into contact with the stool during collection. To reduce the risk of exposure to microbes that occur naturally in stool, you will be given special collection containers and gloves to minimize direct contact with the sample.

You might not benefit from being in this research study. A potential benefit to you from being in this study might be receiving a printout of results from this study. In order to receive results from the overall study, when they are available, you must notify the study staff of your interest.

Payments: You will receive $20 in cash for participating.

Subject Consent: I have read (or have had it read to me) the information about the devilWASTE section and have received answers to my questions. I agree to participate in the devilWASTE section. I have received (or will receive) a signed copy of this form for my records and future reference.

_____ _____ _____

Subject's Signature Printed Name Date

INVESTIGATOR'S STATEMENT: "I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____
Date_____

APPENDIX C

SCREENING FORM AND FEMALE PARTICIPANT DATA COLLECTION SHEET

Name / Subject ID: _____ Date: _____ Staff Initials: _____

Screening Form

Study Title: devilWASTE Study

Inclusion Criteria:

___ English-speaking male or female

___ Enrolled in devilSPARC study

___ Older than 18 years of age

Exclusion Criteria:

___ History of eating disorders

___ History of malabsorption diseases,

___ HIV infection

___ High blood pressure

___ Diabetes

___ Taking probiotics, antibiotics and/or antifungals during the last 2-3 months

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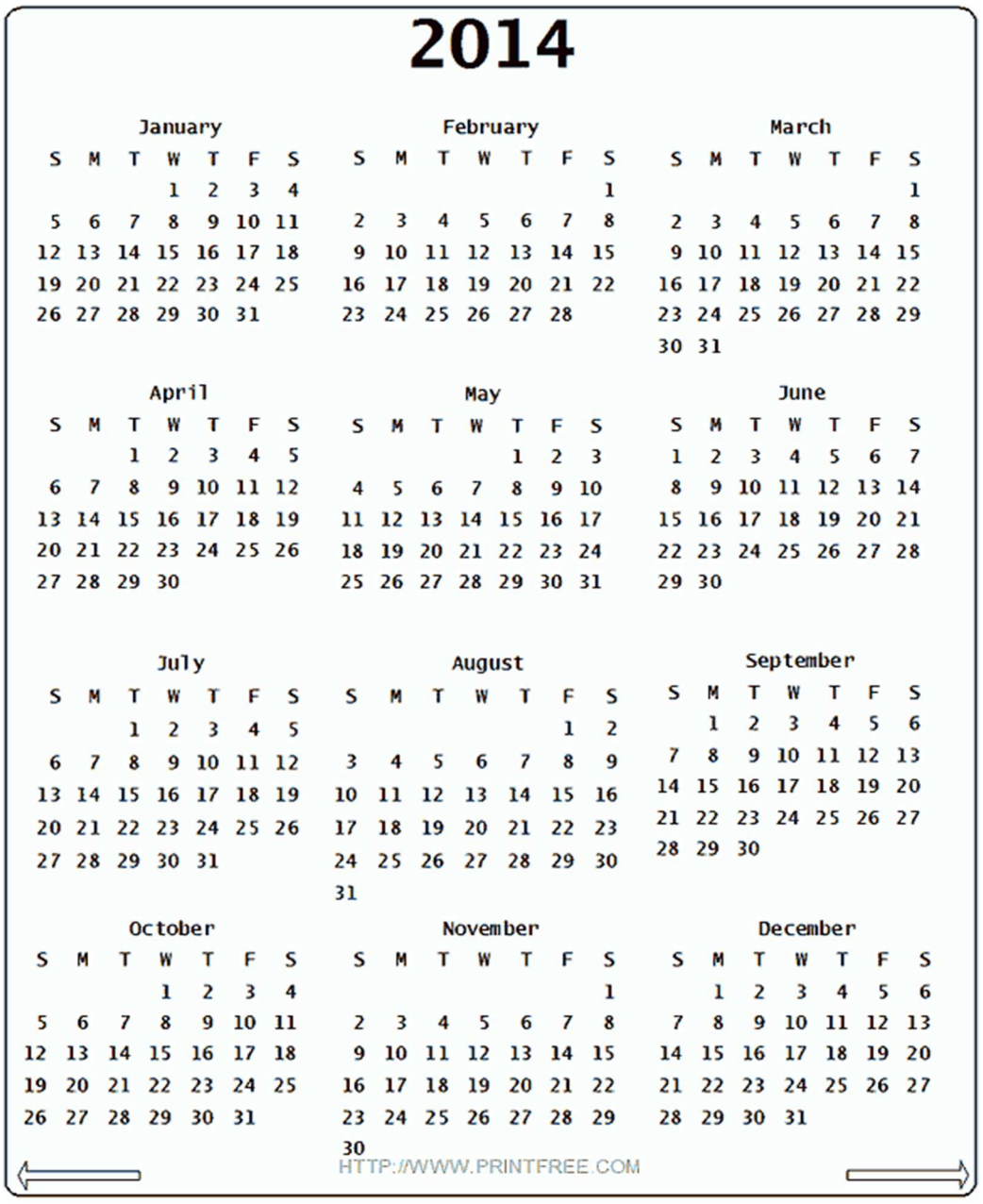
___ Eligible
___ Not Eligible

