Microbial Diversity in the Gut Microbiome in Relation to Weight Gain of Freshman Adolescents at Arizona State

by

Mary Ahern

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved January 2021 by the
Graduate Supervisory Committee:

Corrie Whisner, Chair
Meg Bruening
Dorothy Sears

ARIZONA STATE UNIVERSITY

May 2021

ABSTRACT

Historically, researchers in the gut microbiome have deemed the composition of the microbiome as being adult by the age of two. However, recent studies have contradicted this, demonstrating statistically significant differences in the microbiome even through childhood and adolescence. This difference is important in the field of microbiome research, particularly in studies examining this relationship with weight, because even though there have been significant associations between the gut microbiome and weight, they have been largely studied in adults. The freshman year of college is an interesting time to study this relationship in younger populations, due to the lifestyle changes that make them vulnerable to weight gain. This study included N=139 participants, a majority female (N=97, 69.8%), white (N=59, 42.4%), and non-Hispanic (N=89, 64%). Participants were only included in this analysis if they gave 2 or more fecal samples over the 4 timepoint study. Samples were sequenced using the Illumina MiSeq instrument after polymerase chain reaction (PCR) amplification was performed on the V4 region of the 16S rRNA gene sequence. Statistical analysis was performed using the longitudinal plugin of QIIME2. Results demonstrate that low abundance features seemed to drive a majority of the differences in variability between those who maintained their weight over the course of the study and those who gained weight. This was demonstrated through many significant Unweighted UniFrac results with corresponding nonsignificant Weighted UniFrac data. This study demonstrated that changes in lower abundance features may have driven the significant differences in weight status in this study. This study emphasized the importance of low abundance features and how this relates to

changes in weight status during a period of major lifestyle changes. Further work is needed to confirm these findings and explore how gut microbes change in free-living individuals gaining weight over time.

ACKNOWLEDGMENTS

First, I would like to thank Dr. Whisner, my mentor throughout this whole process. Thank you for the countless hours you have spent meeting with me, answering questions, and reviewing all my writing I have sent to you to review. I could not have done with without your guidance and mentoring.

To my committee, thank you for being a wonderful part of my master's experience here at ASU. Thank you for making time in your busy schedules for me and this project. Your encouragement and expertise have been invaluable.

Finally, I would like to thank my family and friends for helping me throughout the process.

TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

College is a defining moment in one's life. It is the first-time many adolescents are on their own, away from their parents and their home. While this is an exciting time, it can also be detrimental to health. College students are also at particular risk for weight gain, so much so that this phenomenon was even coined "the freshman 15"(Corder et al., 2019). The lifestyle of a college freshman can be characterized by low levels of physical activity, increased alcohol consumption, poor diet quality, increased stress, decreased sleep, and an environment that does not promote healthy habits (Chen et al., 2008; Laska et al., 2009; Levitsky et al., 2004; Nelson et al., 2008). Lifestyle factors contribute to about 70% of students gaining weight during their freshman year, with studies reporting an average weight gain of 4-5 lbs (Gropper et al., 2012; Racette et al., 2005, 2008). Students who gain weight during their freshman year are also more likely to continue gaining weight throughout college (Gropper et al., 2012; Pope et al., 2017). This weight gain in adolescents can continue into adulthood, as overweight or obese adolescents are more likely to be overweight and obese adults (Deshmukh-Taskar et al., 2006). This can lead to chronic health conditions and a myriad of adverse physical, psychological, and economic outcomes (Dietz, 1998; Pan et al., 2013; Reilly & Kelly, 2011; Russell-Mayhew et al., 2012; *Weighing Down America*, n.d.).

With this burgeoning problem of excessive weight gain and obesity, it is in our best public health interest to try to mitigate this trend. Weight loss interventions are widely ineffective in adults with long term success as a rare outcome, indicating prevention may

be a more effective strategy (Anderson et al., 2003; Booth et al., 2014; Lawlor & Chaturvedi, 2006; Miller, 1999). Adolescent populations are an ideal cohort to target with prevention interventions as adolescence has been identified as a time in life where one sets up health related habits and skills that will follow them the rest of their lives (Anderson et al., 2003; Lau et al., 1990; Nelson et al., 2008; Truesdale et al., 2006). Research has also shown that adolescent populations, in particular, are an effective population to target for preventative weight interventions and in extreme circumstances weight loss (Anderson et al., 2003; Boff et al., 2017; Truesdale et al., 2006; Wing et al., 2017). Weight gain and obesity is a multifactorial problem involving the food environment, genetics, physical activity, lifestyle, demographics, and diet ("A Framework for Human Microbiome Research," 2012; *Trends in Intake of Energy and Macronutrients --- United States, 1971--2000*, n.d.; Biro & Wien, 2010; Chung & Leibel, 2008; Hales, 2017; Reilly & Kelly, 2011; Twenge & Campbell, 2018). The complexity of the problem of excessive weight gain has led professionals to seek novel approaches. Researchers and clinicians in the emerging field of gut microbiome research can address the etiology of adolescent obesity and weight gain, particularly in this vulnerable population of college freshmen.

Microbes make up a significant part of this world, especially within the human body (Turnbaugh et al., 2007). Microbial communities inhabit locations in the human body including the skin, vagina, and mouth with the highest concentration found in the digestive system (Conlon & Bird, 2014). In the digestive system, they support a multitude of functions that would not be possible without the interactions that they foster including; metabolism of indigestible carbohydrates and proteins, production of short-chain fatty

acids (SCFAs), enzyme production, modulating inflammation, interacting with the immune system, and synthesizing vitamins (Allaband et al., 2019; Belizário et al., 2018; Cani et al., 2007; Conlon & Bird, 2014; Thursby & Juge, 2017). Diversity of the gut microbiome can be an indicator of overall health of the microbiome ("Structure, Function and Diversity of the Healthy Human Microbiome," 2012). Studies have shown there are a multitude of factors affecting the diversity of the microbiome, particularly diet, physical activity, physiology, antibiotic use, age and overall health (Agans et al., 2011; Allaband et al., 2019; Clarke et al., 2014b; Cotillard et al., 2013; Dethlefsen & Relman, 2011; Ley et al., 2006; Turnbaugh et al., 2009). Change in diversity can be favorable or pathogenic to the human host as increases in diversity of the microbial communities of the vagina can lead to bacterial vaginosis, and decreases in diversity in the gut microbiome can lead to irritable bowel disorder (IBD) or obesity ("Structure, Function and Diversity of the Healthy Human Microbiome," 2012). Research into the relationship between microbial diversity and weight is still a relatively new field, and a majority of the available research has utilized cross-sectional study designs (Wagner et al., 2018).

Studies linking the microbiome and obesity were first conducted in mice (Ley et al., 2006). In Turnbaugh et al, germ-free mice were given fecal transplants from obese and lean individuals fed the same amount and type of food (Ley et al., 2006). Mice that received microbial communities from obese individuals gained significantly more weight than those with microbes from lean individuals (Ley et al., 2006). Subsequent studies testing manipulations of the microbial communities, whether from obese donors or tests on germ-free (GF) mice, have shown interactions inducing weight gain (Bäckhed et al., 2004, 2007;

Ley et al., 2005; Murphy et al., 2010; Ridaura et al., 2013). Among humans, differences in the composition of gut microbial communities have been observed between obese and lean individuals (Angelakis et al., 2011; Ley et al., 2005; Turnbaugh et al., 2009). While early studies focused on the compositional changes between phyla such as the ratio of Firmicutes to Bacteroidetes, or the F/B ratio, research is now focusing on the dynamic changes in diversity at lower taxonomic levels within the individual microbiome as an indication of health (Flores et al., 2014). Flores et al suggests that longitudinal studies which examine temporal changes in the composition and diversity of microbial taxa are more useful indications of health (Flores et al., 2014). With regards to weight gain however, it is difficult to determine if it is weight gain that impacts the microbiome or vice versa as a causal relationship is hard to determine in humans (Harley & Karp, 2012). Results from animal models have implied that it is the microbes influencing the weight gain, but this relationship has not been shown in humans (Harley & Karp, 2012).

Age is an important contributor to changes in microbial diversity (Agans et al., 2011; H.-J. Hu et al., 2015; Hollister et al., 2015; Palmer et al., 2007; Yatsunenko et al., 2012). It was initially believed that the microbiome reached maturity by the age of three, and thus children over that age were characterized as having a mature and stable adult-like microbiome (H.-J. Hu et al., 2015; Palmer et al., 2007; Yatsunenko et al., 2012). However, there is new research suggesting that the microbiome continues to change throughout adolescence (Agans et al., 2011; Hollister et al., 2015). Statistically significant differences in abundance and diversity have been seen in the gut microbiome of children and adolescents compared to adults (Agans et al., 2011; Hollister et al., 2015). While core

microbial communities as well as the presence of species is similar between all three aforementioned age groups, it is suggested that it is abundance that drives the differences (Agans et al., 2011; Ringel-Kulka et al., 2013). Children and Adolescents demonstrated a higher abundance of certain genera such as *Clostridium, Lachnospiraceae,* and *Bifidobacterium*, while adults tended to have a higher abundance of *Bacteroides* spp (Agans et al., 2011; Hollister et al., 2015). With the implications of the microbiome's relationship to obesity and weight gain, understanding this distinction and thus being able to use this information for targeted interventions may be the future of obesity interventions.

Since 1994, the prevalence of obesity in adolescents has doubled (Hales, 2017). However, in that time the field of obesity research and prevention has exploded throughout the world. Adolescents, in particular college students, are not only a vulnerable group for weight gain but also a group where weight mitigation interventions can be efficacious. The interaction between obesity and the microbiome is a blossoming field of study. However, little research has been conducted looking at how the gut microbiome changes in free-living populations during periods of expected weight gain. Specifically, longitudinal study designs are lacking in microbiome research, especially in populations where there is evidence of gain weight over time (Flores et al., 2014; Gropper et al., 2012; Pope et al., 2017; Wagner et al., 2018). Information is also sparse when it comes to the microbiome of adolescent populations, as the composition of their microbiome has largely been viewed as similar to communities observed in adults (Agans et al., 2011; Hollister et al., 2015; H.-J. Hu et al., 2015; Palmer et al., 2007; Yatsunenko et al., 2012). This combination reveals

late adolescence as an ideal time for weight intervention, and study of the microbiome as an effective tool for understanding the mechanisms behind weight gain.

Purpose of Study

The purpose of this study is to better understand the mechanisms of weight gain in a college-aged population vulnerable to weight gain. Specifically, this study will evaluate changes in weight over the freshman year in relation to changes in the gut microbiome community structure and diversity.

Research Aims and Hypothesis

The aim of this study is to assess changes in the gut microbiome over the freshmen year in relation to changes in weight status.

H1: Weight gain will be associated with a decrease in the alpha diversity (Shannon diversity index) of the microbiome from the beginning to the end of freshman year in college students at a large Southwestern University.

H2: Weight gain will be associated with a decrease in the beta diversity (Jaccard and Unweighted UniFrac) of the microbiome from the beginning to the end of freshman year in college students at a large Southwestern University.

CHAPTER 2

REVIEW OF THE LITERATURE

Obesity and Weight Gain

Overweight/ Obesity in the United States

The rates of obesity and its associations to adverse health outcomes is a well-studied, multifactorial problem. As obesity reaches epidemic incidence in all age groups, the rates of obesity and overweight in adolescents is particularly alarming (Hales, 2017). In 1994, 10% of adolescents aged 12-19 were obese (Hales, 2017). According to the CDC, in 2018 over 20% of adolescents were obese (Hales, 2017). Unfortunately, this trend is expected to increase in coming years (Hales, 2017). Obesity is especially dangerous when diagnosed during adolescence as it is hard for adolescents to return to a normal weight by adulthood (The et al., 2010). In fact, nearly 50% of overweight adults were overweight children (Deshmukh-Taskar et al., 2006). A meta-analysis from 2002 to 2010 demonstrated that people who were overweight or obese in childhood/adolescence were at a higher risk for premature mortality, and occurrence of cardiometabolic disease including heart attack, stroke, diabetes, and hypertension in adulthood (Reilly & Kelly, 2011). With rates of adolescent obesity rising, and its association with a significant disease burden in adulthood established, interventions and research should focus on this population and mitigate excessive weight gain.

The definition of obesity and overweight differ slightly between age groups. In adults, weight categories are defined by BMI, with a BMI of 25 to 30 being overweight and above 30 as obesity (*Obesity and Overweight*, n.d.). For children and adolescents 5-

19 there is a growth chart for reference (*Obesity and Overweight*, n.d.). Therefore, diagnosis of overweight or obesity relies on where the child or adolescent falls on this chart with regards to their height and weight (*Obesity and Overweight*, n.d.). An overweight diagnosis is given if the child or adolescent is one standard deviation above the median growth on the WHO chart with an obese diagnosis is if the child or adolescent is 2 standard deviations above the reference median on the chart (Barlow & Expert Committee, 2007). Children under 5 have a separate growth chart but are still measured regarding this chart (*Obesity and Overweight*, n.d.). An overweight diagnosis is given if the child falls 2 standard deviations above the median or between the 85th and 95th percentiles, and obese if they are 3 or more above the median, or in the 95th percentile (Barlow & Expert Committee, 2007).

Consequences of obesity are also monetary (*Weighing Down America*, n.d.). Not only do people with obesity spend more annually on personal healthcare, but as a nation we spend more than 10% of all healthcare expenses directly on obesity and diseases linked to obesity (Dietz, 1998). In 2014, the U.S. healthcare system spent $427 billion on treating conditions linked to overweight or obesity, in addition to the almost $1 trillion in lost wages, deaths, and lost productivity (Dietz, 1998).

Weight Gain Etiologies

Weight gain, especially to the point of obesity, is a complex and multifactorial problem. General mechanisms include decreased energy expenditure due to inactivity or sedentary lifestyle, increased consumption of energy dense foods and calories, genetic predispositions, accessibility to energy dense foods, demographics, behaviors involving

food, and our environment (Biro & Wien, 2010; Chung & Leibel, 2008; Hales, 2017; Reilly & Kelly, 2011; Story et al., 2008; *Trends in Intake of Energy and Macronutrients --- United States, 1971--2000*, n.d.; Twenge & Campbell, 2018). The amount of time people spend doing sedentary activities such as watching tv, driving or commuting, or on computers or cellphones, commonly referred to as "screen time" has increased substantially in recent decades (Twenge & Campbell, 2018).

Not only has overall physical activity decreased dramatically, but processed, calorie dense foods have become readily available, leading the average American to consume more calories and expend less energy than previous generations (Chung & Leibel, 2008; Story et al., 2008). Increased portion sizes have also been associated with obesity due, in large part, to the fact that increased portion sizes have led to greater food consumption (Livingstone & Pourshahidi, 2014). According to NHANES, or the National Health and Nutrition Examination Survey, the average caloric intake for a man in the U.S. increased 200 calories, and over 300 calories for women, when compared to the first NHANES study conducted in the 1970s (Chung & Leibel, 2008).

Aspects of metabolism like regulation of fat storage, components of energy expenditure, and activity of hormones associated with obesity are determined through genetics and have been associated with weight gain (Catalano et al., 2009). Genetics can also play a role in satiety cues, overall body composition, and individual phenotypes (Chung & Leibel, 2008). Genetics is also important in controlling energy balance and susceptibility to weight gain or weight maintenance (Bouchard et al., 1994). Epigenetics can also play a role in weight gain, as risk children and adolescents becoming obese can

increase if they are born premature or if their mother had gestational diabetes (Biro & Wien, 2010).

After the baby is born, demographic characteristics such as gender, socioeconomic status, ethnicity, and geographic location can increase their likelihood of gaining weight throughout childhood (Biro & Wien, 2010). Minority populations, as well as populations of lower socioeconomic status, have a higher prevalence of excessive weight (Hales, 2017). As demonstrated by the CDC, the highest growing population for overweight and obesity is female minorities (Hales, 2017). The environment around food has also changed significantly in recent decades, making energy dense foods readily available, abundant, and inexpensive (Rendina et al., 2019). Fast food consumption is high, particularly in locations considered to be food deserts (Boone-Heinonen et al., 2011). As demonstrated above, weight gain is a multifactorial problem.

Weight Gain in Adolescents

Weight gain in adolescent's has some distinguishing characteristics. Specifically in adolescents, lifestyle factors attributed to weight gain include increased screen time, decreased physical activity, low consumption of fruits and veggies, and high intake of sugary beverages (Aaron et al., 2002). Physical activity, or lack thereof, is also an important component of increased weight gain in this population as most adolescents are not participating in the 60 minutes of daily physical activity recommended by the USDA (Rendina et al., 2019). The amount of time children spend being physically active is declining, while rates of screen time are increasing (*Weighing Down America*, n.d.). This is particularly troublesome in children and adolescence, with the average amount of screen

time reported in high school students as 4 or more hours a day (Twenge & Campbell, 2018). Increases in screen time often replace physical activity (Twenge & Campbell, 2018). Increased energy consumption in adolescents has been attributed to an increase in energy dense snacks and the increased availability and consumption of fast food (Rendina et al., 2019). Parenting styles and home life can also be contributing factors to weight gain in this age group (Aaron et al., 2002). The home food environment is not the only potentially obesogenic environmental factor that affects adolescents. The community environment plays a pivotal role in healthy diet and physical activity levels of the adolescent with regards to proximity and abundance of fast food restaurants, supermarkets, or outdoor recreation areas being associated with weight (Campbell, 2016). Weight and behavioral choices of the parents can also influence the weight of the adolescent (Campbell, 2016).

Causal relationships with one of these many factors are difficult to determine in obesity studies (Pontzer et al., 2012; Westerterp & Speakman, 2008). Westerterp et al. argues that as a society we are not less physically active, concluding that being less physically active is not the reason for the obesity epidemic that surged from 1980 to 2005 (Westerterp & Speakman, 2008). The causal relationship between portion size and obesity has not been determined as overfeeding studies can have many confounding factors and associations that have been found are weak (Herman et al., 2016; Livingstone & Pourshahidi, 2014). Studies demonstrating causation between excessive sugar consumption and weight gain in humans are weak, especially in isocaloric feeding studies. A meta-analysis by Malik et al. demonstrates an average weight gain of roughly a pound or two. Even the genetic etiology is difficult to defend, with monozygotic twin studies

showing that there can be significant variation in weight gain between twins even when given the same number of calories and similar exercise regimens (Bouchard et al., 1994, 1996). As mentioned above, obesity is a complicated problem with causality being very difficult to prove in any of the confounding factors and a myriad of factors that should also be examined.

Overweight/ Obesity Consequences and Outcomes in Adolescents

Adults who are overweight or obese are at a higher risk for a myriad of comorbid conditions including type 2 diabetes, osteoarthritis, pancreatic and prostate cancer, stroke, chronic back pain, and cardiovascular disease (Dietz, 1998). Two major ways obesity affects the body is through the endocrine and immune systems (Singla et al., 2010; Wellen & Hotamisligil, 2005). Adipose tissue can be regarded as an endocrine organ, regulating hormones such as leptin and adiponectin that in turn negatively affect metabolism (Singla et al., 2010). Obesity can also lead to adverse changes in serum lipids including an increase in free fatty acids (FFA), triglycerides (TAGs), and changes in cholesterol including a decrease in HDL with an increase in LDL (Klop et al., 2013). Another major bodily system affected by obesity is the immune system, as obesity can also be characterized by chronic, low-grade inflammation (Wellen & Hotamisligil, 2005). When inflammatory cytokines are overexpressed in the adipose tissue obese individuals, this can result in inflammation and insulin resistance (Wellen & Hotamisligil, 2005). This subsequent insulin resistance can be a potential mechanism for the development of type 2 diabetes mellitus (Wellen & Hotamisligil, 2005).

Consequences of obesity and weight gain on adolescents can be dangerous for their physical as well as mental health. Overweight and obese adolescents are more likely to be diagnosed with chronic conditions typically acquired in adulthood, such as diabetes, hyperinsulinemia, hypertension, pancreatitis, and liver disease (Russell-Mayhew et al., 2012). Adolescents in this upper weight category also experience depression, sleep apnea, social isolation, anxiety, and the presence of an eating disorder at higher rates than their normal weight counterparts (Russell-Mayhew et al., 2012). This is detrimental to the mental health of the adolescent in a time of their life that should be characterized by fun, youth, and curiosity (Russell-Mayhew et al., 2012). They are also more likely to hit puberty earlier, thereby increasing their body size and bringing with it a host of mental health struggles (Russell-Mayhew et al., 2012). Obese and overweight adolescents are also more likely to miss school, having almost 40% more sick days than students of normal weight which can negatively impact their academic performance (Pan et al., 2013). Not only is it imperative to prevent obesity and overweight in this age group for their physical health, but also for their mental health (Truesdale et al., 2006).

Importance of Focusing on this Age Cohort

As demonstrated above, the consequences of excessive weight gain can be dire, regardless of age. Weight gain, even in adolescents, has shown a significant increase in CVD risk factors, regardless of the baseline weight (Gropper et al., 2012). Weight loss, although beneficial, is not an easy outcome. Wing et al. conducted a weight loss survey in adults and found that a weight decrease of 5% is enough to see significant changes in CVD risk and improvements in lipids and glycemic control measures such as insulin and insulin

resistance (Wing et al., 2017). Unfortunately, adult weight loss interventions are rarely effective long term, with a majority of participants gaining back their weight in 5 years or less (Wing et al., 2017). Adolescent studies, on the other hand, have been successful, especially interventions that address the causes of obesity and include a behavioral component such as diet and physical activity implemented with the whole family (Boff et al., 2017; Miller, 1999). Similar interventions involving behavior change have not been as effective in adults, even when administered in the clinical setting (Booth et al., 2014). Therefore, preventing weight gain, or addressing it in the adolescent population is important for cardiometabolic health (Gropper et al., 2012).

