Changes in Weight Status and the Intestinal Microbiota among College Students

by

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ABSTRACT

The transition to college has been identified as a vulnerable period for weight gain and the onset of obesity. Research has shown that the gut microbiota is different in obese compared to lean individuals, but a period of weight gain has never been studied in free-living individuals. The objective of this longitudinal, observational study was to assess the association between changes in the intestinal microbiota and weight-related outcomes in healthy college students living in on-campus dormitories at Arizona State University (n=39). Anthropometric measures and fecal samples were collected at the beginning and end of the school year, and microbial relative abundance for *A. muciniphila, F. prausnitzii, R. gnavus,* and *L. acidophilus* was measured through qPCR analyses. In this population, body mass index (BMI) and waist circumference (WC) increased by 0.97 ± 1.28 kg/m2 and 2.64 ± 4.90 cm, respectively. Wilcoxon-Rank tests revealed that *R. gnavus* fold change was significantly different between groups of weight loss/maintenance and weight gain ≥ 5% body weight (0.14 [-0.21, 0.64], n=24 vs. -0.14 [-0.92, 0.05], n=15, respectively; p=0.028). Correlation analyses suggested a significant negative association between *A. muciniphila* fold change and both % WC change and % BMI change (r= -0.66; p<0.01 and r= -0.33; p=0.04, respectively). However, multivariate regression analysis controlling for sex and race/ethnicity showed a significant association between *A. muciniphila* and % WC change, but not % BMI change (R^2= 0.53; p<0.01 and R^2= 0.24; p=0.15). *F. prausnitzii* was not associated with weight-related outcomes in this sample. *L. acidophilus* was excluded from study analyses after subsequent qPCR trials revealed no amplification in participant samples. Overall, this was the first study to show a relationship between *A. muciniphila* fold change and weight-related outcomes over a period of weight gain. Specifically, *A. muciniphila* was strongly negatively associated with WC in this sample. Further research is needed to

more accurately describe these associations and potential mechanisms associated with the shift in gut microbiota observed with weight gain. Findings from future research may be used to develop interventions for college students aiming to shift the gut microbiota to prevent weight gain.

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CHAPTER 1
INTRODUCTION

Obesity is now an epidemic with 78.6 million adults in the United States population classified as obese.[1] Emerging adulthood has been identified as a vulnerable period for weight gain and is a period marked by vast social and environmental changes that parallel with the transition to college. Specifically, college freshmen must adapt to changes in diet, physical activity, and peer influences. Unfortunately, many freshmen gain weight during the year and adopt unhealthy behaviors that continue to persist throughout college.[2–7] Research has sought to understand this weight gain from social and environmental perspectives, but metabolic changes are also occurring. Previous research has shown that the intestinal microbiota is different in obese compared to lean individuals, and is thought to play a role in energy utilization from the host's diet.[8,9] Intestinal bacteria have the potential to extract more energy from the diet, possibly making it easier to gain weight.[8] Because of this, understanding the role of the microbiota as it relates to weight gain and obesity in college students is vital.

The human intestinal microbiota is made up of over 100 trillion cells with 500 different species represented, and is referred to as a metabolically active organ.[10,11] These microbes utilize undigested fiber and resistant starches for energy.[10] Although the onset of obesity is complex, evidence suggests a connection between excess weight and the intestinal microbiota. Studies in both mice and humans have shown there is a shift in abundance at the phylum level, favoring Firmicutes over Bacteroidetes, with the onset of obesity.[8,12] Landmark studies in mice have shown that germ-free mice, or mice raised in the absence of any microorganisms, colonized with microbiota from obese donors showed an increase in body fat beyond their germ-free counterparts despite being fed identically.[8] A similar study showed a significantly increased level of total body fat in

conventionally raised mice, or mice harboring a microbiota beginning at birth, versus germ free mice, despite the conventional mice being fed 29% less of standard rodent chow than the germ-free mice.[13] These studies suggest that obesity-associated microbiota have an increased ability to harvest energy from the host diet, thereby increasing the total number of calories obtained from food.[8] Although the increases in energy harvest are small, changes in energy balance over time can result in weight gain. The literature also suggests that obesity associated microbiota may promote lipogenesis and increase triglyceride storage in adipocytes.[13]

Human studies support these findings and show that obesity is associated with a decrease in the diversity of intestinal microbes, and a shift in phylum level microbial proportions, favoring Firmicutes over Bacteroides.[12,14] Previous studies have indicated both BMI and WC in associations with gut microbial abundance.[15–17] WC is a simple and inexpensive way to assess central adiposity, with one study suggesting that WC is the best surrogate of visceral adiposity across a wide range of ages.[18,19] BMI has also been used as a measure of obesity and body fatness, but may not be a good indicator of visceral fat.[19,20] However, BMI and WC are often correlated at the population level.[21]

Lactobacillus acidophilus, Ruminococcus gnavus, Akkermansia muciniphila, and *Faecalibacterium prausnitzii* have all been studied in the literature for their association with either health or metabolic disease states.

Lactobacillus acidophilus is a member of the Firmicutes phylum and has been associated with weight gain and obesity in the literature in both humans and animals.[22–24] However, there is also conflicting evidence to link *Lactobacillus acidophilus* with health, showing an increase in abundance with weight loss.[25]

Akkermansia muciniphila is a mucin-degrading bacteria in the Verrucomicrobia phylum that is associated with health.[26,27] The mucus layer in the intestine provides a

barrier between the epithelial cells and the intestinal content and microbes.[26] The mucus gel is made up of glycoproteins called mucins, and as a mucin-degrader *A. muciniphila* has been shown to play a role in the turnover of mucus, improving gut barrier function.[28] Increased gut permeability has been shown to cause inflammation, metabolic endotoxemia, increases in fat mass, and insulin resistance.[29] A study in mice published in 2013 showed that *A. muciniphila* can reduce these outcomes by improving gut barrier function.[28] *A. muciniphila* has also shown to be inversely associated with obesity and type 2 diabetes mellitus in mice.[28]

Ruminococcus gnavus is a gram-positive anaerobic bacteria belonging to the Firmicutes phylum.[30] *R. gnavus* has been associated with inflammatory bowel diseases and is a mucin-degrading bacteria, potentially contributing to a damaged mucosal epithelial barrier and chronic inflammation.[31] Despite this, *R. gnavus* is listed as one of the most common 57 bacterial species in the human gut and is present in over 90% of individuals.[32] Examination of the bacterial diversity of Danish adults revealed that *R. gnavus* was associated with low bacterial richness or diversity.[33] Individuals with low bacterial diversity had greater BMI, overall adiposity, insulin resistance and inflammation.[33]

Faecalibacterium prausnitzii, a member of the Firmicutes phylum, has been associated with health and has been shown to have a lower abundance in obese individuals.[34] It is negatively associated with inflammatory markers, and may be beneficial to the lining of the intestine as a producer of butyrate, a short chain fatty acid that is the main source of energy for the cells lining the intestine.[15] A recent study in mice showed that *F. prausnitzii* may also play a role in improving gut barrier function.[35] This may be due to the production of butyrate and its role, not only in providing energy for epithelial cells, but in gene expression and proliferation of intestinal cells.[35]

Overall, research on the intestinal microbiome and its role in host pathology is in its infancy. Many studies have been done in animal models, and most of the human studies have utilized cross-sectional designs, which lack robust information on the changes in microbes over time. The goal of future research is the ability to manipulate intestinal microbial communities to minimize weight gain from excess energy harvest. However, the first step is to understand how microbial communities change with weight gain and the mechanisms by which these microbes increase energy harvest and adiposity. There are currently no longitudinal human studies that observe changes in the microbiome during a period of increased susceptibility to weight gain. Moreover, the intestinal microbiome has not been closely studied in a college population, making this research project particularly unique.

This study contributes to the body of literature regarding the human intestinal microbiome by providing the first data on changes in bacterial species over time, with specific focus on bacteria known to be associated with metabolic diseases or health. This study also examined the correlations between weight gain and the microbiome through the use of both body mass index and waist circumference to determine which was more closely associated with changes in specific members of the microbiome.

Purpose of the Study

The purpose of this study was to assess changes in the intestinal microbiota of college students living in on-campus dormitories at a large southwestern university and to examine if these changes were associated with changing weight status and anthropometric measurements.

Research Aim and Hypotheses

Research question 1: Is there an association between change in weight related anthropometrics and change in microbial abundance?

H1: Percent weight change (gain, maintain, or lost) during the year will be related to change in relative microbial abundance.

H2: Change in abundance of *L. acidophilus* and *R. gnavus* will be positively correlated with percent change in waist circumference during the year.

H3: Change in abundance of *A. muciniphila* and *F. prausnitzii* will be negatively correlated with percent change in waist circumference during the year.

H4: Percent change in waist circumference during the year will have a stronger correlation with microbial abundance than percent change in BMI.

Definition of Terms

- **Emerging adulthood**: The transition period between 18-25 years of age. For our purposes this refers specifically to college students living on-campus.[7]
- **Germ-free mice**: Mice that are raised without any microorganisms present in their intestines. They are often used to measure the impact on the host after colonizing the intestines with specific bacteria.[11]
- **Microbiota**: The collection of microbes colonizing the host intestinal lining. This is often used interchangeably with microflora.[11]
- **Microbiome**: This refers to the collective genes and genomes of the microbiota.[11]

CHAPTER 2

REVIEW OF LITERATURE

Obesity

Prevalence in the United States

Obesity has become a major public health concern in the United States, and it has staggering implications for future health care costs, morbidity and mortality risks, and overall quality of life.[36] The prevalence of obesity has increased dramatically in the United States since the 1970s.[37] One of the drivers for this change was the mass production of high-energy, processed foods that were affordable and effectively marketed to consumers.[37] These changes in the food supply contributed to the obesity epidemic, coupled with both increased energy intake and decreased energy expenditure due to the built environment.[37] By the year 2000, the prevalence of those who were overweight or obese outnumbered those who were underweight or malnourished for the first time in history.[38] As of 2012, more than one-third of U.S. adults were obese, which is equivalent to 78.6 million adults.[1,36]

Between 1980 and 1999 there were substantial increases in the prevalence of obesity in the United States, but beginning in 2003, data from the National Health and Nutrition Examination Survey (NHANES) showed slowing in the prevalence rates of obesity.[39] No significant changes in obesity prevalence for men and women were observed over the 10 year period from 2003-2008 to 2011-2012.[36,39] However, newly published data from the 2013-2014 NHANES survey shows that the prevalence of obesity has increased significantly from 1999-2000.[40] The percent of those obese increased from 30.5% in 1999-2000 to 37.7% in 2013-2014.[40] Moreover, the prevalence of severe obesity (BMI over 40 kg/m^2) has increased faster than moderate obesity.[41] This is alarming because severe obesity is associated with many serious health conditions, and treatment

options have not shown widespread success.[41] With increasing rates of obesity, there is an increased risk of comorbidities, associated complications, and a lack of widely effective interventions.[42] Because of this, primary prevention of obesity remains critical.[42]

Measures for Obesity

The National Center for Health Statistics, as a part of the U.S. Department of Health and Human Services, defines obesity as a calculated body mass index (BMI) of greater than or equal to 30 kg/m^2.[36] A BMI of 25.0 – 29.9 kg/m^2 is classified as overweight, 18.5 – 24.9 kg/m^2 is classified as normal weight, and a BMI of below 18.5 kg/m^2 is classified as underweight.[43] For children and teens aged 2-20 years, overweight is defined as BMI between the 85th and 95th percentile for the same age and sex, and obesity is defined as BMI greater than or equal to the 95th percentile.[44] BMI is inexpensive, easy to perform, and may be used to indicate high levels of adiposity.[43] While BMI is an indirect measure of adiposity, it has been shown to correlate with other measures of body fat such as skinfold thickness measurements, dual energy x-ray absorptiometry, bioelectrical impedance, and densitometry.[43] However, using BMI as a measure of obesity does have limitations. For example, normal weight individuals with excess body fat may not be diagnosed as overweight, and adults with high amounts of lean body mass may be misclassified as overweight or even obese.[19] Moreover, BMI may not always correlate with body fat in some age and ethnic groups.[19] Despite these limitations, the American Heart Association recommends the use of BMI as a primary tool for the assessment of body fatness in the clinical setting.[19]

