THE IMPACT OF DIET QUALITY ON MICROBIAL DIVERSITY THROUGHOUT PREGNANCY IN AN ETHNICALLY DIVERSE COHORT IN HAWAIʻI

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Abstract

Background:

The gastrointestinal (GIT) microbiome influences metabolism and modulates inflammation during pregnancy, influencing energy regulation, gestational weight gain, and risk of adverse pregnancy outcomes. Manipulating the GIT microbiota has been an area of earnest research interest, with hopes that manipulating an aberrant microbial community may translate to improved pregnancy outcomes. While some dietary patterns have been associated with improved GIT microbial health, few studies have investigated the impact of comprehensive diet quality on this outcome. Our study aimed to associate Diet Quality as defined by the Healthy Eating Index (HEI) with alpha diversity metrics longitudinally across pregnancy in healthy, low risk pregnant women.

Methods:

Forty-one women were recruited, from the 4 largest ethnic groups in Hawaii. Participants completed Food Frequency Questionnaires (FFQs) during each trimester (12 weeks, 20 weeks and 34-36 weeks), to which HEI Diet Quality Score was assigned. Rectal swabs were collected concomitantly at each time point, from which DNA extraction and 16s rRNA sequencing were performed. Alpha and beta diversity profiles were assigned, and correlated in a linear fashion with HEI Score. Linear regression was also used to account for confounding demographic factors.

Results:

HEI score did not correlate with any alpha diversity metrics. Those with the highest HEI score had greater amounts of lactobacillaceae (not significant), and several species were associated with higher diet quality. Native Hawaiians had the highest HEI score compared to Filipina

participants, and had higher amounts of Acidaminoaceae and lactobacillaceae, respectively. There were no differences observed with microbial diversity among the 4 ethnic groups. All alpha diversity indices decreased from the first to third trimester, as expected from published literature in other cohorts.

Conclusion:

Alpha diversity decreased throughout pregnancy in this cohort, commensurate with other studies. Hormonal changes of pregnancy had the largest influence in change over time of microbial composition in this cohort, greater than dietary intake, ethnicity, body mass index, or parity.

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1. BACKGROUND

The Human Microbiome Project (HMP), completed in 2007, revealed that the normal bacteria residing in various organ systems has a functional and symbiotic relationship with human health (1). The majority of these microbes cannot be cultured for classification, and thus newer genetic technologies to sequence bacterial genomes are integral to identifying microbial composition. The HMP used high-throughput sequencing methods to identify bacterial composition in 5 body sites among 300 healthy individuals, creating a reference of normal phyla among people of various ethnicities, ages, genders, and body composition.

The HMP demonstrated that distinct microbial profiles exist among different ethnic groups (2-6). Other external factors can shift normal flora of the skin, gut, vagina, and oral cavity. Changes in age, diet, body composition, and hormones are all known to influence the types of bacteria present (7). Microbial composition across multiple body sites make a physiologic shift during pregnancy due to hormonal fluctuations. The first trimester intestinal microbiome resembles the non-pregnant state with many bacterial types present; toward the end of gestation there is reduced diversity of species. Similar shifts are seen in the vaginal microbiome throughout pregnancy, and variations from this normal trajectory may be associated with adverse pregnancy outcomes. For instance, the differences in vaginal lactobacillus community composition of pregnant Black women compared to Non-Hispanic White women is independently associated with preterm birth (3). It is theorized that dysbiotic profiles within the gastrointestinal tract (GIT) induce bacterial translocation and allow endotoxins create to systemic inflammation which predisposes to preterm labor (8).

Long-term diet, or dietary patterns, are some of the strongest effectors of GIT microbial diversity (9, 10). The proportions of fiber, proteins, or fat regulate bacterial abundance. Diets

composed of lower-fat, protein, unsaturated fats; and higher fiber content promote GIT microbial health (11, 12) and functionally help reduce inflammation. Beneficial diet patterns also prevent bacterial translocation by eliminating gap junctions between mucosal cells (13-15). Omega 3 polyunsaturated fatty acids (PUFAs) similarly protect the intestinal wall by increasing cellular connections, and Mediterranean diet patterns has been associated with higher content of PUFAs (14). Less inflammation translates into associated health benefits in the general population, including less risk of inflammatory bowel disease, metabolic syndrome, and diabetes (16).

Several studies have demonstrated equivalent benefit during pregnancy. Diets rich in probiotics or with large consumption of vegetables affect vaginal and gut microbiomes during pregnancy and decrease adverse pregnancy outcomes (17-20). There appears to be a link between these diets and increased intestinal microbial diversity, which is associated with lower systemic inflammation and a decreased risk of preterm birth (21). Proving this mechanism or the overall contribution of this relationship to pregnancy health is still in its infancy.

To date, researchers have queried how diet and nutrition affect the microbiome of populations of European and African ancestry. However, there is limited data regarding these components of human health in the Native Hawaiian, Asian, and Pacific Islander population here in Hawai‘i . There is a paucity of research on the diet patterns and diet quality during pregnancy in Hawai‘i, as well as a limited understanding of the effect of diet patterns on the microbiome in our unique multi-ethnic community. Many unique foods, such as fermented poi or natto, are consumed in Hawai‘i as well as preserved canned foods, and these may also impact microbial composition.

Understanding how nutrition and the microbiome interact during pregnancy can have important health implications for women disproportionately affected by adverse pregnancy

outcomes. Native Hawaiian and part-Hawaiian women are at higher risk for adverse pregnancy outcomes such as preterm labor (Odds Ratio - OR=1.73) compared to white women(22, 23). Asian and Pacific Islander women are at increased risk of having gestational diabetes (OR 2.05), placenta previa (OR 1.57), and preterm rupture of membranes (OR 1.26)(24). As a result of higher preterm birth rates in this population, premature birth is the leading cause of infant death in Hawaiʻi (25). The infant mortality ratio (IMR) is higher for Native Hawaiians (7.4/1,000) compared to Filipinos (5.4), Japanese (3.1) or Non-Hispanic Whites (5.2) in Hawaiʻi (25, 26). While the preterm birth rate has been declining across the US, it has remained the same for several years in Hawaiʻi (27). Thus, there is an impetus for translational, meaningful interventions to decrease these risks. Identifying cultural dietary habits that potentially influence biodiversity may be an opportunity to target particularly beneficial diets.

1.1 NUTRITION AND DIET QUALITY IN PREGNANCY

Nutrition in pregnancy is an important contributor to pregnancy outcomes (22, 28). From supplementation of folic acid in preventing spina bifida (29) to carbohydrate-controlled diets for gestational diabetes, various macro and micronutrients affect the health of the mother and the fetus (30). Several nutritional determinants of pregnancy outcomes have been identified. For instance, low glycemic index diets have been linked to lower gestational weight gain and decreased serum glucose (31). Probiotic-rich dairy products are associated with lower rates of pre-eclampsia (19). High consumption of fried and processed meats has been linked to an increased risk of preterm birth (32, 33), while diets rich in vegetables and fruits are associated with a lower incidence of preterm birth (34). Finally, the dietary pattern of eating mostly rice, fish, and vegetables in Japan is correlated with higher birth weight (35).

Food groupings commonly eaten together are called diet patterns. Dietary recommendations are easier to follow if made in accordance with dietary patterns. Dietary patterns during pregnancy may shift throughout gestation in accordance with cultural tradition. Many Asian Americans adhere to traditional practices of eating mainly cold and sour (fermented) foods throughout the pregnancy to balance the "hot state" of pregnancy. Hot foods are then consumed during the third trimester to "expel the fetus (36)" and replenish the "chi" that is lost during the birth process. As such, pregnancy is an opportune time to intervene and modify dietary patterns because women are motivated to improve their health for the benefit of the fetus. If these beneficial changes in diet are continued after pregnancy, they may have long-term benefits for the woman's health and her offspring (37, 38).

Using a standardized tool, the *quality* of diet patterns can be assessed by a composite score of all foods and supplements consumed. Such tools, based on national recommendations, are useful for evaluating overall diet quality of pregnant women and can help identify culturally appropriate areas of improvement. Diet Quality Measurement Tools or Indices are developed by researchers and scientists asking patients to perform a dietary recall either through Food Frequency Questionnaire or a 24-hour recall diary. Components of consumed foods are calculated into an algorithm to assign a diet quality score. The Diet Quality Index for Pregnancy (DQIP) was the first described tool used in nutrition research for pregnancy (39). However, the DQIP does not differentiate among types of fats. This is important because the ratio of polyunsaturated fats to saturated fats has been linked to gestational diabetes and preterm birth (40, 41).

More modern tools were developed that are commensurate with current dietary recommendations, such as the Healthy Eating Index (HEI). The HEI is a tool that incorporates

the Dietary Guidelines for Americans (DGA) and evaluates conformance to these recommendations (42) (See Table 1). Higher HEI scores have been validated to be associated with several health outcomes outside of pregnancy (such as cancer, mortality, hypertension, and diabetes mellitus (43)), and are also used to demonstrate association with pregnancy outcomes (44). A higher HEI score was noted to be protective of developing pre-eclampsia and having lower blood glucose levels (45). Other studies have demonstrated associations with gestational diabetes (41, 46), preterm birth (47, 48) neonatal birth weight (44, 49), and gestational weight gain (50). Li et al demonstrated higher HEI scores after pregnancies affected by gestational diabetes had a lower risk of chronic hypertension (51). Yet, the majority of the studies were performed in populations of Non-Hispanic White women, and did not look at the change in diet quality across a gestation. Little research has been done to characterize diet patterns and diet quality during pregnancy in Hawai‘i and evaluate its impact on obstetric outcomes.

HEI–2010[1] Components & Scoring Standards

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
Adequacy:			
Total Fruit[2]	5	≥0.8 cup equiv. per 1,000 kcal	No Fruit
Whole Fruit[3]	5	≥0.4 cup equiv. per 1,000 kcal	No Whole Fruit
Total Vegetables[4]	5	≥1.1 cup equiv. per 1,000 kcal	No Vegetables
Greens and Beans[4]	5	≥0.2 cup equiv. per 1,000 kcal	No Dark Green Vegetables or Beans and Peas
Whole Grains	10	≥1.5 oz equiv. per 1,000 kcal	No Whole Grains
Dairy[5]	10	≥1.3 cup equiv. per 1,000 kcal	No Dairy
Total Protein Foods[6]	5	≥2.5 oz equiv. per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins[6,7]	5	≥0.8 oz equiv. per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids[8]	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 oz equiv. per 1,000 kcal	≥4.3 oz equiv. per 1,000 kcal
Sodium	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Empty Calories[9]	20	≤19% of energy	≥50% of energy

1: Intakes between the minimum and maximum standards are scored proportionately.
2: Includes 100% fruit juice.
3: Includes all forms except juice.
4: Includes any beans and peas not counted as Total Protein Foods.
5: Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.
6: Beans and peas are included here (and not with vegetables) when the Total Protein Foods standard is otherwise not met.
7: Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.
8: Ratio of poly- and monounsaturated fatty acids (PUFAs and MUFAs) to saturated fatty acids (SFAs).
9: Calories from solid fats, alcohol, and added sugars; threshold for counting alcohol is >13 grams/1000 kcal.