Overweight adolescents are likely to remain overweight into adulthood (Deshmukh-Taskar et al., 2006). It is important to mitigate weight gain in adolescence because this is a time where they are creating habits for the rest of their lives that can have implications for health and reduced disease risk (Lau et al., 1990; Nelson et al., 2008). Healthy habits, beliefs, and behaviors formed at home through parental modeling have been associated with healthier choices throughout adolescence, and particularly in the early college years (Lau et al., 1990). This suggests a relationship between habits formed in childhood and health behaviors in late adolescence, and the same can be said about physical activity. Higher rates of participation in physical activity during youth is associated with higher rates of physical activity in adulthood (Corder et al., 2019). This further points out that childhood and adolescence is a pivotal time for strengthening health behaviors. A particularly vulnerable cohort with regards to weight gain are college students. The freshman year of college has been distinguished as a critical period for weight gain and

thus is an ideal time for overweight and obesity prevention interventions due to their unique lifestyles (Anderson et al., 2003).

Weight Gain and College Students

College Student Lifestyle

College is an important transition period in life marked by increased independence, autonomy, and unique experiences. Unfortunately, it is also a time characterized by unhealthy lifestyles and poor health choices. College freshmen lifestyles are unfortunately characterized by decreased levels of physical activity, poor dietary habits, immersion in environments that do not promote healthy habits, inadequate sleep, and increased participation in risky behaviors like alcohol consumption and smoking (Chen et al., 2008; Corder et al., 2019; Laska et al., 2009; Levitsky et al., 2004; Nelson et al., 2008). Each of these factors can be uniquely tied to weight gain.

This transition period, from late adolescence to early adulthood, is marked by a decrease in overall physical activity, with only 12.7-50% of young adults reaching the desired weekly physical activity levels (Gordon-Larsen et al., 2004; McArthur, 2009; Nelson et al., 2008; Racette et al., 2008). Characteristics of insufficient physical activity differ with gender, with low rates of MVPA for females and high rates of leisure time computer use by males (Nelson et al., 2009). College-aged adolescents also experience a decline in overall diet quality (Nelson et al., 2008). In fact, fast food consumption, sugar sweetened beverages intake, and added sugar consumption is highest among the young adult age group (Briefel & Johnson, 2004; Nelson et al., 2009). During the transition from high school into college, there is also a decrease in fruit and vegetable intake, and increased

consumption of salty snacks and beef (Nelson et al., 2009). A reported 33% of college freshmen meet the minimum requirements for fruits and vegetables a day (McArthur, 2009). Poor dietary habits such as high fast food consumption are associated with higher weight in this population (Larson et al., 2011).

Environmental factors also play a role in weight gain for this age group (Levitsky et al., 2004). Food availability like 'all-you-can-eat' dining options, high rates of snacking, eating high-fat foods, general consumption of junk food, and meal frequency are associated with weight gain in college students (Levitsky et al., 2004). Living in a dorm has also been associated with weight gain when compared to students who live at home their first year (de Vos et al., 2015; Nelson & Story, 2009). Food typically kept in dorms is indicative of the reported diet of early college students; salty snacks, sugar-sweetened beverages, processed foods with added sugars, and granola bars, resulting in the average dorm room containing approximately 22,888 kcals (Nelson et al., 2009; Nelson & Story, 2009). The college campus environment also provides easy access to junk food, snacks, and dining halls that favor over consumption of food (Levitsky et al., 2004).

Suboptimal sleep patterns, poor stress management, and an increase in risky behaviors are also part of the college lifestyle, although their relationship to weight gain in this cohort is less well defined (Chen et al., 2008; Laska et al., 2009; Pelletier et al., 2016). Sleep duration can affect weight as there seems to be an inverse relationship between sleep duration and likelihood of obesity (Chen et al., 2008). In the adolescent population specifically, this association has held true for males, but results are mixed with females (Chen et al., 2008). College lifestyles are associated with an uptick in risky lifestyle

behaviors including binge drinking, tobacco smoking, unsafe sex, and illicit drug use (Laska et al., 2009). Participation in high risk behaviors is associated with other poor lifestyle choices in college students (Laska et al., 2009). Lastly, higher than average stress levels and poor stress management have been reported in first year college students (Pelletier et al., 2016). The relationship between stress and weight gain in college students is not well understood, with studies reporting conflicting results (Aceijas et al., 2017; Pelletier et al., 2016).

Perceived barriers are reported as a deterrent to a healthy lifestyle in college underclassmen and can be associated with weight gain (Greaney et al., 2009; Nelson et al., 2009; Yan & Harrington, 2019). These include lack of availability and accessibility to healthy foods or recreation centers, lack of social support for a healthy lifestyle, lack of motivation, not possessing the necessary time management skills, stress eating, lack of time, and alcohol-related eating (Greaney et al., 2009; Nelson et al., 2009; Yan & Harrington, 2019). College student knowledge of physical activity recommendations is low with only 40% reporting awareness that the recommended amount of physical activity is 30 minutes of moderate to vigorous physical activity 5 days a week (McArthur, 2009). The most important motivation for exercise was health followed by appearance and mental health (McArthur, 2009). Like the etiologies for obesity in general, the relationship between weight gain and the lifestyle of college freshman is a multifactorial problem.

Weight Gain Trajectory in College Students

The age-old anecdote of the "freshman fifteen" has been tested by scientists and effectively debunked in recent years (Vadeboncoeur et al., 2015). While most freshmen

don't gain 15 pounds over their freshman year, they do gain some weight, with about 70%-80% of freshman gaining weight (Racette et al., 2005; Thursby & Juge, 2017). After conducting a meta-analysis of articles from 1985-2011, Racette et al. found that the results are closer to a mean weight of 1.75 kg or about 4 lbs during a student's freshman year of college (Racette et al., 2008; Williamson et al., 1990). This average can also be partitioned by gender, as one study reported that by the end of freshman year, females had gained an average of 1.7 kg +/- 4.5 kg and males averaged a weight gain of 4.2 kg +/- 6.4 kg (Racette et al., 2008). While this doesn't sound like much, even 1.7 kg of weight gain over less than a year is a statistically significant amount over that period of time (Racette et al., 2008; Williamson et al., 1990).

Significant weight gain throughout the freshman year is also an indication of weight gain throughout college (Gropper et al., 2012; Pope et al., 2017). While the average student's weight gain slowed down after freshman year, the students who gained the most weight during their freshman year were likely to continue gaining weight, gaining almost 5 kgs (11 lbs.), by the end of their college tenure (Palmer et al., 2007; Racette et al., 2008). This rate of almost 7 kgs per year is higher than what previous studies have indicated as an average weight gain for young adults per year, which is closer to 2.2 to 4.4 kgs (Gropper et al., 2012; J. Hu et al., 2013). In one study, the percent of students in the overweight and obese category went from 18% freshman year, to 31% in their senior year (Thursby & Juge, 2017). In another study, a modest but statistically significant weight gain during freshman year of just 2.3 kg for a cohort of students was enough to double the number of participants who were overweight or obese (Anderson et al., 2003). Weight gain trajectory in college

students is also highly variable. A meta-analysis by Racette et al. reported a range of -13.2 kg to +20.9 kg (Racette et al., 2008). Most troubling, is that researchers have not been able to come to a consensus as to the cause of weight gain in college freshman (Palmer et al., 2007; Racette et al., 2005; Thursby & Juge, 2017).

The Microbiome

Introduction to the Microbiome

Microbes make up a large part of our world. The phrase, "the human microbiome" refers to the collection of microorganisms that inhabit the human body (Group et al., 2009). The main places microbes inhabit are the skin, mouth, vagina, and the gastrointestinal tract with each location consisting of its own unique combination of microbes and diverse functions (Group et al., 2009). In these environments, a mutualistic relationship takes place, as these microbes are essential for our bodies to perform certain tasks while our bodies provide a constant supply of nutrients to the microbes (Group et al., 2009; "Structure, Function and Diversity of the Healthy Human Microbiome," 2012). The microbes that live on our skin and inside our bodies have implications for the function of our immune system, metabolism, mental health, and even in our development of diseases ("Structure, Function and Diversity of the Healthy Human Microbiome," 2012; Thursby & Juge, 2017). When babies are born, their first contact with the outside world is through inoculation of their mother's microbes through the vaginal opening (Ma et al., 2012). Humans are in continuous contact with microbes, and they are important for health but also have potential for disease.

While the roles of the microbes are unique in each region of the body, important similarities are the ability of microbes to protect against pathogen invasion, and that dysbiosis can be a contributing factor in disease (Bäckhed et al., 2007; De Filippo et al., 2010; Kilian et al., 2016; Ma et al., 2012; Sanford & Gallo, 2013). Dysbiosis is defined as a disturbance in organisms composing the microflora of the gut community (Marchesi, 2011). The microbiota which inhabit the mouth are crucial for homeostasis as well as protecting against disease as dysbiosis of the oral microbiome is associated with dental diseases such as cavities, periodontitis, and gingivitis (Kilian et al., 2016). In the vagina, a community dominated by *Lactobacillus,* microbes contribute by maintaining the pH of the environment and by protecting the community against invasion by potential pathogens that cause yeast infections, urinary tract infections, and vaginosis (Ma et al., 2012). The skin, which is considered the largest organ of the immune system, contains millions of microbes that contribute to immunity as well as health and disease such as psoriasis, dandruff, eczema, and acne (Sanford & Gallo, 2013). As part of the gastrointestinal (GI) tract, microbes enhance metabolic functions such as increased energy extraction from foods called energy harvesting, immune functions, GI functions, and protection against pathogens (Bäckhed et al., 2004; De Filippo et al., 2010; Ley et al., 2006; Turnbaugh et al., 2006).

Specifically, in the gut, the microbes have a myriad of functions that contribute to the host in essential ways. Most microbial biomass and therefore metabolism occur in the luminal contents of the large intestine, as compared to the stomach or duodenum (Allaband et al., 2019; Belizário et al., 2018). Through the GI tract, microbes play a vital role in

metabolism, synthesis of vitamins, immunological functions, and inflammation (Belizário et al., 2018; Conlon & Bird, 2014). Fermentation of carbohydrates by microbes in the large intestine creates SCFA, or short chain fatty acids, that are essential in maintaining the health of the colon (Belizário et al., 2018; Conlon & Bird, 2014). As part of the immune response, microbial metabolites are important signalers helping the immune system distinguish self from nonself (Peterson et al., 2015). Aspects of gut permeability are maintained through the gut microbiome and disruptions can lead to chronic inflammation as potential pathogens enter the circulation (Belizário et al., 2018; Cani et al., 2007; Conlon & Bird, 2014; Thursby & Juge, 2017). Systemic and chronic inflammation have been associated with diseases (Belizário et al., 2018; Cani et al., 2007).

Microbial Diversity and Implications in Health

When it comes to stability of any environment, diversity is critical (McCann, 2000). Diversity within a community has ecological advantages, including functional redundancy and resistance to invasion (McCann, 2000). In the gut microbiome specifically, that can also mean increased resilience, improved immune function, and decreased susceptibility to GI related disorders as low diversity has been associated with obesity and IBD (Group et al., 2009). Diversity is also key in the gut microbiome as many different microbes are needed to break down most substrates (Conlon & Bird, 2014). Diversity in the gut microbiome is location dependent, with the large intestine and colon being the most diverse and small intestine being the least (Group et al., 2009; Turnbaugh et al., 2009). Diversity in the microbiome is also not specific to microbes, as there are also archaea, viruses and eukaryotic cells present that work together (Segata, 2015).

Stability within a human host, especially in the gut microbiome is associated with health and is measured through diversity (Group et al., 2009; "Structure, Function and Diversity of the Healthy Human Microbiome," 2012). Two ways to measure microbial diversity are alpha and beta diversity (Whittaker, 1972). Alpha diversity is the diversity within one sample and is a measurement of the richness and evenness of unique members present (Whittaker, 1972). These measurements will yield information about the number of species at a given time and are important measurements of what is present (Group et al., 2009; Whittaker, 1972). Microbial diversity between samples, or beta diversity, yields information on differences and dissimilarities between samples (Goodrich et al., 2014). This can demonstrate differences between geographic regions, in disease and non-disease states or between regions on the body (Goodrich et al., 2014).

While a healthy microbiome can be hard to define, scientists have leaned towards a broad characterization. A healthy gut can be characterized by stability, particularly in both richness and diversity (Cotillard et al., 2013). Chronic conditions such as inflammatory bowel disease (IBD), obesity, and other GI disruptions can affect the health, and thus stabilization of diversity of the microbiome. Cross sectional analysis indicates that conditions such as IBD, obesity and psychiatric conditions such as attention deficit hyperactivity disorder, or ADHD, have been associated with low diversity measurements in the gut microbiome (Prehn-Kristensen et al., 2018). An instability or imbalance in microbial diversity is referred to as dysbiosis and can have potentially harmful consequences for the host (Belizário et al., 2018). Dysbiosis can cause interruptions in gut permeability, host metabolism, inflammation, and in turn can lead to adverse health

outcomes such as IBD, IBS, obesity, diabetes, and colorectal cancer (Belizário et al., 2018; Peterson et al., 2015). Functional redundancy is an important, protective aspect of diversity as it ensures that if there is a change in diversity that key functions and processes remain uninterrupted (Bäckhed et al., 2007).

Lifestyle and the Microbiome

Modifiable lifestyle factors such as diet and exercise. It is suggested that diet accounts for 20% of microbial composition (Leeming et al., 2019). The relationship between the microbiome and diet was initially demonstrated in mice (David et al., 2014; Turnbaugh et al., 2009; Wu et al., 2011). In mice, these changes can occur almost instantly, within hours to a couple days (David et al., 2014; Turnbaugh et al., 2006). When mice are fed either a "Western" diet high in fat and sugar, or a high fiber, plant-based diet, rapid changes are seen in their microbial communities (Turnbaugh et al., 2006; Wu et al., 2011). However, these changes were not stable and once put on a normal diet their microbes returned to normal, thus demonstrating that a short-term dietary change did not change the composition permanently (Wu et al., 2011). Studies conducted on humans, demonstrate changes that occur at a much slower rate (David et al., 2014). These studies demonstrated that changes in microbial composition due to diet can take weeks if not months in humans, and consistent changes in taxa have not been demonstrated (Allaband et al., 2019; David et al., 2014). This demonstrates that the timescale and exact mechanism of how diet changes the microbiome is still inconclusive.

In the human gut microbiome, compositional changes of the microbes depend on the macronutrient composition of the meals and overall diet (Allaband et al., 2019; Conlon

& Bird, 2014; David et al., 2014; Wu et al., 2011). The nutrients that are found in the diet will fuel microbes that run on those specific nutrients (Cotillard et al., 2013). Microbes allow humans to break down carbohydrates by producing enzymes that humans do not produce, such as glucoside hydrolase and polysaccharide lyases that are used in the metabolism of fiber (Belizário et al., 2018). High fiber and mostly vegetarian diets are associated with a higher abundance of *Prevotella, Xylanibacter* (Bacteroidetes), and *Treponema* (Spirochetes) and subsequently a higher proportion of short chain fatty acids (SCFA), greater richness, and enhanced diversity of the microbiome (De Filippo et al., 2010). Greater dietary fiber intake is associated with higher levels of alpha diversity and is slightly protective against weight gain (Baxter et al., 2019; Holscher et al., 2018; Menni et al., 2017; Van Hul & Cani, 2019). Diets high in animal fat, sugar, processed foods, and starch but low in fiber tend to be composed of more Firmicutes and Bacteroidetes, especially when compared to the previously mentioned high fiber diet (Cani et al., 2007; Da Silva et al., 2020; De Filippo et al., 2010). These aforementioned diets high in animal fat, sugar, processed foods and starch, are associated with decreases in diversity of the gut microbiome (Da Silva et al., 2020; Rampelli et al., 2015).

As mentioned above, exercise has been shown to affect the composition and diversity of the microbiome. Changes in abundance of microbes in the microbiome have been shown to be associated with exercise in human and animal models, though the exact measurements, such as changes in alpha diversity, beta diversity, and abundance have been inconsistent (Mitchell et al., 2019). This may be due to the effects of two important confounding factors, diet and weight status (Allen et al., 2018; Clarke et al., 2014a;

Mitchell et al., 2019; Whisner et al., 2018). While Clarke et al demonstrated significant differences in diversity between athletes and healthy controls, it may be due to the high protein diet of the athletes (Clarke et al., 2014a). The effects of exercise on the microbiome can be independent of diet, but not always independent of weight (Allen et al., 2018; Mitchell et al., 2019). Lean individuals may respond better to exercise induced changes in the microbiome when compared to their overweight or obese counterparts (Allen et al., 2018). This was associated through changes in species that produce SCFA such as *Lachnospira* and *Faecalibacterium* were increased in lean but not obese adults (Allen et al., 2018; Clarke et al., 2014a; Mitchell et al., 2019). *Lachnospira,* as well as *Paraprevotellacae* and *Lachnospiaceae* have been associated with physically active youth (Whisner et al., 2018).

Antibiotic Use and The Microbiome

Antibiotic use can also contribute to differences in the composition of the microbial communities in an individual. Typically, the microbial diversity within a given individual remains steady once they reach adulthood (Dethlefsen & Relman, 2011; H.-J. Hu et al., 2015; Palmer et al., 2007; Yatsunenko et al., 2012). Once given antibiotics however, this diversity decreases rapidly and dramatically, with studies showing a significant change anywhere from 1-4 days after the first ingestion of an antibiotic (Dethlefsen & Relman, 2011). Microbial communities begin to shift back to normal after a week, but in some patients, microbes never return to normal (Dethlefsen & Relman, 2011). Interestingly, antibiotic use has also been associated with improved metabolic and hormonal markers in

obese rats, possibly due to diminishing the gap between pro-inflammatory and anti-inflammatory microbes associated with the obese microbiome (Cotillard et al., 2013).

Changes in Microbial Diversity Throughout Life

Age has a huge impact on the compositional and functional characterization of the microbiome (Palmer et al., 2007). The gut microbiome is an important contributor to human metabolism beginning at birth and assisting individuals with vital tasks throughout life (Palmer et al., 2007). In early life, the composition of the infant's microbiome is highly variable and constantly changing in response to the environment and diet (J. Hu et al., 2013; Palmer et al., 2007). It has been shown to be less diverse and more variable when compared to the microbiome of an adult (J. Hu et al., 2013). The microbiome of an infant consists mostly of two phyla: Actinobacteria and Proteobacteria (Thursby & Juge, 2017). Two important and abundant genera, specifically for infants, are *Lactobacilius* (from the phylum Firmicutes), and *Bifidobacterium* (from the Actinobacteria phylum) (Conlon & Bird, 2014). These genera are abundant in infants because their main source of fuel is the oligosaccharides present in human breast milk (Conlon & Bird, 2014). Infants who are formula fed and not breastfed have less *Bifidobacterium* and greater microbial diversity (Thursby & Juge, 2017). Microbes also serve an important role in the development of the immune system (Conlon & Bird, 2014). As the infant weans from breast milk to solid food, the abundance of *Lactobacillus and Bifidobacteria* decreases as the functional diversity of the microbiome increases to increase the functions (Thursby & Juge, 2017). This is just one example of the dynamic way the microbiome adjusts to age and functional needs. It is believed that the convergence of the youth and adult microbiome is complete by ages 1-3

years, where the child's abundance of the aforementioned genera is closer to that of an adult and begins to remain relatively stable until late adulthood (J. Hu et al., 2013; Palmer et al., 2007; Yatsunenko et al., 2012).

It can be argued that there are interesting changes that take place in the microbiome between the age of three to adolescence (Agans et al., 2011; Avershina et al., 2014; Cheng et al., 2016; Conlon & Bird, 2014; Hollister et al., 2015; Odamaki et al., 2016). This is due to the continued dynamic nature of the gut microbiome into adolescence and even early adulthood (Avershina et al., 2014; Cheng et al., 2016; Odamaki et al., 2016). The gut microbiome of an adolescence can be characterized by the changing relationship of Firmicutes to Bacteroidetes (Cheng et al., 2016; Conlon & Bird, 2014). In a study conducted by Hollister et al, researchers found that the gut microbiome of children, as well as adults, contained a majority of Bacteroidetes and Firmicutes (Hollister et al., 2015). It was at the genus level, however, where statistically significant differences were observed. Children's microbiomes had greater abundance of *Bifidobacterium, Faecalibacterium, and Lachnospiraceae* while adults had higher amounts of *Bacteroides spp* (Hollister et al., 2015). Upon comparing the microbiome composition of adolescents to adults, adolescents had a statistically significant difference in microbiome composition, with adolescent's having a higher abundance of genera *Clostridium* and *Bifidobacterium* while adults had a greater abundance of *Bacteroides spp* (Agans et al., 2011; Hollister et al., 2015). Specifically, they found a similar abundance of *Faecalibacterium* in adolescents and adults in the study, contrasting a key discovery in Hollister et al (Agans et al., 2011; Hollister et al., 2015).

Odamaki et al. argues that microbial composition can be grouped into five different clusters by age, with significant overlap due to individual uniqueness (Odamaki et al., 2016). There are certain compositional changes that occur in trends, for example, Proteobacteria increases until the age of three, decreases, then increases again in the 70s, making the microbiome of infants similar in ways to the microbiome of the elderly (Agans et al., 2011). Actinobacteria, which characterizes the infant microbiome, steadily decreases once weaning begins, and never regains its numbers held in infancy (Odamaki et al., 2016; Palmer et al., 2007). However, other studies have shown that Actinobacteria retain these high abundances until about five years of age (Cheng et al., 2016). Throughout the aging process, Bacteroidetes increase and Firmicutes decrease (Cheng et al., 2016; Odamaki et al., 2016). This is another way that the infant microbiome is like the elderly microbiome aged 70 and up, with numbers of Bacteroidetes becoming closer to Firmicutes, which is a relationship associated with aging (Odamaki et al., 2016; Palmer et al., 2007).

A majority of studies pertaining to the composition of the microbiome in humans in health and disease assess the microbiome of adults (Agans et al., 2011; Conlon & Bird, 2014; David et al., 2014; Hollister et al., 2015). However, the composition and diversity of an adult's microbiome is not as comparable to adolescents as most studies have assumed (J. Hu et al., 2013; Palmer et al., 2007). Hollister et al. concluded that even though the number and taxa were comparable between adults and children, there was a significant difference at the genus level that indicated potential functional differences (Hollister et al., 2015). Studies discussed above have shown that the abundance within the species and not

the presence of the species changes as a function of age (Agans et al., 2011; Hollister et al., 2015; Ringel-Kulka et al., 2013).