Female Participant Data Collection Sheet

Please circle the first date of your last menstrual period (If not shown, please write date on the line below):

Date: _____



APPENDIX D

STOOL SPECIMEN COLLECTION STUDY PROTOCOL PROCEDURES AND DEVILWASTE STUDY STOOL COLLECTION INSTRUCTIONS

ARIZONA STATE UNIVERSITY

	Standard Operating Procedures **SNHP Research Facility**	Effective Date:
SUBJECT:	**STOOL SPECIMEN COLLECTION STUDY PROTOCOL PROCEDURES**	Revised/Reviewed: 9/25/14

Purpose:
The purpose of this Standard Operating Procedure is to provide guidelines for training laboratory personnel in instructing subjects to collect feces in the clinical facility or at home over a period of time and on processing fecal collections when returned to the lab.

FECAL COLLECTION AND PROCESSING INSTRUCTIONS

Supplies:

- 1 Commode Specimen Collection System (e.g. Fisherbrand) that includes a sealable plastic collection container with tightly fitting, snap-on (leak resistant) lid and collection frame
- 1 Ziploc bag
- 1 small bag liner for collection bowl
- 1 pair of latex or nitrile gloves
- 1 instant ice pack
- 1 Small cooler for transport

Instructions to the patient:

1. Raise the toilet seat. Place the stool collection frame on the back of the toilet bowl (Figure 1). All four corners of the collection frame should be supported by the toilet bowl. Place collection bowl in the center of frame (Figure 2) and lower the toilet seat back down.

Figure 1 

 **Figure 2**

2. Open up the small scented bag liner. Place the open bag inside of the collection bowl so that the entire bottom of the bag lines the bottom of the bowl and the edges of the bag rise up and over the edges of the bowl. This will protect the surfaces of the collection bowl from getting stool on them and also allow you to close up the bag around your sample before snapping on the lid.
3. Do **NOT** urinate into the collection container. Deposit your stool directly into the bag lining the collection bowl.
4. After collection. Pull the edges of the bag liner up above the sample and twist the bag closed. You may put a **LOOSE** knot in the bag but do **NOT** tie it too tightly. After securing the bag, snap the plastic lid tightly onto the collection bowl.
5. Place the closed container into the Ziploc bag and seal the bag (Figure 3).

Figure 3

6. Transfer the enclosed container into the cooler and dispose of the collection frame in the regular trash.
7. Activate the instant ice pack by following the instructions on the pack (usually squeezing and shaking). Once the ice pack is cold, place it with the stool sample inside the cooler. Write the time (hrs:min AM/PM) of collection on the cooler label which also has your research participant ID on it.
8. Transport the cooler and sample to ABC-1 on ASU's downtown campus: 425 North 5th Street, Phoenix, AZ 85004 between 8:00 am and 5:00 pm.
9. The sample must be delivered within 24 hours of the collection time noted on the cooler label.