Waist circumference (WC) is another tool used to assess adiposity, especially central adiposity. WC is simple to perform and very inexpensive, only requiring a

measuring tape. Moreover, WC correlates highly with abdominal adiposity, as measured by imaging methods, and is also recommended as a primary tool for assessing adiposity by the American Heart Association.[19,45] In fact, one study showed that WC was a better indicator of abdominal visceral adipose tissue and more closely related to metabolic variables than the commonly used waist-to-hip ratio (WHR).[46] WHR has been used as a measure of central adiposity, but the usefulness of this measure has been debated.[19] Previous research has shown that WC provided a better indication of central fat in children and adolescents than did WHR.[47] Other studies support this claim and state that WC is the best anthropometric indicator of abdominal visceral adiposity.[48,49] Additionally, a year-long study in college freshmen showed that changes in weight correlated to changes in WC, but not changes in WHR.[50] The greatest limitation with using WC is that visceral adipose tissue cannot be measured, but rather all abdominal adipose including subcutaneous and visceral adipose depots.[19,51] Computer tomography or magnetic resonance imaging can distinguish between adipose stores, but these methods are time-consuming, costly, and unlikely to be used on a large scale in clinical practice.[19]

BMI and WC are especially clinically relevant when used together because research has indicated that an increased WC indicates a greater accumulation of visceral adipose tissue and a greater risk of comorbidities, even with a normal BMI classification.[19,51] For individuals with a normal BMI but higher than normal WC, other cardiometabolic risk factors should be evaluated to determine the overall risk for disease.[19]

Weight Gain in College Students

Recently, there has been increased interest in college students and the transition that occurs from high school to increasing independence in college. The freshman year of college has been identified as a critical period for lifestyle changes and weight gain, making it a potential target as an intervention period for obesity prevention.[2,4] This transition period is usually marked by leaving home for the first time, a new environment, building new friendships and social networks, and greater independence in overall decision making.[7] The phrase 'Freshmen 15' is often used to describe a 15 pound (6.8kg) weight gain during the freshman year, but most studies to-date have shown much lower weight gains.[4,52,53] A study recruiting 764 students from Washington University showed that 70% gained an average of 4.1 ± 3.6 kg.[6] However, most studies show a smaller increase in weight from 1-3.5 kg.[2–5] A recent meta-analysis of studies on weight gain during the freshman year showed a mean weight gain of 1.75 kg (3.86 lbs).[54] This analysis also indicates that weight gain increases over the course of the freshman year, suggesting that longer studies will show greater gains in weight from students.[54]

Weight gain is often a slow process because of a slight positive energy balance. However, this positive energy balance produces a cumulative effect over time that is hard to combat due to its relative inability to be detected.[5] In fact, one study showed that a 1.9 ± 2.4 kg increase in weight was attributed to an increase of only 174 kcal/day.[5]

In 2004, a nationally representative longitudinal study was published that evaluated the incidence of obesity occurring during the transition from adolescence to adulthood.[55] This study showed a high incidence of obesity during this transition period, and a high persistence of obesity into adulthood.[55] Although an increase in BMI might be expected as this developmental period is also marked by upward growth, the changes observed in BMI were greater than predicted for age related changes.[55] This raises a

major concern that the onset of obesity during adolescence is maintained into adulthood, raising the risk for chronic diseases.

Links to Disease Risk

According to the Systematic Evidence Review from the Obesity Expert Panel at the U.S. Department of Health and Human Services, obesity increases the risk for an array of diseases including hypertension, dyslipidemia, type 2 diabetes mellitus, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea, respiratory problems, and even cancer.[56] Also, a longer duration of obesity is associated with higher risks.[57] The Coronary Artery Risk Development in Young Adults (CARDIA) prospective study followed non-obese participants for 25 years and found that the incidence of obesity was 40.4%.[57] Throughout this study, a longer duration of obesity was associated with higher levels of blood pressure, glucose, insulin, C-reactive protein, and triglycerides.[57] In particular, there was a strong relationship observed for obesity with type 2 diabetes mellitus and hypertension.[58] The CARDIA study also showed that a longer duration of obesity beginning in young adulthood was associated with coronary artery calcification, which is a measure for atherosclerosis that can predict the development of coronary heart disease events.[57] These findings are supported throughout the literature, with an increase in adverse health conditions observed with increasing severity of weight gain.[58]

These diseases not only decrease the overall quality of life, but they also come with the burden of increased medical expenses. It is estimated that the cost of healthcare is $1,429 - $1723 higher per year for an obese individual than for a person of normal weight.[59,60] Overall, the medical costs associated with obesity have been estimated at $147 billion per year.[59] A quantitative review of 33 studies published in 2011 estimated

that total spending on obesity was 4.8% - 9.1% of national health care expenditures.[60] Furthermore, it is estimated that 35% of the total cost of obesity comes from the morbidly obese with a BMI ≥ 40 kg/m^2.[60]

Although there is not an infallible way to measure the direct and indirect costs of obesity, the statistics are compelling that obesity causes a major financial burden. In the United States, it is estimated that the financial burden of obesity is two to three times greater than in other industrialized countries.[60] As the prevalence of obesity remains high, the incidence of obesity-related comorbidities will increase along with medical costs. Because the transition to college has been identified as a vulnerable stage for weight gain and the onset of obesity, prevention strategies should be targeted toward this population in order to promote healthy lifestyles and eating habits throughout life.

Lifestyle Factors Affecting Obesity

Diet

The onset of obesity is influenced by many different factors including diet, physical activity, environment, and the intestinal microbiome. These factors influence the population continuously, but this section will focus on the influences specific to college students, as the onset of obesity during this time period is a rising concern.

A study including 68 freshmen students at Cornell University identified many dietary factors which could be causing weight gain in students, including 'all-you-can-eat' facilities, evening snacks, high-fat food consumption, junk food consumption, and recent dieting.[5] Regression models linked a large majority of the weight gain to 'all-you-can-eat' dining halls and high-fat snack foods.[5] Survey answers from the students suggested that these buffet style dining halls allowed for larger portions, and they often left feeling they had overeaten.[5] Moreover, literature suggests that students are not just

eating more, but that food choices shift away from nutrient-dense foods and toward energy-dense alternatives.[6]

Recent literature has shown major shifts in the consumption of all food groups from childhood into early adulthood, specifically a decrease in the consumption of nutrient-dense foods.[61] Young adults consumed more sweetened beverages, salty snacks, and beef than did children, and did not appear to limit the consumption of fried and fast foods.[6,61] One study showed over half of the student participants reported eating fast food or high-fat fried foods at least 3 times in the past week.[6] Additionally, a study in 738 college students showed that 69% of participants consumed less than 5 servings of fruits and vegetables per day.[62] Moreover, the most recent data from the National College Health Assessment (ACHA-NCHA) in 2014 found that 58.6% of all college students consumed only 1-2 servings of fruit and vegetables per day.[63]

Physical Activity

The statistics for physical activity in college freshmen remain bleak. A sample of college freshmen in a focus group discussing changes during the freshman year described the difficulties of the transition to college, especially as it relates to health and physical activity.[3] Students described a lack of established routines, and many of them cited losing organized sports involvement in high school as detrimental to their level of physical activity.[3] Moreover, despite having more free time to exercise, many students lacked the intrinsic motivation to do so and were unconcerned about the long-term impact of their behaviors.[3]

According to the Centers for Disease Control, adults need at least 150 minutes of moderate-intensity aerobic activity or 75 minutes of vigorous-intensity aerobic activity each week and muscle-strengthening activities on 2 or more days of the week.[64]

However, most studies of college freshmen note a lack of physical activity.[6,63,65,66] This is concerning because physical activity, especially strength building and stretching, will continue to decline with age.[65] In a sample of 764 students from Washington University, researchers found that only half of the students participated in regular aerobic exercise, and 30% did not regularly participate in any form of exercise.[6] A recent undergraduate summary from ACHA-NCHA estimated that within the week prior to the survey 22% of all college students did not participate in any form of moderate-intensity cardio or aerobic exercise for at least 30 minutes, and 36.1% did not participate in any vigorous-intensity cardio or aerobic exercise for at least 20 minutes.[63]

One study showed that of the adolescents meeting physical activity requirements, one-third did not meet requirements by the time they reached young adulthood.[66] Moreover, close to 25% of adolescents engaged in over 14 hours of screen time per week and continued this into adulthood.[66] This study also found that Hispanic and Black females were less likely to meet physical activity guidelines compared with Caucasian and Asian-American females.[66]

Additionally, a decrease in physical activity changes body composition.[67] Despite energy consumption remaining somewhat constant in a sample of female freshmen students, a lack of exercise caused an increase in fat mass and a decrease in lean body mass.[67] This suggests that changes in body composition may be a sensitive indicator of changes during the freshman year. Weight, although useful, cannot account for changes in body composition that could be affected by both diet and physical activity.

Environment

The development of healthy eating and physical activity patterns is influenced by many things in the environment. The 'built environment' is the environment for working

and living that is created by a society and can heavily influence food consumption and physical activity.[38] In recent decades, the term 'obesogenic environment' has been used to describe a built environment that promotes obesity of individuals and populations through surroundings, opportunities, or conditions.[68] In the college setting, this obesity-promoting environment can be seen most readily in the availability of food choices near campus.[69]

In 2013, a collaborative study was published that evaluated the restaurant and dining venues on post-secondary campuses and within a 1.5 mile radius.[69] Thirteen university campuses, one residential post-secondary training program, and one technical college were assessed.[69] These schools varied in size, and a total of 68 dining venues on-campus and 175 restaurants off-campus were evaluated. The results showed that dining halls on campus provided the greatest number of healthy options, but also had the most barriers to healthy eating. [69] The Nutrition Environment Measures Survey – Campus Dining (NEMS-CD) scale was used to evaluate restaurants, and a compilation of all dining facilities showed a very low score.[69] This implies that dining facilities had many barriers to healthy eating. The report showed that restaurants were identifying healthy entrees, providing nutrition facts, and offering reduced portion sizes. However, many restaurants also offered larger portion sizes, often at a value price, and none of the restaurants encouraged healthy eating.[69] Overall, the results from this study show that the dining environment on or near college campuses does not facilitate or support healthy eating.[69] This obesogenic environment should be a high priority in obesity prevention during the years of college when students are vulnerable to weight gain.

Gut Microbiome

The human intestinal microbiota and its collective genes, or microbiome, are spectacularly diverse. There are at least 500 species and 100 trillion microbes present in the human intestinal lining.[10,11] Over 97% of intestinal microbes are from four dominant phyla groups including Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, listed in order of prevalence.[70] The microbiome contains at least 150 times more genetic material than is contained in the entire human genome.[70] In adults, the microbiota community is influenced by the genotype of the host as well as environmental factors such as diet, medication, exercise, weight, and energy balance.[11,70] Moreover, scientific literature has consistently implicated that the microbiota in both mice and humans plays a role in weight change and obesity.[8,9,12–14,16,17,22,24,25,71–79] The intestinal microbiota has emerged as a modifiable risk factor for obesity and is a potential target for preventing weight gain and obesity in college students.