TABLE 1: Healthy Eating Index by the Dietary Guidelines for Americans, from the National Cancer Institute's HEI-2010 Components & Scoring Standards Table (52).

1.2 GASTROINTESTINAL MICROBIOME IN PREGNANCY

The significance of microbial diversity in pregnancy is just beginning to be understood. Dysbiosis of normal microbial communities in the oral-intestinal tract and vagina has been implicated in several poor pregnancy outcomes including preterm labor. The microbial community that has the strongest effect on pregnancy outcomes is unknown at this time. Originally, vaginal microbiota was thought to be a key contributor to preterm labor. However, it has also been shown that the oral-intestinal tract flora has a similar profile to the placental microbiome and is believed to be populated via hematogenous spread.

In studying microbial composition, *microbiota* refer to the organisms present, comprised of various phyla, species, and families present. The *microbiome* defines the genetic material used to identify these microbes (53). For research and clinical purposes, the types of bacteria present within a microbiome are represented by operational taxonomic units (OTUs). OTUs refer to defined levels of resolution such as phylum, class, order, family, genus, or species.

To characterize the types of OTUs present, *richness* is used to describe the *number* of OTUs within a sample; *evenness* compares the relative *size* or the contribution of those OTUs within a sample. Diversity indices have been created to describe a sample's richness and evenness. Alpha diversity refers to the diversity (variation in richness and evenness) within a sample, while beta diversity compares components among various samples. Popular diversity indices include the Simpson Index, Chao1 Index, and Shannon-Weaver Index. These indices are derived from equations to have a set continuous variable that represents more or less diversity (generally averaging 0-100) (54). Beta diversity compares how different one sample is from another and is often described in principal coordinate analysis (PCA) from a type of distance matrix. The distance matrix may be calculated from a variety or a-prior selected algorithms looking how abundant or how related OTUs are, as such as is the case with Bray-Curtis, or Uni-Frac methods, respectively (55). If two individual's samples appear very similar, they are graphed closely together, while if the composition is fundamentally different, they would be graphed more distantly. No one index is tested to be better than another, and they all serve the purpose of being able to uniformly compare community composition in various studies.

Since 2007 at the time of the HMP, several studies have looked at what 'normal' microbiota is during pregnancy in both the vaginal and gastrointestinal tract (GIT). During a normal pregnancy, the maternal GIT microbiota shifts from first to third trimester. There is a

gradual decline in butyrate-producing bacteria from the Firmicutes (Coprococci, Eubacterium, Roseburia, and Faecalibacterium genera) and Bacteroidetes (Odoribacter and Alistipes genera) phyla (56). There is an increase in Bifidobacteria, Proteobacteria, and lactic acid–producing bacteria. This process is thought to help facilitate the normally observed increase inflammation and weight gain to increase energy supply for the fetus (57). The end result is less alpha diversity and OTU richness by the end of the third trimester. Several studies demonstrate that at this point, the composition resembles that of an individual with metabolic syndrome, with increased Actinobacteria and Proteobacteria, and decreased Faecalibacterium (57, 58).

As observed in non-pregnant populations, the hormonal shifts associated with obesity alter microbial composition of the GIT in pregnant women. Gomez-Arango (59) compared the intestinal microbiomes of obese and overweight women at 16 weeks' gestation. They noted several differences between the two groups: Obese women had a higher ratio of Firmicutes:Bacteroidetes of 3:1 than overweight women. Obese women had higher abundance of actinobacteria as well. Overall, the microbial composition did not differ in those who did and did not develop gestational diabetes. Overweight women had higher richness and evenness than obese women (57).

Other studies have shown an association with the types of bacteria present in the gut of pregnant women and the development of gestational diabetes. Crussell also noted that the microbiome of women with GDM is similar to those with Type 2 DM (60). Unfortunately, adding probiotics in attempts to change the microbiota composition of those at risk of gestational diabetes did not produce a risk reduction(61). Interestingly, Gomez-Arrango and colleagues linked types of enterotypes of the genus Odoribacter to decreased blood pressure and postulated that increases in butyrate led to decreases PAI-1 and systolic blood pressure. (56).

1.3 DIETARY QUALITY IMPACT ON MICROBIOTA

As previously mentioned, long-term diet, or dietary patterns, are strong effectors of microbial diversity (9, 10). This is in part due to proportions non-digestible fermentable dietary carbohydrates consumed, leading to metabolism of short-chain fatty acids (SCFA) and modulation of bacterial abundance (7). Understanding and manipulating dietary intake has been area of interest by several investigators hoping to understand how this modifiable lifestyle component can translate to improvements in human health.

While particular food groups or diet patterns are most commonly studied, there have been a few studies looking at overall diet quality through diet quality indexes, and their association with microbial health. Bowyer et al., compared HEI, a Mediterranean dietary score, and the "Healthy food diversity index." The study determined that among these diet quality scores, HEI was the best dietary index to attribute variation among environmental factors affecting microbial composition (62). In a large study of the multi-ethnic, non-pregnant, cohort from Hawai‘i and California, 4 diet quality scores were compared against microbial diversity. The study compared of 858 men and 877 women of white, Japanese American, Latino, Native Hawaiian, and African American ancestry. Alpha diversity was compared across terciles of 4 diet indexes (HEI, Alternate Healthy Eating Index (AHEI), Alternate Mediterranean Diet Score, and Dietary Approaches to Stop Hypertension). The authors noted an increase in Shannon diversity by 1-2% across each tercile of improved diet quality. They also noted mean relative abundance of the phylum Actinobacteria was 13–19% lower with higher diet quality across all 4 indexes (63). Laitinen et al., looked a similar association in 84 overweight and obese pregnant women in Finland. They also noted that higher dietary quality was related to higher gut microbiota diversity in each of the three indexes evaluated. Daily consumption of whole grains and

vegetables had the largest impact, and a high dietary quality score was related to particular microbial abundances, primarily to the genus Coprococcus and species Faecalibacterium prausnitzii, whereas an unknown species of the genus Sutterella was related to a lower dietary quality (64). Studies such as these demonstrate that diet quality can be used as an objective measure of comprehensive nutrient intake to understand the relationship with food and GIT microbiota.

1.4 OBJECTIVES AND HYPOTHESIS

Taking this foundational information into consideration, this study aims to measure the impact of diet quality on microbial diversity throughout pregnancy. **We hypothesize that lower HEI scores are associated with lower microbial diversity during pregnancy.** This will be evaluated through three main objectives:

Objective 1:	**Compare dietary quality indices in 4 major ethnic groups during pregnancy in Hawai‘i.**
Objective 2:	**Assess the intestinal microbiome of pregnant women concomitantly in this cohort.**
Objective 3:	**Correlate the relationship of microbial changes and dietary quality across each trimester of pregnancy and between ethnic groups.**

This study evaluates pregnant women, but does attempt to investigate the association of nutrition, microbial dysbiosis and adverse pregnancy outcomes. Future studies will aim to associate these patterns with adverse pregnancy outcomes and discover methods to modify high-risk profiles.

2. METHODS AND PROCEDURES

2.1. STUDY SUBJECTS AND RECRUITMENT

This study was approved by the Western IRB in compliance with Hawai‘i Pacific Health IRB protocol. This study is a longitudinal cohort pilot study. Ten women were recruited from the 4 most common ethnic groups in Hawai‘i – Japanese, Filipino, Native Hawaiian, and non-Hispanic White (65). Participants were approached to enroll in this study at the Fetal Diagnostic Center at Kapiolani Medical Center for Women and Children while waiting for an ultrasound appointment, after being identified by medical chart review as potentially eligible. Inclusion Criteria were as such: women aged 18-45 years old, primarily English Speaking and English literate, self-identified as Asian, Non-Hispanic White, or Native Hawaiian on intake registration information form and in their first trimester of pregnancy (<14 weeks 0 days gestation). While many individuals in Hawai‘i have a multiethnic background, participants had to identify as 50% or greater (having one parent that is 100% of their reported heritage) to participate in the study. Native Hawaiians of any percent ethnicity were eligible for participation. Participants that identified as 50% one ethnicity and 50% of another ethnicity were excluded.

Other exclusion criteria included: plans to move out of the area prior to delivery, plan to deliver at another hospital other than Kapiolani Medical Center, multiple gestation, pre-existing diabetes or hypertension, heart disease, chronic renal disease, systemic lupus erythematosus, hypothyroidism, history of bariatric surgery, history of an eating disorder, or inflammatory bowel disease, and women who are currently incarcerated.

2.2 STUDY PROTOCOL

2.2a DIET QUALITY DATA

The Multi-ethnic Food Frequency Questionnaire (MEC-FFQ) was developed and

validated in a large healthy adult population from 1993-1996 in Hawai‘i and California (66). Participants were followed for decades and the tool has proven effective in associating diet with oncologic outcomes and cardiovascular risk. It has not yet been used in pregnant women, but would be an ideal tool to characterize dietary patterns in our patient population. The FFQ includes 182 specific food items uniquely associated with the traditional diets such as poi, taro, spam, tofu, salted fish, miso soup, saimin, and fermented foods, presumably high in probiotics.

Once a participant was approached to enroll and provided consent to participate in the study, participation included: (1) Completing the Multiethnic Cohort Food Frequency Questionnaire (MEC FFQ) during the first trimester and second trimester ultrasound visits; (2) Collecting microbiome samples via rectal swab at the same time points in each trimester. Third trimester data was collected via mail. While many gut microbiome studies use fecal samples, the compliance with returning stool samples is low and rectal swabs generally have the same genetic yield (67), and thus rectal swabs were chosen as the sampling method. The first FFQ and bacterial swab collection was completed at time of enrollment, around 11 – 13 weeks' gestation. The second collection occurred in the second trimester at the time of their anatomy ultrasound at 18-20 weeks' gestation. Third trimester samples were collected from 34-36 weeks' gestation.