Weight Gain and the Microbiome

Microbes Associated with Weight Gain

There are five main phyla that make up a majority of the human gut microbiome; Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia (Castaner et al., 2018; Chakraborti, 2015; Clarke et al., 2012; Group et al., 2009). When discussing the relationship between weight gain and microbes, the two main phyla are Bacteroidetes and Firmicutes as they make up about 60%-90% of the gastrointestinal communities (Castaner et al., 2018; Conlon & Bird, 2014). The Human Microbiome Project indicates that there is wide compositional variety between people (Group et al., 2009). Experimental studies conducted in mice initially demonstrated the relationship between these phyla and the substantial role they play in energy harvesting and regulation of weight gain (Bäckhed et al., 2007; Turnbaugh et al., 2006). The relationship between these phyla has been discussed in regard to abundance, with a high ratio of Firmicutes to Bacteroidetes (F/B ratio) being associated with obesity and increased energy harvest from the diet (Turnbaugh et al., 2006). Preliminary studies in humans show similar results with obese humans having more Firmicutes than Bacteroidetes when compared to lean controls (Ley et al., 2005, 2006). When weight loss was induced, this ratio began to become closer to that of a lean individual, further supporting this high F/B ratio as potentially obesogenic (Ley et al., 2005, 2006).

However, experimental studies in humans do not consistently demonstrate a relationship between the F/B ratio and weight gain. Contradicting studies describing the relationship between these two phyla and weight report no difference (Arumugam et al., 2011; Da Silva et al., 2020; Fleissner et al., 2010; Ley et al., 2005). With the advent of higher sensitivity genetic testing, interactions at the family, genus, and even species levels are being reported (Group et al., 2009). Lower level classifications of Firmicutes and Bacteroidetes have been shown to be affected by weight gain (Armougom et al., 2009; Menni et al., 2017; Remely et al., 2015). An increase in *Lactobacillus,* a genus in the Firmicutes phylum, has been associated with weight gain in mice, children, and adults (Armougom et al., 2009). Individuals with a higher abundance of *Ruminococcaceae* are thought to have a lower risk for weight gain, suggesting a potential protective effect against obesity for this Firmicutes family member (Menni et al., 2017).

Weight loss also has an impact on lower level classifications of microbes in the Firmicutes and Bacteroidetes phyla (Remely et al., 2015). As one would expect, during times of induced weight loss, the F/B ratio decreased (Remely et al., 2015). Weight loss is also associated with an increase in abundance of the genus *Lactobacilius* and species *Faecalibacterium prausnitzii* that also belongs to the Firmicutes phyla (Remely et al., 2015). The family *Lachnospiraceae,* from the Firmicutes phylum, is associated with a lower weight, lower energy consumption, and higher levels of leptin (Remely et al., 2015). Medically induced weight loss such as bariatric surgery also affects the microbial community in the gut, by increasing alpha and beta diversity up to one year after surgery (Shen et al., 2019). After bariatric surgery, the abundance of *Akkermansia muciniphilia*

increased in patients, demonstrating a positive change in the gut microbiome (Roopchand et al., 2015).

Firmicutes and Bacteroidetes are not the only phyla observed to impact weight gain. Two other abundant phyla in the gut microbiome that have shown a relationship to obesity and weight gain are Verrucimicrobia and Actinobacteria (Chakraborti, 2015; Group et al., 2009; Naito et al., 2018). The Verrucomicrobia phylum, in particular *Akkermansia muciniphila* sp., is present in low levels in people with obesity and metabolic syndrome (Chakraborti, 2015; Naito et al., 2018). *Akkermansia muciniphilia* sp. degrades mucin in the intestine, eventually leading to increased mucus production and thickening the intestinal barrier (Naito et al., 2018). This species is thought to promote mucus layer turnover in the intestinal lumen and prevent translocation of endotoxins and its subsequent role in inflammation (Naito et al., 2018). Actinobacteria has also been noted in associations between obesity and the microbiome. In particular, rats supplemented with the genus *Bifidobacterium,* from the Actinobacteria phylum, had better metabolic outcomes and lower weight suggesting that this bacterium has anti-obesogenic effects (Yin et al., 2010). However, a higher abundance of Actinobacteria has been associated with obesity (Chakraborti, 2015; Turnbaugh et al., 2009).

Most studies on the relationship between obesity and the microbiome are done on adults, not adolescents or children (Castaner et al., 2018). Recall there are statistically significant differences between the two age groups and the composition of the microbiome (Agans et al., 2011; Hollister et al., 2015; Palmer et al., 2007). However, there are similar inconsistencies to adults as some studies demonstrate a relationship between the F/B ratio

and weight, in children and adolescents, while others do not (Bervoets et al., 2013; Da Silva et al., 2020; J. Hu et al., 2013; Ignacio et al., 2016; Murugesan et al., 2015; Whisner et al., 2018). Da Silva et al, like other studies, also demonstrated a positive linear relationship between Firmicutes and weight and an inverse relationship between Bacteroidetes and Firmicutes in children (Da Silva et al., 2020). A significantly lower number of Bacteroidetes and *Bifidobacterium* were observed in the overweight cohort when compared to normal weight children (Da Silva et al., 2020). In two studies out of South America, increased abundance of the family *Enterobacteriaceae* from the Firmicutes phyla, was associated with obesity and overweight (Karlsson et al., 2012; Murugesan et al., 2015). However, in Hu et al, no relationship was found between F/B ratio and weight in adolescents (J. Hu et al., 2013). Another report by Ignacio et al only found a slight, not statistically significant increase in Firmicutes with weight (Ignacio et al., 2016). Bacteroidetes appeared to decrease between normal weight and overweight, but then increase between overweight to obese, contradicting results mentioned above (Da Silva et al., 2020; Ignacio et al., 2016).

Again, interesting differences can also be seen at the family, genus, or species level, with weight being associated with a myriad of different taxa (Da Silva et al., 2020; J. Hu et al., 2013). A higher abundance of the family *Bifidobacteriaceae* and genus *Bifidobacterium* have been associated with normal weight children and may have potential anti-obesogenic effects through reducing body fat stores (Da Silva et al., 2020). Another bacterium reported in higher abundance in normal weight youth when compared to obese was *Akkermansia muciniphila* (Journey et al., 2020; Karlsson et al., 2012). On the other

hand, *Lactobacilius* was associated with overweight and obesity in children (Bervoets et al., 2013; Da Silva et al., 2020). Bervoets et al reported a positive linear relationship between C reactive protein (CRP) concentrations and abundance of *Lactobacilius* in overweight children, suggesting a potential role of this bacteria in low grade inflammation (Bervoets et al., 2013). The phyla Proteobacteria is also higher in normal weight children when compared to overweight or obese (Murugesan et al., 2015). *Faecalibacterium* sp. and *Roseburia* sp. have also been seen in a higher abundance in overweight or obese youth (Murugesan et al., 2015). However, Hu et al reported no changes in abundance of *Faecalibacterium* sp. in normal weight compared to overweight or obese adolescents (J. Hu et al., 2013).

Potential Mechanisms of Action

The main mechanisms by which the gut microbiome affects weight gain are energy harvesting, inflammation and gut permeability, regulation of fat storage, and production of microbial metabolites such as short chain fatty acids (Bäckhed et al., 2004, 2007; Cani et al., 2007; Conlon & Bird, 2014; Harley & Karp, 2012; Heiss & Olofsson, 2018; Lin et al., 2012; Turnbaugh et al., 2006, 2009). The relationship between weight gain and microbes inhabiting the gut was first discovered in mice (Turnbaugh et al., 2006). A pivotal study by Turnbaugh et al. in 2006 discovered the transmissibility of the "obese microbiome" as well as the concept of energy harvesting (Turnbaugh et al., 2006). Once transplanted with microbes from the gut of overweight individuals, mice gained significantly more weight than mice transplanted with microbes from lean individuals, leading to the conclusion that microbes from obese individuals must be more efficient at harvesting energy from the diet

(Turnbaugh et al., 2006). These results are not always reproducible however, with no significant difference being observed between obese and lean transplanted mice (Bäckhed et al., 2007). Due to inconsistencies in results, the suggestion has been made that the concept of energy harvesting may be more complicated than initially thought (Murphy et al., 2010). Microbes inhabiting the intestines are also associated with an increased uptake of glucose from the diet, leading to an increase in the macronutrient content of the diet and strengthening the concept of energy harvesting (Bäckhed et al., 2004). One of the key roles of the microbes that inhabit the gut is to break down the contents of the diet that the human body is not naturally equipped to do through fermentation which results in the production of SCFA (Heiss & Olofsson, 2018).

Recall above that important byproducts of microbial metabolism are SCFAs which are used by colonocytes in the GI tract for energy, maintenance of luminal tissue, or for delivery to the liver (Chittim et al., 2018; Conlon & Bird, 2014). The three main SCFA produced through microbial fermentation are acetate, propionate and butyrate (Chittim et al., 2018; Conlon & Bird, 2014; Le Chatelier et al., 2013; Lin et al., 2012). The role of short-chain fatty acids, or SCFA, and weight maintenance has been studied extensively in rat models. One study by Lin et al. found that mice supplemented with butyrate and propionate completely avoided weight gain while acetate-fed mice had 40% less weight gain than the controls (Lin et al., 2012). Rats were fed high fat diets, HFD, to try to induce weight gain (Lin et al., 2012). Other metrics such as fasting glucose, glucose tolerance, fasting insulin and leptin levels all improved in each group (Lin et al., 2012). Another study by the same authors found that supplementation with SCFAs only resulted in reduced food

intake when butyrate was given (Lin et al., 2012). After conducting these studies the authors concluded that the SCFAs butyrate, acetate, and propionate protected the mice in these studies from the obesogenic effects of a HFD (Lin et al., 2012).

Another potential mechanism is the potential proinflammatory effect of the microbiome (Verdam et al., 2013). Inflammation is regulated through the immune system and is associated with an increase in proinflammatory cytokines (Cani et al., 2007). These are modulated through bacterial lipopolysaccharides, or LPS, in which a higher amounts of this endotoxin can cause inflammation in the tissues (Cani et al., 2007; Conlon & Bird, 2014; Naito et al., 2018). Obesity, particularly related to high fat diets, has been associated with an increased amount of LPS (Cani et al., 2007). Individuals with greater alpha diversity present with more *Faecalibacterium prausnitzii,* a species that is associated with anti-inflammatory properties, whereas individuals with a lower alpha diversity had more proinflammatory *Bacteroides* (Le Chatelier et al., 2013). Mechanisms through which low alpha diversity can contribute to the proinflammatory effects of the microbiome include; increased mucus degradation of the gut through a decrease in *Akkermansia* and decrease in butyrate-producing bacteria (Le Chatelier et al., 2013).

There are two main mechanisms by which fat storage is regulated through the microbiome, fat induced adipose factor (FIAF) and AMP-activated protein kinase (AMPK) (Bäckhed et al., 2007). FIAF is a circulating lipoprotein lipase (LPL) inhibitor (Bäckhed et al., 2004). Bacterial reduction in FIAF activity promotes adiposity and subsequent weight gain by upregulating LPL and thus triacylglycerol (TAG) storage in adipose tissue (Bäckhed et al., 2004). This suggests that an increase in FIAF activity could increase

leanness (Bäckhed et al., 2004). Another metabolite that is modulated through the gut microbiome is AMPK. This signaling molecule is associated with leanness (Bäckhed et al., 2007). AMPK is a key metabolite in fatty acid oxidation (Bäckhed et al., 2007). As a result of energy harvesting through the microbiome, microbes are thought to have a role in decreasing AMPK and thus decreasing fatty acid oxidation (Bäckhed et al., 2007). When GF mice are inoculated with microbes from conventionally raised mice, they became hyperglycemic and hyperinsulinemia, both of which stimulate lipogenesis and de novo lipogenesis in the liver; this resulted in body fat gain and increased insulin resistance (Bäckhed et al., 2004). The relationship between fat storage regulation and the microbiome is not always clear, with some studies reporting increases in adiposity that are not associated with microbes (Fleissner et al., 2010).

Therapeutic Applications Using the Microbiome

Understanding the microbiome and the way it changes and responds to the health of the human host can have potential therapeutic and public health implications for a multitude of conditions such as obesity, IBD, or other metabolic conditions (Brusaferro et al., 2018; Van Hul & Cani, 2019; Wang et al., 2019). Therapeutic ways in which the microbiome can be used include probiotics, prebiotics, polyphenol supplementation, and fecal microbiota Transplants, or FMT (Belizário et al., 2018; Brusaferro et al., 2018; Harley & Karp, 2012; Van Hul & Cani, 2019; Wang et al., 2019). Polyphenols are chemical compounds found in plants that are beneficial to humans (Van Hul & Cani, 2019). Probiotics supplements are live microorganisms while prebiotics are composed of selectively fermented nondigestible substrate provided by food, both of which have been

shown, along with polyphenols, to have positive health benefits (Everard et al., 2011; Harley & Karp, 2012; Marchesi, 2011; Van Hul & Cani, 2019).

The use of probiotics to modulate the microbiome has been used for thousands of years with yogurt and other fermented foods (Harley & Karp, 2012). Prebiotics have been associated with an increase in alpha diversity, as well as a decrease in the F/B ratio, which in turn can affect fat storage and inflammation (Everard et al., 2011). Studies using probiotic or prebiotic supplementation have shown positive results in energy homeostasis, metabolism, and maintaining a healthy weight (Cani et al., 2007; Harley & Karp, 2012). Supplementing with polyphenols has also demonstrated positive outcomes on the composition of the gut microbiome including an increase in the abundance of *Akkermansia muciniphilia* and increase in alpha diversity (Anhê et al., 2015; Roopchand et al., 2015). However, no one strain of probiotic demonstrated the same metabolic effect on every rat in one review (Cani et al., 2007). Probiotic, prebiotic, and polyphenol supplementation offer exciting ways to manipulate the microbiome, but more research is needed (Cani & Van Hul, 2015; Harley & Karp, 2012).

While the use of probiotics is a promising therapy, little is known about duration, frequency, dosage, and long-term effects of probiotic supplementation (Brusaferro et al., 2018). In addition to the potentially therapeutic uses of probiotics, fecal microbiota transplants have been used in the 21st century as an incredibly effective treatment for the pathogenic and deadly infection of *Clostridium difficile* (Wang et al., 2019). A fecal microbiota transplant, or FMT, is when a healthy person donates a sample of their stool which is then transferred to a sick person as a means of therapy (Wang et al., 2019). The

reported effectiveness of this treatment is roughly 90%, whereas the other typical treatment option of multiple antibiotics has an effectiveness rate closer to 20-30% (Wang et al., 2019). However, like probiotics, long-term effects of FMT are also unknown, and more research is needed (Brusaferro et al., 2018; Wang et al., 2019). Modulation and manipulation of the gut microbiome can have exciting implications in weight loss interventions, as they are usually ineffective over the long term (Van Hul & Cani, 2019).

CHAPTER 3

METHODS

Participants and Study Design

Participants for devilWASTE were recruited through a larger study, Social impact of Physical Activity and nutRition in College, or SPARC, which sought to analyze the influence between lifestyle factors, health, and the social networks of college freshman (Bruening et al., 2016). Participants were recruited for SPARC from six residence halls across three different Arizona State University campuses with the help of residence hall employees, flyers, and emails (Bruening et al., 2016). Monetary incentives were used to increase participation and retention rates throughout the study (Bruening et al., 2016). Once eligible students were enrolled in the larger study, they were given the opportunity to enroll in the devilWASTE study (Bruening et al., 2016). The exclusion criteria for devilWASTE included being under the age of 18, certain GI conditions such as malabsorptive disease, history of an eating disorder, antibiotic use 2-3 months prior, and current conditions that affect the microbiome including HIV infection, diabetes, or high blood pressure (Bruening et al., 2016). Inclusion criteria were living in a residence hall at ASU, English speaking, and participation in SPARC study (Bruening et al., 2016).

Informed consent was obtained from those who met the inclusion and exclusion criteria for the microbiome study. The devilWASTE study, as well as the parent SPARC study were approved by the Arizona State University Institutional Review Board. The study design for devilWASTE was a longitudinal observational design.

Study Measures and Methods

Recruitment for devilWASTE took place in August 2015 and longitudinal data collection continued through May 2016. There were four collection time points offered at the beginning and end of each semester. However, participants only selected three to complete the longitudinal nature of the study. The third collection time was added as an additional mid-point collection to increase participation rates due to low turnout for the second time point as it was during finals. At each time point, anthropometrics was taken as well as web-based self-reported data for dietary intake and physical activity. Anthropometrics were taken by trained research staff using instruments such as scales (SECA, USA) for weight, stadiometers (SECA, USA) for height, and flexible, spring loaded measuring tapes for waist circumference measurements. These measurements were completed up to three times to ensure accuracy. The two measures that were in 0.5 kg and 0.5 cm were averaged to get a measurement. This measurement was subsequently taken down and used for BMI calculations and waist circumference references. Demographics were self-reported.

Dietary Intake

Diet data were obtained using a validated National Cancer Institute Dietary Screener Questionnaire or DSQ (*Dietary Screener Questionnaire*, n.d.). This questionnaire does not determine caloric intake for study participants; however, it can be used to ascertain intake of certain food groups such as fruits, vegetables, or meat over the past month (*Dietary Screener Questionnaire in the National Health Interview Survey Cancer Control Supplement 2010: Overview*, n.d.). Validation of the DSQ in this population was conducted

during the pilot study using a 24-hour recall as suggested in Thompson et al when validating the DSQ for study populations (Bruening et al., 2016; Thompson et al., 2017). The correlation between 24-hr recall and DSQ in child and adolescent populations is reported between 0.34 and 0.50 depending on the type of food in the adolescent population (Hewawitharana et al., 2018). In an adult population, the correlation has been reported between 0.5-0.8 (Thompson et al., 2004). It is a validated measurement to "screen" for consumption of certain nutrients or components of the diet, mentioned above, and not as an indicator of overall diet (Thompson et al., 2017). Strengths of a dietary screener are that it is self-administered, low cost, and convenient (*Dietary Screener Questionnaire in the National Health Interview Survey Cancer Control Supplement 2010: Overview*, n.d.). This screener is an asset in studies where general diet data is all that is needed (Thompson et al., 2017). Considered a more accurate measure of overall diet, the 24-hour recall is preferred in studies where diet and caloric intake of study participants is scrutinized (Thompson et al., 2017).

Physical Activity and Sedentary Behavior

The Godin-Shephard Leisure-Time Physical Activity Questionnaire was used to measure the physical activity of the study participants. This is an assessment that asks questions regarding the strenuousness of exercise categorized in terms of vigorous, moderate, or light, and the number of hours per week spent doing each of these activities (Godin, n.d.). An additional question was included to assess sedentary time in hours per day (Bruening et al., 2016). These numbers were then combined, and a score was given for both leisure-time physical activity and sedentary time for study participants (Bruening et

al., 2016). The Godin-Shephard questionnaire has been validated against an accelerometer in adolescent populations with a correlation of 0.81 (Sirard et al., 2013). Actigraph accelerometers are a quantitative measurement device of physical activity in individuals wearing the equipment and are considered accurate (Sirard et al., 2013). A questionnaire like the Godin-Shephard is advantageous in this study due to the ease of administration, reliability for study population, and cost-effectiveness when compared to accelerometers (Sirard et al., 2013).

Fecal Collection

After anthropometrics and assessments were completed, participants were given a stool sample collection kit (Commode Specimen Collection Kit, Fisher Scientific, Anthem, AZ) and a brief demonstration on how to properly collect a sample. This kit was labeled with a devilWASTE specific participant ID as well as contact information for the study staff and written instructions. Contents of the kit included a collection bowl and bag that were pre-weighed as well as a cooler and ice pack to keep the sample cold until it reached the Healthy Lifestyles Research Center on the Downtown ASU campus. Participants were instructed to call research staff within 30-60 minutes to pick up their samples to avoid bacterial growth and changes in microbial communities. Fecal samples were then processed within 24 hours of collection and stored at -80 degree Celsius until extraction. Samples were processed using our unique processing protocol which included defrosting samples and completing the information on the "Sample Processing Log". This included weighing the sample and taking the pH of the stool in three separate locations. Then, between 0.150-0.250 g of sample was put into a 2mL PowerBead tube (Catalog No. 12888-

100-5, QIAGEN, Germantown, MD, USA) and stored in a -80-degrees Celsius freezer until extraction. Weight of the tube and weight of the tube plus the fecal sample were also recorded in a sample processing log.

DNA Extraction

DNeasy Powersoil Isolation Kits (Catalog No. 12888-100, QIAGEN, Germantown, MD, USA) were used to extract microbial DNA from fecal samples. These kits combine a series of salt and ethanol-based solutions as well as heating, cooling, filtering, and centrifugation methods to first decrease the amount of fecal matter in the sample then break the cell membranes of microbial cells to release the DNA. In addition to the manufacturer steps, a heating step was incorporated at the beginning of the protocol as well as an additional cleaning protocol to ensure no fecal inhibitors remained in the final DNA sample. Once the DNA was isolated, it was tested using a QIAGEN spectrophotometer machine (Catalog No. 9002340, QIAGEN, Germantown, MD, USA) to test for the appropriate quality and concentration of the samples. A quality of at least 1.7 (ng/microL) and a concentration of roughly 10 (A260/A280) were considered adequate. Samples were tested for quality and concentration by putting 2 microliters of the DNA solution into a QIAxpert Slide-40 (Catalog No. 990700, QIAGEN, Germantown, MD, USA) and inserted into the QIAxpert spectrophotometer. If samples were of the appropriate quality and concentration, they were placed into the DNA box and stored at -80 degrees Celsius. If they were not, it was noted, and they were later reprocessed and extracted again. A total of 491 samples from 262 participants were extracted and sent for sequencing.