Influences on Intestinal Microbiota

Birth and Development

Most evidence supports that the intestinal lining is sterile at birth.[11,80,81] However, recent evidence suggests that bacteria may be present on the placenta and in the amniotic fluid in utero.[82,83] In fact, the meconium of 21 healthy newborns revealed bacterial presence, but the overall diversity was low.[83] The mode of delivery also influences the development of the microbiota. Vaginal-born infants have gut microbiota similar to the vaginal microbiota of their mothers, whereas those delivered by Caesarean are first exposed to bacteria from the environment, hospital workers and the skin of their mother.[81,84,85]

Variation in the microbiota is greater in infants than in adults as the microbiota is volatile in infancy.[86] The microbiota is affected not only by the mode of delivery, but also by type of infant feeding, gestational age, hospitalization, and antibiotic usage.[81] Breast fed infants have a more heterogeneous, taxonomically diverse microbiota than formula-fed infants.[87] Overall, infants who were born full-term, vaginally at home, and were exclusively breastfed seemed to have the most beneficial intestinal microbiota composition.[81] Over time, a core microbiota develops that remains relatively stable throughout adulthood.[85] Data from a large cohort of geographically diverse babies suggests that microbiota stability does not occur until at least age 3.[88] Moreover, this cohort showed that infants and children in the United States had very different microbiota from populations in Malawi and Venezuela, suggesting that geographic location and cultural factors play a role in the early development of the microbiota.[88]

Antibiotics

Antibiotics have been used to treat bacterial infections and prevent the spread of infectious agents for over half a century.[89,90] However, antibiotics may also lead to bacterial resistance and the disruption of the microbiota.[90] Disruption of the microbiota may change the normal microbiota-mediated colonization and result in the growth of resistant pathogens.[91] Because selective antibiotics do not exist, it becomes hard to decrease pathogenic bacteria while simultaneously increasing beneficial bacteria.[91] Moreover, antibacterial resistance is increasing while the discovery of new antibiotics is slowing.[92]

During infancy, antibiotic usage can have irreparable effects, as vaginally-born infants are unlikely to be recolonized by vaginal microbiota.[92] Analysis of the fecal microbiota of 84 preterm infants who received antibiotics shortly after birth showed an

increase in opportunistic pathogens and a reduction in species richness.[93] Similarly, a study in 142 Finnish children aged 2 – 7 showed that treatment with a common antibiotic decreased richness and changed the microbiota composition, with effects still apparent two years after treatment ended.[94] Other evidence supports the notion that early administration of antibiotics is associated with overweight or obesity later in childhood, especially for children given antibiotics in the first 6 months of life or receiving multiple rounds in the first 2 years of life.[95–98]

Treatment with antibiotics decreases the diversity of the microbiota, and some bacterial species do not return to pre-treatment levels.[89] Moreover, even bacterial species that are not specifically targeted by the antibiotic may be affected due to the co-dependence of the microbiota community.[89] The resulting dysbiosis of the microbiome may lead to a decreased ability to supply nutrients, produce vitamins, and protect the intestinal lining from pathogens.[92] The direct and indirect effects of antibiotics are complex, but other treatment options are limited.[89]

Due to increasing antibiotic resistance and undesirable collateral damage to the intestinal microbiota caused by antibiotic usage, new treatment therapies are needed that target only specific infectious pathogens.[89] Probiotics and fecal transplants may hold promise in restoring the dysbiotic microbiota.[92]

Probiotics

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts.[99] These microorganisms are generally part of the normal microflora and are administered as a supplement or in foods such as yogurt.[11] Probiotics may displace pathogenic bacteria, increase the barrier function of the gut mucosa, and modulate the immune system.[100] However, in clinical studies examining the

effectiveness of probiotics in attenuating effects of diarrhea, irritable bowel syndrome, *Helicobacter pylori*, and allergies, the evidence has been insufficient to compel widespread recommendations for probiotic usage.[100] Some studies have shown that probiotics may affect the outcomes of type 2 diabetes mellitus and cardiovascular disease by changing the gut microbiota composition, regulating insulin signaling, and lowering cholesterol. However, the evidence is not sufficient and the mechanisms of action have not been elucidated.[101]

Prebiotics

Prebiotics are non-digestible food ingredients that selectively stimulate the growth of microbial genera or species in the intestinal microbiota that confer health benefits to the host.[99] Prebiotics are not digested by endogenous enzymes, but are fermented by bacteria in the intestines. Many prebiotics are digested by *Bifidobacterium* and stimulate the growth of *Bifidobacterium* colonies.[99,101] Short chain fatty acids (SCFA) are the end product of fermentation, and are a major source of energy for intestinal epithelial cells.[101] SCFAs in the intestinal lumen lower the pH and favor butyrate-producing bacteria such as *Roseburia* spp. and *F. prausnitzii*.[102]

Diet

Recent studies have shown that shifting dietary macronutrients can rapidly alter the gut microbiome.[103] An animal study in which mice were "humanized" to represent the bacterial diversity of the human gut ecosystem showed that switching from a low-fat, plant based diet to a high-fat/high-sugar diet shifted the microbiota composition within a single day.[103,104] These results were affirmed in a human study comparing the effects of a plant-based diet and an animal-based diet after 5 days in healthy young adults. The

results showed the diets altered the microbial community structure, with greater changes observed after the animal-based diet.[104] The animal-based diet produced a decrease in Firmicutes, specifically *Roseburia, Eubacterium rectale, and Ruminococcus bromii* which metabolize plant polysaccharides, and an increase in bile-tolerant microbes such as *Alistipes, Bilophila,* and *Bacteroides*.[104]

A study examining the gut microbiota of children aged 1-6 in Western Europe (EU) and Burkina Faso (BF) found significant differences in the microbiota composition.[105] These changes were attributed to the differences in diet between groups, with EU children consuming a diet low in fiber and high in animal protein, starch, sugar, and fat, and BF children consuming a diet high in fiber and consisting mostly of cereals, legumes, and vegetables.[105] The ratio of Firmicutes to Bacteroidetes (F:B) was significantly different between groups, with those from BF having a greater representation of Bacteroidetes. Moreover, the BF children had greater amounts of SCFA present, possibly due to greater fiber intake and the higher presence of bacteria that ferment fiber.[105]

This study comparing the gut microbiota of children in EU to BF is consistent with a study examining enterotypes of the gut microbiota. The gut microbiota of 10 subjects was characterized before and after a controlled-feeding study of either a high-fat/low-fiber or low-fat/high-fiber diet.[106] Before the feeding trial, most subjects had an enterotype with high levels of *Bacteroides*, associated with animal fat and protein, compared to *Prevotella*, associated with carbohydrates. Changes in the microbiota were detected after only 24 hours in the controlled feeding trial, but the enterotype classification remained stable throughout the entire 10-day feeding trial.[106] In this study, the modest changes in the gut microbiota in response to dietary changes suggests that

the enterotype of the gut microbiota is associated with the long-term diet of an individual.[106]

Furthermore, altering the overall caloric load, not specific macronutrients, also resulted in changes to the microbiota composition.[75] In a randomized, crossover design trial, 12 lean and 9 obese adults consumed a 2,400 kcal diet and a 3,400 kcal diet for 3 days each, separated by a 3 day wash-out period.[75] Changes to the microbiota were apparent and correlated with stool energy loss in lean individuals despite the short treatment period. There was a 20% increase in the Firmicutes phyla, with a corresponding decrease in Bacteroidetes, which correlated to an increased energy harvest of approximately 150 kcal.[75] This is consistent with studies showing that obese have more Firmicutes and less Bacteroidetes.[12,107]

Overall, the literature does support the relationship between diet and changes to the intestinal microbiota. However, the relationship between diet, intestinal microbiota, and body fat is still very ambiguous. Some investigators propose that diet independently affects both the gut microbiota and levels of body fat, but that interrelationships also exist.[108] A recent controlled feeding trial with a randomized, crossover design showed that changes to the diet only accounted for 10% of the total variation in microbiota composition.[109] This study used a standard diet for baseline, two diets comparable in calories with either added resistant starch or non-starch polysaccharides, and a calorically restricted diet. The results showed differences in microbiota composition between diets, but most of the variation was due to the individual.[109] In mice, dietary changes accounted for 57% of the variation in intestinal microbiota, but this is expected since mice used in experimental settings are genetically and environmentally similar.[109,110] Because humans display wide differences in genetics, habitual diet, and

medication, a 10% variation due to diet is actually quite substantial and warrants further research.[109]

Exercise

Overall, there has been a lack of evidence on the link between exercise and changes to the intestinal microbiota, and a direct causal relationship has not been established.[111] Most evidence has been observed in animal models due to the ease of simulating a controlled environment and the difficulty in separating the effects of diet and exercise in humans.[112] Studies in mice have shown that exercise produces significant changes in the intestinal microbiota when compared to non-exercised mice. Twelve mice were assigned to either an exercise group or a sedentary group for 5 weeks, and the activity level of the mice correlated to shifts in abundance and composition of the intestinal microbiota, and there was a distinct categorization between groups using principal coordinate analysis.[113]

Similarly, another study in mice showed that both diet and exercise influence the intestinal microbiota, but the changes were independent of each other. This study included two high-fat diet groups, with and without exercise, and two normal-diet groups, with and without exercise.[114] The results showed that exercise alone modified the intestinal microbial community, but these modifications did not completely attenuate the effects from the high-fat diet.[114] This suggests that examining both the diet and physical activity levels of participants is vital to correctly interpreting the data.

A human study specifically examining the relationship between exercise and the intestinal microbiota was published by Clarke, et al. in 2014. This study examined the intestinal microbiota of 40 professional rugby athletes compared to two control groups matched for size and for age and gender.[115] The athletes not only had a lower

inflammatory status, but had a more diverse intestinal microbiota than the controls.[115] The protein consumption of the athletes correlated with the microbiota diversity. However, the diet of the rugby players was much different than the control groups, which potentially confounds the relationship between exercise and the intestinal microbiota.[111,115] This study is careful to describe the results as preliminary and correlational, as research should be initiated to further examine these relationships.[111,115]

Host-Microbe Interactions, Metabolism, and Disease

Nutrient Utilization

In the gastrointestinal tract, most carbohydrates are hydrolyzed by host enzymes and absorbed in the small intestine. However, the human body is not capable of hydrolyzing many polysaccharides, particularly from plants. These polysaccharides pass undigested from the small intestine into the large intestine where they are acted on by resident bacteria.[11] These could include cellulose, xylan, and pectin, as well as inulin, non-digestible oligosaccharides, resistant starches, and even host glycoconjugates.[10,11] A highly symbiotic relationship exists between the host and the microbiota, as the microbiota has access to a variety of energy sources that would be useless to the host.[11] Because resident bacteria possess enzymes that can hydrolyze non-digestible polysaccharides, the host is able to absorb products of microbial fermentation that otherwise would not be present.[11]

After the polysaccharides are hydrolyzed by bacteria in the colon, glucose and other monosaccharides enter glycolysis to produce pyruvate and ATP. In the anaerobic environment of the intestine, fermentation occurs to produce short chain fatty acids which are absorbed and used by the host.[116] Propionate is produced from pyruvate, and butyrate and acetate are produced from acetyl-CoA.[11] Glycolysis and fermentation also

produce ATP which is used by the microbiota. Production of short chain fatty acids generally favors acetate at 70%, with propionate at 20% and butyrate at 10%, however this ratio can vary between individuals and is influenced by diet.[11] Generally, propionate is taken up by the liver, butyrate is used by colonic epithelial cells for energy, and acetate is taken up by peripheral tissues or used for lipogenesis.[116]

The intestinal microbiota also plays a role in synthesizing Vitamin K and possibly Vitamin B12, biotin, folic acid, and pantothenate. [11] However, levels of these vitamins in the feces suggests that they may be associated with the microbes and unavailable for host use.[11] Moreover, intestinal microbes also ferment amino acids, degrade oxalate, activate or inactive bioactive food components, and deconjugate metabolites that may then be reabsorbed and recycled.[80]

Inflammation and Intestinal Barrier Integrity

Recent evidence suggests that inflammation is related to the permeability of the intestinal barrier. The intestinal barrier is composed of an inner mucus layer and an outer layer where the commensal bacteria are found.[117] This barrier protects the intestinal cells from the bacteria and inter-luminal contents through mucus turnover, the production of immunoglobulin A, and the secretion of antimicrobial peptides.[117] Inflammation can cause a reduction in the thickness of the intestinal mucus layer, and pro-inflammatory cytokines can increase the permeability of the normally tight junctions between the intestinal epithelial cells.[118] The increased permeability allows both lipopolysaccharides (LPS) and peptidoglycans to be absorbed.[99] LPS is a component of gram-negative bacteria that is continually produced in the gut with the turnover of bacterial cells, and peptidoglycans are structural units in bacterial cells.[119,120] Both LPS

and peptidoglycans are endotoxins that activate pro-inflammatory signaling cascades in the body.[99]

Recently, evidence has been published suggesting that a high-fat diet may cause changes to the intestinal microbiota, influencing the amount of LPS found in circulation.[72,121] A study in mice examined the possible relationship between circulating LPS, metabolic endotoxemia, and changes in the intestinal microbiota.[72] The authors hypothesized that changes in the intestinal microbial composition due to a high-fat diet could cause metabolic endotoxemia, triggering obesity and possibly diabetes.[72] The investigators showed that a high fat diet would increase the amount of LPS containing bacteria in the intestine, and that a 4-week high fat diet would increase plasma LPS concentrations by two- to three-fold.[121] This level of LPS in the plasma was termed metabolic endotoxemia, characterized by an increase in intestinal permeability, resulting hyperglycemia, hyperinsulinemia, and an increase in whole body adipose tissue.[121] These investigators also showed that antibiotic treatment to reduce bacterial count would reduce circulating LPS levels and the effects of metabolic endotoxemia such as glucose intolerance, weight and fat gain, inflammation, and oxidative stress.[72]

Studies in humans have confirmed these results, showing that plasma levels of LPS seem to rise with higher fat intake.[122,123] Moreover, another study in humans showed that WC was positively correlated with permeability of the lower gastrointestinal tract.[124] Although the study did not directly measure LPS levels, the investigators hypothesized that higher LPS concentrations in plasma could be responsible for the adipose tissue hyperplasia.[124] Overall, these studies show that intestinal bacteria play a role in the inflammatory process and the modulation of the intestinal barrier permeability.