The MEC FFQ asks participants to mark how often and in what portion size they consume 182 various foods. They are asked recall a typical diet for the previous month. Pictures are provided to help assess portion size. Additional questions were included such as duration of residence in Hawai‘i, place of birth, and whether the participant felt that their diet matched their culture of their self-reported ethnicity. The data extracted from the MEC FFQ was analyzed by the University of Hawai‘i Cancer Center Nutrition Shared Support Resource. The resource provides information on 54 nutrients from food, energy, macronutrients, and 24 nutrients from

supplements. The analysis also provided HEI Diet Quality Scores for each questionnaire that is completed, so that each participant had up to three scores.

2.2b MICROBIOME COLLECTION AND SEQUENCING

DNA isolation was performed using the Qiagen AllPrep DNA/RNA Extraction Kit. 16S rRNA primers were then used to create bacterial DNA libraries for sequencing. The Epigenomics Core performs metagenomic sequencing on the Ion Genestudio S5 Sequencer. V2-4-8, V3-6, V7-9 primers are used to amplify the hypervariable regions of the 16S rRNA gene from bacteria. Amplified fragments are then sequenced and analyzed using the Ion 16S™ metagenomics analyses module within the Ion Reporter™ software. The primers allow for broad, yet high-throughput sequencing to identify bacteria down to the genus or species level among complex polybacterial samples. The software output uses Greengenes and MicroSEQ ID 16S rRNA reference databases to identify classify the relative proportions of microbes present. The Ion Torrent Data analysis platform aligns sequence fragments and provides OTUs at the family, genus, and species level. These reads were used to assign alpha and beta diversity scores through the Ion Torrent Software.

2.3 DATA ANALYSIS

The longitudinal cohort of 41 participants was recruited according to a sample size of convenience with the limited resources and funding for the project. There were not studies with similar design at the time of planning this study, and thus not sufficient literature to guide a power calculation. A theoretical a-prior power calculation looking to detect a linear correlation between two continuous variables, with a rho or correlation factor of 0.3 with 80% power, and type 1 error of <0.05 would require 85 samples. It was anticipated that the cohort would possibly

yield up to 120 samples, but with loss to follow-up or difficulty with the assays, that 100 samples would be sufficient to find such an association.

Characteristics of the participants were summarized by mean and standard deviation for continuous variables and frequencies and percentages for categorical variables. Two-tailed Student's t test and the χ^2 test were used to test the differences of these variables respectively. Variables were log transformed to improve normality and homoscedasticity where appropriate. A Mann Whitney U test was applied for non-normally distributed data. Repeated Measures ANOVA with multiple comparison test as post-hoc analysis was used to compare HEI Scores and alpha diversity (Shannon Index, Chao 1 Scores, Simpson and Observed number of Species) indexes as well as means of other characteristics among ethnic groups ethnicity aggregately among trimesters. A linear mixed model was used to compare change of alpha diversity throughout pregnancy by HEI Score and other covariates (age, parity, obesity, and ethnicity).

The α-diversity indexes were computed after rarefraction was performed, using the average value of the 10 rarefied value at sequence number 15927 (to keep maximum samples). Beta diversity profiles were analyzed with PCA among each ethnic group during each trimester after Euclidean Distance Matrix was developed. The primary outcome measures of correlation of HEI score with alpha diversity score were compared with Pearson correlation as well as multivariate linear regression while accounting for confounders such as age, BMI, and parity. Linear mixed models were used to compare HEI scores and alpha diversity indices independently from first to third trimester. All data analyses were performed using R Studio version 1.0.136 (http://www.r-project.org/) and a two-tailed p-value of less than 0.05 was be regarded as statistically significant.

3. RESULTS

3.1 PARTICIPANT RECRUITMENT AND FOLLOW UP

From August 28th, 2019 to November 26th, 2019, 504 women were screened for eligibility. 327 women were deemed ineligible due to: age (6), current medical condition (105), ethnicity (172), non-English speaking (11), not planning on delivering at Kapiolani (20), did not show up to appointment to be approached (13), missed abortion (4), multiple gestation (2), or found to be out of the gestational age window. Of 177 women who were deemed eligible, 41 participants were enrolled, 67 declined, and 61 were missed. 41 participants were enrolled, which is greater than the planned 40 participants, as Participant #40 and #41 were enrolled simultaneously on the same day by two different study investigators, and thus it was decided to keep them both in the study.

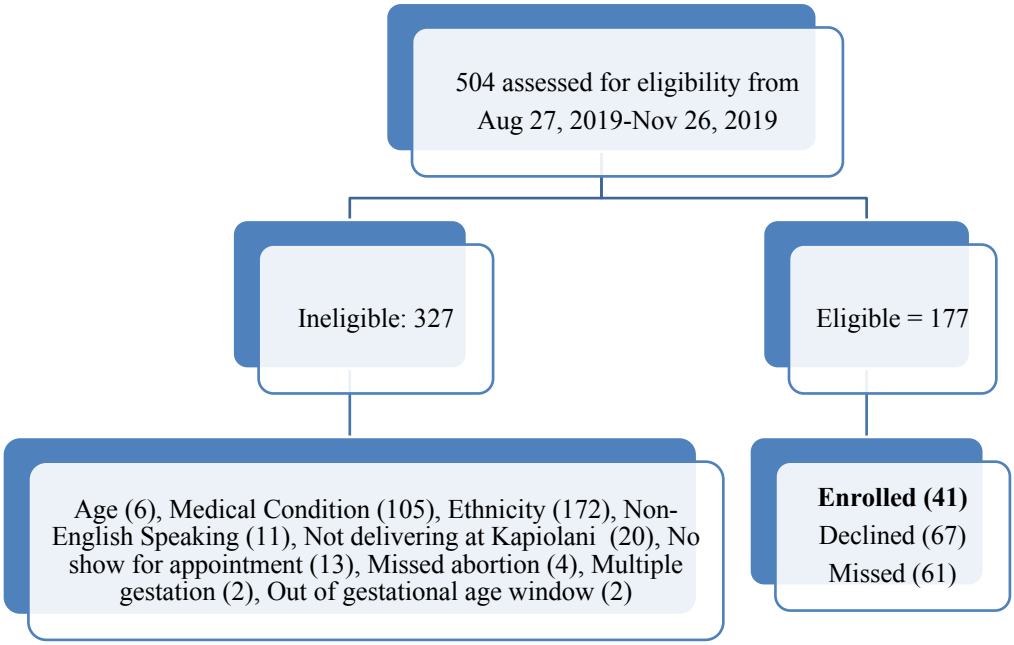

FIGURE 1: Recruitment Scheme for participants approached to enroll in the study

FFQs results were available for 40 participants during the first trimester, 37 for the second trimester, and 33 for the third trimester. Loss to follow-up or withdrawal from the study occurred for 1 patient after the first trimester (termination of pregnancy), and 4 participants after the second trimester collection (1 termination due to severe preeclampsia, 1 second trimester loss, 1elective termination and 1 participant moved away). During the third trimester, there were an additional 8 participants who were lost to follow up and did not fill out their survey in the mail, with results for 33 participants in the third trimester.

DNA quality and sequencing results were available for 35 participants from the first trimester, 36 from the second, and 30 from the third trimester. DNA extraction methods were optimized after the first batch of extractions, and thus approximately 5 samples from the first trimester did not have enough quality DNA to be processed and sequenced. These methods were revised to aliquot and bank material for future study.

3.2 DEMOGRAPHICS

Patient demographics are displayed in Table 2. There were 10 participants each of Non-Hispanic White, Filipino, Japanese and 11 participants of Native Hawaiian descent. The average age of the cohort was 29, and the majority were nulliparous (56%). Average BMI was 27.2 kg/m2, with only 17% being obese.

Baseline Demographics		
Ethnicity	Japanese Filipino Non-Hispanic White Native Hawaiian	10 (24.4%) 10 (24.4%) 11 (26.8%) 10 (24.4%)
BMI	Obese Overweight Normal	10 (24.4%) 11 (26.8%) 20 (48.8%)
Parity	Nulliparous Primaparous Multiparous	15 (36.6%) 21 (51.2%) 5 (12.2%)
	Age (median)	29

TABLE 2: Baseline demographics of the cohort

3.3 DIET QUALITY

The aggregate distribution of the HEI scores among all three trimesters is shown in Figure 2.

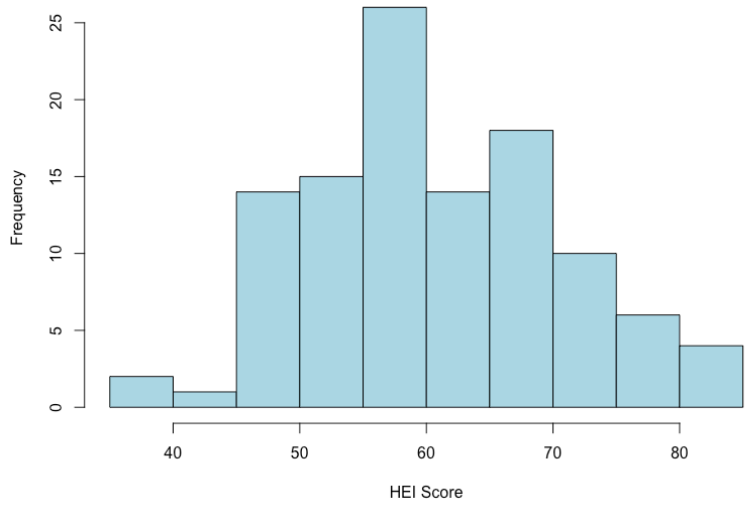

FIGURE 2: Histogram showing the distribution of all HEI scores throughout gestation.

Table 3 shows the demographic make-up of participants by HEI quartiles, as well as energy components.