DNA Sequencing

Samples were sequenced at The Biodesign Institute at Arizona State University Tempe Campus in the Genomics Core Lab. At the lab, sequences were quantified using Quant-iT PicoGreen/ assay (Catalog No. P7589, Invitrogen, Carlsbad, CA, USA). Sequencing methods began with amplification through triplicate PCR in 96 well plates to distinguish the presence of archaea from the bacteria, and next generation sequencing to identify bacterial species. This was done through amplification of the 16S rRNA gene sequence using primers for the conserved V4 region of the bacterial genome. The V4 region was identified through the use of the forward 515F primers and 806R reverse primers containing Illumina adaptor sequences (Turnbaugh et al., 2007). Purification and quantification materials used for PCR in the Genomics Core Lab included QIAquick PCR Purification Kit (Catalog No. 28106, Qiagen, Germantown, MD, USA), and the KAPA Library Quantification Kit (Catalog No. KK4824, Kapa Biosystems, Wilmington, MA, USA). After PCR was completed, the Illumina MiSeq instrument, (Catalog No. SY-410-1003, Illumina, Inc., San Diego, CA) was used for sequencing. All protocols were completed in accordance with best practices established by the Human Microbiome Project guidelines.

Sequence Analysis

Quantitative Insights Into Microbial Ecology 2 (QIIME2) was the bioinformatics software in which statistics were performed on the sequences (Bolyen et al., 2019). After sequences were demultiplexed, they were added into the QIIME2 pipeline where they were denoised by using the DADA2 command to account for inherent errors produced through

sequencing. Samples were then rarified to determine a workable sequencing depth. Phylogeny and taxonomy were performed next. The FastTree command was used to analyze phylogeny of the sequences while a naive-Bayes classifier from the GreenGenes 13.8 database was used to assess taxonomy (DeSantis et al., 2006; *FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments*, n.d.). With sequences now categorized, diversity measures and statistics were performed to test the hypothesis.

Statistical Analysis

A convenience sample was used in this study and thus the sample size was not powered. Metadata for these analyses were taken from the parent, SPARC Study (Bruening et al., 2016). The mean and standard deviation were assessed in SPSS for the descriptive statistics including gender, race/ethnicity, age, height, weight, BMI, waist circumference, PA level, diet, date of last period, and antibiotic use within the last 2-3 months. Normality was assessed using the Shapiro-Wilk test. The independent variable of weight will be assessed as a categorical and continuous variable. Change in weight will be categorized as weight loss, weight gain and weight maintenance defined as a loss of more than 3% participants baseline, a gain of more than 3% and in between, respectively.

The QIIME2 platform was used to run diversity statistics. Longitudinal analysis was performed using QIIME2 Longitudinal plugin which assessed the volatility of the community's abundance over time (Bokulich et al., 2018). Features within this plugin that were used include feature volatility, first differences and first distances (Bokulich et al., 2018). Linear effects modeling (LME) was used to analyze changes using multiple variables, such as time (random effect), subject ID (random effect), and baseline BMI

(fixed effect), and sex (fixed effect). LME modeling can take into account fixed effects such as sex, baseline BMI, timepoint and random effects such as changes in weight. These data were then visualized using the Vega, QIIME2's visualization feature, through volatility plots (Bokulich et al., 2018). A volatility plot will be able to visualize changes in abundance and potential perturbation between an individual or categories of weight gain over time (Bokulich et al., 2018). A significance value of 0.05 was used.

Statistical Analysis by Hypothesis

H1: Longitudinal changes in alpha diversity will be assessed using the linear model above, incorporating feature volatility as the outcome measure, which takes into account changes in relative abundance of microbial taxa between samples over time using machine learning. LME modeling will account for the covariates sex, baseline BMI, subject ID, and time.

H2: Longitudinal changes in beta diversity will be assessed using the linear model above, incorporating first differences and first distances to assess broad community structure changes over time. First differences measures magnitude of change between communities over time whereas first distances measures rate of change. Unweighted UniFrac will be one of the beta diversity metrics used in this model which accounts for taxa phylogeny between subject samples. This will be able to demonstrate if there are differences in rate of phylogenetic transitions of microbial taxa between weight groups. Jaccard will also be used as a metric of beta diversity; this measure accounts for dissimilarity between communities using the presence/absence of taxa. LME modeling will account for the covariates sex, baseline BMI, subject ID, and time.

CHAPTER 4

RESULTS

Participant Characteristics

A total of 231 participants were included in the devilWASTE study; n=139 that provided two or more stool samples, n=90 provided only one and were excluded from this analysis. Of the 139 participants, 69.8% were female and 30.2% were male. A majority of participants were white (N=59, 42.4%) and non-Hispanic (N=89, 64%). A further breakdown of the race/ethnicity of participants can be found in **Table 1**. A majority of participants (N=114, 82%) reported more than 30 minutes of vigorous physical activity a week. In response to the Godin-Shepard Physical Activity Questionnaire, which measures moderate-to-vigorous physical activity in hours per week, 64.7% of participants were grouped into the "Active" category with a score of 24 or higher. Moderately active, defined as a score of 14-23, accounted for 18.7% of participants and lastly, 16.6% of participants were grouped into the sedentary category with a score of less than 14. A majority of participants (N=102, 73.4%) reported over 360 minutes of screen time per week**.** The average number of days from their first stool collection date to their last was 216 (median) with an IQR of 77 days. At baseline, a majority of participants were in the normal weight or underweight BMI category, 25 kg/m^2 or below (N=91, 65.5%), with the underweight category including 4.3% (N=6) and the normal weight group including 61.2% (N=85) of the study cohort. There were 48 (34.5%) participants that were overweight or obese, with a BMI of over 25 kg/m^2.

Participants were distributed into three weight change groups that included weight loss (N=13, 9.4%), weight maintenance (N=59, 42.4%), and weight gain (N=67, 48.2%).

Table 1

Sociodemographic and Key Variables of Participants (n=139)

[1]Age (years) mean ± SD	18.6 ± 0.7
[1]Sex, %	
Male	30.2
Female	69.8
[1]Race/ ethnicity, %	
White	42.4
Black	12.9
Hispanic	25.9
Other	18.7
[1]Height (cm) median (IQR)	165.6 (14.3)
[1]Weight (kg) median (IQR)	66.2 (22.9)
[1]Waist circumference (cm) median (IQR)	79 (16.8)
[1]Median weight change (kg) (Range)	1.8 (-9.3- 16.8)
[1]Body Mass Index (BMI) Category, %	
<18.5 kg/m^2 (underweight)	4.3
18.5-24.9 kg/m^2 (normal)	61.2
25-29.9 kg/m^2 (overweight)	23.7
≥30 kg/m^2 (obese)	10.8
[1]BMI measured (kg/m^2) median (IQR)	23.4 (5)
[1]BMI Dichotomized, %	
Not overweight/ obese	65.5
Overweight/ obese	34.5
[1]Weight change between timepoint 1 and 2, %	
Weight maintenance	42.4
Weight gain	48.2
Weight loss	9.4
[1]Screen time (minutes per week) dichotomized, %	
≥360	26.6
<360	73.4
[1]Screen time (minutes per week) categorical, %	
0-75	26.6
195	35.3
315-360	38.1
[1]Moderate to vigorous activity (based on mean score from Godin-Shepard Questionnaire, %	
Active (score of ≥24)	64.7
Moderately active (score of 14-23)	18.7
Sedentary (score <14)	16.6
[1]Days between timepoint 1 and 2, median (IQR)	216 (77)

Note: *[1]N=139*

Gut Microbiome Analysis

Sampling depth was based on the minimum frequency for the samples (7335) then confirmed using an alpha rarefaction curve for OTUs (**Figure 1**). A depth of 7335 included all samples as well as a majority of their OTUs. Increasing the depth to 10,000 would have excluded 4 participants. By 25,000, which is where OTU counts increased, the sample size of the weight gain group would have gone from 180 to 40 (**Figure 2**).

Figure 1

Observed OTUs by Sampling Depth Across Weight Categories

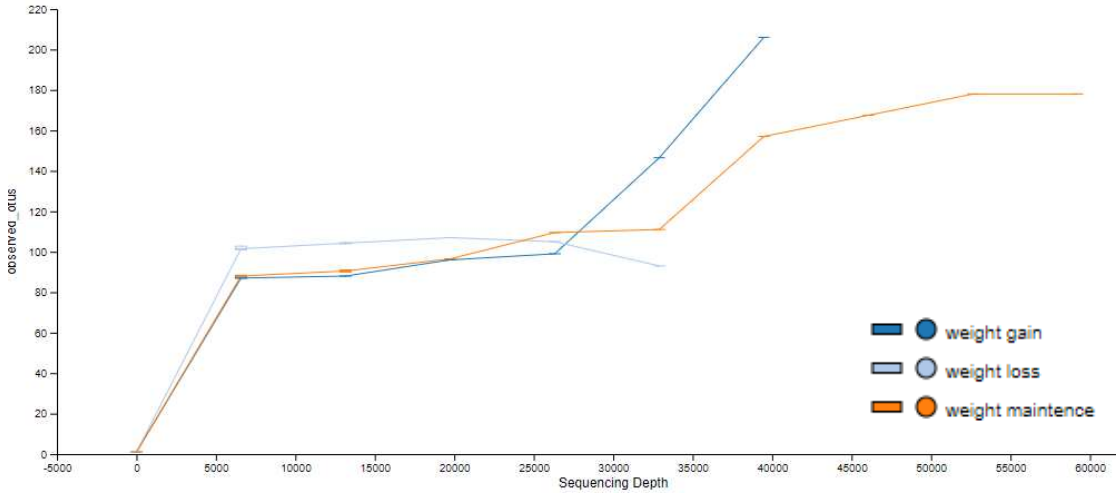
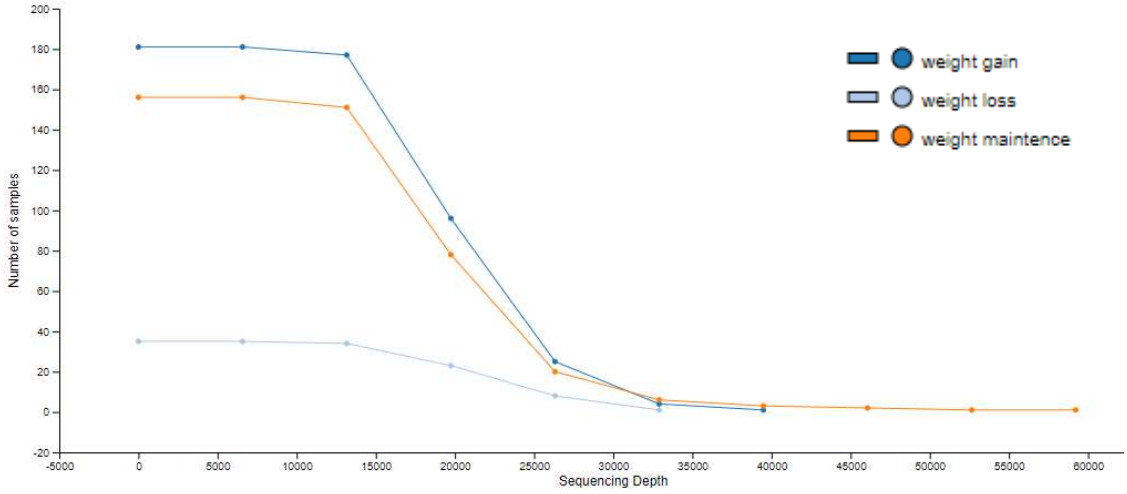


Figure 2

Number of Samples by Sampling Depth Across Weight Categories



Feature Volatility

Feature volatility can be used to assess the volatility of a feature over time. The family *Lachnospiraceae*, genus *Ruminococcus* (from *Lachnospiraceae* family), and species *lavalense* were all found to be important drivers of volatility. (**Figure 3**). The features used for subsequent analysis were determined according to the high importance and global mean on (**Figure 3**). Feature volatility for *Lachnospiraceae*, *Ruminococcus*, and *lavalense* were then included in linear mixed effects models to test the impact of weight change and covariates of interest such as time, baseline BMI, gender, and race/ethnicity. Relative abundance of *Lachnospiraceae* was significantly impacted by race/ethnicity, with white participants having a 1181.807 unit greater increase (P>|z|= 0.016) in this feature when compared to students classified as Other. Significance with race ethnicity was also demonstrated in feature volatility in *Ruminococcus,* with black

race/ethnicity associated with a 306.286 unit decrease (P>|z|= 0.022) and Hispanic race/ethnicity associated with a 223.696 unit decrease (P>|z|= 0.046) in feature abundance over time. Lastly, the feature volatility for *lavalense* was significantly impacted by time (P>|z|= 0.001) measured in days from the first time point to the last time point, demonstrating that a one-unit change in days leads to a 0.023 decrease in this species. It should be noted that results for *Lachnospiraceae* and *Ruminococcus* should be interpreted with caution due to high group variance variable (model error) of 1,343,652.014 and 110,752.998, respectively. See (**Table 2**) below for all results from the Feature Volatility Linear Mixed Effects model for *Lachnospiraceae*. Similar results were seen in genus *Ruminococcus* (**Table 3)** and species *lavalense* (**Table 4**).

Figure 3

Feature Volatility Plot with Importance and Global Mean for lavalense

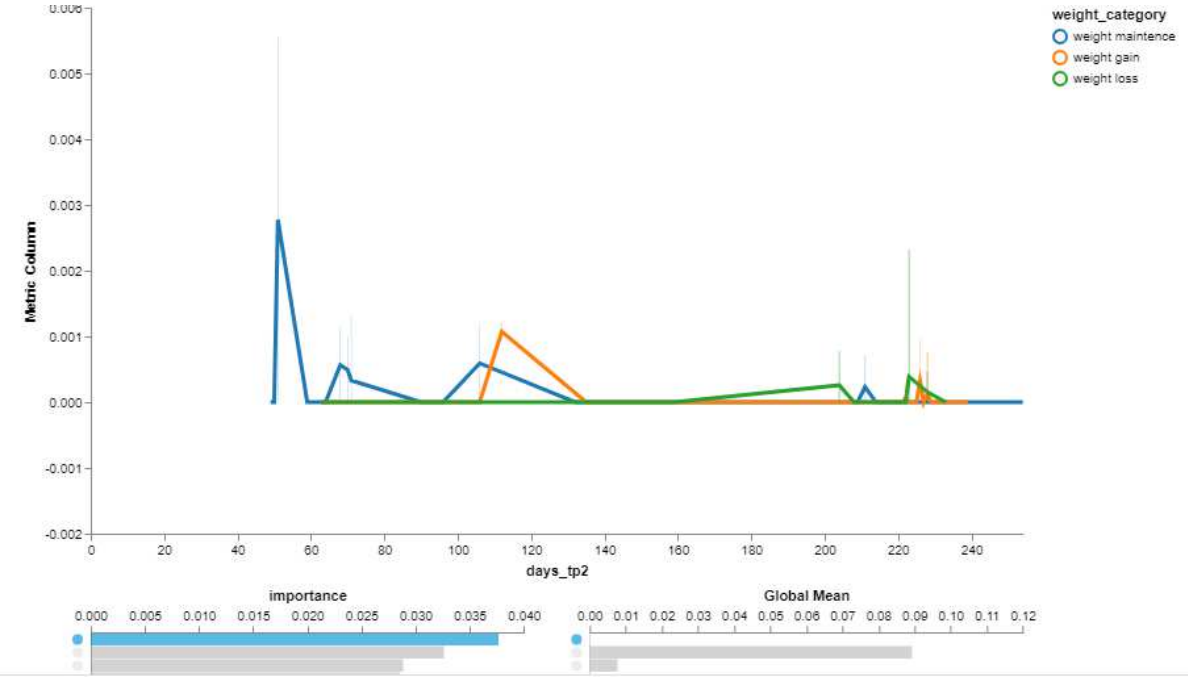


Table 2

Linear Mixed Model for Feature Volatility Lachnospiraceae

	Coefficient	Standard Error	z	P>|z|	[0.025	0.975]
Intercept	6073.155	782.186	7.764	**0.000**	4540.099	7606.211
Baseline BMI [Obese]	148.397	558.918	0.266	0.791	-947.062	1243.856
Baseline BMI [Overweight]	-78.711	415.739	-0.189	0.850	-893.545	736.122
Baseline BMI [Underweight]	-65.689	842.978	-0.078	0.938	-1717.895	1586.518
Race/ Ethnicity [Black]	-1063.293	638.234	-1.666	0.096	-2314.209	187.623
Race/ Ethnicity [Hispanic]	-25.621	534.662	-0.048	0.962	-1073.539	1022.297
Race/ Ethnicity [White]	1181.807	492.427	2.400	**0.016**	216.667	2146.947
Weight category [weight loss]	-711.072	632.158	-1.125	0.261	-1950.080	527.936
Weight category [weight maintenance]	-315.858	363.050	-0.870	0.384	-1027.423	395.706
Days in study	2.385	3.140	0.760	0.448	-3.769	8.539
Gender[male]	-74.438	379.497	-0.196	0.844	-818.238	669.363
Group Variance	1343652.044	234.710				

Note: Numbers are bolded to indicate significant results.

Table 3

Linear Mixed Model for Feature Volatility Ruminococcus

	Coefficient	Standard Error	z	P>|z|	[0.025	0.975]
Intercept	581.010	161.150	3.605	**0.000**	265.162	896.859
Baseline BMI [Obese]	-104.771	118.261	-0.886	0.376	-336.558	127.016
Baseline BMI [Overweight]	12.833	87.425	0.147	0.883	-158.517	184.182
Baseline BMI [Underweight]	-160.063	178.670	-0.896	0.370	-510.250	190.125
Race/ Ethnicity [Black]	-306.286	133.776	-2.290	**0.022**	-568.482	-44.091
Race/ Ethnicity [Hispanic]	-223.696	112.061	-1.996	**0.046**	-443.332	-4.061
Race/ Ethnicity [White]	-130.745	103.421	-1.264	0.206	-333.447	71.957
Weight category [weight loss]	-115.668	80.052	-1.445	0.148	-272.567	41.231
Weight category [weight maintenance]	-154.006	133.026	-1.158	0.247	-414.733	106.721
Days in study	-52.712	76.539	-0.689	0.491	-202.725	97.302
Gender[male]	0.236	0.646	0.366	0.715	-1.029	1.501
Group Variance	110752.998	69.493				

Note: Numbers are bolded to indicate significant results.

Table 4

Linear Mixed Model for Feature Volatility lavalense

	Coefficient	Standard Error	z	P>|z|	[0.025	0.975]
Intercept	5.153	1.743	2.956	**0.003**	1.736	8.569
Baseline BMI [Obese]	-0.766	1.405	-0.545	0.586	-3.521	1.988
Baseline BMI [Overweight]	-0.437	1.178	-0.371	0.710	-2.745	1.871
Baseline BMI [Underweight]	0.398	1.085	0.367	0.713	-1.728	2.525
Race/ Ethnicity [Black]	-0.375	0.831	-0.451	0.652	-2.004	1.254
Race/ Ethnicity [Hispanic]	1.508	1.389	1.085	0.278	-1.215	4.231
Race/ Ethnicity [White]	0.555	0.797	0.696	0.486	-1.007	2.118
Weight category [weight loss]	0.504	1.222	0.412	0.680	-1.891	2.900
Weight category [weight maintenance]	0.511	0.914	0.559	0.576	-1.280	2.302
Days in study	1.887	1.844	1.024	0.306	-1.727	5.501
Gender[male]	-0.024	0.007	-3.389	**0.001**	-0.037	-0.010
Group Variance	2.742	0.666				

Note: Numbers are bolded to indicate significant results.

Pairwise Differences and Pairwise Distances

The next metric used to assess diversity was pairwise differences. According to Shannon pairwise differences (**Figure 4**), the weight maintenance group experienced a statistically significant decrease in Shannon diversity (richness) over time (p=0.036), but this significant change was lost when adjusting for multiple comparisons (FDR= 0.108). There were no significant differences in the weight gain (p=0.51) and weight loss

(p=0.97) groups over time. The omnibus Kruskal-Wallis test to compare the deltas between these groups was not significant (p=0.102. H= 4.55) indicating that changes across groups did not differ. Statistical significance was not observed following pairwise difference analyses using Faith's PD. However, evenness, which was measured using Pielou's evenness metric (**Figure 5**), demonstrated that the weight maintenance group experienced statistically significant changes in microbial taxa evenness over time following a Wilcoxon signed-rank test (W=601.0, p=0.032), but this was no longer significant after adjustment for multiple comparisons (FDR p=0.096). When comparing the delta values for each weight change group, no significant differences were observed (Kruskal-Wallis omnibus test p=0.209, H=3.13).