STOOL COLLECTION PROCESSING

1. Wear appropriate PPE to process fecal samples. This includes disposable gloves, lab coat and protective eye wear.
2. In the lab, record the weight of the fecal collection container with sample inside and lid closed.
3. Record the weight of the lid, collection container and sample bag inside the container and subtract these weights from the weight calculated in number 2; this is the weight of the fecal sample.
4. Transfer the fecal sample to a sterile stomacher bag which will be used to homogenize the sample.
5. Assuming that 1 ml of water has a mass of 1 g, add autoclaved, double-deionized water at two times the weight of the stool sample. (example: if stool weights 100 g then add 200 ml of water to the sterile stomacher bag).
6. Place the water and stool-filled stomacher bag into the stomacher, allowing approximately 6 inches of the bag to stick out above the top of the machine.
7. Pinch the bag closed with the stomacher door while holding onto the top of the bag with one hand. Pull the door handle/lever down to lock the door in the closed position.
8. Keep one hand on the bag while using the other hand to turn on the machine. Homogenize the sample for 2-3 minutes or longer until the sample has a homogeneous consistency (fecal slurry).
9. Once homogenized, transfer up to 50 ml of the liquid sample from the bag into a 50 ml falcon tube. Leave about a centimeter at the top of the tube empty to allow space for expansion during freezing.
10. Tightly seal the falcon tube and store in the freezer until time for analysis.
11. Dump remaining fecal slurry into the toilet for disposal and place the used stomacher bag in with the bio-waste.
12. Fecal collection containers can be disposed of in the regular trash. If feces is present on the container, place used bowl and lid in the bio-waste instead.
13. Wash equipment used for the collection and processing in the following manner:
    a. Spray down the inside and outside surfaces of the stomacher and the toilet bowl with a 10% bleach solution and soak for 10 minutes.
    b. Use paper towels to wipe up the bleach solution and dry the equipment.
14. Clean the processing area (e.g. counter space) with appropriate disinfectant as described in the Exposure Control Plan SOP.

devilWASTE Study: Stool Collection Instructions

Purpose:

The purpose of this Standard Operating Procedure is to provide guidelines for training laboratory personnel in instructing subjects to collect feces in the clinical facility or at home over a period of time and on processing fecal collections when returned to the lab.

FECAL COLLECTION INSTRUCTIONS

Supplies:

- 1 Commode Specimen Collection System (e.g. Fisherbrand) that includes a sealable plastic collection container with tightly fitting, snap-on (leak resistant) lid and collection frame
- 1 Ziploc bag
- 2 small bag liner for collection bowl (one is extra in case of hole or tear in bag)
- 1 pair of latex or nitrile gloves
- 1 instant ice pack
- 1 rectangular freezer pack (must be placed in freezer 12 h in advance of sample collection)
- 1 Small cooler for transport

Instructions to the patient:

1. Place the rectangular freezer pack in the freezer 10-12 h prior to sample collection.

2. Raise the toilet seat. Place the stool collection frame on the back of the toilet bowl (Figure 1). All four corners of the collection frame should be supported by the toilet bowl. Place collection bowl in the center of frame (Figure 2) and lower the toilet seat back down.

Figure 1

Figure 2

3. Open up the small scented bag liner. Place the open bag inside of the collection bowl so that the entire bottom of the bag lines the bottom of the bowl and the edges of the bag rise up and over the edges of the bowl. This will protect the surfaces of the collection bowl from getting stool on them and also allow you to close up the bag around your sample before snapping on the lid.

4. Do **NOT** urinate into the collection container. Deposit your stool directly into the bag lining the collection bowl.

5. After collection. Pull the edges of the bag liner up above the sample and twist the bag closed. You may put a **LOOSE** knot in the bag but do **NOT** tie it too tightly. After securing the bag, snap the plastic lid tightly onto the collection bowl.

6. Place the closed container into the Ziploc bag and seal the bag (Figure 3).

Figure 3

7. Place the frozen rectangular ice pack in the bottom of the cooler. Transfer the enclosed container into the cooler and dispose of the collection frame in the regular trash.

8. Activate the instant ice pack by following the instructions on the pack (usually squeezing and shaking). Once the ice pack is cold, place it with the stool sample inside the cooler. Write the time (hrs:min AM/PM) of collection on the cooler label which also has your research participant ID on it.

9. Transport the cooler and sample to the **Biodesign Institute on ASU's Tempe Campus at 727 E. Tyler Street, Tempe, AZ 85287-5001 (preferred)** or the ABC-1 Building on ASU's downtown campus at 425 North 5th Street, Phoenix, AZ 85004 **between 8:00 am and 5:00 pm**.

   You may also email or call us to have your sample picked up from your dorm using the following: cwhisner@asu.edu or 602-827-2261. Please include your contact information in messages so that we can coordinate the best time for pick-up of your sample.

10. The sample must be delivered within 24 hours of the collection time noted on the cooler label.