Specific microbial species may play a role in maintaining the intestinal barrier. *Akkermansia muciniphila* promotes intestinal barrier integrity through involvement

with mucus turnover and anti-inflammatory pathways.[117] Also, patients with inflammatory bowel diseases (IBD) had reduced levels of *A. muciniphila*.[117,125] In mice, feeding a high-fat diet produced a 46% thinner mucus layer as well as metabolic endotoxemia, adipose tissue inflammation, fat mass gain, and insulin resistance.[28] However, these metabolic disorders induced by the high-fat diet were reversed with *A. muciniphila* treatment. This evidence shows that *A. muciniphila* plays a critical role in the maintenance of a healthy intestinal barrier and the modulation of host-microbiota interactions affecting inflammation and weight gain.

Another mucolytic bacteria, *Ruminococcus gnavus*, was increased in both the feces and mucosa biopsy samples of patients with Crohn's disease (CD) primarily in the ileum.[126] Moreover, a study including 46 patients with IBD and 20 healthy controls found that patients with IBD had a shift in the dominant mucosa-associated mucolytic bacteria.[125] Patients with IBD had increased prevalence of mucosa-associated bacteria, with at least a 4-fold increase in *R. gnavus* compared to controls.[125] This increase was observed in both non-inflamed CD and ulcerative colitis patients as well as inflamed CD patients.[125] These studies indicate that *R. gnavus* has potentially inflammatory properties.

Weight Gain and Obesity and the Gut Microbiota

In recent years, both animal and human studies have shown connections between alterations in gut microbiota and obesity. [8,9,12–14,16,17,22,25,71–78,127] Obesity has been associated with a decrease in the level of diversity within the microbiota and with an increased ability for energy harvesting from the host diet.[14] Although obesity is caused by a positive energy balance, gut microbial ecology may also affect energy homeostasis through promoting energy extraction from the host diet and storage within the host.[9] In

other words, the caloric value on a food package is not necessarily definite but is influenced by the composition and efficiency of intestinal microbes.[128] Understanding the interactions between the microbiome and the host could bring considerable implications for the prevention and treatment of obesity. As obesity continues to be a major concern in the United States, alterations in gut microbiota influencing energy intake, absorption, or storage of nutrients could be an indispensable component in alleviating obesity.[13]

Studying the intestinal microbiome in humans is daunting due to a fluid environment and a plethora of factors that can influence the microbiota. However, studying the microbiome in animals is much easier due to use of germ-free mice (GF). GF mice are raised without any resident microorganisms and can be used in tests to define the impact on the host of colonizing the intestine with specific bacteria.[11] Determining specific functions of bacteria in humans is very difficult due to the dynamic nature of the microbiota and the environment, so using GF mice that may or may not be genetically modified presents a unique case for scientific experiments.

In 2004, a landmark study was published by Fredrik Bäckhed and colleagues giving the first empirical evidence that the gut microbiota plays a role in host adiposity.[13] In this study, GF mice were conventionalized, or exposed to the microbiota of conventionally raised mice, and then compared to both GF mice and conventionally raised mice. The results showed that previously GF mice which had been conventionalized ended the study with a 57% increase in total body fat content.[13] Conventional mice had 42% more body fat than GF mice even when consuming 29% less of a standard rodent chow diet.[13] Additionally, multiple studies have shown that GF were protected from obesity even when consuming a high-fat, high-sugar Westernized diet, while conventional mice experienced restructuring of microbiota communities.[71,77]

Specifically, Firmicutes increased with the Western diet, while Bacteroidetes decreased.[71] These studies also demonstrated that gut microbiota were involved in regulating the metabolism and storage of fat through the modulation of host genes involved in adipocyte uptake.[77] These results support that the intestinal microbiota plays a role in weight gain and the onset of obesity, because GF mice did not gain any weight.

Studies published over the next several years, primarily originating at Washington University in St. Louis, were in agreement on the connection between obesity and the microbiome, and further examined the changes that occurred in the microbiota that coincided with obesity. In 2005, Ruth Ley and colleagues published a study that proposed a division level shift in the proportion of Bacteroidetes to Firmicutes with the onset of obesity.[9] Mice in this study showed a 50% reduction in Bacteroidetes with the onset of obesity, and a proportionate increase in Firmicutes.[9] This was supported by a study published in 2006 showing similar phylum level shifts with obesity, but went further to show that the obese microbiome had an increased ability to extract energy from the host diet, and that this was transmissible.[8] GF mice colonized with the obese microbiota showed significant increases in total body fat over those GF mice colonized with a lean microbiota.[8] The ability to extract more energy from the diet would have been highly useful to our ancient ancestors who lacked readily available food sources, but is seemingly detrimental in our modern society.[13]

In 2014, an article was published showing that mice colonized with fecal microbiota from human adult female twins, one lean and one obese, showed changes in phenotype in response to the colonization of microbes.[129] The mice colonized with microbiota from the obese twin gained considerable overall body mass and adipose tissue, whereas the mice colonized with microbiota from the lean twin did not gain weight.[129]

Moreover as sequencing technology has progressed in recent years, namely in 16s rRNA high-throughput sequencing, current studies are looking past phylum level changes and more toward genus and species level changes.[79] A recent study showed a reduction in the abundance of *Lactobacillus* spp., and an increase in *Ruminococcaceae, Rikenellacacea,* and *Enterobacteriaceae* when consuming a high fat diet to induce obesity.[79] In contrast to earlier studies, this study also shows that the Bacteroidetes to Firmicutes ratio is not the most important shift in microbial ecology. Contrary to other studies, Bacteroidetes were increased with the onset of obesity.[79]

Studies in humans have produced less consistent results due to greater individual variation between subjects. Human studies have confirmed the results of early mice studies showing the link between the intestinal microbiome and obesity, but most studies are cross-sectional analyses comparing obese participants to lean counterparts. These studies provide a baseline for knowledge about the gut microbiome in an obese state, but lack information about changes occurring during weight gain. Future studies should examine this period of weight gain in order to target preventing weight gain.

In 2009 a study was published confirming that obesity is associated with phylum-level changes in the microbiota of humans.[103] This cross-sectional study examined the intestinal microbiota of twins concordant for obesity, and found that obesity was associated with a significant decrease in diversity and a decrease in the proportion of Bacteroidetes when compared to lean.[14] Due to this evidence, the ratio of Bacteroidetes to Firmicutes (B/F) became a focus of further studies, but produced conflicting results. For example, some studies have shown no differences in the B/F ratio between lean and obese subjects,[130,131] while another study showed an increase in Bacteroidetes in overweight subjects, and a higher ratio of B/F in overweight and obese subjects.[132]

Despite this, studies published recently have supported the notion that obesity produces phylum level changes in the intestinal microbiota.[17,22,74] A cross-sectional study in 13 non-obese and 15 obese showed a decreased B/F ratio in obese, where the Bacteroidetes phylum was 3-fold less abundant in obese subjects.[17] The B/F ratio was strongly and negatively associated with BMI.[17]

In Japan, investigators showed a decreased B/F ratio between 33 obese and 23 non-obese subjects, but also showed that the obese subjects had increased intestinal microbiota diversity.[74] This contradicts many other studies, but may be due to the way investigators in Japan classified participants as non-obese or obese, with BMI>25 classified as obese and BMI<20 classified as non-obese.[12,14,17] Admittedly, all of the participants in the non-obese group had a BMI<18.[74]

Inconsistent results at the phyla level are also followed by inconsistencies at the genus and species level. For example, at the genus level *Lactobacilli* are generally thought of as promoting health and are often consumed as probiotics.[133] However, a recent study showed a higher *Lactobacillus* concentration in obese compared to lean patients.[23] Different *Lactobacillus* species have shown to have different effects on weight change.[24] For example, obese subjects with abdominal visceral fat who received the probiotic *Lactobacillus gasseri* as part of a double-blind, parallel arm, randomized, placebo-controlled trial showed reductions in abdominal visceral fat, subcutaneous fat and body weight after 12 weeks.[134] However, *Lactobacillus acidophilus*, was associated with weight gain in humans and animals in a recent meta-analysis of randomized-controlled trials.[78]

The species *Faecalibacterium prausnitzii* is recognized as a healthy component of the gut microbiota, and represents 5%- 15% of the total bacterial population.[135] *F. prausnitzii* is a butyrate producer, and has been shown to have anti-inflammatory

properties.[73,135,136] In recent studies, *F. prausnitzii* has been negatively correlated with WC, plasma triglycerides, and inflammatory markers.[34,73] However, the relationship to weight remains unclear as one recent study showed that *F. prausnitzii* was greater in obese Indian children compared to lean.[137]

Proposed Mechanisms between the Gut Microbiota and Obesity

Based on the available evidence, there are a few proposed mechanisms by which the intestinal microbiota influences weight. First, intestinal microbiota are thought to increase the energy harvested from the diet due to the degradation and absorption of normally non-digestible polysaccharides.[99,119,138,139] This has been clearly demonstrated in mice but not in humans.[99] The processing of these polysaccharides stimulates hepatic triglyceride production through the transactivation of carbohydrate response element binding protein and sterol response element binding protein 1.[13,119] Also, intestinal microbiota are thought to suppress Fasting-induced adipocyte factor (Fiaf), a lipoprotein lipase (LPL) inhibitor.[13] In the absence of Fiaf, LPL activity increases, promoting the uptake of triglycerides into adipose tissue.[13] With the increased production of triglycerides in the liver and the increase in LPL activity, triglyceride storage in adipocytes increases.[13]

Studies in mice have also shown that fatty acid oxidation may be affected by gut microbiota through regulation of AMP-activated protein kinase (AMPK) activity.[77] Because AMPK is a monitor of cellular energy levels, a reduction in AMPK may lead to decreased fatty acid oxidation and decreased insulin sensitivity.[77,140]

Finally, weight may be influenced by the activation of G-protein-coupled receptors (GPCR) by SCFAs produced from bacterial fermentation of carbohydrates.[140] In mice deficient in GPCRs, specifically Gpr 41, a decreased expression of peptide YY

(PYY) was observed, along with an increased intestinal transit time and decreased hepatic lipogenesis.[140] PYY may decrease appetite as well as inhibit gastric motility and gastric and pancreatic secretions.[140] This evidence provides speculation that an upregulation of GPCRs by SCFAs may influence weight gain and the onset of obesity, but further research is needed to confirm this mechanism.

Weight Loss

Apart from examining the microbiota community of lean and obese subjects, there have also been studies focusing on the effects of weight loss on the microbiota. In 2006, a study was published showing distinct differences in the microbiota composition of lean and obese people.[12] In 12 obese participants, phylum level analysis showed decreased Bacteroidetes and increased Firmicutes relative to lean. After one year of either a fat-restricted or carbohydrate-restricted low calorie diet, the abundance of Bacteroidetes increased and Firmicutes decreased, shifting the microbiota composition to resemble that of lean counterparts. These results confirmed early mice studies showing a decrease in Bacteroidetes and increase in Firmicutes in obese mice relative to lean counterparts.[8,9,12]

A cross-sectional study with 3 normal weight subjects, 3 obese subjects, and 3 post-gastric bypass subjects revealed a decrease in the proportion of Firmicutes after gastric bypass.[141] There was also a relationship between obesity and methanogenic *Archaea* present in the intestines. The investigators hypothesized that methanogenic *Archaea* use the H_2 produced from bacteria such as *Prevotellaceae* to increase the metabolism of plant polysaccharides and dietary fibers in the gut, producing more SCFAs.[141] These SCFAs can then be absorbed and used for energy by the host.