Figure 4

Shannon Pairwise Differences by Weight Category

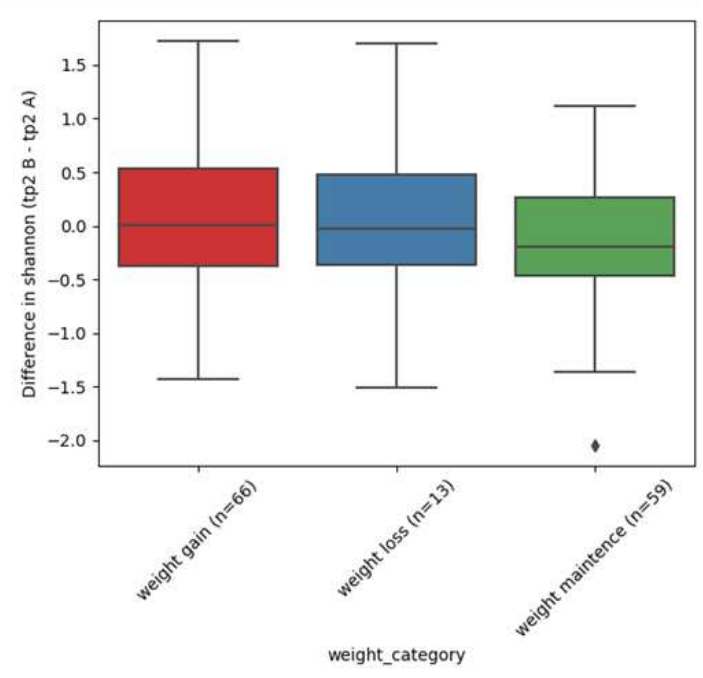
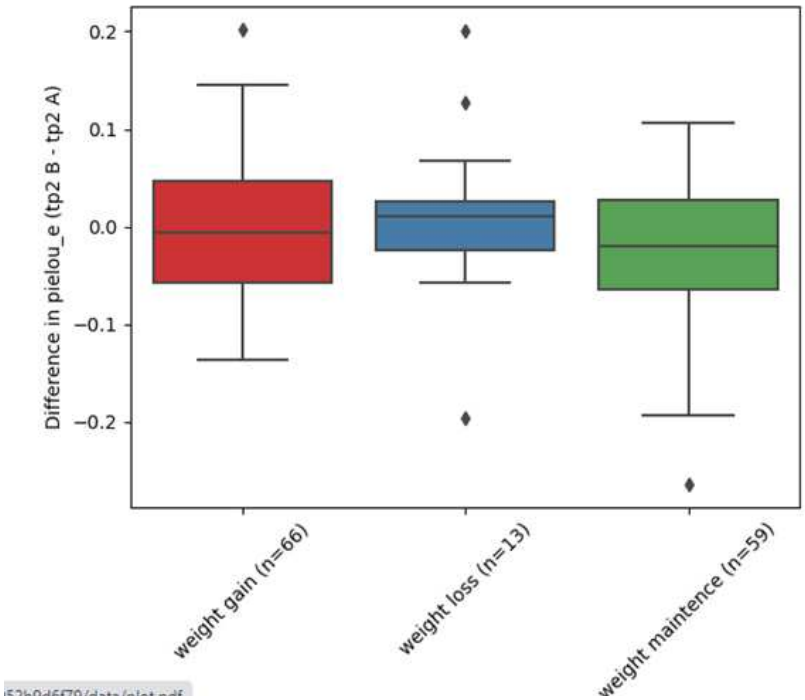


Figure 5

Pielou's Evenness Pairwise Differences by Weight Category



Pairwise differences were also investigated using beta diversity inputs to compare weight change categories. No significant differences were found for Jaccard and Bray-Curtis diversity metrics. In the pairwise difference tests for changes within each weight change group by time using the Unweighted UniFrac metric (**Figure 6**), the weight gain (W=802.0, p=0.053, FDR p=0.083) and weight maintenance (W=631.0, p=0.055, FDR p=0.083) groups experienced a slight increase and decrease, respectively. Overall, the changes in the multiple group Kruskal-Wallis test were significant as well (p=0.029, omnibus H= 7.07). This was driven by the significant pairwise, post-hoc group comparison between the weight maintenance and weight gain groups which remained

significant after adjustment for multiple comparisons (Mann-Whitney U=1410.0, p=0.008, FDR p=0.024). Results between weight gain and weight loss (p=0.797) or weight loss and weight maintenance (p=0.272) groups were not significant. Following pairwise differences analysis using the Weighted UniFrac metric, trends were observed for all three weight change groups (**Figure 7**) with all three groups experiencing a small decrease in this metric over time. This trend was most pronounced for the weight gain group, but the pre-post difference was not significant after FDR adjustment (W=749, p=0.023, FDR p=0.068). The Kruskal-Wallis test comparing the differences between weight change groups was not significant when using the Weighted UniFrac metric (p=0.431, H= 1.68).

Figure 6

Unweighted UniFrac Pairwise Differences by Weight Category

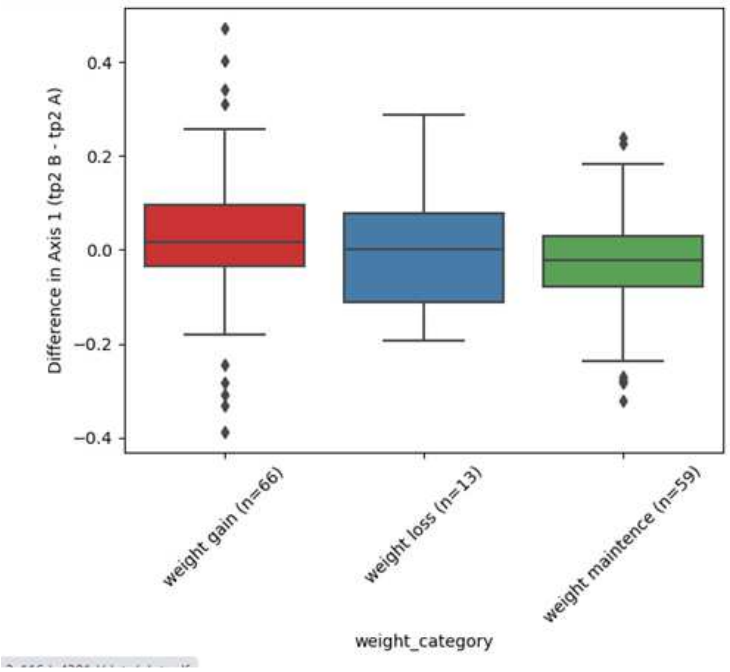
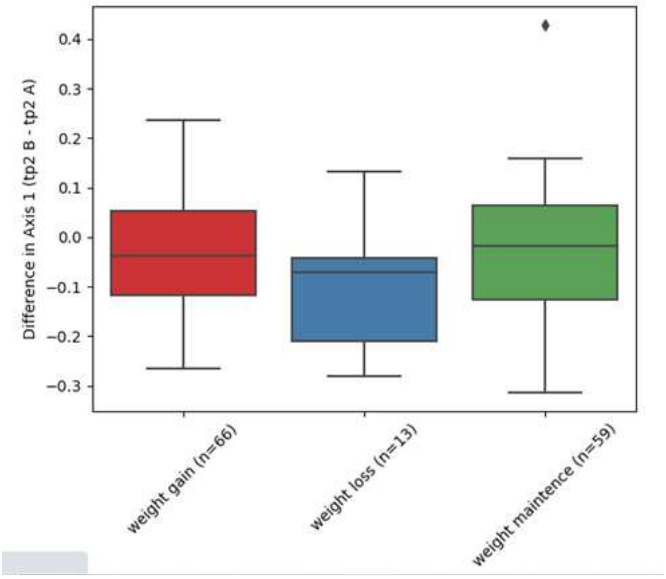


Figure 7

Weighted UniFrac Pairwise Differences by Weight Category



Beta diversity metrics were the only inputs into the pairwise distances metric to evaluate the stability of the gut microbiome community over time. The Kruskal-Wallis test for multiple group comparisons were not significant for any of the beta diversity metrics including Jaccard (p= 0.455, H=1.58), Bray-Curtis (p=0.454, H=1.58), Unweighted UniFrac (p=0.474, H= 1.49) and Weighted UniFrac (p=0.127, H=4.12). This suggests that the overall community structure remains stable over time relative to how weight status changed.

First Differences and First Distances

Lastly, differences over time were assessed by calculating first differences and first distances. Once calculated these values were input into linear mixed models to determine significance and relationships with covariates. For alpha diversity metrics with first differences, Shannon diversity (**Table 5**) was associated with change in weight such

that a statistically significant decrease of 0.235 units was observed in the weight maintenance category (P>|z|= 0.030) when compared to the weight gain group (reference). While the linear mixed model for Faith's PD demonstrated no relationship with any of the covariates, first differences using Pielou's Evenness demonstrated a decreasing trend (P>|z|= 0.085) in the weight maintenance group, relative to the weight gain group, as well. Meaning, people who maintained their weight over the course of the study had less change in Shannon and Pielou's in relation to those who gained weight.

Table 5

Linear Mixed Model for First Difference Shannon

	Coefficient	Standard Error	z	P>|z|	[0.025	0.975]
Intercept	-0.167	0.247	-0.674	0.500	-0.652	0.318
Baseline BMI [Obese]	-0.032	0.165	-0.194	0.846	-0.355	0.291
Baseline BMI [Overweight]	0.024	0.131	0.181	0.856	-0.233	0.281
Baseline BMI [Underweight]	0.124	0.254	0.489	0.625	-0.374	0.622
Race/ Ethnicity [Black]	0.193	0.209	0.920	0.358	-0.218	0.445
Race/ Ethnicity [Hispanic]	0.109	0.171	0.639	0.523	-0.226	0.445
Race/ Ethnicity [White]	0.040	0.159	0.249	0.803	-0.273	0.352
Weight category [weight loss]	-0.103	0.195	-0.531	0.596	-0.485	0.278
Weight category [weight maintenance]	-0.235	0.113	-2.076	**0.038**	-0.458	-0.013
Days in study	0.001	0.001	1.419	0.156	-0.001	0.003
Gender[male]	-0.152	0.117	-1.292	0.196	-0.382	0.078
Group Variance	0.000	0.230				

Note: Numbers are bolded to indicate significant results.

For beta diversity measures, PCoA plots, like **Figure 8** below for Bray-Curtis, were used in the model and demonstrate change around the primary axis. Both Bray-Curtis and Jaccard demonstrated significance in relation to weight in these linear mixed models. First differences Bray- Curtis was associated with a significant decrease of 0.077 units in students with a baseline BMI classifying as overweight (P>|z|= 0.039) and first differences Jaccard was associated with a decrease of 0.044 units in the weight maintenance group (P>|z|= 0.014) relative to the normal weight BMI group and weight gain groups, respectively. On the other hand, first differences analysis using the Unweighted UniFrac metric revealed a small but statistically significant increase over time of less than 1 unit per day (P>|z|= 0.016). This significance in time however was lost with the Weighted UniFrac (P>|z|= 0.518) metric which adjusts for microbial abundance, which may suggest the importance of abundance in driving differences over time. Lastly, beta diversity could also be measured using first distances which uses a distance metric as the input, then again everything was input into a linear mixed effects model. First distances using the Jaccard diversity metric (**Table 6**) was significantly associated with a 0.095 increase in weight loss (P>|z|= 0.020) relative to weight gain. This metric was also significantly associated with time as there was a decrease of 0.001 units per day (P>|z|= 0.000). Like first differences, first distances Unweighted UniFrac was significantly associated with time (P>|z|= 0.000) although in this case it demonstrated a decrease by 0.001 units per day. However, as demonstrated above, Weighted UniFrac was not significantly associated with time (P>|z|= 0.511).

Figure 8

PCoA Plot for Bray-Curtis Distinguished by Weight Category

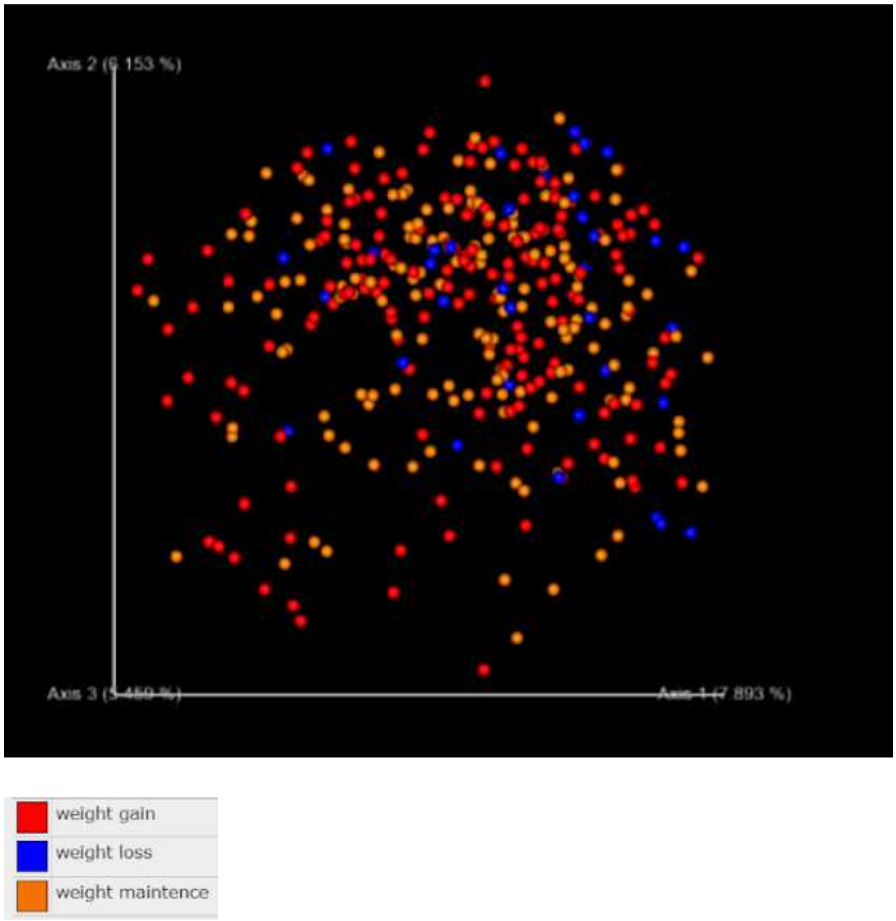


Table 6

Linear Mixed Model for First Distances Jaccard

	Coefficient	Standard Error	z	P>|z|	[0.025	0.975]
Intercept	0.649	0.053	12.166	0.000	0.544	0.753
Baseline BMI [Obese]	0.025	0.035	0.711	0.477	-0.044	0.094
Baseline BMI [Overweight]	-0.037	0.028	-1.319	0.187	-0.093	0.018
Baseline BMI [Underweight]	-0.054	0.055	-0.987	0.324	-0.161	0.053
Race/ Ethnicity [Black]	0.073	0.044	1.636	0.102	-0.014	0.160
Race/ Ethnicity [Hispanic]	0.024	0.037	0.660	0.509	-0.048	0.097
Race/ Ethnicity [White]	0.037	0.034	1.075	0.282	-0.030	0.104
Weight category [weight loss]	0.095	0.041	2.332	**0.020**	0.015	0.174
Weight category [weight maintenance]	0.020	0.024	0.835	0.404	-0.028	0.068
Days in study	-0.001	0.000	-3.583	**0.000**	-0.001	-0.000
Gender[male]	0.021	0.025	0.821	0.412	-0.029	0.071
Group Variance	0.000	0.038				

Note: Numbers are bolded to indicate significant results.

CHAPTER 5

DISCUSSION

The aim of this study was to assess the changes in community structure and diversity of the gut microbiome in relation to changes in weight status in an adolescent population from the beginning to the end of freshman year. The average weight change of participants over this time was +1.8 kg, or 3.96 lbs., with almost half of the participants gaining weight. This is just under previously reported averages of weight gain over the freshman year of 4-5 lbs (Gropper et al., 2012; Racette et al., 2005, 2008). In this study cohort, both time and weight had an impact on the diversity and variability of the gut microbiome. Linear mixed models were used to test the impact of time and weight as variables, while adjusting for covariates. Several results were deemed to be significantly influenced by either time or weight status in these models, but no results demonstrated a significant interaction between the two. Alpha diversity was only significantly associated with weight in the first differences Shannon diversity test while beta diversity seemed to be most significantly associated with both weight and time. Particularly, Unweighted UniFrac analysis illustrated that inter-individual changes were greater than intra-individual changes in microbial community structure when considering weight and time as a variable.

Weight, the first outcome of interest, had statistically significant results in both alpha and beta diversity measurements. With regards to alpha diversity metrics and their relation to weight or time metrics, there were only a couple relevant results. Weight was significantly related to microbial richness (Shannon Diversity First Differences) such that

a decrease in richness was observed in the weight maintenance category relative to the weight gain group. A trend for microbial evenness (Pielou's Diversity First Differences) was also observed when the weight maintenance group was compared to the reference weight gain group. Together, these findings demonstrate that the participants who maintained their weight over the course of the study had less change in richness and evenness when compared to those who gained weight. Few human studies have evaluated changes in microbial diversity in relation to weight change over time which makes comparison to other studies difficult. While cross-sectional studies have observed differences in richness and evenness between normal weight adults and those with overweight or obesity (Sze, Schloss), a large cohort study of school-aged children (KOALA Cohort Study) found no differences in microbial diversity and richness when comparing children of different weight status (Mbakwa et al., 2018; Sze & Schloss, 2016).

Regarding gut microbial community similarity, Jaccard metric first difference analysis revealed that the weight maintenance group was different from weight gain and loss groups. Meaning that, when compared to weight gain, those who maintained their weight from the beginning to the end of the study had less variability in community similarities. Interestingly, in this study, the First Distances Jaccard linear mixed model results showed that those who lost weight had a higher microbial turnover than those who gained weight. In longitudinal studies by Frost et al and Shen et al, increases in beta diversity were reported with weight loss among obese adults (Frost et al., 2019; Shen et al., 2019). However, the significant metrics were Bray-Curtis in Frost et al. and UniFrac

in Shen et al (Frost et al., 2019; Shen et al., 2019). Compared to results from our study, the results from Shen et al. indicated that the significance was in phylogeny whereas the results from Frost et al., much like the results in our study, were driven by either similarity of dissimilarity indexes. Another significant result in our study is related to First Differences Bray-Curtis which was significantly associated with the overweight baseline BMI group. These results may be interpreted as the overweight baseline BMI group had less change in variability on Axis 1 relative to the normal weight group. Bray-Curtis takes into account presence and absence with abundance while Jaccard is related to presence and absence. A significant result in Bray-Curtis but not Jaccard may indicate that these differences were driven by abundance. Again, the meta-analysis by Sze and Schloss et al. reaffirms this result, as they also found that Bray-Curtis was significantly different in obese populations when compared to non-obese, but were unable to find the directionality of this change as this meta-analysis was only of cross-sectional studies (Sze & Schloss, 2016). Again, in our study, abundance was also seen to impact weight groups in the UniFrac Pairwise Differences tests. The significant omnibus test for pairwise differences in Unweighted UniFrac, was driven by the significant post-hoc pairwise test between the weight maintenance and weight gain groups. Meaning that the difference from baseline to the end of the study was significantly lower for those who maintained their weight when compared to those who gained weight. Significance was lost in the Weighted UniFrac test, which may signify that this result was driven by low abundance features.

Temporal variability in microbial communities is well known, and while communities are mostly stable, some change over time is expected. Time, the other outcome of interest, was a statistically significant predictor of qualitative first differences using the Unweighted UniFrac metric, but not in the quantitative first differences linear model using the Weighted UniFrac metric. This potentially demonstrates that the increase in microbial composition variability over time may be driven by low abundance features. A similar trend was seen in the first distances linear mixed effect model for Unweighted versus Weighted UniFrac. However, in the first differences model time is shown to increase Unweighted UniFrac whereas in the first distances model, time is shown to decrease Unweighted UniFrac. This difference may be due to the nature of the tests, first differences measure the rate of change from first to last sample whereas first distances measure the difference between successive samples. This may make first distances susceptible to extenuating circumstances, while first differences specifically measures beginning and end. Lastly, time was also statistically significant in the First Distances Bray-Curtis and the First Distances Jaccard linear mixed models, showing decreased diversity over time. Significance in both tests may indicate that time not only impacts the overall community structure, or turnover, but abundance as well. Similar results were seen in Fu et al. and Marti et al (Fu et al., 2019; Marti et al., 2017). Results in these longitudinal studies indicated stability in high abundance features and more variability in the lower abundance features over time (Fu et al., 2019; Marti et al., 2017). These two studies, along with our study, were the only studies found emphasizing the importance of low abundance features and time. However, both Fu et al. and Marti et al. were

conducted in adult populations, indicating that studies in youth, focusing on changes in low abundance features over time, are an area for further investigation.

To our knowledge, this is the first longitudinal study to date that has found a significant relationship between weight gain in adolescents and low abundance features in a myriad of tests. Whisner et al, using data from a cross-sectional study of first-year college students, also found significant relationships with low abundance features but only in relation to self-reported physical activity levels and not overweight/obesity status (Whisner et al., 2018). The importance of low abundance features is a different focus than a majority of studies have taken on the relationship between weight and diversity of the microbiome. Historically, studies have focused on two of the most common and abundant phyla in the gut microbiome, Firmicutes and Bacteroidetes (Ley et al., 2005; Turnbaugh et al., 2009). While the gut microbiome is largely composed of high abundance phyla members from Firmicutes and Bacteroidetes, there is also a large amount of low abundant features which may also be important (Claussen et al., 2017). Parsing out low abundance features for analysis is difficult, as most analyses are not sensitive to low abundance features (Claussen et al., 2017). It is suggested that these lower abundant features may contribute to more specific functions in the body, and the absence of these low abundance features may leave a specific but vital function incomplete thereby leaving the host vulnerable adverse health outcomes (e.g. obesity, cardiometabolic disease) (Claussen et al., 2017; Shafquat et al., 2014).

A majority of existing studies examining the changes in diversity of the gut microbiome in relation to weight are done in adult populations. These studies have

demonstrated that diversity and species richness can be associated with obesity or weight gain over time (Le Chatelier et al., 2013). However, there are significant differences between the adult and adolescent microbiome and it has been suggested that abundance may drive these differences in community composition (Agans et al., 2011; Hollister et al., 2015). For example, Agans et al. reported in their cross-sectional analysis that, in both adult and adolescent samples, high abundance features were relatively similar but the low abundance features had high between-sample variation (Agans et al., 2011). Without adult samples in the current study, it is hard to say whether the information determined in this study marks a significant difference between adult and adolescent microbial composition. As demonstrated above, there were statistically significant differences with regards to abundance in both weight and time in this adolescent group. Also taking into account the fact that abundance may be what drives the significant differences between adult and adolescent microbiomes, it is difficult to apply findings in adult populations, with regards to changes in the gut microbiome diversity and weight across time, to adolescents. Again, this demonstrates the need for further investigation into this population.

There were several variables that did not seem to impact the gut microbiome but have been reported in other studies. Initially, the linear mixed models were adjusted with weight and time as an interaction. However, this interaction was not significant in any of the models. Other than the feature volatility analysis, race/ethnicity, as well as gender, were not significant predictors of gut microbial diversity. However, Menni et al. found that *Lachnospiraceae*, which was identified in this study as being associated with

race/ethnicity, was associated with lower weight gain (Menni et al., 2017). Overall, many studies have identified significant results in relation to differences in race/ethnicity and are well summarized in a review by Gupta et al (Gupta et al., 2017). This study also did not take into account diet or physical activity to avoid over burdening the linear mixed models. Another study done on a similar college-aged population found that fiber consumption as well as moderate to vigorous physical activity played a role in differential abundance (Whisner et al., 2018). The effect of exercise as a modulator of changes in the gut microbiome is becoming increasingly studied, and may induce changes in the gut microbiome in adolescents that were previously sedentary, especially in terms of beta diversity which were the most significant results in our study (Allen et al., 2018).