HEI Quartile	First	Second	Third	Fourth	p-value
Range:	[37.49 - 53.1] (n=11)	[53.2 - 59.4] (n=8)	[59.5 - 67.1] (n=9)	[67.2 - 81.54] (n=12)	
Age					
Median [Min, Max]	25.0 [19.0, 38.0]	27.5 [22.0, 37.0]	33.0 [23.0, 38.0]	32.5 [24.0, 40.0]	
Obesity					0.2789
Normal	4 (36.4%)	3 (37.5%)	6 (66.7%)	6 (50.0%)	
Overweight	6 (54.5%)	2 (25.0%)	1 (11.1%)	2 (16.7%)	
Obese	1 (9.1%)	3 (37.5%)	2 (22.2%)	4 (33.3%)	
Ethnicity					0.1023
Filipino	3 (27.3%)	5 (62.5%)	1 (11.1%)	1 (8.3%)	
Japanese	3 (27.3%)	0 (0%)	3 (33.3%)	3 (25.0%)	
Native Hawai‘i an	3 (27.3%)	1 (12.5%)	1 (11.1%)	6 (50.0%)	
Non-Hispanic White	2 (18.2%)	2 (25.0%)	4 (44.4%)	2 (16.7%)	
Parity					0.3544
Multiparous	1 (9.1%)	1 (12.5%)	0 (0%)	3 (25.0%)	
Nulliparous	4 (36.4%)	5 (62.5%)	6 (66.7%)	7 (58.3%)	
Primaparous	6 (54.5%)	2 (25.0%)	3 (33.3%)	2 (16.7%)	
Aggregate Nutrient Consumption					
Total Energy (kcal)	2090 (2460)	2010 (1180)	1550 (630)	2560 (1180)	0.103
% carbohydrates from total energy	46.3 (6.36)*	47.6 (5.75)^	49.1 (4.51)	51.5 (6.96)*^	**0.00748**
% protein from total energy	16.3 (2.29)	16.1 (2.37)	15.8 (2.34)	15.9 (2.53)	0.832
% fat from total energy	37.4 (5.05)*	36.2 (4.09)^	35.1 (3.63)	32.6 (5.42)*^	**0.00104**
Monounsaturated fat (g)	33.9 (39.9)	32.4 (20.0)	23.7 (10.1)	35.9 (18.1)	0.281
Polyunsaturated fat (g)	15.8 (17.8)	15.6 (9.79)	11.8 (4.84)*	20.6 (10.5)*	**0.0441**
Cholesterol (mg)	343 (387)	276 (176)	231 (142)	330 (202)	0.32
Sodium (mg)	3520 (3430)	3290 (1660)	2770 (1090)	4170 (1930)	0.123
Fiber (g)	14.8 (17.3)*	14.8 (7.54)*	17.9 (7.41)*	34.4 (18.8)*	**2.58E-07**
Calcium (mg)	834 (1010)	675 (516)^	754 (298)*	1170 (591)*^	**0.028**
Folate (Mg)	446 (449)	400 (225)*	474 (228)*	850 (480)*	**2.14E-05**
Iron (mg)	13.2 (12.6)*	12.8 (7.85)*	13.1 (6.00)*	22.8 (11.9)*	**0.0002**

TABLE 3: Aggregate results from all three trimesters for all participants who completed FFQs. Aggregate Nutrient Consumption is displayed as mean (SD).
ANOVA with multiple comparisons Tukey HSD test used to compare means of components between the 4 groups. Significant differences between groups are indicated by an * or ^.

Native Hawaiians had the highest HEI scores aggregately and in each trimester (Not significant (NS) (Table 4, Figure 3), and this was primarily due to the difference between the scores of Native Hawaiians and Filipino participants in the third trimester (p=0.03).

HEI Score by trimester	**1st Trimester (n=40)**	**2nd Trimester (n=37)**	**3rd Trimester (n=33)**
Filipino	53.54 (8.3)	58.2 (6.96)	54.02 (5.17)*
Japanese	61.86 (12.75)	59.53 (8.9)	61.78 (10)
Native Hawaiian	62.21 (11)	67.29 (11.6)	67.35(12.2)*
Non-Hispanic White	60.74 (9.4)	58.63(7.81)	61.66 (5.9)
p-value	0.224	0.111	**0.03**

TABLE 4: HEI Scores across three trimesters in each ethnic group

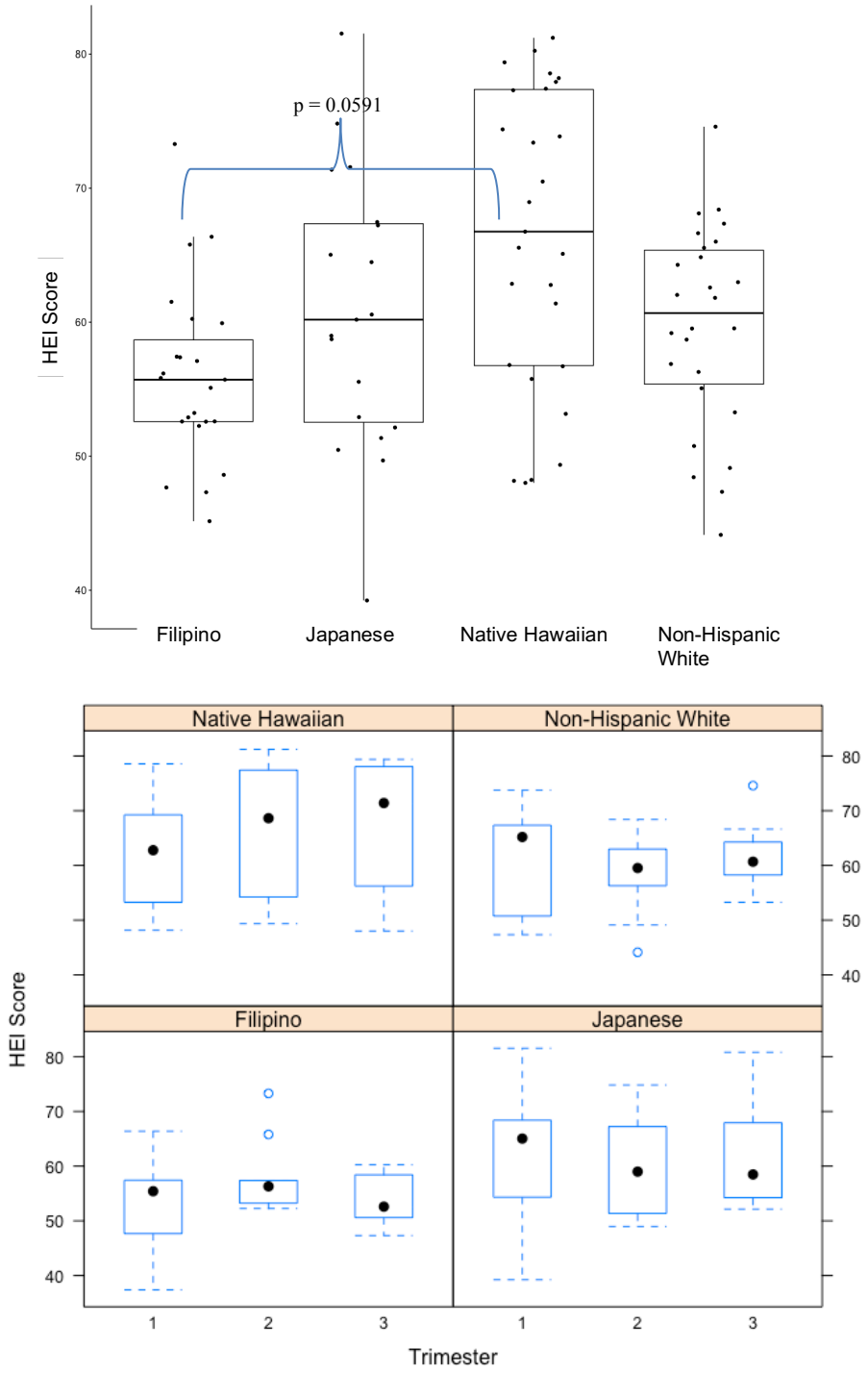

FIGURE 3: Box plot of HEI scores for each ethnic group aggregately for all trimesters (PANEL A) (mean – solid line, SD- whisker, and according to each trimester (PANEL B) showing the mean (•) and Standard deviations (dashed lines).

3.4 MICROBIOME DATA

After filtering for DNA quality and samples that produces greater than 10,000 reads, results were available for 35 participants from the first trimester, 36 from the second, and 30 from the third trimester.

3.4a OBSERVED TAXA

Overall, 577 different OTUs were identified. The most abundant phyla and families according to trimester are demonstrated in Figure 4. There is a shift in the types and abundance of microbes present between the first and second trimesters to the third trimester. There are no statistical differences at this level among ethnicities, but trends are seen at the family level (Figure 5).

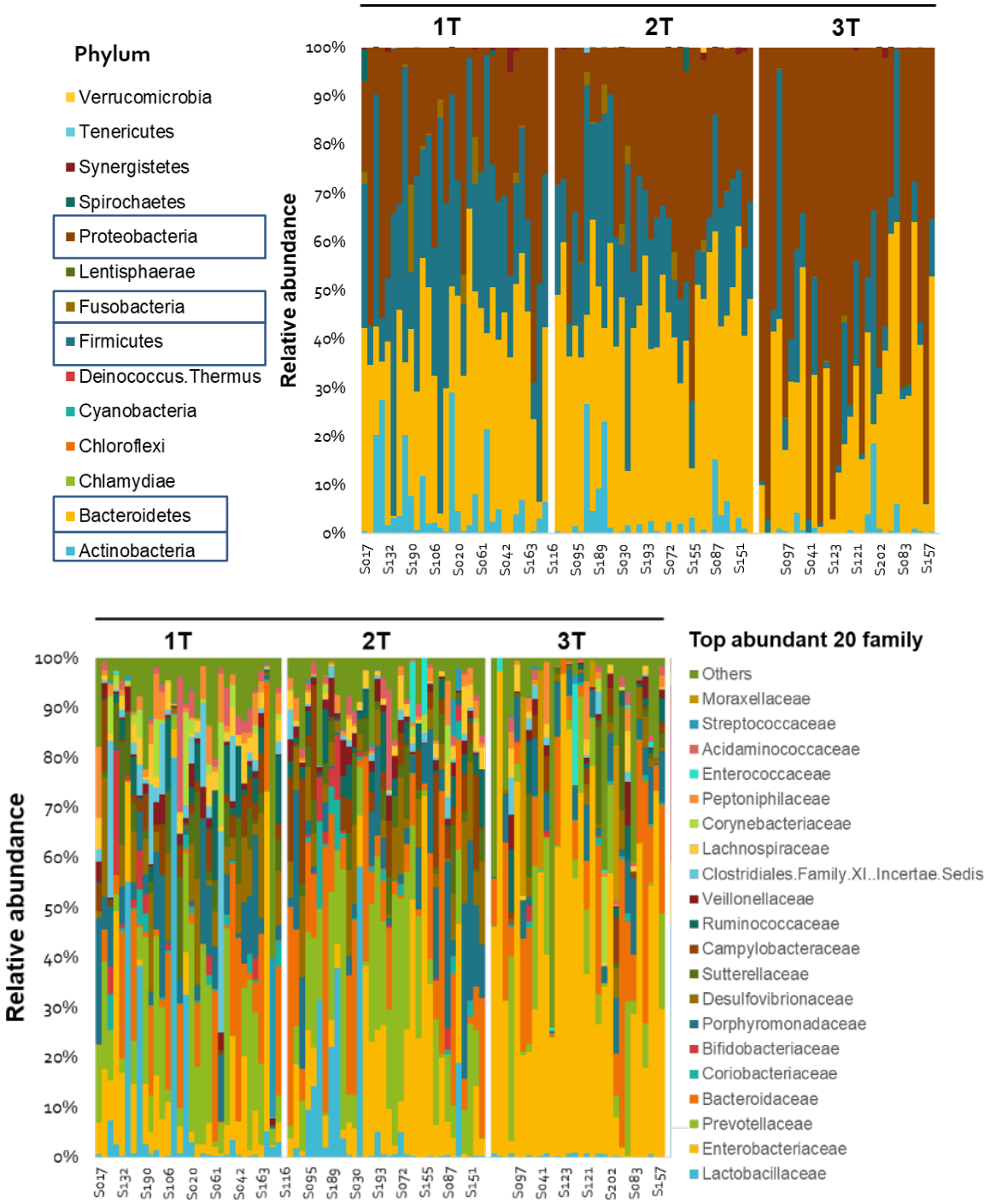

FIGURE 4: Phylum (Top panel) and Family (bottom panel) distribution across all samples according to trimester. The most abundant phyla are Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes, and Actinobacteria.