Additionally, after a 4-month weight loss intervention in 33 obese individuals, a significant increase was observed in total bacterial abundance, and the B/F ratio increased due to a decrease in Firmicutes with no change in Bacteroidetes.[15] Moreover, at the genus and species level both *Akkermansia* and *Faecalibacterium prausnitzii* increased, respectively, with weight loss.[15] However, other studies have shown no difference in the B/F ratio after weight loss.[131] As with the connection to obesity, studies examining the effects of weight loss on the intestinal microbiota should consider shifts in composition at a lower taxonomic level.

Summary

Obesity continues to be a major problem in the United States today and contributes to the development of comorbidities that negatively impact quality of life.[36,56] The transition period that occurs as freshmen enter college has been identified as a vulnerable period for weight gain and the onset of obesity, as gaining independence and changing social influences often affect lifestyle, dietary patterns, and exercise habits.[2–7,54] There is also considerable evidence that the intestinal microbiota may change with obesity and play a role in nutrient utilization. However, the role of the gut microbiota in obesity has mostly been studied through cross-sectional analyses in humans or through weight loss trials. Because of this, changes that occur to the gut microbiota during weight gain are largely unknown in humans. Moreover, because college freshmen are vulnerable to weight gain, they represent a unique time period in which changes to the gut microbiota might be documented.

CHAPTER 3

METHODS

Participants

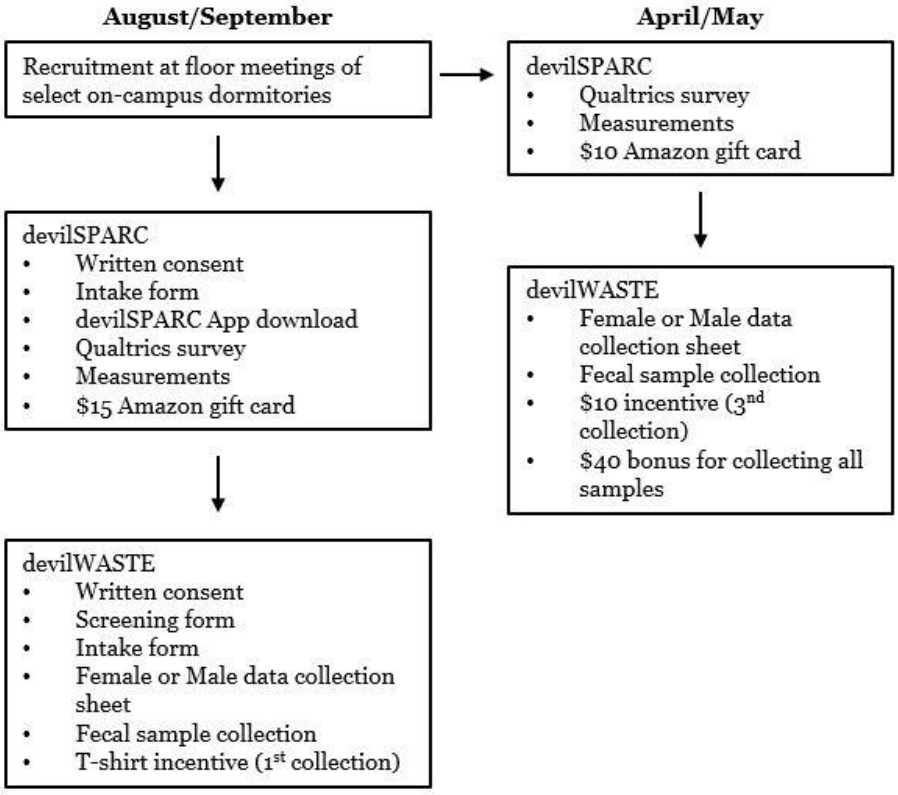
College students were recruited from floor meetings in select on-campus dormitories in the fall of 2015 at Arizona State University. After being recruited for a larger parent study, Social Impact of Physical Activity and Nutrition in College (SPARC), participants were then given the option to enroll in this sub-study, called devilWASTE. Students were considered eligible for the sub-study if they were English speaking males or females and if they were enrolled in the parent study. Enrollment in devilWASTE was contingent on continued enrollment in the parent study. Students were excluded from the study if they had a history of eating disorders, malabsorption diseases, HIV infection, high blood pressure, diabetes, or were taking prebiotics, probiotics, antibiotics or antifungal treatments regularly. Participants received up to $110 in gift cards for participating in the parent study, and a t-shirt and up to $60 for participating in devilWASTE. All study protocols were approved by the Arizona State University Institutional Review Board (Appendix A). Written informed consent was obtained from each participant (Appendix B). Enrollment forms are included in Appendix C.

Study Design and Variables

This longitudinal and observational study took place between August 2015 and May 2016 at Arizona State University. The study timeline and procedures are outlined in Figure 1. This project includes only data from time point 1 (August/September) and time point 3 (April/May), not time point 2 (November/December). For this study, data from time point 2 was excluded in order to detect the greatest change in weight-related

measures over the entire year. Participants completed an initial survey that included demographic information such as age, race/ethnicity, and gender, and met with researchers at each time point for anthropometric measurements and to collect fecal samples. BMI was calculated as kg/m^2, with height measured on a SECA stadiometer and weight measured on a high-precision SECA calibrated scale. Waist circumference (WC) was measured at the umbilicus with a spring-loaded, tension measuring tape. Height, weight, and WC were measured up to three times for each participant to obtain two measures within 0.5 cm, 0.5 kg, and 0.5 cm of each other, respectively. All researchers/staff were trained using validated measurement techniques.

Figure 1. *Procedures for each phase of the study.*



Fecal Sample Processing and DNA Extraction

Participants were given discrete collection kits and detailed instructions on how to collect fecal samples. Upon collection, fecal samples were picked up as soon as possible from participants, the goal being less than 30 minutes. Fecal samples were frozen at -80°C until processing. Frozen fecal samples were thawed at 4°C and wet weight was recorded to the nearest 0.01 g after subtracting the weight of collection materials. DNA was extracted from 200-300 mg of feces, collected from the center of the sample, using a modified version of the protocol outlined in the MoBio Power Soil DNA Isolation Kit (12888-100, MoBio, Carlsbad, CA). A heating step of 65°C for 10 minutes was added to the original protocol, per manufacturer recommendations, to increase DNA yield. DNA concentration and quality were checked and quantified using a QIAxpert System (QIAGEN, Venlo, NL) according to manufacturer instructions.

Quantitative Real-time PCR Analysis

Microbial targets were quantified through quantitative real-time PCR analysis with microbial DNA qPCR Assay kits (Qiagen, Venlo, NL) for the following targets: *Lactobacillus acidophilus (*BBID00184A*), Ruminococcus gnavus* (BBID00299A*), Faecalibacterium prausnitzii* (BBID00299A*,* and *Akkermansia muciniphilia* (BBID00026A). Primers used for qPCR were proprietary (Qiagen). The PCR reactions were performed using a CFX Connect thermocycler (Bio-Rad). Each reaction was run in duplicate with a final volume of 25 µl with 12.5µl of microbial qPCR mastermix, 1µl of microbial DNA qPCR primers, 5ng of genomic DNA from fecal samples, and microbial DNA-free water as specified per microbial target. Each plate contained a no template control, healthy control sample, and *Pan Bacteria 3* (BPCL00362A) as a reference microbe for each participant. For all reactions, samples were activated at 95°C for 10

minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 2 minutes. Relative quantification of target microbes was calculated by using the $2^{(-\Delta\Delta CT)}$ method.[142]

Statistical Analysis

Statistical analysis was carried out using IBM SPSS Version 24 (SPSS Inc., Chicago, IL) and JMP (SAS Institute). Prior to analyses, data were organized and cleaned by removing outliers >3 SD from the mean. All data were checked for normality using the Shapiro-Wilk test, and $p>0.05$ was considered normally distributed. Appropriate transformations were performed. Spearman correlation tests were used to examine correlations between microbial fold change and both percent change in WC and percent change in BMI. Variables were independent of one another and were non-parametric. To further assess these associations, multivariate regression models examined the association between percent change in WC/BMI on microbial fold change with the addition of sex and race/ethnicity as covariates. All data was presented as mean ± SD or median (25, 75 IQR) for parametric and non-parametric variables, respectively. P values <0.05 were considered significant.

The fold change for each microbial target was compared across groups according to percent weight change (loss, maintain, gain). Although weight maintenance implies no change in weight, fluctuations in weight were expected due to hydration status, equipment, clothing, and food consumption.[143] Because of this, the literature was consulted to determine a standard definition for weight maintenance and the amount of weight change that is clinically relevant. A recent meta-analysis indicated that there is no standard definition for weight maintenance, but expert consensus recommends that weight maintenance be considered a weight change of ± 3% of starting body weight.[143]

Weight change of 3% to less than 5% should be considered small weight fluctuations, and changes of 5% or greater should be considered clinically relevant.[143] Using percent weight change is preferred over absolute weight change as it accounts for baseline body size.[143] Based on this consensus, data were analyzed using weight maintenance defined as both a change of ±3% body weight and ±5% body weight. Logarithmic transformations were used for microbial data due to skew and kurtosis, but data remained non-parametric. Groups were compared using the Wilcoxon Rank test because variables were independent of one another, included two categorical groups, and the data was non-parametric.

CHAPTER 4

DATA AND RESULTS

Participant Characteristics

A total of 262 students participated in this longitudinal gut microbiome study with 110 completing both the first and last study visits. Forty-two students living in dormitories at Arizona State University were included in this pilot analysis because they had adequate sample for both qPCR assessments and high-throughput sequencing. Each participant provided informed consent, and met with researchers up to three times for anthropometric measurements and to collect fecal samples. During data analysis, 3 participants were excluded from analysis as outliers (>3 SD from the mean), leaving 39 total participants included in all analyses.

Participant characteristics are described in Table 1. At baseline, participants had an average BMI of 24.46 ± 4.24 kg/m^2, weight of 69.76 ± 14.84 kg, and waist circumference (WC) of 80.63 ± 11.19 cm. At the last measurement in April/May, BMI had increased by an average of 0.97 ± 1.28 kg/m^2, weight by 2.89 ± 3.74 kg, and WC by 2.64 ± 4.90 cm. Overall, 38.5% (n=15) of participants increased their weight by at least 5%, and 59.0% (n=23) of participants increased their weight by at least 3%.

Table 1. Baseline characteristics of study participants (n=39)	
Characteristic	*Result*
	% (n)
Gender	
Female	61.5 (24)
Male	38.5 (15)
Race/Ethnicity	
Native American/Mixed	12.8 (5)
Asian	5.1 (2)
Black	12.8 (5)
Hispanic	23.1 (9)
White	46.2 (18)
	Mean ± SD
Age, *y*	18.54 ± 0.67
Start of school year	
BMI, kg/m^2	24.46 ± 4.24
Weight, *kg*	69.76 ± 14.84
Waist circumference, *cm*	80.63 ± 11.19
End of school year	
BMI, kg/m^2	25.44 ± 4.68
Weight, *kg*	72.65 ± 16.10
Waist circumference, *cm*	83.27 ± 12.39
Abbreviations: BMI, body mass index.	

Weight Change Groups and Microbial Abundance

L. acidophilus trial qPCR runs produced no amplification on subsequent samples in the presence of positive control amplification. Due to these findings, *L. acidophilus* was excluded from all further analyses. Results include findings after relative amplification of *A. muciniphila, F. prausnitzii,* and *R. gnavus*. Participants were divided into groups based on percent weight gain, weight maintenance, and weight loss to determine if categorical groups would differ in microbial fold change over the year.

Due to the small number of participant who lost >3% body weight (n=2), these participants were included in the group with individuals who maintained weight over the year. When classifying participants based on ±3% change in body weight, fold change for *A. muciniphila* (p=0.13), *F. prausnitzii* (p=0.65), and *R. gnavus* (p=0.06) were not significant between groups of weight loss/ maintenance (<3% body weight; n=16), and weight gain (≥3% body weight; n=23).

When participants were classified according to ±5% change in body weight, fold change for *A. muciniphila* (p=0.057) and *F. prausnitzii* (p=1.00) were not significant between groups of weight loss/maintenance (n=24) and ≥5% weight gain (n=15). However, *R. gnavus* fold change was significantly different between groups of weight loss/maintenance and weight gain (0.14 [-0.21, 0.64], n=24 vs. -0.14 [-0.92, 0.05], n=15, respectively; p=0.028). Findings based on participant classification of weight loss/maintenance and weight gain ≥5% are shown in Figure 2.