As noted above, there are many factors known to influence the diversity and community structure of the microbiome, some that were addressed in this study, but many that were not. A majority of current research has focused thus far on cross-sectional analysis of the gut microbiome in relation to weight status, and few longitudinal studies have been done in humans. In addition, the adolescent microbiome has been greatly under researched since prior consensus has been that the gut microbiome reaches maturity by childhood. This highlights two major strengths of this study: the adolescent population and the longitudinal study design. Additional strengths include the larger sample size and the racial and ethnic diversity of the sample. There were also several limitations to the study design including a limited sample size in certain groups such as the weight loss group leading to low power; a convenience sample; high attrition rates;

and limited range in participant age. The specific age range does not make it generalizable to the broader public; however, the racial/ethnic diversity and size of the study sample makes it a good representation of college freshmen. While this research is not enough to determine exact differences between the gut microbiome of adults versus adolescents in weight change, it may be enough to warrant future studies to try to determine if differences in the gut microbiome occur at different ages experiences changes in weight.

Previous studies have reported that weight is negatively associated with microbial diversity, but few studies have looked at this relationship longitudinally, especially in a weight gain vulnerable adolescent population. This study demonstrated that over time, those who maintained their weight had less variability in beta diversity when compared to those who gained weight. These findings were particularly significant in Unweighted UniFrac analyses and this metric is particularly sensitive to low abundance features. This contradicts many reported outcomes in relation to weight and microbial diversity of the gut microbiome that primarily focuses on phylum-level changes. Since most of this work was done on adult populations, these findings suggest that there may be another mechanism at work in the weight gain of the adolescent microbiome. Future directions should focus on addressing these changes in microbial abundance in longitudinal studies and may include trying to determine which low abundant features are the ones driving these changes. Researchers may also try to include both adults and adolescents in future longitudinal studies analyzing changes in the gut microbiome in relation to changes in weight.

REFERENCES

A framework for human microbiome research. (2012). *Nature*, *486*(7402), 215–221. https://doi.org/10.1038/nature11209

Aaron, D. J., Storti, K. L., Robertson, R. J., Kriska, A. M., & LaPorte, R. E. (2002). Longitudinal study of the number and choice of leisure time physical activities from mid to late adolescence: Implications for school curricula and community recreation programs. *Archives of Pediatrics & Adolescent Medicine*, *156*(11), 1075–1080. https://doi.org/10.1001/archpedi.156.11.1075

Aceijas, C., Waldhäusl, S., Lambert, N., Cassar, S., & Bello-Corassa, R. (2017). Determinants of health-related lifestyles among university students. *Perspectives in Public Health*, *137*(4), 227–236. https://doi.org/10.1177/1757913916666875

Agans, R., Rigsbee, L., Kenche, H., Michail, S., Khamis, H. J., & Paliy, O. (2011). DISTAL GUT MICROBIOTA OF ADOLESCENT CHILDREN IS DIFFERENT FROM THAT OF ADULTS. *FEMS Microbiology Ecology*, *77*(2), 404–412. https://doi.org/10.1111/j.1574-6941.2011.01120.x

Allaband, C., McDonald, D., Vázquez-Baeza, Y., Minich, J. J., Tripathi, A., Brenner, D. A., Loomba, R., Smarr, L., Sandborn, W. J., Schnabl, B., Dorrestein, P., Zarrinpar, A., & Knight, R. (2019). Microbiome 101: Studying, Analyzing, and Interpreting Gut Microbiome Data for Clinicians. *Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association*, *17*(2), 218–230. https://doi.org/10.1016/j.cgh.2018.09.017

Allen, J. M., Mailing, L. J., Niemiro, G. M., Moore, R., Cook, M. D., White, B. A., Holscher, H. D., & Woods, J. A. (2018). Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Medicine & Science in Sports & Exercise*, *50*(4), 747–757. https://doi.org/10.1249/MSS.0000000000001495

Anderson, D. A., Shapiro, J. R., & Lundgren, J. D. (2003). The freshman year of college as a critical period for weight gain: An initial evaluation. *Eating Behaviors*, *4*(4), 363–367. https://doi.org/10.1016/S1471-0153(03)00030-8

Angelakis, E., Armougom, F., Million, M., & Raoult, D. (2011). The relationship between gut microbiota and weight gain in humans. *Future Microbiology*, *7*(1), 91–109. https://doi.org/10.2217/fmb.11.142

Anhê, F. F., Roy, D., Pilon, G., Dudonné, S., Matamoros, S., Varin, T. V., Garofalo, C., Moine, Q., Desjardins, Y., Levy, E., & Marette, A. (2015). A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp.

Population in the gut microbiota of mice. *Gut*, *64*(6), 872–883. https://doi.org/10.1136/gutjnl-2014-307142

Armougom, F., Henry, M., Vialettes, B., Raccah, D., & Raoult, D. (2009). Monitoring Bacterial Community of Human Gut Microbiota Reveals an Increase in Lactobacillus in Obese Patients and Methanogens in Anorexic Patients. *PLoS ONE*, *4*(9). https://doi.org/10.1371/journal.pone.0007125

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J.-M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., … Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180. https://doi.org/10.1038/nature09944

Avershina, E., Storrø, O., Øien, T., Johnsen, R., Pope, P., & Rudi, K. (2014). Major faecal microbiota shifts in composition and diversity with age in a geographically restricted cohort of mothers and their children. *FEMS Microbiology Ecology*, *87*(1), 280–290. https://doi.org/10.1111/1574-6941.12223

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718–15723. https://doi.org/10.1073/pnas.0407076101

Bäckhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 979–984. https://doi.org/10.1073/pnas.0605374104

Barlow, S. E., & Expert Committee. (2007). Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: Summary report. *Pediatrics*, *120 Suppl 4*, S164-192. https://doi.org/10.1542/peds.2007-2329C

Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. (2019). Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. *MBio*, *10*(1). https://doi.org/10.1128/mBio.02566-18

Belizário, J. E., Faintuch, J., & Garay-Malpartida, M. (2018). Gut Microbiome Dysbiosis and Immunometabolism: New Frontiers for Treatment of Metabolic Diseases. *Mediators of Inflammation*, *2018*, 2037838. https://doi.org/10.1155/2018/2037838

Bervoets, L., Van Hoorenbeeck, K., Kortleven, I., Van Noten, C., Hens, N., Vael, C., Goossens, H., Desager, K. N., & Vankerckhoven, V. (2013). Differences in gut

microbiota composition between obese and lean children: A cross-sectional study. *Gut Pathogens*, *5*, 10. https://doi.org/10.1186/1757-4749-5-10

Biro, F. M., & Wien, M. (2010). Childhood obesity and adult morbidities. *The American Journal of Clinical Nutrition*, *91*(5), 1499S-1505S. https://doi.org/10.3945/ajcn.2010.28701B

Boff, R. de M., Liboni, R. P. A., Batista, I. P. de A., de Souza, L. H., & Oliveira, M. da S. (2017). Weight loss interventions for overweight and obese adolescents: A systematic review. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity*, *22*(2), 211–229. https://doi.org/10.1007/s40519-016-0309-1

Bokulich, N. A., Dillon, M. R., Zhang, Y., Rideout, J. R., Bolyen, E., Li, H., Albert, P. S., & Caporaso, J. G. (2018). q2-longitudinal: Longitudinal and Paired-Sample Analyses of Microbiome Data. *MSystems*, *3*(6). https://doi.org/10.1128/mSystems.00219-18

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Boone-Heinonen, J., Gordon-Larsen, P., Kiefe, C. I., Shikany, J. M., Lewis, C. E., & Popkin, B. M. (2011). Fast food restaurants and food stores: Longitudinal associations with diet in young adults: The CARDIA Study. *Archives of Internal Medicine*, *171*(13), 1162–1170. https://doi.org/10.1001/archinternmed.2011.283

Booth, H. P., Prevost, T. A., Wright, A. J., & Gulliford, M. C. (2014). Effectiveness of behavioural weight loss interventions delivered in a primary care setting: A systematic review and meta-analysis. *Family Practice*, *31*(6), 643–653. https://doi.org/10.1093/fampra/cmu064

Bouchard, C., Tremblay, A., Després, J.-P., Nadeau, A., Lupien, P. J., Moorjan, S., Thériault, G., & Kim, S. Y. (1996). Overfeeding in identical twins: 5-year postoverfeeding results. *Metabolism*, *45*(8), 1042–1050. https://doi.org/10.1016/S0026-0495(96)90277-2

Bouchard, C., Tremblay, A., Després, J.-P., Thériault, G., Nadeauf, A., Lupien, P. J., Moorjani, S., Prudhomme, D., & Fournier, G. (1994). The Response to Exercise with Constant Energy Intake in Identical Twins. *Obesity Research*, *2*(5), 400–410. https://doi.org/10.1002/j.1550-8528.1994.tb00087.x

Briefel, R. R., & Johnson, C. L. (2004). SECULAR TRENDS IN DIETARY INTAKE IN THE UNITED STATES. *Annual Review of Nutrition*, *24*(1), 401–431. https://doi.org/10.1146/annurev.nutr.23.011702.073349

Bruening, M., Ohri-Vachaspati, P., Brewis, A., Laska, M., Todd, M., Hruschka, D., Schaefer, D. R., Whisner, C. M., & Dunton, G. (2016). Longitudinal social networks impacts on weight and weight-related behaviors assessed using mobile-based ecological momentary assessments: Study Protocols for the SPARC study. *BMC Public Health*, *16*(1). https://doi.org/10.1186/s12889-016-3536-5

Brusaferro, A., Cozzali, R., Orabona, C., Biscarini, A., Farinelli, E., Cavalli, E., Grohmann, U., Principi, N., & Esposito, S. (2018). Is It Time to Use Probiotics to Prevent or Treat Obesity? *Nutrients*, *10*(11). https://doi.org/10.3390/nu10111613

Campbell, M. K. (2016). Biological, environmental, and social influences on childhood obesity. *Pediatric Research*, *79*(1), 205–211. https://doi.org/10.1038/pr.2015.208

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A. M., Fava, F., Tuohy, K. M., Chabo, C., Waget, A., Delmée, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrières, J., Tanti, J.-F., Gibson, G. R., Casteilla, L., … Burcelin, R. (2007). Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. *Diabetes*, *56*(7), 1761–1772. https://doi.org/10.2337/db06-1491

Cani, P. D., & Van Hul, M. (2015). Novel opportunities for next-generation probiotics targeting metabolic syndrome. *Current Opinion in Biotechnology*, *32*, 21–27. https://doi.org/10.1016/j.copbio.2014.10.006

Castaner, O., Goday, A., Park, Y.-M., Lee, S.-H., Magkos, F., Shiow, S.-A. T. E., & Schröder, H. (2018). The Gut Microbiome Profile in Obesity: A Systematic Review. *International Journal of Endocrinology*, *2018*. https://doi.org/10.1155/2018/4095789

Catalano, P. M., Farrell, K., Thomas, A., Huston-Presley, L., Mencin, P., de Mouzon, S. H., & Amini, S. B. (2009). Perinatal risk factors for childhood obesity and metabolic dysregulation. *The American Journal of Clinical Nutrition*, *90*(5), 1303–1313. https://doi.org/10.3945/ajcn.2008.27416

Chakraborti, C. K. (2015). New-found link between microbiota and obesity. *World Journal of Gastrointestinal Pathophysiology*, *6*(4), 110–119. https://doi.org/10.4291/wjgp.v6.i4.110

Chen, X., Beydoun, M. A., & Wang, Y. (2008). Is Sleep Duration Associated With Childhood Obesity? A Systematic Review and Meta-analysis. *Obesity*, *16*(2), 265–274. https://doi.org/10.1038/oby.2007.63

Cheng, H. L., Medlow, S., & Steinbeck, K. (2016). The Health Consequences of Obesity in Young Adulthood. *Current Obesity Reports*, *5*(1), 30–37. https://doi.org/10.1007/s13679-016-0190-2

Chittim, C. L., Irwin, S. M., & Balskus, E. P. (2018). Deciphering Human Gut Microbiota-Nutrient Interactions: A Role for Biochemistry. *Biochemistry*, *57*(18), 2567–2577. https://doi.org/10.1021/acs.biochem.7b01277

Chung, W. K., & Leibel, R. L. (2008). Considerations Regarding the Genetics of Obesity. *Obesity (Silver Spring, Md.)*, *16*(Suppl 3), S33–S39. https://doi.org/10.1038/oby.2008.514

Clarke, S. F., Murphy, E. F., Nilaweera, K., Ross, P. R., Shanahan, F., O'Toole, P. W., & Cotter, P. D. (2012). The gut microbiota and its relationship to diet and obesity. *Gut Microbes*, *3*(3), 186–202. https://doi.org/10.4161/gmic.20168

Clarke, S. F., Murphy, E. F., O'Sullivan, O., Lucey, A. J., Humphreys, M., Hogan, A., Hayes, P., O'Reilly, M., Jeffery, I. B., Wood-Martin, R., Kerins, D. M., Quigley, E., Ross, R. P., O'Toole, P. W., Molloy, M. G., Falvey, E., Shanahan, F., & Cotter, P. D. (2014a). Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*, *63*(12), 1913–1920. https://doi.org/10.1136/gutjnl-2013-306541

Claussen, J., Skiecevičienė, J., Wang, J., Rausch, P., Karlsen, T., Lieb, W., . . . Hütt, M. (2017). Boolean analysis reveals systematic interactions among low-abundance species in the human gut microbiome. Retrieved January, from https://journals.plos.org/ploscompbiol/article?id=10.1371%2Fjournal.pcbi.1005361

Conlon, M. A., & Bird, A. R. (2014). The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. *Nutrients*, *7*(1), 17–44. https://doi.org/10.3390/nu7010017

Corder, K., Winpenny, E., Love, R., Brown, H. E., White, M., & Sluijs, E. van. (2019). Change in physical activity from adolescence to early adulthood: A systematic review and meta-analysis of longitudinal cohort studies. *British Journal of Sports Medicine*, *53*(8), 496–503. https://doi.org/10.1136/bjsports-2016-097330

Cotillard, A., Kennedy, S. P., Kong, L. C., Prifti, E., Pons, N., Le Chatelier, E., Almeida, M., Quinquis, B., Levenez, F., Galleron, N., Gougis, S., Rizkalla, S., Batto, J.-M., Renault, P., Doré, J., Zucker, J.-D., Clément, K., & Ehrlich, S. D. (2013). Dietary intervention impact on gut microbial gene richness. *Nature*, *500*(7464), 585–588. https://doi.org/10.1038/nature12480

Da Silva, C. C., Monteil, M. A., & Davis, E. M. (2020). Overweight and Obesity in Children Are Associated with an Abundance of Firmicutes and Reduction of

Bifidobacterium in Their Gastrointestinal Microbiota. *Childhood Obesity*, chi.2019.0280. https://doi.org/10.1089/chi.2019.0280

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J., & Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14691–14696. https://doi.org/10.1073/pnas.1005963107

de Vos, P., Hanck, C., Neisingh, M., Prak, D., Groen, H., & Faas, M. M. (2015). Weight gain in freshman college students and perceived health. *Preventive Medicine Reports*, *2*, 229–234. https://doi.org/10.1016/j.pmedr.2015.03.008

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Applied and Environmental Microbiology*, *72*(7), 5069–5072. https://doi.org/10.1128/AEM.03006-05

Deshmukh-Taskar, P., Nicklas, T. A., Morales, M., Yang, S.-J., Zakeri, I., & Berenson, G. S. (2006). Tracking of overweight status from childhood to young adulthood: The Bogalusa Heart Study. *European Journal of Clinical Nutrition*, *60*(1), 48–57. https://doi.org/10.1038/sj.ejcn.1602266

Dethlefsen, L., & Relman, D. A. (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proceedings of the National Academy of Sciences*, *108*(Supplement 1), 4554–4561. https://doi.org/10.1073/pnas.1000087107

Dietary Screener Questionnaire. (n.d.). 5.

Dietary Screener Questionnaire in the National Health Interview Survey Cancer Control Supplement 2010: Overview. (n.d.). Retrieved December 27, 2019, from https://epi.grants.cancer.gov/nhis/2010-screener/

Dietz, W. H. (1998). Health Consequences of Obesity in Youth: Childhood Predictors of Adult Disease. *Pediatrics*, *101*(Supplement 2), 518–525.

Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G. M., Neyrinck, A. M., Possemiers, S., Van Holle, A., François, P., de Vos, W. M., Delzenne, N. M.,

Schrenzel, J., & Cani, P. D. (2011). Responses of Gut Microbiota and Glucose and Lipid Metabolism to Prebiotics in Genetic Obese and Diet-Induced Leptin-Resistant Mice. *Diabetes*, *60*(11), 2775–2786. https://doi.org/10.2337/db11-0227

FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. (n.d.). Retrieved April 15, 2020, from https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0009490

Fleissner, C. K., Huebel, N., Abd El-Bary, M. M., Loh, G., Klaus, S., & Blaut, M. (2010). Absence of intestinal microbiota does not protect mice from diet-induced obesity. *The British Journal of Nutrition*, *104*(6), 919–929. https://doi.org/10.1017/S0007114510001303

Flores, G. E., Caporaso, J. G., Henley, J. B., Rideout, J. R., Domogala, D., Chase, J., Leff, J. W., Vázquez-Baeza, Y., Gonzalez, A., Knight, R., Dunn, R. R., & Fierer, N. (2014). Temporal variability is a personalized feature of the human microbiome. *Genome Biology*, *15*(12), 531. https://doi.org/10.1186/s13059-014-0531-y

Frost, F., Storck, L. J., Kacprowski, T., Gärtner, S., Rühlemann, M., Bang, C., Franke, A., Völker, U., Aghdassi, A. A., Steveling, A., Mayerle, J., Weiss, F. U., Homuth, G., & Lerch, M. M. (2019). A structured weight loss program increases gut microbiota phylogenetic diversity and reduces levels of Collinsella in obese type 2 diabetics: A pilot study. *PLOS ONE*, *14*(7), e0219489. https://doi.org/10.1371/journal.pone.0219489

Fu, B. C., Randolph, T. W., Lim, U., Monroe, K. R., Cheng, I., Wilkens, L. R., Le Marchand, L., Lampe, J. W., & Hullar, M. A. J. (2019). Temporal Variability and Stability of the Fecal Microbiome: The Multiethnic Cohort Study. *Cancer Epidemiology Biomarkers & Prevention*, *28*(1), 154–162. https://doi.org/10.1158/1055-9965.EPI-18-0348

Godin, G. (n.d.). *A Simple Method to Assess Exercise Behavior in the Community*. 7.

Goodrich, J. K., Di Rienzi, S. C., Poole, A. C., Koren, O., Walters, W. A., Caporaso, J. G., Knight, R., & Ley, R. E. (2014). Conducting a Microbiome Study. *Cell*, *158*(2), 250–262. https://doi.org/10.1016/j.cell.2014.06.037

Gordon-Larsen, P., Adair, L. S., Nelson, M. C., & Popkin, B. M. (2004). Five-year obesity incidence in the transition period between adolescence and adulthood: The National Longitudinal Study of Adolescent Health. *The American Journal of Clinical Nutrition*, *80*(3), 569–575. https://doi.org/10.1093/ajcn/80.3.569

Greaney, M. L., Less, F. D., White, A. A., Dayton, S. F., Riebe, D., Blissmer, B., Shoff, S., Walsh, J. R., & Greene, G. W. (2009). College Students' Barriers and Enablers for Healthful Weight Management: A Qualitative Study. *Journal of Nutrition*

Education and Behavior, *41*(4), 281–286. https://doi.org/10.1016/j.jneb.2008.04.354

Gropper, S. S., Simmons, K. P., Connell, L. J., & Ulrich, P. V. (2012). Changes in body weight, composition, and shape: A 4-year study of college students. *Applied Physiology, Nutrition, and Metabolism*, *37*(6), 1118–1123. https://doi.org/10.1139/h2012-139

Group, T. N. H. W., Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., Schloss, J. A., Bonazzi, V., McEwen, J. E., Wetterstrand, K. A., Deal, C., Baker, C. C., Francesco, V. D., Howcroft, T. K., Karp, R. W., Lunsford, R. D., Wellington, C. R., Belachew, T., Wright, M., … Guyer, M. (2009). The NIH Human Microbiome Project. *Genome Research*, *19*(12), 2317–2323. https://doi.org/10.1101/gr.096651.109

Gupta, V. K., Paul, S., & Dutta, C. (2017). Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.01162

Hales, C. M. (2017). *Prevalence of Obesity Among Adults and Youth: United States, 2015–2016*. *288*, 8.