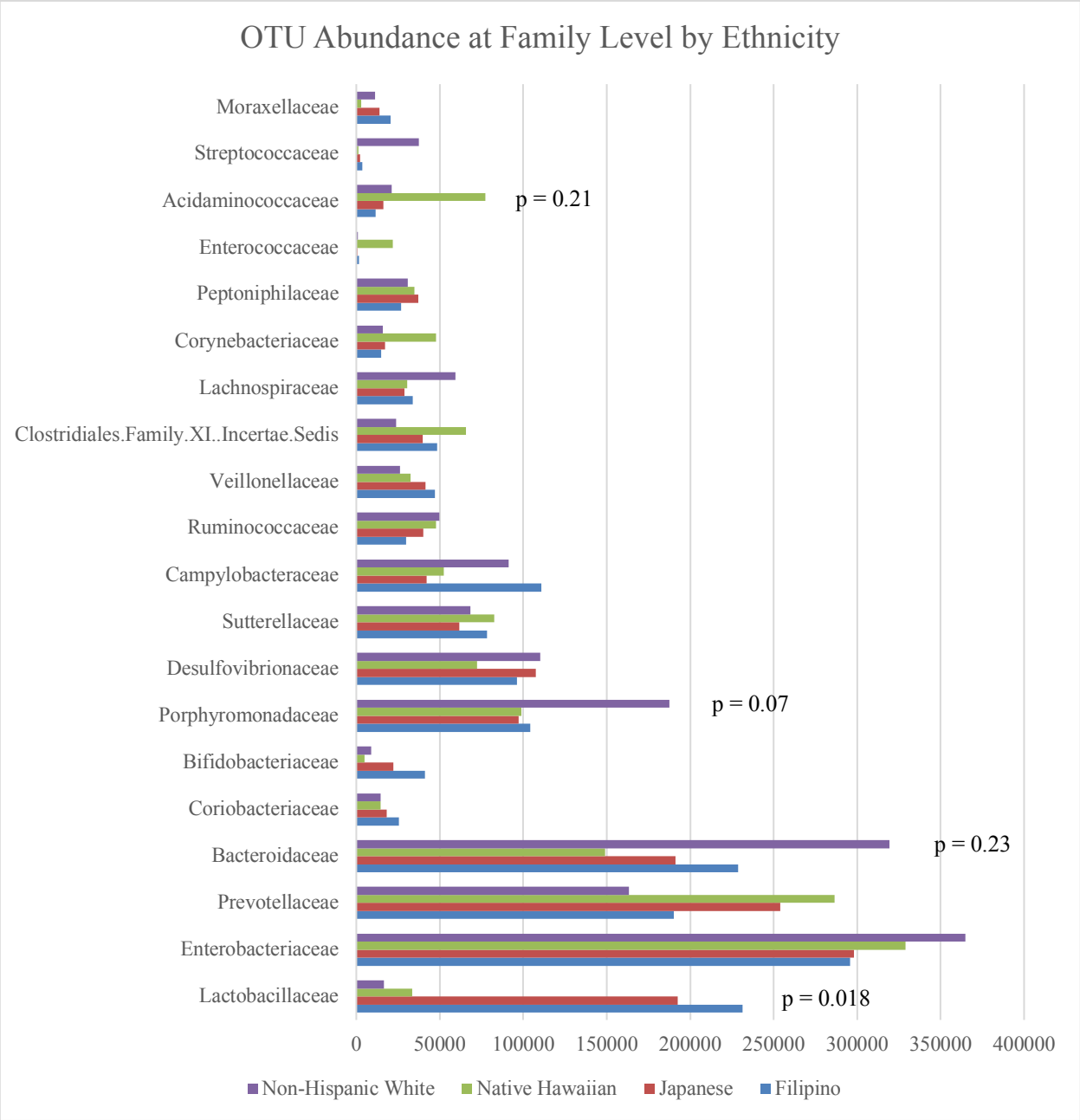

FIGURE 5: Relative abundance of the top 10 most abundant families by Ethnicity

We observed greater abundance of lactobacillaceae in Japanese and Filipino compared to Non-Hispanic White and Native Hawaiian participants (p = 0.018). Non-Hispanic White women tended to have higher Porphyromonadaceae (not significant (NS)). Native Hawaiians had higher

levels of Acidaminococcaceae (NS). Native Hawaiians also had the highest ratios of Prevotellaceae to Bacteroidaceae, vs. Non-Hispanic white women have the lowest ratios (NS).

Specific species were noted to be more abundant among particular ethnic groups, as listed in Table 5.

Species	p-value	Non-Hispanic White	Native Hawaiian	Japanese	Filipino
Flavonifractor_plautii	0.015	ab	b	a	b
Bacteroides_fragilis	0.012	b	b	ab	a
Peptostreptococcus_anaerobius	0.016	b	b	a	ab
Megasphaera_sp.	0.024	ab	b	a	ab
Bacteroides_coprophilus	0.027	a	a	a	a
Arcanobacterium_sp.	0.027	b	ab	ab	a
Butyricicoccus_pullicaecorum	0.026	b	ab	a	ab
Porphyromonas_asaccharolytica	0.013	a	b	ab	b
Phascolarctobacterium_faecium	0.036	a	ab	ab	b
Prevotella_sp.	0.008	ab	b	a	b
Prevotella_copri	0.023	b	a	ab	b
Bacteroides_caccae	0.024	a	b	b	ab
Streptococcus_intermedius	0.046	a	a	a	a
Eggerthella_lenta	0.037	a	a	a	a

TABLE 5: The relationship between species and ethnicity was evaluated using ANOVA test. Only species with significant relationships with the ethnicity groups are listed here, with ANOVA test, p-value <0.05. The letters in the table represents the groups of the ethnicity (if no letter is shared between two groups, then the species are significantly different among these two groups)

3.4b ALPHA DIVERSITY

Alpha diversity or within sample diversity was compared using Chao 1, Observed number of Taxa (OTUs), Simpson Index and Shannon Index. There were no differences in alpha diversity metrics among participants of obese versus non-obese participants, or among the different ethnic groups. Cohesively, there was a shift among all participants to have less diverse GIT microbial diversity in the third trimester. This was true for Chao1 Index, Shannon, Simpson and overall number of OTU abundance.

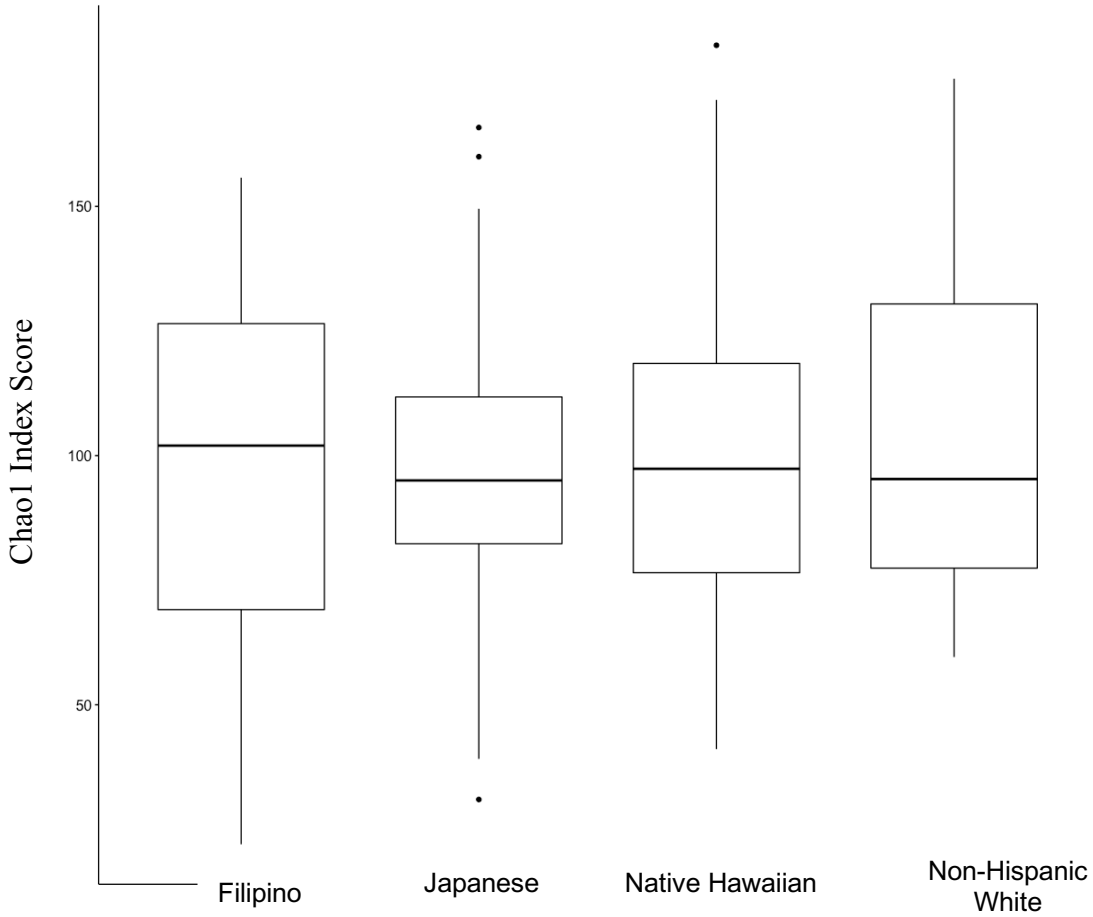

Figure 6 (A): Aggregate Chao1 Scores according to each ethnic group, (ANOVA p = 0.94)