In order to examine the effects of the two individuals who lost weight on the outcomes, these individuals were excluded and the analyses repeated. Between groups of weight maintenance (≤4.99% body weight, n=22) and weight gain (≥5% body weight, n=15), *A. muciniphila* fold change and *F. prausnitzii* fold change were not significantly different (p=0.11; p=0.95, respectively). However, with the exclusion of the individuals

who lost weight, the association strengthened between groups of weight maintenance and weight gain for *R. gnavus* fold change (0.19 [-0.12, 0.71], n=22 vs. -0.14 [-0.92, 0.05], n=15, respectively; p=0.019).

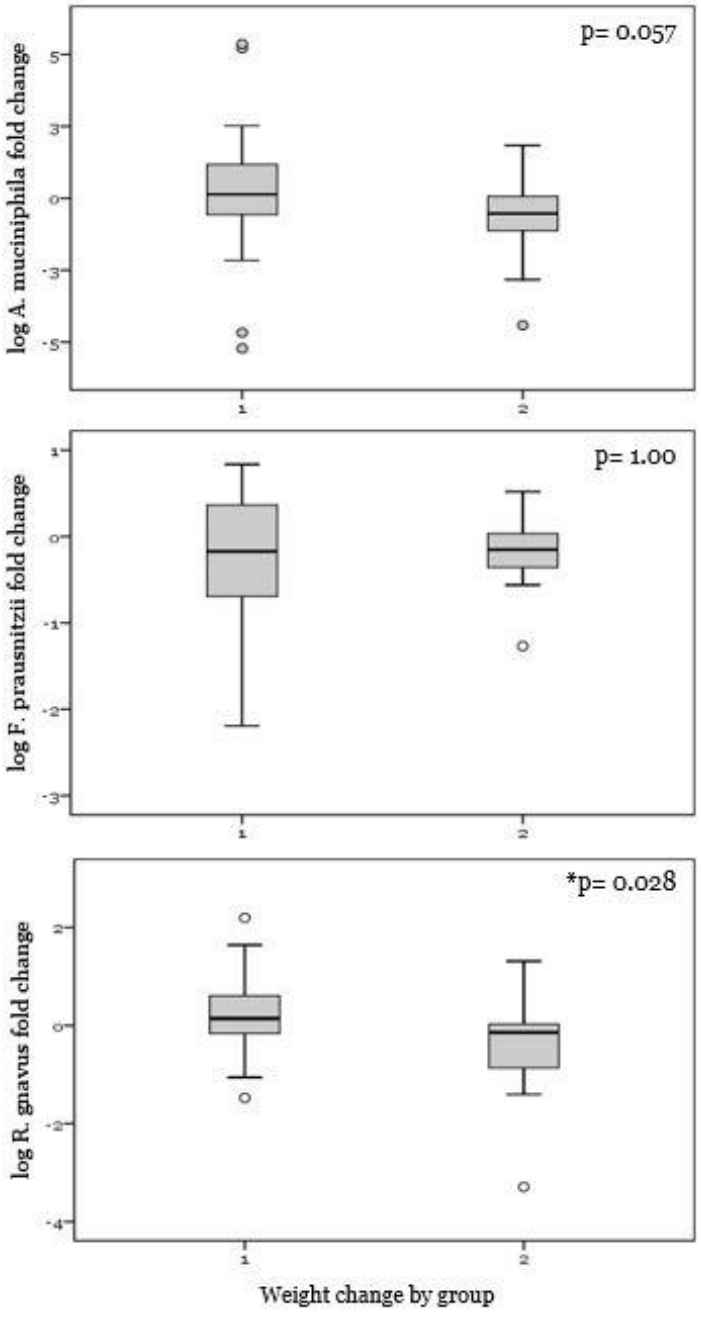


Figure 2. *Microbial fold change by groups of weight change. 1: weight loss/ maintenance (weight change <5%; n=24); 2: weight gain ≥5% body weight (n=15).*
* p< 0.05

Waist Circumference, BMI, and Microbial Abundance

Spearman's correlation was used to examine the association between log-transformed microbial data and both % change in waist circumference (WC) and % change in BMI (Figure 3). Results suggest a significant negative correlation between *A. muciniphila* fold change and % change in WC (r= -0.66, p<0.01), but the correlation was not significant between *F. prausnitzii* (r=0.18, p=0.26) or *R. gnavus* (r= -0.10, p=0.54) and % change in WC.

Regarding % change in BMI, a significant negative correlation was found with *A. muciniphila* fold change (r= -0.33, p=0.04), but the correlation was not significant between *F. prausnitzii* (r= -0.15, p=0.37) or *R. gnavus* (r= -0.17, p=0.31). Taken together, these results suggest that *A. muciniphila* fold change had a stronger association with % change in WC than % change in BMI.

In order to further explore the correlations between BMI/WC and microbial fold change, multivariate regressions (Tables 2, 3, 4) were used to evaluate associations between these variables while controlling for covariates known to influence the gut microbiome. For each table, Model 1 examines the association between % WC change and Model 2 examines % BMI change, both including covariates of sex and race/ethnicity. For *A. muciniphila*, Model 1 was significant (R^2=0.53, p<0.01), with % WC change having a negative influence on microbial abundance (Estimate= -0.22, p<0.01). Additional covariates did not significantly affect this association. However, the results were not significant in Model 2 for *A. muciniphila* and % BMI change (R^2=0.24, p=0.15).

Neither Model 1 nor Model 2 had significant findings for *F. prausnitzii* fold change (R^2=0.10, p=0.72; R^2=0.06, p=0.90, respectively) or *R. gnavus* fold change (R^2=0.29, p=0.07; R^2=0.27, p=0.09, respectively). However, for *R. gnavus* fold change,

the influence of % WC change approached significance (Estimate= -0.05, p=0.08). Notably, self-reporting as Black race/ethnicity had a negative influence on the change in *R. gnavus* that approached significance for both Model 1 and Model 2 (Estimate= -0.69, p=0.09; Estimate= -0.78, p=0.06, respectively).

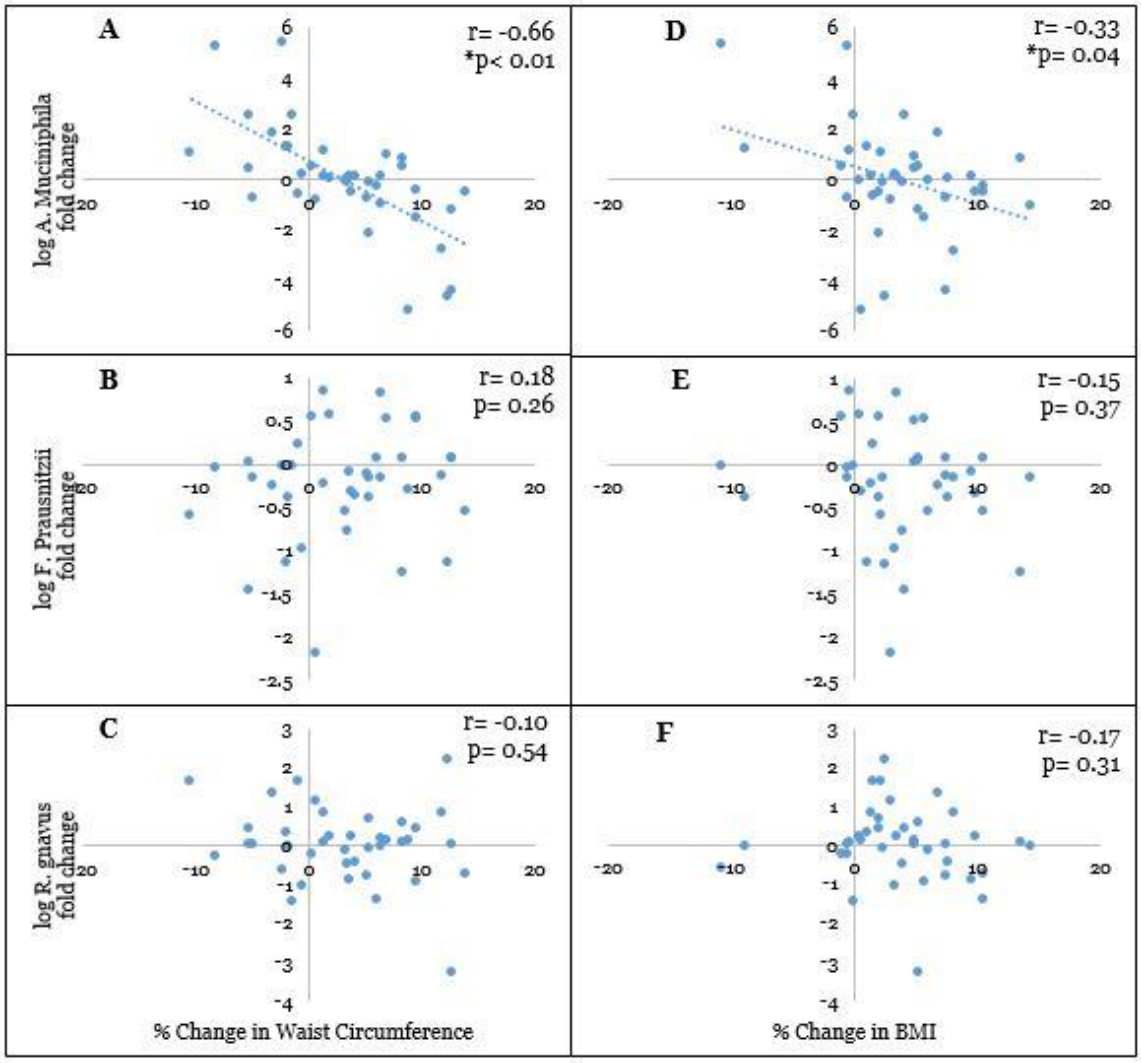


Figure 3. *Associations between log-transformed microbial fold change and % change in waist circumference (left) and % change in BMI (right) for participants (n=39). Log transformed microbial expression of A. muciniphila, F. prausnitzii, and R. gnavus are provided in panels A and D, B and E, and C and F, respectively. The Spearman correlation coefficient indicated a significant negative correlation for A. muciniphila fold change and % change in waist circumference (r= -0.66; p< 0.0001) and % change in BMI (r= -0.33; p= 0.04). The line of best fit for linear function is included on both graphs (Panels A and D).*

* p< 0.05

Table 2. Multivariate regression for BMI/WC change and *A. muciniphila* fold change (n=39).

A. muciniphila

Model 1 (R^2=0.53; p<0.01*)				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob > |t|**
Intercept	0.99	0.36	2.75	0.01*
% WC change	-0.22	0.05	-4.76	<0.01*
Sex	-0.03	0.32	-0.09	0.93
Native American/Mixed	0.06	0.65	0.09	0.93
Asian	1.60	1.02	1.57	0.13
Black	-1.04	0.70	-1.48	0.15
Hispanic	-0.07	0.53	-0.13	0.90
Model 2 (R^2=0.24; p=0.15)				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob > |t|**
Intercept	0.56	0.45	1.25	0.22
% BMI change	-0.11	0.07	-1.52	0.14
Sex	0.50	0.37	1.35	0.19
Native American/Mixed	-0.01	0.82	-0.01	0.99
Asian	2.03	1.38	1.48	0.15
Black	-1.65	0.87	-1.9	0.07
Hispanic	0.12	0.72	0.16	0.87

Abbreviations: BMI, Body Mass Index; WC, waist circumference.

* Significant p< 0.05

Table 3. Multivariate regression for BMI/WC change and *F. prausnitzii* fold change (n=39).

F. prausnitzii

Model 1 (R^2=0.10; p=0.72)				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob > |t|**
Intercept	-0.23	0.15	-1.50	0.14
% WC change	0.02	0.02	1.20	0.24
Sex	0.08	0.13	0.61	0.55
Native American/Mixed	-0.13	0.27	-0.47	0.64
Asian	0.58	0.43	1.36	0.18
Black	-0.05	0.29	-0.18	0.85
Hispanic	-0.31	0.22	-1.41	0.17
Model 2 (R^2=0.06; p=0.90)				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob > |t|**
Intercept	-0.15	0.15	-0.97	0.34
% BMI change	0.00	0.02	-0.12	0.91
Sex	0.02	0.12	0.13	0.90
Native American/Mixed	-0.11	0.28	-0.40	0.69
Asian	0.42	0.46	0.90	0.37
Black	0.03	0.29	0.1	0.92
Hispanic	-0.27	0.27	-1.12	0.27

Abbreviations: BMI, Body Mass Index; WC, waist circumference.