Harley, I. T. W., & Karp, C. L. (2012). Obesity and the gut microbiome: Striving for causality. *Molecular Metabolism*, *1*(1–2), 21–31. https://doi.org/10.1016/j.molmet.2012.07.002

Heiss, C. N., & Olofsson, L. E. (2018). Gut Microbiota-Dependent Modulation of Energy Metabolism. *Journal of Innate Immunity*, *10*(3), 163–171. https://doi.org/10.1159/000481519

Herman, C. P., Polivy, J., Vartanian, L. R., & Pliner, P. (2016). Are large portions responsible for the obesity epidemic? *Physiology & Behavior*, *156*, 177–181. https://doi.org/10.1016/j.physbeh.2016.01.024

Hewawitharana, S. C., Thompson, F. E., Loria, C. M., Strauss, W., Nagaraja, J., Ritchie, L., & Webb, K. L. (2018). Comparison of the NHANES dietary screener questionnaire to the Automated Self-Administered 24-Hour Recall for Children in the Healthy Communities Study. *Nutrition Journal*, *17*. https://doi.org/10.1186/s12937-018-0415-1

Hollister, E. B., Riehle, K., Luna, R. A., Weidler, E. M., Rubio-Gonzales, M., Mistretta, T.-A., Raza, S., Doddapaneni, H. V., Metcalf, G. A., Muzny, D. M., Gibbs, R. A., Petrosino, J. F., Shulman, R. J., & Versalovic, J. (2015). Structure and function of the healthy pre-adolescent pediatric gut microbiome. *Microbiome*, *3*. https://doi.org/10.1186/s40168-015-0101-x

Holscher, H. D., Taylor, A. M., Swanson, K. S., Novotny, J. A., & Baer, D. J. (2018). Almond Consumption and Processing Affects the Composition of the Gastrointestinal Microbiota of Healthy Adult Men and Women: A Randomized Controlled Trial. *Nutrients*, *10*(2). https://doi.org/10.3390/nu10020126

Hu, H.-J., Park, S.-G., Jang, H. B., Choi, M.-G., Park, K.-H., Kang, J. H., Park, S. I., Lee, H.-J., & Cho, S.-H. (2015). Obesity Alters the Microbial Community Profile in Korean Adolescents. *PLoS ONE*, *10*(7). https://doi.org/10.1371/journal.pone.0134333

Hu, J., Nomura, Y., Bashir, A., Fernandez-Hernandez, H., Itzkowitz, S., Pei, Z., Stone, J., Loudon, H., & Peter, I. (2013). Diversified Microbiota of Meconium Is Affected by Maternal Diabetes Status. *PLoS ONE*, *8*(11). https://doi.org/10.1371/journal.pone.0078257

Ignacio, A., Fernandes, M. R., Rodrigues, V. a. A., Groppo, F. C., Cardoso, A. L., Avila-Campos, M. J., & Nakano, V. (2016). Correlation between body mass index and faecal microbiota from children. *Clinical Microbiology and Infection*, *22*(3), 258.e1-258.e8. https://doi.org/10.1016/j.cmi.2015.10.031

Journey, E. K., Ortega-Santos, C. P., Bruening, M., & Whisner, C. M. (2020). Changes in Weight Status and the Intestinal Microbiota among Adolescent College Freshman. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *66*(2), 166–171. https://doi.org/10.1016/j.jadohealth.2019.06.005

Karlsson, C. L. J., Önnerfält, J., Xu, J., Molin, G., Ahrné, S., & Thorngren Jerneck, K. (2012). The Microbiota of the Gut in Preschool Children With Normal and Excessive Body Weight. *Obesity*, *20*(11), 2257–2261. https://doi.org/10.1038/oby.2012.110

Kilian, M., Chapple, I. L. C., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L., Tonetti, M. S., Wade, W. G., & Zaura, E. (2016). The oral microbiome – an update for oral healthcare professionals. *British Dental Journal*, *221*(10), 657–666. https://doi.org/10.1038/sj.bdj.2016.865

Klop, B., Elte, J. W. F., & Castro Cabezas, M. (2013). Dyslipidemia in Obesity: Mechanisms and Potential Targets. *Nutrients*, *5*(4), 1218–1240. https://doi.org/10.3390/nu5041218

Larson, N., Neumark-Sztainer, D., Laska, M. N., & Story, M. (2011). Young adults and eating away from home: Associations with dietary intake patterns and weight status differ by choice of restaurant. *Journal of the American Dietetic Association*, *111*(11), 1696–1703. https://doi.org/10.1016/j.jada.2011.08.007

Laska, M. N., Pasch, K. E., Lust, K., Story, M., & Ehlinger, E. (2009). Latent Class Analysis of Lifestyle Characteristics and Health Risk Behaviors among College Youth. *Prevention Science : The Official Journal of the Society for Prevention Research*, *10*(4), 376–386. https://doi.org/10.1007/s11121-009-0140-2

Lau, R. R., Quadrel, M. J., & Hartman, K. A. (1990). Development and change of young adults' preventive health beliefs and behavior: Influence from parents and peers. *Journal of Health and Social Behavior*, *31*(3), 240–259.

Lawlor, D. A., & Chaturvedi, N. (2006). Treatment and prevention of obesity—Are there critical periods for intervention? *International Journal of Epidemiology*, *35*(1), 3–9. https://doi.org/10.1093/ije/dyi309

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.-M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jørgensen, T., Brandslund, I., Nielsen, H. B., Juncker, A. S., Bertalan, M., … Pedersen, O. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, *500*(7464), 541–546. https://doi.org/10.1038/nature12506

Leeming, E. R., Johnson, A. J., Spector, T. D., & Le Roy, C. I. (2019). Effect of Diet on the Gut Microbiota: Rethinking Intervention Duration. *Nutrients*, *11*(12), 2862. https://doi.org/10.3390/nu11122862

Levitsky, D. A., Halbmaier, C. A., & Mrdjenovic, G. (2004). The freshman weight gain: A model for the study of the epidemic of obesity. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, *28*(11), 1435–1442. https://doi.org/10.1038/sj.ijo.0802776

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences*, *102*(31), 11070–11075. https://doi.org/10.1073/pnas.0504978102

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023. https://doi.org/10.1038/4441022a

Lin, H. V., Frassetto, A., Kowalik, E. J., Nawrocki, A. R., Lu, M. M., Kosinski, J. R., Hubert, J. A., Szeto, D., Yao, X., Forrest, G., & Marsh, D. J. (2012). Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PloS One*, *7*(4), e35240. https://doi.org/10.1371/journal.pone.0035240

Livingstone, M. B. E., & Pourshahidi, L. K. (2014). Portion Size and Obesity123. *Advances in Nutrition*, *5*(6), 829–834. https://doi.org/10.3945/an.114.007104

Ma, B., Forney, L. J., & Ravel, J. (2012). The vaginal microbiome: Rethinking health and diseases. *Annual Review of Microbiology*, *66*, 371–389. https://doi.org/10.1146/annurev-micro-092611-150157

Marchesi, J. R. (2011). Human distal gut microbiome. *Environmental Microbiology*, *13*(12), 3088–3102. https://doi.org/10.1111/j.1462-2920.2011.02574.x

Mbakwa, C. A., Hermes, G. D. A., Penders, J., Savelkoul, P. H. M., Thijs, C., Dagnelie, P. C., Mommers, M., Zoetendal, E. G., Smidt, H., & Arts, I. C. W. (2018). Gut Microbiota and Body Weight in School□Aged Children: The KOALA Birth Cohort Study. *Obesity*, *26*(11), 1767–1776. https://doi.org/10.1002/oby.22320

McArthur, L. (2009). Race and Sex Differences in College Student Physical Activity Correlates. *American Journal of Health Behavior*, *33*(1). https://doi.org/10.5993/AJHB.33.1.8

McCann, K. S. (2000). The diversity-stability debate. *Nature*, *405*(6783), 228–233. https://doi.org/10.1038/35012234

Menni, C., Jackson, M. A., Pallister, T., Steves, C. J., Spector, T. D., & Valdes, A. M. (2017). Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. *International Journal of Obesity (2005)*, *41*(7), 1099–1105. https://doi.org/10.1038/ijo.2017.66

Miller, W. C. (1999). How effective are traditional dietary and exercise interventions for weight loss? *Medicine & Science in Sports & Exercise*, *31*(8), 1129.

Mitchell, C. M., Davy, B. M., Hulver, M. W., Neilson, A. P., Bennett, B. J., & Davy, K. P. (2019). Does Exercise Alter Gut Microbial Composition? A Systematic Review. *Medicine & Science in Sports & Exercise*, *51*(1), 160–167. https://doi.org/10.1249/MSS.0000000000001760

Murphy, E. F., Cotter, P. D., Healy, S., Marques, T. M., O'Sullivan, O., Fouhy, F., Clarke, S. F., O'Toole, P. W., Quigley, E. M., Stanton, C., Ross, P. R., O'Doherty, R. M., & Shanahan, F. (2010). Composition and energy harvesting capacity of the gut microbiota: Relationship to diet, obesity and time in mouse models. *Gut*, *59*(12), 1635–1642. https://doi.org/10.1136/gut.2010.215665

Murugesan, S., Ulloa-Martínez, M., Martínez-Rojano, H., Galván-Rodríguez, F. M., Miranda-Brito, C., Romano, M. C., Piña-Escobedo, A., Pizano-Zárate, M. L., Hoyo-Vadillo, C., & García-Mena, J. (2015). Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. *European Journal of Clinical Microbiology & Infectious Diseases*, *34*(7), 1337–1346. https://doi.org/10.1007/s10096-015-2355-4

Naito, Y., Uchiyama, K., & Takagi, T. (2018). A next-generation beneficial microbe: Akkermansia muciniphila. *Journal of Clinical Biochemistry and Nutrition*, *63*(1), 33–35. https://doi.org/10.3164/jcbn.18-57

Nelson, M. C., Kocos, R., Lytle, L. A., & Perry, C. L. (2009). Understanding the Perceived Determinants of Weight-related Behaviors in Late Adolescence: A Qualitative Analysis among College Youth. *Journal of Nutrition Education and Behavior*, *41*(4), 287–292. https://doi.org/10.1016/j.jneb.2008.05.005

Nelson, M. C., & Story, M. (2009). Food Environments in University Dorms: 20,000 Calories per Dorm Room and Counting. *American Journal of Preventive Medicine*, *36*(6), 523–526. https://doi.org/10.1016/j.amepre.2009.01.030

Nelson, M. C., Story, M., Larson, N. I., Neumark-Sztainer, D., & Lytle, L. A. (2008). Emerging Adulthood and College-aged Youth: An Overlooked Age for Weight-related Behavior Change. *Obesity*, *16*(10), 2205–2211. https://doi.org/10.1038/oby.2008.365

Obesity and overweight. (n.d.). Retrieved January 18, 2020, from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J., Abe, F., & Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiology*, *16*(1), 90. https://doi.org/10.1186/s12866-016-0708-5

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the Human Infant Intestinal Microbiota. *PLoS Biology*, *5*(7). https://doi.org/10.1371/journal.pbio.0050177

Pan, L., Sherry, B., Park, S., & Blanck, H. M. (2013). The Association of Obesity and School Absenteeism Attributed to Illness or Injury Among Adolescents in the United States, 2009. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *52*(1), 64–69. https://doi.org/10.1016/j.jadohealth.2012.04.003

Pelletier, J. E., Lytle, L. A., & Laska, M. N. (2016). Stress, Health Risk Behaviors, and Weight Status Among Community College Students. *Health Education & Behavior*, *43*(2), 139–144. https://doi.org/10.1177/1090198115598983

Peterson, C. T., Sharma, V., Elmén, L., & Peterson, S. N. (2015). Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. *Clinical and Experimental Immunology*, *179*(3), 363–377. https://doi.org/10.1111/cei.12474

Pontzer, H., Raichlen, D. A., Wood, B. M., Mabulla, A. Z. P., Racette, S. B., & Marlowe, F. W. (2012). Hunter-Gatherer Energetics and Human Obesity. *PLOS ONE*, *7*(7), e40503. https://doi.org/10.1371/journal.pone.0040503

Pope, L., Hansen, D., & Harvey, J. (2017). Examining the Weight Trajectory of College Students. *Journal of Nutrition Education and Behavior*, *49*(2), 137-141.e1. https://doi.org/10.1016/j.jneb.2016.10.014

Prehn-Kristensen, A., Zimmermann, A., Tittmann, L., Lieb, W., Schreiber, S., Baving, L., & Fischer, A. (2018). Reduced microbiome alpha diversity in young patients with ADHD. *PLoS ONE*, *13*(7). https://doi.org/10.1371/journal.pone.0200728

Racette, S. B., Deusinger, S. S., Strube, M. J., Highstein, G. R., & Deusinger, R. H. (2005). Weight Changes, Exercise, and Dietary Patterns During Freshman and Sophomore Years of College. *Journal of American College Health*, *53*(6), 245–251. https://doi.org/10.3200/JACH.53.6.245-251

Racette, S. B., Deusinger, S. S., Strube, M. J., Highstein, G. R., & Deusinger, R. H. (2008). Changes in Weight and Health Behaviors from Freshman through Senior Year of College. *Journal of Nutrition Education and Behavior*, *40*(1), 39–42. https://doi.org/10.1016/j.jneb.2007.01.001

Rampelli, S., Schnorr, S. L., Consolandi, C., Turroni, S., Severgnini, M., Peano, C., Brigidi, P., Crittenden, A. N., Henry, A. G., & Candela, M. (2015). Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota. *Current Biology*, *25*(13), 1682–1693. https://doi.org/10.1016/j.cub.2015.04.055

Reilly, J. J., & Kelly, J. (2011). Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: Systematic review. *International Journal of Obesity*, *35*(7), 891–898. https://doi.org/10.1038/ijo.2010.222

Remely, M., Tesar, I., Hippe, B., Gnauer, S., Rust, P., & Haslberger, A. G. (2015). Gut microbiota composition correlates with changes in body fat content due to weight loss. *Beneficial Microbes*, *6*(4), 431–439. https://doi.org/10.3920/BM2014.0104

Rendina, D., Campanozzi, A., & De Filippo, G. (2019). Methodological approach to the assessment of the obesogenic environment in children and adolescents: A review of the literature. *Nutrition, Metabolism and Cardiovascular Diseases*, *29*(6), 561–571. https://doi.org/10.1016/j.numecd.2019.02.009

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., Griffin, N. W., Lombard, V., Henrissat, B., Bain, J. R., Muehlbauer, M. J., Ilkayeva, O., Semenkovich, C. F., Funai, K., Hayashi, D. K., Lyle, B. J., Martini, M. C., Ursell, L. K., Clemente, J. C., … Gordon, J. I. (2013). Cultured gut microbiota from twins discordant for obesity modulate adiposity and metabolic phenotypes in

mice. *Science (New York, N.Y.)*, *341*(6150). https://doi.org/10.1126/science.1241214

Ringel-Kulka, T., Cheng, J., Ringel, Y., Salojärvi, J., Carroll, I., Palva, A., Vos, W. M. de, & Satokari, R. (2013). Intestinal Microbiota in Healthy U.S. Young Children and Adults—A High Throughput Microarray Analysis. *PLOS ONE*, *8*(5), e64315. https://doi.org/10.1371/journal.pone.0064315

Roopchand, D. E., Carmody, R. N., Kuhn, P., Moskal, K., Rojas-Silva, P., Turnbaugh, P. J., & Raskin, I. (2015). Dietary Polyphenols Promote Growth of the Gut Bacterium Akkermansia muciniphila and Attenuate High-Fat Diet–Induced Metabolic Syndrome. *Diabetes*, *64*(8), 2847–2858. https://doi.org/10.2337/db14-1916

Russell-Mayhew, S., McVey, G., Bardick, A., & Ireland, A. (2012). Mental Health, Wellness, and Childhood Overweight/Obesity. *Journal of Obesity*, *2012*. https://doi.org/10.1155/2012/281801

Sanford, J. A., & Gallo, R. L. (2013). Functions of the skin microbiota in health and disease. *Seminars in Immunology*, *25*(5), 370–377. https://doi.org/10.1016/j.smim.2013.09.005

Segata, N. (2015). Gut Microbiome: Westernization and the Disappearance of Intestinal Diversity. *Current Biology*, *25*(14), R611–R613. https://doi.org/10.1016/j.cub.2015.05.040

Shafquat, A., Joice, R., Simmons, S. L., & Huttenhower, C. (2014). Functional and phylogenetic assembly of microbial communities in the human microbiome. *Trends in Microbiology*, *22*(5), 261–266. https://doi.org/10.1016/j.tim.2014.01.011

Shen, N., Caixàs, A., Ahlers, M., Patel, K., Gao, Z., Dutia, R., Blaser, M. J., Clemente, J. C., & Laferrère, B. (2019). Longitudinal changes of microbiome composition and microbial metabolomics after surgical weight loss in individuals with obesity. *Surgery for Obesity and Related Diseases*, *15*(8), 1367–1373. https://doi.org/10.1016/j.soard.2019.05.038

Singla, P., Bardoloi, A., & Parkash, A. A. (2010). Metabolic effects of obesity: A review. *World Journal of Diabetes*, *1*(3), 76–88. https://doi.org/10.4239/wjd.v1.i3.76

Sirard, J. R., Hannan, P. J., Cutler, G. J., Graham, D. J., & Neumark-Sztainer, D. (2013). Evaluation of two self-report measures of physical activity with accelerometry in young adults. *Journal of Physical Activity & Health*, *10*(1), 85–96.

Story, M., Kaphingst, K. M., Robinson-O'Brien, R., & Glanz, K. (2008). Creating Healthy Food and Eating Environments: Policy and Environmental Approaches.

Annual Review of Public Health, *29*(1), 253–272. https://doi.org/10.1146/annurev.publhealth.29.020907.090926

Structure, Function and Diversity of the Healthy Human Microbiome. (2012). *Nature*, *486*(7402), 207–214. https://doi.org/10.1038/nature11234

Sze, M. A., & Schloss, P. D. (2016). Looking for a Signal in the Noise: Revisiting Obesity and the Microbiome. *MBio*, *7*(4), e01018-16, /mbio/7/4/e01018-16.atom. https://doi.org/10.1128/mBio.01018-16

The, N. S., Suchindran, C., North, K. E., Popkin, B. M., & Gordon-Larsen, P. (2010). Association of adolescent obesity with risk of severe obesity in adulthood. *JAMA*, *304*(18), 2042–2047. https://doi.org/10.1001/jama.2010.1635

Thompson, F. E., Midthune, D., Kahle, L., & Dodd, K. W. (2017). Development and Evaluation of the National Cancer Institute's Dietary Screener Questionnaire Scoring Algorithms123. *The Journal of Nutrition*, *147*(6), 1226–1233. https://doi.org/10.3945/jn.116.246058

Thompson, F. E., Midthune, D., Subar, A. F., Kahle, L. L., Schatzkin, A., & Kipnis, V. (2004). Performance of a short tool to assess dietary intakes of fruits and vegetables, percentage energy from fat and fibre. *Public Health Nutrition*, *7*(8), 1097–1106. https://doi.org/10.1079/PHN2004642

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, *474*(11), 1823–1836. https://doi.org/10.1042/BCJ20160510

Trends in Intake of Energy and Macronutrients—United States, 1971—2000. (n.d.). Retrieved November 23, 2019, from https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5304a3.htm

Truesdale, K., Stevens, J., Lewis, C., Schreiner, P., Loria, C., & Cai, J. (2006). Changes in risk factors for cardiovascular disease by baseline weight status in young adults who maintain or gain weight over 15 years: The CARDIA study. *International Journal of Obesity (2005)*, *30*(9), 1397–1407. https://doi.org/10.1038/sj.ijo.0803307

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. A., Affourtit, J. P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R., & Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484. https://doi.org/10.1038/nature07540

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C., Knight, R., & Gordon, J. I. (2007). The human microbiome project: Exploring the microbial part of ourselves in a changing world. *Nature*, *449*(7164), 804–810. https://doi.org/10.1038/nature06244

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*(7122), 1027–1031. https://doi.org/10.1038/nature05414

Twenge, J. M., & Campbell, W. K. (2018). Associations between screen time and lower psychological well-being among children and adolescents: Evidence from a population-based study. *Preventive Medicine Reports*, *12*, 271–283. https://doi.org/10.1016/j.pmedr.2018.10.003

Vadeboncoeur, C., Townsend, N., & Foster, C. (2015). A meta-analysis of weight gain in first year university students: Is freshman 15 a myth? *BMC Obesity*, *2*(1), 22. https://doi.org/10.1186/s40608-015-0051-7

Van Hul, M., & Cani, P. D. (2019). Targeting Carbohydrates and Polyphenols for a Healthy Microbiome and Healthy Weight. *Current Nutrition Reports*, *8*(4), 307–316. https://doi.org/10.1007/s13668-019-00281-5

Verdam, F. J., Fuentes, S., Jonge, C. de, Zoetendal, E. G., Erbil, R., Greve, J. W., Buurman, W. A., Vos, W. M. de, & Rensen, S. S. (2013). Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity*, *21*(12), E607–E615. https://doi.org/10.1002/oby.20466

Wagner, B. D., Grunwald, G. K., Zerbe, G. O., Mikulich-Gilbertson, S. K., Robertson, C. E., Zemanick, E. T., & Harris, J. K. (2018). On the Use of Diversity Measures in Longitudinal Sequencing Studies of Microbial Communities. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01037

Wang, J.-W., Kuo, C.-H., Kuo, F.-C., Wang, Y.-K., Hsu, W.-H., Yu, F.-J., Hu, H.-M., Hsu, P.-I., Wang, J.-Y., & Wu, D.-C. (2019). Fecal microbiota transplantation: Review and update. *Journal of the Formosan Medical Association*, *118*, S23–S31. https://doi.org/10.1016/j.jfma.2018.08.011

Weighing Down America: The Health and Economic Impact of Obesity | Milken Institute. (n.d.). Retrieved December 2, 2019, from http://milkeninstitute.org/reports/weighing-down-america-health-and-economic-impact-obesity

Wellen, K. E., & Hotamisligil, G. S. (2005). Inflammation, stress, and diabetes. *Journal of Clinical Investigation*, *115*(5), 1111–1119. https://doi.org/10.1172/JCI200525102

Westerterp, K. R., & Speakman, J. R. (2008). Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. *International Journal of Obesity (2005)*, *32*(8), 1256–1263. https://doi.org/10.1038/ijo.2008.74

Whisner, C. M., Maldonado, J., Dente, B., Krajmalnik-Brown, R., & Bruening, M. (2018). Diet, physical activity and screen time but not body mass index are associated with the gut microbiome of a diverse cohort of college students living in university housing: A cross-sectional study. *BMC Microbiology*, *18*(1), 210. https://doi.org/10.1186/s12866-018-1362-x

Whittaker, R. H. (1972). Evolution and Measurement of Species Diversity. *TAXON*, *21*(2–3), 213–251. https://doi.org/10.2307/1218190

Williamson, D. F., Kahn, H. S., Remington, P. L., & Anda, R. F. (1990). The 10-Year Incidence of Overweight and Major Weight Gain in US Adults. *Archives of Internal Medicine*, *150*(3), 665–672. https://doi.org/10.1001/archinte.1990.00390150135026

Wing, R. R., Tate, D. F., Garcia, K. R., Bahnson, J., Lewis, C. E., & Espeland, M. A. (2017). Improvements in Cardiovascular Risk Factors in Young Adults in a Randomized Trial of Approaches to Weight Gain Prevention. *Obesity (Silver Spring, Md.)*, *25*(10), 1660–1666. https://doi.org/10.1002/oby.21917

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science (New York, N.y.)*, *334*(6052), 105–108. https://doi.org/10.1126/science.1208344

Yan, Z., & Harrington, A. (2019). Factors that predict weight gain among first-year college students. *Health Education Journal*, 0017896919865758. https://doi.org/10.1177/0017896919865758

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222–227. https://doi.org/10.1038/nature11053

Yin, Y.-N., Yu, Q.-F., Fu, N., Liu, X.-W., & Lu, F.-G. (2010). Effects of four Bifidobacteria on obesity in high-fat diet induced rats. *World Journal of Gastroenterology : WJG*, *16*(27), 3394–3401. https://doi.org/10.3748/wjg.v16.i27.3394

APPENDIX A

APPROVAL DOCUMENT FROM THE IRB

APPROVAL: MODIFICATION

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

Dear Meredith Bruening:

On 10/16/2014 the ASU IRB reviewed the following protocol:

Type of Review:	Modification
Title:	The Role of Friendship Networks on BMI and Behaviors among College Freshmen
Investigator:	Meredith Bruening
IRB ID:	1309009596
Funding:	Name: NIH: National Institutes of Health; Funding Source ID: HHS-NIH-National Institutes of Health,
Grant Title:	None
Grant ID:	None

Documents Reviewed:	• Microbiome pilot consent form, Category: Consent Form; • consent without track changes , Category: Consent Form; • ValidationConsentForm--Revised, Category: Consent Form; • Response to 9/23 modification request, Dr.docx, Category: IRB Protocol; • irb_microbiome-mod_091714 (1).docx, Category: IRB Protocol; • ApprovedIRBProtocol, Category: IRB Protocol; • Web-based survey: Revisions in list form, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Web-based survey (no comments), Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Response to 9/23 modification request, Dr.pdf,

	Category: Other (to reflect anything not captured above); • Microbiome pilot flyer, Category: Recruitment Materials; • ValidationFlyer, Category: Recruitment Materials; • Validation Study Flyer (half-page), Design 1, Category: Recruitment Materials; • Validation Study Flyer (half-page), Design 2, Category: Recruitment Materials; • Validation Study Flyer (quarter-page), Design 2, Category: Recruitment Materials; • Validation Study Flyer (quarter-page), Design 1, Category: Recruitment Materials; • ValidationEmail, Category: Recruitment Materials;

The IRB approved the modification.