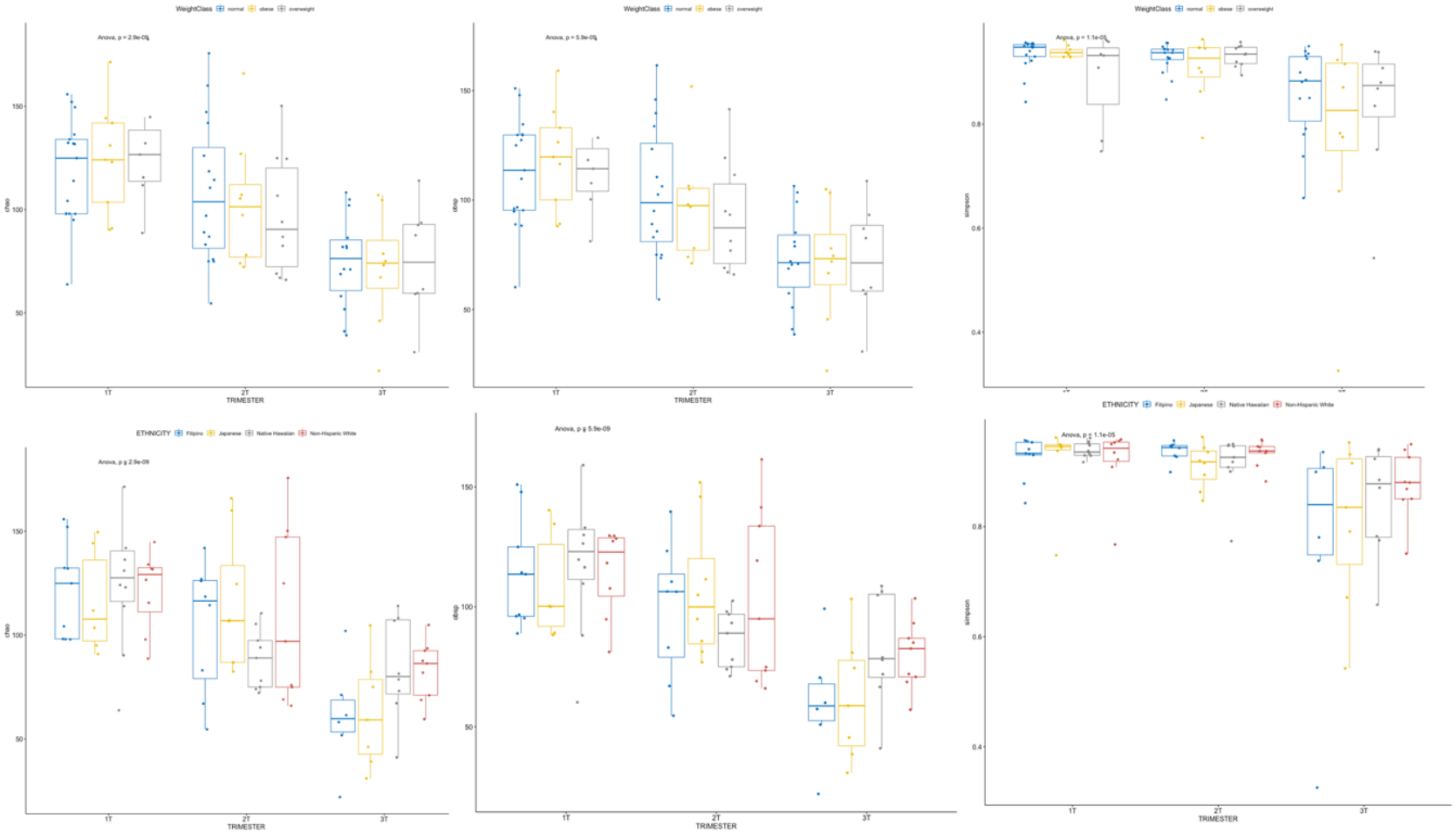
WeightClass normal obese overweight
Anova, p = 2.9e-09
Anova, p = 5.9e-09
Anova, p = 1.1e-05
ETHNICITY Filipino Japanese Native Hawaiian Non-Hispanic White
Anova, p = 2.9e-09
Anova, p = 5.9e-09
Anova, p = 1.1e-05
chao
obsp
simpson
TRIMESTER
1T
2T
3T

FIGURE 6 (B): Alpha Diversity profiles according to trimester for different ethnic groups and weight classes.

3.4c BETA DIVERSITY

Principal Component Analysis plots are shown to compare samples among different ethnic groups, among those who are obese vs. non-obese. There are no clear differences among ethnic groups or those who are obese versus non-obese. Third trimester samples do show a separate distance matrix in the PCA plot (Figure 7), demonstrating a shift of microbial composition at the end of gestation.

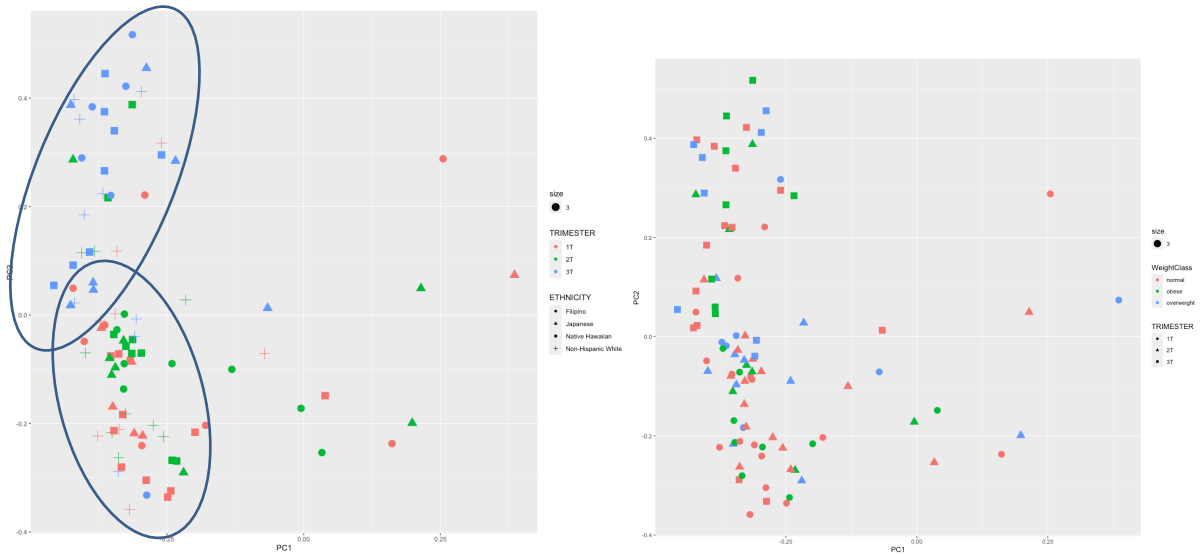

FIGURE 7: Principal Component Analysis according to Ethnicity (Panel A) and BMI (Panel B).

3.5 THE RELATIONSHIP OF DIET QUALITY TO MICROBIAL DIVERSITY

The primary planned outcome was linear correlation with alpha diversity metrics and HEI Score. There was no correlation with any of the diversity quality metrics, in either a linear fashion or by bivariate comparison of the highest and lowest HEI quartiles (not shown). Table 6

shows the correlation coefficient for all diversity metrics with combined HEI scores, irrespective of trimester. Figure 8 demonstrates this comparison in a scatterplot, with HEI score on the Y axis, and number of observed species on the x-axis. A multivariate linear mixed regression model was also performed, including covariates of age, parity, BMI and ethnicity with Observed Species being the dependent variable, and HEI score as the exposure with an interaction of time (Trimester). No significant correlation or covariate was produced from the model. Finally, Figure 9 demonstrates that there was no grouping of similar bacteria in those who had similar HEI scores.

Diversity index	**Rectal**	
	r	**p**
Chao1	0.104	0.316
Observed Species #	0.099	0.341
Shannon	-0.019	0.858
Simpson	0.034	0.740

TABLE 6. Correlation between HEI score and α-diversity indexes

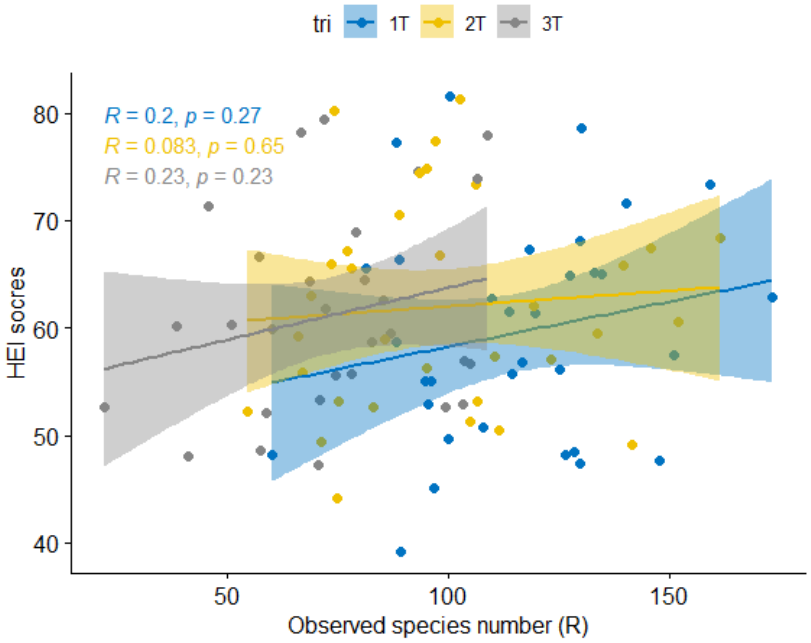

FIGURE 8. Linear relationship between observed species and HEI score, separated by trimester, where R is the correlation coefficient and p is the corresponding p-value.