* Significant p< 0.05

Table 4. Multivariate regression for BMI/WC change and *R. gnavus* fold change (n=39).

R. gnavus

Model 1 (R^2=0.29; p=0.07)				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob > |t|**
Intercept	-0.03	0.20	-0.15	0.88
% WC change	-0.05	0.03	-1.80	0.08
Sex	-0.21	0.18	-1.21	0.24
Native American/Mixed	0.17	0.37	0.46	0.65
Asian	-0.40	0.58	-0.69	0.49
Black	-0.69	0.39	-1.74	0.09
Hispanic	0.45	0.30	1.52	0.14
Model 2 (R^2=0.27; p=0.09)				
Term	**Estimate**	**Std Error**	**t Ratio**	**Prob > |t|**
Intercept	-0.06	0.20	-0.30	0.76
% BMI change	-0.05	0.03	-1.52	0.14
Sex	-0.12	0.17	-0.71	0.48
Native American/Mixed	0.17	0.37	0.46	0.65
Asian	-0.52	0.62	-0.84	0.41
Black	-0.78	0.39	-1.99	0.06
Hispanic	0.59	0.33	1.82	0.08

Abbreviations: BMI, Body Mass Index; WC, waist circumference.

* Significant p< 0.05

CHAPTER 5

DISCUSSION

The purpose of this longitudinal study was to assess changes in the intestinal microbiota of college students living in the dorms, and to examine if these changes were associated with changing weight status and anthropometric measurements. This study involved 39 students and provides insight into a period of life commonly associated with weight gain. Overall, nearly 39% of this cohort experienced clinically significant weight gain (≥5% body weight) with 59% gaining at least 3% of their baseline body weight.

When participants were divided into groups of weight loss/maintenance (<5% weight gain) and weight gain (≥5% body weight), *R. gnavus* fold change was significantly different between groups. This finding suggests that a significant change in body weight (±5%) may be associated with changes in microbial abundance. Correlation analyses suggested that there were no associations between % change in WC and either *R. gnavus* fold change or *F. prausnitzii* fold change. However, *A. muciniphila* fold change was significantly negatively correlated with % change in WC and % change in BMI. *A. muciniphila* fold change had a stronger correlation to % change in WC than % change in BMI. Further analysis with multivariate regressions suggested that % WC change had a significant influence on *A. muciniphila* fold change after accounting for sex, race, and ethnicity. However, the association between % BMI change and *A. muciniphila* fold change lost significance with the addition of covariates. These findings suggest that in this population, changes in WC have a greater influence on *A. muciniphila* fold change than changes in BMI.

Overall, these findings provide insight into the associations between gut microbiota and weight-related outcomes in college students living on campus. Because of

interest in defining an obesity-associated microbiota, information collected during weight gain is particularly useful in exploring the changes that occur in the microbiota along with an increase in weight.

A. muciniphila Related to WC and BMI

This study was the first to show that *A. muciniphila* had a significant negative correlation with % change in WC and % change in BMI during a period of weight gain. This suggests that as participants increased in WC and BMI over the year, the abundance of *A. muciniphila* decreased. The relationship was stronger for WC, suggesting that WC may have a greater effect than BMI on *A. muciniphila* fold change.

These findings are in agreement with previous cross-sectional studies showing that *Akkermansia* (genus) was negatively correlated with BMI in a group of 30 Colombian adults, and that there was a significant difference between *A. muciniphila* abundance in 20 overweight/obese children compared to 20 normal weight children aged 4-5 years.[16,144] Moreover, another cross-sectional study showed a significant difference between *Akkermansia* abundance in obese adults compared to a separate group of adults having lost weight after gastric bypass surgery.[141] Additionally, a study published in 2016 examined gut microbial composition and function in sub-clinical type 2 diabetes mellitus and suggested that *A. muciniphila* may have a decreased abundance before the onset of disease.[145] In the cohort of 10 monozygotic twin pairs included in this study, *A. muciniphila* was negatively correlated with BMI, fasting blood sugar, and insulin levels.[145] Overall, these cross-sectional studies suggest that *A. muciniphila* abundance is associated with weight-related outcomes, and the current study supports these results with longitudinal findings.

However, a study published in 2016 in overweight and obese adults showed that waist circumference was not significantly different between groups of high *A. muciniphila* abundance and low *A. muciniphila* abundance.[146] The abundance of *A. muciniphila* was inversely associated with subcutaneous white adipose tissue diameter, but not total fat mass.[146] Further, this study showed that overweight/obese participants who had higher *A. muciniphila* abundance had a healthier metabolic status, as evidenced by a lower waist-to-hip ratio and higher insulin sensitivity.[146]

In this study, *A. muciniphila* fold change was correlated with % BMI change, but lost significance in the multivariate regression with the addition of covariates. Meanwhile, % WC change remained highly correlated with *A. muciniphila* fold change, even with the addition of covariates. WC is highly correlated with abdominal adiposity, and BMI is often used as a measure of obesity, but has limitations due to its inability to distinguish between fat mass and lean body mass.[19]

A. muciniphila is a mucin-degrading bacteria and has been shown to improve gut barrier function.[28] In mice fed a high-fat diet, treatment with *A. muciniphila* counteracted a decrease in thickness of the inner mucus layer, as well as reversed fat mass gain, adipose tissue inflammation, and insulin resistance.[28] In 2017 the same research group published another study in mice fed a high-fat diet and treated with either live *A. muciniphila*, pasteurized *A. muciniphila,* or Amuc_110, a protein derived from the outer membrane of *A. muciniphila*. The results suggested that treatment with either of the three forms of *A. muciniphila* increased the expression of genes encoding tight-junction proteins in the jejunum and ileum, thereby strengthening gut barrier function and reducing the leakage of LPS into circulation.[147] *A. muciniphila* treatment also attenuated fat mass gain, insulin resistance, and dyslipidemia in mice fed a high-fat diet, suggesting that *A. muciniphila* may help prevent these inflammatory states.[147]

These results have not been shown in humans.[28,147] However, in humans, increased permeability of the lower gastrointestinal tract has been positively correlated with both waist circumference and visceral fat measured by CT scan.[124] Further research is needed in humans to explore the relationships between *A. muciniphila*, permeability of the gastrointestinal tract, and visceral fat in humans.

F. prausnitzii is Not Related to Weight-Related Outcomes

Contrary to other published studies, this study showed no correlation between *F. prausnitzii* abundance and weight-related outcomes of WC, BMI, and weight. *F. prausnitzii* fold change was not different between groups of participants who lost/maintained weight or gained >5% body weight over the year. *F. prausnitzii* fold change was not correlated to % change in WC or % in BMI.

These results are not consistent with previous studies showing that *F. prausnitzii* is related to weight-related outcomes. In 2013, a cross-sectional study with 28 participants showed that *F.* prausnitzii relative abundance was negatively correlated with BMI.[17] In 2015, a weight loss study with a dietary intervention showed a significant increase in *F. prausnitzii* after an average weight loss of 7.44±4.9 kg.[15] Comparatively, the average weight gain in this study was 2.89±3.74 kg, which may imply that a greater change in weight is necessary in order to affect the abundance of this microbe.

Furthermore, a randomized intervention study showed that WC was negatively correlated with *F. prausnitzii* relative abundance in participants with metabolic syndrome (MetS).[34] Additionally, *F. prausnitzii* relative abundance significantly increased in participants with MetS after 2 years consuming a Mediterranean diet.[34] However, these studies indicate that while F. prausnitzii has been associated with weight-related outcomes, dietary changes may also have an impact on the abundance of

F. prausnitzii. This suggests that including dietary information in the present study could have influenced the outcomes for this target of interest.

However, in a cross-sectional study with 28 Indian children, the relative abundance of *F. prausnitzii* was positively associated with obesity.[137] *F. prausnitzii* is a member of the Firmicutes phylum, which has frequently been found to increase with obesity.[9,13,17,22,71] However, many studies have linked *F. prausnitzii* with health, showing that those who have inflammatory bowel diseases or diabetes, are elderly and frail, as well as those with chronic idiopathic diarrhea and malnutrition have a lower abundance of *F. prausnitzii*.[73,148,149] Moreover, another study showed that *F. prausnitzii* abundance was negatively correlated with inflammatory markers after gastric bypass surgery independent of changes in food intake.[73]

Overall, it seems that *F. prausnitzii* may have more complex relationships than these studies have assessed. *F. prausnitzii* was not related to clinically significant weight changes (≥5% weight change) in this population, but previous research suggests that larger changes in weight-related outcomes may be necessary to affect abundance of this microbe. *F. prausnitzii* has been negatively associated with disease states, inflammation, waist circumference, and dietary changes, but most studies lack causal inference in their ability to make conclusions regarding this microbe. The results of the current study, in view of previous research, suggest that *F. prausnitzii* may have been influenced by dietary factors or inflammatory states that this study was not able to assess.

R. gnavus Related to Weight Change

This study was the first to show that *R. gnavus* fold change was related to clinically significant weight change over time. *R. gnavus* fold change was significantly different between groups of participants who lost/maintained weight and gained ≥5%

body weight. The group that gained clinically significant weight over the year had a lower average fold change in *R. gnavus* over the academic year compared to the other group. These findings suggest that there may be an association between weight gain and *R. gnavus* abundance. However, linear models of *R. gnavus* suggested that this microbe was not associated with either % change in WC or % change in BMI.

Few studies have shown relationships between *R. gnavus* and weight-related outcomes. Contrary to the present study's findings, a cross-sectional study in 2013 showed that *R. gnavus* was positively correlated with BMI in 20 adults.[17] In 2009, a weight-loss intervention study in 36 adolescents showed a decrease in *Clostridium* cluster XIVa that correlated with weight loss.[25] *R. gnavus* is a member of *Clostridium* cluster XIVa along with other genera such as *Coprococcus, Eubacterium,* and *Lachnospira*.[25] *R. gnavus* is a mucolytic bacteria that has been linked to inflammatory bowel diseases, with one study showing a 4 fold increase *R. gnavus* in the intestinal epithelium of both Crohn's and ulcerative colitis patients.[125] The present study was not able to examine inflammatory states, which could have affected *R. gnavus* relative abundance.

In the multivariate regression, findings approached significance. Specifically, % WC change had a negative influence on *R. gnavus*, and the addition of covariates helped to explain more of the variation. Specifically, the addition of race/ethnicity as a covariate impacted the model, with self-identifying as Black race/ethnicity having a negative effect within both regression models and almost reaching significance. These findings suggest that race/ethnicity may influence gut microbial communities. In support of this, an article published in 2015 showed that microbial profiles between 20 individuals differed by race/ethnicity groups.[150] Specifically, African Americans had more Firmicutes than Whites, and were specifically enriched in the *Ruminococcacea* family.[150] *R. gnavus*

belongs to the *Ruminococcacea* family, but the present study shows the opposite effect, with Black race/ethnicity exerting a negative influence on *R. gnavus* fold change.[150]

Overall, the findings of this study contradict previous studies involving *R. gnavus*. This could be due to limitations of the present study in lacking measures of inflammation and using anthropometric measures instead of more sophisticated body composition measures. However, given the lack of research on *R. gnavus* and its association with weight-related outcomes, more research is needed.

L. Acidophilus

During preparation for qPCR, tests were run for each microbial kit to ensure a proper testing environment and controls. After subsequent qPCR runs, *L. acidophilus* showed no amplification for each of the test samples. There has been considerable research on *L. acidophilus* and its relation to weight-related outcomes and its function as a probiotic.[22–24,78] However, other research has shown that most *L. acidophilus* is actually allochthonous, or formed in another place, derived from fermented food, the oral cavity, or more proximal parts of the gastrointestinal tract.[151] Research in the late 1800's and early 1900's gave Lactobacilli a reputation as a dominant member of the intestinal microbiota, but these findings were likely related to the absence of anaerobic culture techniques.[151] In reality, *L. acidophilus* may represent 0.01% of cultivable counts, and Lactobacilli may not be detected at all in 25% of fecal samples.[151] Due to these findings and a lack of amplification from test samples, *L. acidophilus* was excluded in all final study analyses.