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

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

Sincerely,

IRB Administrator

cc:

APPENDIX B

QIIME2 CODES USED IN ANALYSIS

Preliminary Analysis

Importing

```
qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path /mnt/c/Users/Mary/desktop/devilWASTEfastq-1 \
  --input-format CasavaOneEightSingleLanePerSampleDirFmt \
  --output-path demux-paired-end-1.qza

qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path /mnt/c/Users/Mary/desktop/devilWASTEfastq-2 \
  --input-format CasavaOneEightSingleLanePerSampleDirFmt \
  --output-path demux-paired-end-2.qza
```

Visualized Import

```
qiime demux summarize \
  --i-data demux-paired-end-1.qza \
  --o-visualization devilWASTE-1.qzv

qiime demux summarize \
  --i-data demux-paired-end-2.qza \
  --o-visualization devilWASTE-2.qzv
```

Denoising

```
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs demux-paired-end-1.qza \
  --p-trunc-len-f 250 \
  --p-trunc-len-r 250 \
--p-trim-left-f 14 \
 --p-trim-left-r 14 \
  --p-chimera-method consensus \
  --o-representative-sequences fastq-dada2-1.qza \
  --o-table table-dada2-1.qza \
  --o-denoising-stats stats-dada2-1.qza

qiime dada2 denoise-paired \
  --i-demultiplexed-seqs demux-paired-end-2.qza \
  --p-trunc-len-f 250 \
  --p-trunc-len-r 250 \
--p-trim-left-f 14 \
 --p-trim-left-r 14 \
  --p-chimera-method consensus \
  --o-representative-sequences fastq-dada2-2.qza \
```

```
  --o-table table-dada2-2.qza \
  --o-denoising-stats stats-dada2-2.qza
```

Visualize Denoised Data

```
qiime metadata tabulate \
  --m-input-file stats-dada2-1.qza \
  --o-visualization denoising-stats-1.qzv
qiime metadata tabulate \
  --m-input-file stats-dada2-2.qza \
  --o-visualization denoising-stats-2.qzv
```

Merge Denoised Data

```
qiime feature-table merge \
  --i-tables table-dada2-1.qza \
  --i-tables table-dada2-2.qza \
--p-overlap-method sum \
  --o-merged-table tableDW.qza

qiime feature-table merge-seqs \
  --i-data fastq-dada2-1.qza \
  --i-data fastq-dada2-2.qza \
  --o-merged-data rep-seqsDW.qza
```

Filtering

```
qiime feature-table filter-samples \
  --i-table tableDW.qza \
  --m-metadata-file DWmetadata.txt \
  --o-filtered-table id-filtered-tableDW.qza
```

Visualize

```
qiime feature-table summarize \
  --i-table id-filtered-tableDW.qza \
  --o-visualization tableDW.qzv \
  --m-sample-metadata-file DWmetadata.txt
```

Taxonomy

```
qiime feature-classifier classify-sklearn \
  --i-classifier v3v4-99-gg-nb-classifier.qza \
  --i-reads rep-seqsDW.qza \
```

```
  --o-classification taxonomyDW.qza


qiime metadata tabulate \
  --m-input-file taxonomyDW.qza \
  --o-visualization taxonomyDW.qzv

qiime taxa barplot \
  --i-taxonomy taxonomyDW.qza \
--i-table  id-filtered-tableDW.qza \
  --m-metadata-file DWmetadata.txt \
  --o-visualization taxa-bar-plotsDW.qzv
```

Alpha Rarefaction Curve

```
qiime diversity alpha-rarefaction \
--i-table id-filtered-tableDW.qza \
--i-phylogeny rooted-treeDW.qza \
--p-max-depth 59227 \
--m-metadata-file DWmetadata.txt \
--o-visualization alpha-rarefactionDW.qzv
```

Phylogeny

```
qiime phylogeny align-to-tree-mafft-fasttree \
  --i-sequences rep-seqsDW.qza \
  --o-alignment alignedfastqDW.qza \
  --o-masked-alignment maskedfastqDW.qza \
  --o-tree unrooted-treeDW.qza \
  --o-rooted-tree rooted-treeDW.qza

qiime diversity core-metrics-phylogenetic \
  --i-phylogeny rooted-treeDW.qza \
  --i-table id-filtered-tableDW.qza \
  --p-sampling-depth 7335 \
  --m-metadata-file DWmetadata.txt \
  --output-dir core-metrics-resultsDW
```

Alpha Diversity

```
qiime diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-resultsDW/faith_pd_vector.qza \
  --m-metadata-file DWmetadata.txt \
  --o-visualization core-metrics-resultsDW/faith-pd-group-significance.qzv
```

```
qiime diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-resultsDW/evenness_vector.qza \
  --m-metadata-file DWmetadata.txt \
  --o-visualization core-metrics-resultsDW/evenness-group-significance.qzv

qiime diversity alpha-group-significance \
 --i-alpha-diversity core-metrics-resultsDW/shannon_vector.qza \
 --m-metadata-file DWmetadata.txt \
 --o-visualization core-metrics-resultsDW/shannon_group-significance.qzv
```

Beta Diversity by Variables

```
qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-resultsDW/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-column weight_category \
  --o-visualization core-metrics-resultsDW/unweighted-unifrac-weightcategory.qzv \
--p-pairwise

qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-resultsDW/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-column w_weightstatus4_num \
  --o-visualization core-metrics-resultsDW/unweighted-unifrac-BMI.qzv \
--p-pairwise

qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-resultsDW/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-column w_ovobmeasured2 \
  --o-visualization core-metrics-resultsDW/unweighted-unifrac-overweight.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/jaccard_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column weight_category \
--o-visualization core-metrics-resultsDW/jaccard-distance-matrix-weightcategory.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/jaccard_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column w_weightstatus4_num \
```

```
--o-visualization core-metrics-resultsDW/jaccard-distance-matrix-BMI.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/jaccard_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column w_ovobmeasured2 \
--o-visualization core-metrics-resultsDW/jaccard-distance-matrix-overweight.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/bray_curtis_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column weight_category \
--o-visualization core-metrics-resultsDW/bray-curtis-matrix-weightcategory.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/bray_curtis_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column w_weightstatus4_num \
--o-visualization core-metrics-resultsDW/bray-curtis-matrix-BMI.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/bray_curtis_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column w_ovobmeasured2 \
--o-visualization core-metrics-resultsDW/bray-curtis-matrix-overweight.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/weighted_unifrac_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column weight_category \
--o-visualization core-metrics-resultsDW/weighted-unifrac-weightcategory.qzv \
--p-pairwise

qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/weighted_unifrac_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column w_weightstatus4_num \
--o-visualization core-metrics-resultsDW/weighted-unifrac-BMI.qzv \
--p-pairwise
```

```
qiime diversity beta-group-significance \
--i-distance-matrix core-metrics-resultsDW/weighted_unifrac_distance_matrix.qza \
--m-metadata-file DWmetadata.txt \
--m-metadata-column w_ovobmeasured2 \
--o-visualization core-metrics-resultsDW/weighted-unifrac-overweight.qzv \
--p-pairwise
```

Longitudinal Analysis
Feature Volatility

Taxa Collapse by Family

```
qiime taxa collapse \
--i-table id-filtered-tableDW.qza \
--i-taxonomy taxonomyDW.qza \
--p-level 5 \
--o-collapsed-table DW-family-collasped.qza
```

Feature Volatility- Family

```
qiime longitudinal feature-volatility \
  --i-table DW-family-collasped.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column days_tp2 \
  --p-individual-id-column sparkid \
  --output-dir DW-feat-volatilityFamily
```

Taxa Collapse by Genus

```
qiime taxa collapse \
--i-table id-filtered-tableDW.qza \
--i-taxonomy taxonomyDW.qza \
--p-level 6 \
--o-collapsed-table DW-genus-collasped.qza
```

Feature Volatility- Genus

```
qiime longitudinal feature-volatility \
  --i-table DW-genus-collasped.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column days_tp2 \
  --p-individual-id-column sparkid \
  --output-dir DW-feat-volatilityGenus
```

Taxa Collapse by Species

```
qiime taxa collapse \
--i-table id-filtered-tableDW.qza \
--i-taxonomy taxonomyDW.qza \
--p-level 7 \
--o-collapsed-table DW-species-collasped.qza
```

Feature Volatility- Species

```
qiime longitudinal feature-volatility \
  --i-table DW-species-collasped.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column days_tp2 \
  --p-individual-id-column sparkid \
  --output-dir DW-feat-volatilitySpecies
```

First Differences

First Differences- Shannon

```
qiime longitudinal first-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file shannon_vector.qza \
  --p-state-column FecalTP \
  --p-metric shannon \
  --p-individual-id-column sparkid \
 --p-replicate-handling random \
  --o-first-differences shannon-first-differencesFecalTP.qza
```

First Differences- Faith

```
qiime longitudinal first-differences \
  --m-metadata-file DWmetadata.txt \
   --m-metadata-file faith_pd_vector.qza \
  --p-metric faith_pd \
  --p-state-column FecalTP \
  --p-individual-id-column sparkid \
 --p-replicate-handling random \
  --o-first-differences faiths-first-differencesFecalTP.qza
```

First Differences- Evenness

```
qiime longitudinal first-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file evenness_vector.qza \
  --p-state-column FecalTP \
  --p-metric pielou_e \
  --p-individual-id-column sparkid \
 --p-replicate-handling random \
  --o-first-differences evenness-first-differencesFecalTP.qza
```

First Differences- Bray Curtis

```
 qiime longitudinal first-differences \
--m-metadata-file DWmetadata.txt \
--m-metadata-file bray_curtis_pcoa_results.qza \
--p-state-column FecalTP \--p-metric "Axis 1" \
--p-individual-id-column sparkid \
--p-replicate-handling random \
--o-first-differences braycurtis-first-differencesFecalTP.qza
```

First Differences- Unweighted UniFrac

```
qiime longitudinal first-differences \
--m-metadata-file DWmetadata.txt \
--m-metadata-file unweighted_unifrac_pcoa_results.qza \
--p-state-column FecalTP \--p-metric "Axis 1" \
--p-individual-id-column sparkid \
--p-replicate-handling random \
--o-first-differences unweighted-first-differencesFecalTP.qza
```

First Differences- Weighted UniFrac

```
qiime longitudinal first-differences \
--m-metadata-file DWmetadata.txt \
--m-metadata-file weighted_unifrac_pcoa_results.qza \
--p-state-column FecalTP \
--p-metric "Axis 1" \
--p-individual-id-column sparkid \
--p-replicate-handling random \
--o-first-differences weighted-first-differencesFecalTP.qza
```

First Differences- Jaccard

```
qiime longitudinal first-differences \
--m-metadata-file DWmetadata.txt \
--m-metadata-file jaccard_pcoa_results.qza \
--p-state-column FecalTP \--p-metric "Axis 1" \
--p-individual-id-column sparkid \
--p-replicate-handling random \
--o-first-differences jaccard-first-differencesFecalTP.qza
```

First Distances

First Distances- Bray Curtis

```
qiime longitudinal first-distances \
  --i-distance-matrix bray_curtis_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column FecalTP \
  --p-individual-id-column sparkid \
  --p-replicate-handling random \
  --o-first-distances braycurtis-first-distancesFecalTP.qza
```

First Distances- Unweighted UniFrac

```
qiime longitudinal first-distances \
  --i-distance-matrix unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column FecalTP \
  --p-individual-id-column sparkid \
  --p-replicate-handling random \
  --o-first-distances unweighted-first-distancesFecalTP.qza
```

First Distances- Weighted UniFrac

```
qiime longitudinal first-distances \
  --i-distance-matrix weighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column FecalTP \
  --p-individual-id-column sparkid \
  --p-replicate-handling random \
  --o-first-distances weighted-first-distancesFecalTP.qza
```

First Distances- Jaccard

```
qiime longitudinal first-distances \
  --i-distance-matrix jaccard_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
  --p-state-column FecalTP \
  --p-individual-id-column sparkid \
  --p-replicate-handling random \
  --o-first-distances jaccard-first-distancesFecalTP.qza
```

Pairwise Differences

Pairwise Differences- Shannon

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file shannon_vector.qza \
  --p-metric shannon \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-shannon.qzv
```

Pairwise Differences- Evenness

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file evenness_vector.qza \
  --p-metric pielou_e \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-evenness.qzv
```

Pairwise Differences- Faith

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file faith_pd_vector.qza \
  --p-metric faith_pd \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-faiths-pd.qzv
```

Pairwise Differences- Unweighted UniFrac

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file unweighted_unifrac_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-unweightedUniFrac.qzv
```

Pairwise Differences- Weighted UniFrac

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file weighted_unifrac_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-weightedUniFrac.qzv
```

Pairwise Differences- Bray Curtis

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file bray_curtis_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column weight_category \
```

```
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-bray-curtis.qzv
```

Pairwise Differences- Jaccard

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file jaccard_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-jaccard.qzv
```

Pairwise Differences- Unweighted UniFrac

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file unweighted_unifrac_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column w_weightstatus4_num \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-unweightedUniFracBMI.qzv
```

Pairwise Differences- Weighted UniFrac

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file weighted_unifrac_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column w_weightstatus4_num \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-weightedUniFracBMI.qzv
```

Pairwise Differences- Bray Curtis

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file bray_curtis_pcoa_results.qza \
  --p-metric “Axis 1” \
--p-group-column w_weightstatus4_num \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-bray-curtisBMI.qzv
```

Pairwise Differences- Jaccard

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file jaccard_pcoa_results.qza \
  --p-metric “Axis 1” \
--p-group-column w_weightstatus4_num \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-jaccardBMI.qzv
```

Pairwise Differences- Weighted UniFrac

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file weighted_unifrac_pcoa_results.qza \
  --p-metric “Axis 1” \
--p-group-column w_ovomeasured2 \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-weightedUniFracOvob.qzv
```

Pairwise Differences- Bray Curtis

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
```

```
  --m-metadata-file bray_curtis_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column w_ovomeasured2 \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-bray-curtisOvob.qzv
```

Pairwise Differences- Jaccard

```
qiime longitudinal pairwise-differences \
  --m-metadata-file DWmetadata.txt \
  --m-metadata-file jaccard_pcoa_results.qza \
  --p-metric "Axis 1" \
--p-group-column w_ovomeasured2 \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-differences-jaccardOvob.qzv
```

Pairwise Distances

Pairwise Distances- Unweighted UniFrac

```
qiime longitudinal pairwise-distances \
  --i-distance-matrix unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
 --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-distances-unifrac.qzv
```

Pairwise Distances- Jaccard

```
qiime longitudinal pairwise-distances \
  --i-distance-matrix jaccard_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
--p-group-column weight_category \
```

```
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-distances-jaccard.qzv
```

Pairwise Distances- Weighted UniFrac

```
qiime longitudinal pairwise-distances \
  --i-distance-matrix weighted_unifrac_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-distances-weightedunifrac.qzv
```

Pairwise Distances- Bray Curtis

```
qiime longitudinal pairwise-distances \
  --i-distance-matrix bray_curtis_distance_matrix.qza \
  --m-metadata-file DWmetadata.txt \
--p-group-column weight_category \
  --p-state-column tp2 \
  --p-state-1 A \
  --p-state-2 B \
  --p-individual-id-column sparkid \
  --o-visualization pairwise-distances-bray-curtis.qzv
```

Linear Mixed Effects Models

Feature Volatility LME- Family

```
qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file DW-family-collasped.qza \
--p-state-column days_tp2 \
--p-metric
'k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae' \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--p-formula
'k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae'~days_tp
2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
```

--o-visualization LME-featurevolatility-family_categorical.qzv

Feature Volatility LME- Genus

qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file DW-genus-collasped.qza \
--p-metric
'k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Rum
inococcus]' \
--p-group-columns d_raceeth4_num,d_gen_num,weight_category,baseline_BMI \
--p-formula
'k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Rum
inococcus]'~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--p-state-column days_tp2 \
--p-individual-id-column sparkid \
--o-visualization LME-feature-volatility-genus_categorical.qzv

Feature Volatility LME- Species

qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file DW-species-collasped.qza \
--p-metric
'k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Clost
ridium;s__lavalense' \
--p-group-columns d_raceeth4_num,d_gen_num,weight_category,baseline_BMI \
--p-formula
'k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Clost
ridium;s__lavalense'~days_tp2+d_raceeth4_num+d_gen_num+weight_category+baseline
_BMI \
--p-state-column days_tp2 \
--p-individual-id-column sparkid \
--o-visualization LME-feature-volatility-species_categorical.qzv

First Differences LME- Shannon

qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file shannon-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \

--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--o-visualization LME-firstdiff-shannon-categorical.qzv

First Differences LME- Faith

qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file faiths-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--o-visualization LME-firstdiff-faiths_categorical.qzv

First Differences LME- Evenness
qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file evenness-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--o-visualization LME-firstdiff-evenness_categorical.qzv

First Differences LME- Bray
qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file braycurtis-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--o-visualization LME-firstdiff-braycurtis_categorical.qzv

First Differences LME- Jaccard

qiime longitudinal linear-mixed-effects \

```
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file jaccard-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--o-visualization LME-firstdiff-jaccard_categorical.qzv
```

First Differences LME- Unweighted UniFrac

```
qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file unweighted-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--o-visualization LME-firstdiff-unweighted_categorical.qzv
```

First Differences LME- Weighted UniFrac

```
qiime longitudinal linear-mixed-effects \
--m-metadata-file DWmetadataBaseline_categorical.txt \
--m-metadata-file weighted-first-differencesFecalTP.qza \
--p-state-column days_tp2 \
--p-metric Difference \
--p-individual-id-column sparkid \
--p-group-columns baseline_BMI,d_raceeth4_num,d_gen_num,weight_category \
--p-formula
Difference~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
--o-visualization LME-firstdiff-weighted_categorical.qzv
```

First Distances LME- Bray Curtis

```
qiime longitudinal linear-mixed-effects \
  --m-metadata-file DWmetadataBaseline_categorical.txt \
  --m-metadata-file braycurtis-first-distancesFecalTP.qza \
  --p-state-column days_tp2 \
  --p-metric Distance \
```

```
--p-formula
Distance~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
  --p-individual-id-column sparkid \
--p-group-columns d_raceeth4_num,d_gen_num,weight_category,baseline_BMI \
  --o-visualization LME-firstdist-braycurtis_categorical.qzv
```

First Distances LME- Jaccard

```
qiime longitudinal linear-mixed-effects \
  --m-metadata-file DWmetadataBaseline_categorical.txt \
  --m-metadata-file jaccard-first-distancesFecalTP.qza \
  --p-state-column days_tp2 \
  --p-metric Distance \
--p-formula
Distance~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
  --p-individual-id-column sparkid \
--p-group-columns d_raceeth4_num,d_gen_num,baseline_BMI,weight_category \
  --o-visualization LME-firstdist-jaccard_categorical.qzv
```

First Distances LME- Unweighted Unifrac

```
qiime longitudinal linear-mixed-effects \
  --m-metadata-file DWmetadataBaseline_categorical.txt \
  --m-metadata-file unweighted-first-distancesFecalTP.qza \
  --p-state-column days_tp2 \
  --p-metric Distance \
--p-formula
Distance~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
  --p-individual-id-column sparkid \
--p-group-columns d_raceeth4_num,d_gen_num,baseline_BMI,weight_category \
  --o-visualization LME-firstdist-unwtd_categorical.qzv
```

First Distances LME- Weighted Unifrac

```
qiime longitudinal linear-mixed-effects \
  --m-metadata-file DWmetadataBaseline_categorical.txt \
  --m-metadata-file weighted-first-distancesFecalTP.qza \
  --p-state-column days_tp2 \
  --p-metric Distance \
--p-formula
Distance~days_tp2+baseline_BMI+d_raceeth4_num+d_gen_num+weight_category \
  --p-individual-id-column sparkid \
--p-group-columns d_raceeth4_num,d_gen_num,baseline_BMI,weight_category \
  --o-visualization LME-firstdist-wtd_categorical.qzv
```

```
qiime longitudinal volatility \
  --m-metadata-file DWmetadataBaseline.txt \
  --m-metadata-file jaccard-first-differencesFecalTP.qza \
--m-metadata-file jaccard-first-distancesFecalTP.qza \
   --p-state-column FecalTP \
  --p-individual-id-column sparkid \
  --o-visualization volatilityFD-jaccard-FecalTP.qza
```