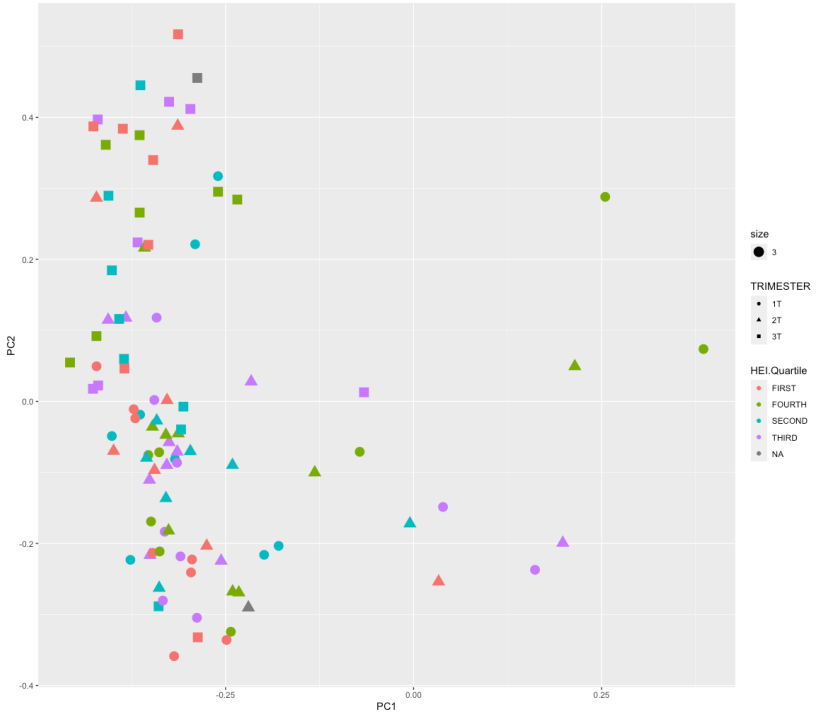

FIGURE 9: PCA Plot of all samples, coded according to HEI Quartile. HEI scores did not delineate similarities between groups.

Figure 10 shows Family abundance by HEI Quartiles. There is a greater abundance of lactobacillaceae in the those that scored the highest compared to than those with the lowest HEI scores. (p=0.02169). There is also a trend for higher diet quality being associated with increased Prevotellaceae. Specific species associated with the highest HEI quartile are listed in Table 7.

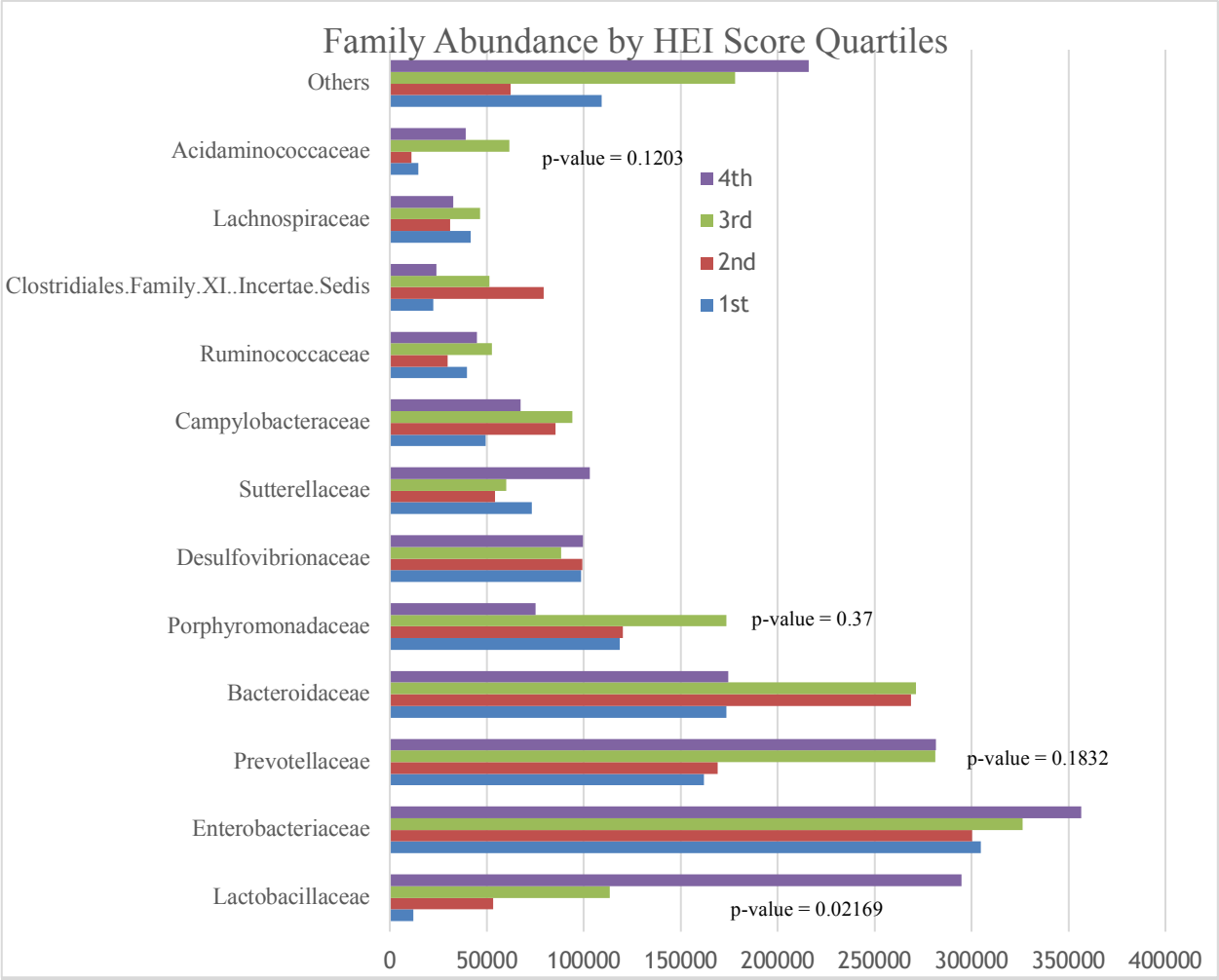

FIGURE 10: Top 10 families by relative abundance according to HEI Score.

Genus_Species	Correlation(r)	*p*-value
Mannheimia_varigena	0.3285	0.0012
Bacteroides_coprophilus	0.2527	0.0135
Haemophilus_parainfluenzae	0.2484	0.0152
Pseudobutyrivibrio_ruminis	0.2442	0.0171
Haemophilus_sputorum	0.2164	0.0352
Megamonas_funiformis	0.2146	0.0368
Prevotella_copri	0.2079	0.0432
Megamonas_rupellensis	0.2078	0.0433

TABLE 7. Species with significant correlations with the highest HEI quartile

4. DISCUSSION

The results demonstrate unique revelations about nutrition and microbial composition during pregnancy in Hawai‘i.

4.1 NUTRITION

Diet quality metrics help evaluate comprehensive attributes of diet, independent of the particular pattern (vegetarian, omnivore, etc). Most studies using diet quality scores in the United States have demonstrated a narrow distribution of scores, mostly dependent on socioeconomic status (68). While some studies compared major ethnicities in the Mainland United States, the unique makeup of Hawai‘i residents is infrequently represented in dietary research of pregnant women. (45, 49, 69). In Hawai‘i, the make-up of immigrants, first generation children, and a large heterogenous population of mixing cultures makes scores more homogeneous when comparing among ethnicities or race. Even so, studies using diet quality indices have used a larger sample size to detect differences between groups in diet quality. Our study showed a difference in aggregate diet quality scores among two of four ethnic populations: Native Hawaiians and Filipino. With further analysis, the largest difference between the two groups was during the third trimester. HEI scores increased throughout gestation in the Native Hawaiian

population versus Filipino population, which stayed stable. The change over time was not statistically different between ethnic groups. This finding differs from findings in the original multi-ethnic cohort, where Non-Hispanic White and Japanese men and women had the highest proportion of participants in the highest quintile of HEI scores (ranging from 79-100), compared to Latino, African American or Native Hawaiians. Lower quintile scores ranged from 21.2-60.3 (43).

Pregnant women in Hawai‘i have similar dietary quality intake as other mainland pregnant study cohorts. In a study by Yee et al., periconception quality was assessed at 6-13 weeks gestation. The ethnic make-of up of the study population was primarily Non-Hispanic White, Black, Hispanic, with only a small percentage self-reporting Asian race. The study population of almost 10,000 women among 8 US medical centers was collected from 2010 to 2013. The mean HEI score of the entire population was 63, with a standard deviation of 13, slightly higher than our cohort, with a mean of 59. They noted that periconceptual diet was associated with a small increase in poor pregnancy outcomes and supported the importance of improving micro and macronutrient intake of women of low socioeconomic status to improve these outcomes.

Rifas-Shiman et al., tracked diet quality in a large cohort of 1,543 women from the first to second trimester (69), using a modified HEI score. Changes observed in scores were attributed primarily to an increase in vitamins or supplements (35%), and not necessarily a change in macronutrients from different food groups. Our study is unique in tracking HEI diet quality over all three trimesters to look for a change during gestation. There are plausible reasons why diet quality would shift throughout gestation. In general, many women report changing their dietary habits during pregnancy, as they may feel affected by nausea or hyperemesis during the first

trimester and report eating more nutritiously later in the gestation. Women of Asian heritage sometimes report changing their diet to favor more hot or spicy foods at the end of pregnancy. Our study sought to determine whether diet quality changed over time. This change was not detected in the cohort aggregately. However, particular groups of participants – namely Native Hawaiians, progressively improved the HEI over time.

Overall, this study describes diet quality of a sampling of healthy pregnant women in Hawai‘i. There were no detectable differences among women of different ethnicities or body weight classes. This may reflect the multicultural influence that island life provides, with women consuming many different types of food that may or not match their cultural heritage. In other studies, the most predictive sociodemographic factor for diet quality in the United States was income status(45). We did not collect this information during our study and thus cannot reflect specifically about this.

4.2 MICROBIOME

The characterization of GIT microbial composition during pregnancy in the literature is varied. A few studies have demonstrated stable diversity throughout the entire pregnancy (70, 71). Yet, the majority of GIT microbiota research during pregnancy shows a shift in the third trimester to less diversity. An increase in abundance of lactic-acid producing bacteria such proteobacteria, bifidobacteria, and actinobacteria (72, 73) and a decline in butyrate-producing bacteria (59) is observed.