Strengths and Limitations

The strengths of this study include the longitudinal design that allowed for the assessment of relationships over time, and the ability to capture a period of time when participants were susceptible to weight gain. Weight gain is difficult to study due to the ethical concerns of imposing weight gain on participants. However, the observational nature of this study allowed weight gain to occur without outside influence.

The limitations of this study include using waist circumference (WC) as a non-invasive measure of visceral adiposity instead of using dual-energy x-ray absorptiometry. This limitation was necessary because of the large sample size in the parent study, logistics scheduling DXA scans, and limited funding. Using weight and calculated BMI as a measure of obesity was also a limitation since weight differences over time may reflect changes in hydration status and clothing, and may vary throughout the day.

Further, physical activity and dietary information were not included in this study, so it is not clear if relationships were related to changes in weight-related outcomes or changes in diet or physical activity. Only one fecal sample was collected at each time point, so it is unclear if results are representative of the normal gut microbial composition or if changes are due to short term changes in diet. Research has shown that gut microbial enterotypes, based on phyla level microbial abundance, remain stable even during dietary changes.[106] However, changes in lower level taxonomy may be detected within 24 hours after initiating a dietary intervention.[106] Moreover, a recent study found a higher abundance of *A. muciniphila* and *F. prausnitzii* in active women when compared to sedentary women.[152] This suggests that abundance of the target microbes in this study could have been influenced by short-term dietary changes or physical activity levels not considered in this analysis.

Lastly, this study included a small sample size that may not be representative of the larger campus. Because of this, the generalizability of results is limited and should be confirmed with further studies.

Summary

This study was the first to examine changes in the intestinal microbiota over a period of weight gain. Results suggest significant associations between species level microbes and weight-related outcomes. Specifically, *A. muciniphila* fold change was negatively associated with % change in WC and % change in BMI. This study was also the first to show that *R. gnavus* fold change was related to weight change. These results underscore the hypothesis that intestinal microbial communities change with weight gain. Because the freshman year of college is often associated with an increase in weight and fat mass, these relationships should be explored further in an effort to attenuate weight gain during college and reduce the risk of obesity and obesity-related comorbidities later in life.

CHAPTER 6

CONCLUSION

The transition to college has been identified as a period of increased susceptibility to weight gain as students adapt to many lifestyle changes.[2–4] The intestinal microbiota has been recognized as a factor influencing metabolic functioning and weight changes.[99] Cross-sectional research has shown that intestinal microbiota is different between lean and obese people, and further research has shown differences in the intestinal microbiota after weight loss.[12,14,15,17] However, this is the first study to examine the intestinal microbiota during a period of weight gain in humans. This study examined changes in abundance of 3 species level microbes in students living in on-campus dorms over an academic year, and found that *A. muciniphila* and *R. gnavus* fold changes were significantly associated with weight-related outcomes. However, contrary to published literature, relationships between *F. prausnitzii* and weight-related outcomes were not significant. More research is needed to confirm these findings and further explore relationships between the intestinal microbiome and weight-related outcomes in college students. A larger sample size and more robust sequencing of microbial communities could provide more information on potential associations and mechanisms. Further work may also result in the identification of interventional strategies that either prevent or treat microbial-related weight changes.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

ASU Knowledge Enterprise Development

APPROVAL: MODIFICATION

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

Dear Meredith Bruening:

On 10/25/2016 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: HHS: National Institutes of Health (NIH), Funding Source ID: HHS-NIH-National Institutes of Health
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• TangoCongratulations.pdf, Category: Participant materials (specific directions for them); • waist_hip_circumference_form.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • devilWASTE Female Participant Data Collection Sheet 2015.pdf, Category: Measures (Survey

	questions/Interview questions /interview guides/focus group questions); • Response to 9/23 modification request, Dr.docx, Category: IRB Protocol; • RecruitQuestionFlyer.pdf, Category: Recruitment Materials; • ReminderFlyers.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • RefusalResponseSheet.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • DidYouMissUs_RecruitmentFlyer.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Participant ID card and reminders, Category: Participant materials (specific directions for them); • App_Description.pdf, Category: Participant materials (specific directions for them); • WelcomeBackSpringEmail, Category: Participant materials (specific directions for them); • ExtraIncentivesEmail100715.pdf, Category: Participant materials (specific directions for them); • Changes in app survey questions, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • NewSpringTeamMembers, Category: Other (to reflect anything not captured above); • devilSPARC messages_072115.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Microbiome pilot consent form, Category: Consent Form; • UpdatedProtocol_MergedWithdevilWASTE_101416.doc, Category: IRB Protocol; • TangoCardAnnouncementEmail.pdf, Category: Participant materials (specific directions for them); • Updated longitudinal survey plan, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • GetInvolvedinScienceRecruitFlyer.pdf, Category: Recruitment Materials; • devilWASTE Screening Form 2015.pdf, Category: Screening forms; • devilWASTE Male Participant Data Collection Sheet 2015.pdf, Category: Measures (Survey

	questions/Interview questions /interview guides/focus group questions); • BoostInEMAIncentives_100715.pdf, Category: Participant materials (specific directions for them); • height_weight_form.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • IRB Protocol for Microbiome Sub-Study, Category: IRB Protocol; • Response to 9/23 modification request, Dr.pdf, Category: Other (to reflect anything not captured above); • Microbiome pilot flyer, Category: Recruitment Materials; • script just devilSPARC, Category: Consent Form; • Flyers for Extra Participants.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • devilWASTE Study Enroll Form 2015.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • devilWASTE script, Category: Consent Form; • devilSPARC_2015ConsentForm_082115.pdf, Category: Consent Form; • ExtraMoneyRecruit.pdf, Category: Recruitment Materials; • Refer-A-Friend Program_100715.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Script explaining the app (goes with the AppdescriptionPDF), Category: Participant materials (specific directions for them); • Tentative devilSPARC data collection schedule.pdf, Category: Other (to reflect anything not captured above);

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:

Stephanie Nelson
Tsung-Yen Yu
Anna Schmeling
David Schaefer
Corrie Whisner
Anna Gianpetro
Daniel Hruschka
Alexandra Slade
Michael Todd
Kevin Hollingshead
Rebecca Bender
Monica Diaz
Peter Pace
Jessica King
Kelly McCormick
Tristan Thibodeau
Elizabeth Journey
Jason Shumberger
Megan Dzurka
Brandon Dente
Alyssa Azuma
Rosa Krajmalnik-Brown
Jose Rosales Chavez
Katy Argo
Juan Maldonado Ortiz
Kara Robertson
Jessica Ashurst
Irene van Woerden
Mariya Voytyuk
Punam Ohri-Vachaspati

APPENDIX B

CONSENT FORM

CONSENT/ASSENT FORM
devilSPARC + devilWASTE, Fall 2015

ASU College of Health Solutions
ARIZONA STATE UNIVERSITY

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.

RESEARCHER(S): Professor Meg Bruening, PhD, MPH, RD from the College of Health Solutions is partnering with the College of Liberal Arts and Sciences to invite you to participate in a research study.

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

DESCRIPTION OF RESEARCH STUDY: If you decide to be a part of this study, you will be asked to complete the following related to eating, physical activity behaviors and weight:

STUDY ACTIVITIES	PARTICIPANT'S INITIALS INDICATING UNDERSTANDING
Check-in survey (4 times throughout the year)	_____
Height, weight, waist, hip measurements (4 times throughout the year)	_____
devilSPARC app surveys (4 times throughout the year. App will be downloaded to your phone) *The mobile app will prompt you to complete a short (1-minute) questionnaire about your current activities. You will be asked to complete these brief surveys randomly 8 times per day (between 9am and 11pm) for a total of 4 days at each time point.*	_____
SunCard *Researchers will have limited access to view your SunCard activity throughout the year, including entrance/exit of ASU's dining halls, food receipt data, and on-campus gym facilities.*	_____
Friends Researchers will ask you to provide the names and contact information of our friends so that we ca invite them to participate in the study	_____

Participation in this study is voluntary. You can choose to stop at any time. Your survey responses will be kept **strictly confidential**, and will only be compiled as a group, not individually. Your decision to participate and your responses, should you choose to participate, will not affect your enrollment status at Arizona State University in any way. If you agree to participate, your time spent participating will total around 5-6 hours.

We may have additional opportunities to participate in other studies. If you are willing to be contacted about these opportunities, please initial here: ____ *(participant's initials indicating willingness)*

RISKS: Once installed, the mobile app will run in the background on your phone. This may lead to battery drain, and will capture some information about your location during participation. We are working with an outside vendor, Twilio, to send you text messages for you to complete the devilSPARC app surveys; we have an agreement to maintain your confidentiality with this company. You should only receive messages from us from this company unless you have signed up for other services through other vendors. Your SunCard activity may also include information about your location. Additionally, you may feel uncomfortable providing personal information about yourself in the study questionnaires. At every point, the researcher will de-identify data so that your questionnaire responses and information about your location will not be linked to you personally. 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 include helping the researcher 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 by name. In order to maintain confidentiality of your records, Dr. Bruening will assure that your name will only appear on this consent form and the intake form. 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 up to $110 in Amazon gift cards for completing this study. In addition, if 60% of the students under the direction of your Community Mentor complete the study, your floor may receive extra incentives such as lanyards, t-shirts and water bottles.

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 researcher, Dr. Meg Bruening at devilSPARC@asu.edu or 480.269.7454.

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 for your records.

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 additional section of the study! If interested, see below.

RESEARCHER(S): Professor Corrie Whisner, PhD from the College of Health Solutions invites you to participate in a research study.

PURPOSE OF SECTION: The goal of this study section, called devilWASTE, is to learn about how your diet, friends and fitness during your first year of college impact the microbes that live in your intestine. Microbes occur naturally within and on your body. Most microbes are not harmful, and may play an important role in your health.

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 three fecal / stool samples during your first year of college. Stool collection kits that contain everything you will need to collect the samples will be provided to you by the study staff. Samples 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 the devilSPARC study. Hormone changes and medications influence intestinal microbes; therefore, female participants will be asked to provide the first date of their last menstrual cycle and all participants will be asked about medication use.

RISKS AND BENEFITS OF PARTICIPATION: There are no major risks associated with collecting stool samples. 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 your gut microbes. In order to receive results from the overall study, when they are available, you must notify the study staff of your interest.

COSTS AND PAYMENTS: You will receive a t-shirt and up to $60 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

ENROLLMENT FORMS (3)

devilSPARC ID: _____ Date: _____ Staff Initials: _____

devilWASTE Screening Form

Inclusion Criteria:

___ English-speaking male or female

___ Enrolled in devilSPARC study

Exclusion Criteria:

___ History of eating disorders

___ History of malabsorption diseases,

___ HIV infection

___ High blood pressure

___ Diabetes

___ Taking prebiotics, probiotics, antibiotics and/or antifungal treatments regularly (if prescribed recently for illness you may still participate in later sample collections). Please specify specific treatment / product use in box below.

___ Eligible

___ Not Eligible

devilWaste Study Intake Form

IMPORTANT: This information will be used during data collection to communicate with you regarding sample collection and pick-up. After completion of the study, this form will be destroyed and all data will be associated with your WASTE and SPARC id numbers (never your name).

WASTE ID #: _____ SPARC ID #: _____

Name: _____

Phone #: _____
(circle one: cell / dorm)

Email: _____
(one that you check regularly)

Preferred contact method: ___ Text ___ Call ___ Email

Would you like to be contacted in order to receive a print out of your intestinal bacteria? (Check one)

____ Yes ____ No

devilWASTE ID: _____ Date: _____ Staff Initials: _____

Male Participant Data Collection Sheet

Have you been consuming / taking prebiotics, probiotics, antibiotics and/or antifungals during the last 2-3 month? ____ Yes ____ No

If yes, please specify the medication, treatment duration and when you began taking the product.

_____
_____
_____
_____

devilWASTE ID: _____ Date: _____ Staff Initials: _____

Female Participant Data Collection Sheet

Have you been consuming / taking prebiotics, probiotics, antibiotics and/or antifungals during the last 2-3 month? ____ Yes ____ No

If yes, please specify the medication, treatment duration and when you began taking the product.

_____

_____

_____

_____

Please circle the first date of your last menstrual period.