Our cohort followed this widely described transition to decreased diversity under the influence of hormonal shifts across gestation. There were significant differences in Shannon, Chao1, Simpson, and observed number of species from first trimester to third trimester. The changes were mostly represented in a difference in total abundance of lactobacillaceae

(p=0.013), prevotellaceae (0.03), and lachnospiracheae (0.003). This is commensurate with other published studies of pregnant populations (72) (57) and is evidence that sample collection and processing were adequately performed.

In examining how this multiethnic cohort compared to other mainland populations, only a few studies have compared microbial diversity of pregnant in various ethnic groups. In study in South Carolina, GIT microbiome was studied in early gestation and late gestation among 28 Non-Hispanic White, Hispanic, and Black women. Alpha diversity (Fischer, Shannon, observed species) were noted to decrease overtime, as expected. White women had greater abundance of Firmicutes and Bacteroidetes compared to non-White women (n=6). The authors also discovered differences in alpha diversity among nulliparous women versus multiparous women (72). Rothenberg and colleagues assessed differences in ethnic groups across gestation and demonstrated that beta diversity patterns differed by ethnicity, with distinct groups in PCA of Caucasian and Hispanic mothers, African-American and Hispanic mothers, and Caucasian and African-American mothers (74). However, PCA is unable to account for confounding variables, and such, differences may be been attributed to other sociodemographic factors. Looking at the gut microbiome observed in the HMP of non-pregnant individuals, alpha diversity varies across ethnicities, with the following ranks: Hispanics > Caucasians > Asian-Pacific Islanders > African Americans. In the HMP, there was a significantly lower Shannon diversity for Asian-Pacific Islanders relative to Caucasians and a trend of lower Shannon diversity for Asian-Pacific Islanders relative to Hispanics (75). Vice versa, our cohort did *not* show distinct grouping according to ethnicity. Participants self-reported their ethnicity, and while inclusion criteria included being 'half' of one particular ethnicity, the majority of participants reported other

smaller ‘proportions’ of ethnic make-up. The multi-ethnic heritage may contribute to a more homogeneous microbiota profile of pregnant women in Hawai‘i.

Compared to other cohorts of different ethnic make-up, the specific taxa of participants in Hawai‘i appears to differ from other mainland populations. The differences seen in our population were greater amounts of lactobacillaecae in Japanese and Filipino compared to Non-Hispanic White and Native Hawaiian participants. Non-Hispanic white women tended to have higher Porphyromonadaceae, and Native Hawaiians had higher levels of Acidaminococcaceae. Native Hawaiians also had the highest ratios of Prevotellaceae to Bacteroidaceae, vs. Non-Hispanic white women have the lowest ratios. Our characterization of the GIT microbiome in pregnancy is unique in that we characterized taxa down to the species level (72). Other studies of the GIT in pregnancy made comparisons at the phylum or family level. There were several species that were unique among two ethnic groups and differed from the other groups. While no one species was unique to a particular ethnic group, these findings help characterize the types of bacteria present in pregnant women of Hawai‘i .

It is difficult to determine if the microbiome of residents of Hawai‘i resemble that of pregnant women in Asia, as there are limited published studies available from across the Pacific. Shiozaki et al., in Japan, investigated the GIT microbiome at 28 weeks in women who did and did not go into preterm labor and have preterm birth. They describe differences in taxa at the family level (76), with those who did not have preterm delivery having high abundance of Clostridium subcluster XVIII, Clostridium cluster IV, Clostridium subcluster XIVa, and Bacteroides, and a significantly lower levels of lactobacillaecae. Interestingly in our study, Japanese women had very high levels of lactobacillaecae.

In discussing environmental factors of microbial diversity, the impact of obesity on the pregnancy microbiome is also influential. The effects of adiposity have been studied extensively and have a direct effect on the composition of gut microbiota. Gomez-Arrango et al., compared overweight (BMI 25-30) to Obese pregnant women (BMI >30). Investigators observed that overweight pregnant women tended to have a higher microbial richness (number of OTUs) and evenness (relative prevalence of the various OTUs within the gut) than obese pregnant women but, there was no difference in the total number of different taxa between the groups. Beta-diversity showed no significant differences between overweight and obese women in this cohort (59) In Finland, similar findings were observed in overweight and obese pregnant women at the phylum level (77). When comparing women of normal weight, to overweight and obese, we did not detect differences in the microbial composition in our cohort either.

4.3 DIETARY INTERACTIONS WITH THE MICROBIOME

Our primary outcome, a linear correlation with diet quality and microbial diversity, was not observed (Table 6). The most likely reason for this negative finding is not enough power to detect a correlation. Previous studies comparing diet quality and microbial diversity have not used continuous variables as the primary outcome, but instead used a binary exposure of high diet quality (or those in the highest quartile or tercile) versus low diet quality (those in the lowest quartile or tercile of scores). These were much larger cohorts and with this methodology, they detected small but significant differences in those with high versus low diet quality. A linear correlation was chosen for this study with the postulation that even within a smaller sample size (due to funding constraints), we would be able to detect a difference. As diversity is known to change at baseline across pregnancy, the correlation was also compared within each trimester (Figure 8), and there was no statistical correlation in any of the trimesters demonstrated.

Looking at secondary analysis of the data collected, we can identify other associations within our cohort of dietary intake and microbial diversity that are unique to pregnant women of Hawai‘i. First, those with the highest diet quality scores (3rd and 4th quartiles) had higher abundance of lactobacillaece, prevotellaceae and acidaminoaceae. Bacteroides were high in the 2nd and 3rd quartiles, with very low abundance for those with the lowest diet quality scores.

At the species level, several species were noted to be more abundant in those with the highest HEI score. While the literature is limited in descriptions of how these organisms impact human health, some of the identified species have been described in patients with autoimmune or inflammatory conditions (78, 79). Haemophilius parainfluenzae, for instance, is generally thought to be pathogenic, but one study looking at GIT microbial dysbiosis in children with Autism Spectrum disorder, noted a lower abundance of this organism in children with ASD versus those without. Megamonas species from the Acidaminococcaceae and Verimellaceae families are known to produce short chain fatty acids (80), and described to be possibly influential in protecting against Methicillin Resistant Staphylococcus Aureus (MRSA) (81). Determining any clinical significance of specific species is limited, as efforts in describing these organisms in human health is still new and exploratory, yet lays the foundation for future areas of research.

Looking at the specific enterotypes represented in this cohort, it is interesting to see that Prevotella was highly abundant in participants with higher diet quality scores (64). A cross-sectional analysis of dietary information and the gut microbiome in humans showed that the Prevotella-dominant enterotype is associated with high intake of fiber, carbohydrate, and simple sugars, whereas Bacteroides-dominant enterotype is associated with the high intake of animal fat and protein (82). De Fillipo et al. demonstrated that the composition of the intestinal microbiota

differs significantly between children living in a rural African village in Burkina Faso and those living in Europe. Children in Burkina Faso, presumably eating a more agrarian diet, had greater amounts of Prevotella, lower amounts of Bacteroides, overall greater microbial richness, and produced higher levels of short-chain fatty acids than the microbiota of European children, who were eating a more westernized diet (83). Martinez et al. compared gut microbiota of people in Papua New Guinea to participants in urban Nebraska. Papua New Guineans had similarities to other subsistence dwellers, such as the presence of a high ratio of Prevotella relative to Bacteriodes, which is common in people consuming traditional diets where there is a high dependence on fibrous, plant-derived foods found in the Pacific (84). Certainly, these comparisons hone in on dietary patterns, such as Westernized diet versus traditional diets comprised of less animal proteins and higher fiber.

This opens up room for further investigation: Native Hawaiians had the highest amounts of Prevotella compared to Bacteroides in our cohort, and had the highest diet quality scores as well. Yet, this population also has some of the highest adverse pregnancy outcomes in our community. Further understanding these relationships may help shed light on systemic inflammation leading to disparate birth outcomes.

5. CONCLUSIONS

5.1 LIMITATIONS

Certainly, the largest limitation of this study was the small sample size, not allowing us to determine if our null hypothesis could truly be accepted. This was due to funding, as well as refining DNA extraction and library preparation in the early stages of the project, thus losing 5 microbiome samples from the first trimester. Other limitations include inherent recall bias by

using a Food Frequency Questionnaire. However, FFQs are often structured to have patients recall what they eat over a predetermined time period. FFQs have been shown to be as accurate as 24-hour recall in correlation with biologic specimens showing metabolites and nutrients (40). The benefit of using this FFQ is it is validated within our unique population, but has not specifically been studied in pregnancy. Finally, it is possible that another diet quality measure may have been more predictive of microbial diversity, such as the Alternate Mediterranean Diet index, which directly assesses the amounts of polyunsaturated and monosaturated fats consumed. However, in a study by Bowyer et al, the HEI was compared to other dietary indices and found to be the most predictive of microbial diversity (62). Latilen found a significant association (spearman correlation rho = 0.3, p=0.003) with their predetermined diet quality index (IDQ) in their validated index of diet quality at 14 weeks gestation (64). Our general findings are in line with expected findings from other populations in terms of range of diet quality and alpha diversity profiles, demonstrating the feasibility and success in executing this project.

Ultimately, microbial diversity is comprised of several environmental factors including adiposity, environment, geography and diet. The only determinant observed in our cohort was pregnancy itself, with the hormonal changes leading to an expected decrease in diversity over time. While other studies have demonstrated that diet has more of an influence on microbial composition than body mass index, this was masked in our study by the pregnant cohort (9).

The strengths of this study include it being the first-time diet quality and microbial composition has been described in pregnant women in Hawai‘i. This study lays a ground work for future investigation.

5.2 FUTURE DIRECTIONS

Ultimately, the goal of these endeavors is to better understand normal pregnancy health, and aberrations that lead to adverse pregnancy outcomes. While gut or other commensal microbes may not be the primary contributor to disease processes, the network that they play into of adiposity, genetics, ethnicity, geography, and other environmental factors may be modifiable and thus translatable into intervention. A recent study to evaluate the relationships among maternal weight, gut microbiome, blood pressure, and plasminogen activator inhibitor-1 (PAI-1) levels in overweight and obese pregnant women found the abundance of butyrate-producing bacteria to be significantly negatively associated with systolic and diastolic blood pressure and PAI-1 levels (56). These findings suggest increasing butyrate-producing bacteria may contribute to lower BP in overweight and obese pregnant women. Studies of omega-3 long-chain polyunsaturated fatty acids (PUFAs) also suggest protection of the intestinal wall through strengthening of cellular connections (85). This project is foundational to refining methodology and understanding the feasibility of enlarging such a cohort to future directions.

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