**Association of Maternal Obesity with Infant Gut Microbiota: Evidence from Cebu, Philippines**

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**Abstract**

**Objective:** The objective of this study was to investigate the correlation between maternal obesity and infant gut microbiota. It was designed to test the hypothesis that infants born from obese mothers have different gut microbiotas than those born from non-obese mothers, which may impact the health of the infant during later stages of their life.

**Methods:** The study was conducted on a cohort of Filipino women and their newborn children taking part in the Cebu Longitudinal Health and Nutrition Survey (CLHNS). Fecal samples from pregnant and non-pregnant "index children" of the cohort were collected from November 2017 through February 2020, resulting in a total of 106 distinct samples. When these pregnant women gave birth, infant fecal samples were taken both at two weeks of age and six months of age for a total of 93 samples. DNA was extracted from ethanol-preserved fecal samples and a two-step PCR was used to amplify the V4 region of the 16S rRNA gene in order to generate amplicon data describing microbial communities. Sequence data were quality-filtered and denoised using the bioinformatic platform QIIME2, and the resulting data were analyzed using the statistical programming software R with the goal of associating infant gut microbiota with maternal obesity.

**Results:** PERMANOVA tests comparing infant microbiome composition across maternal BMI groups (i.e. underweight, normal, overweight/obese) were not statistically significant (p=.28), and the association only slightly increases when controlling for geography (p=.25). ANOVA tests revealed no statistically significant differences in infant gut microbiota diversity between the maternal BMI groups ($F_2 = 0.778$). While these findings do not support a largescale effect of maternal BMI on the infant gut microbiome, a few individual taxa were affected by overweight/obese and underweight maternal BMI status.

**Conclusion:** The effect of maternal BMI on the infant gut microbiome does not appear to be substantial in this sample of Filipino mothers and infants. While some individual taxa do appear to be impacted by maternal BMI status, the mechanisms through which this occurs remains unclear.

## Introduction

Human evolution has taken place within a microbial context, and throughout this coevolution, microbial communities (or microbiota) have become integral components of human biology and physiology.[1, 2] Microbiota in the human gastrointestinal tract (gut) is one such example, where microbiota enable key functions including the development and regulation of the immune system, nutrient absorption and metabolism, and brain function (e.g. cognition and social behavior).[1-5] The delicate symbiotic relationship between gut commensal microbiota and the human host is a reminder of the important role microbial communities play in human health as a result of their abundance, diversity and distribution.[2, 6] However, host-microbiota relationships are not immune to external influence and are easily disrupted by host factors such as diet, lifestyle habits, and antibiotic use.[7, 8] The resulting 'dysbiosis', or microbial imbalance[8] can lead to downstream negative health outcomes in humans.[1, 6-8] For example, a phenomenon known as the "fiber gap" is observable when populations transitioning to an industrialized economy abandon traditional, high-fiber foods for the "Western Diet," which is high in saturated fats and sugar.[1] This "nutritional transition"[9] results in a substantial decline in the diversity of the gut microbiota and the relative abundance of specific microbial taxa and their metabolites, ultimately putting people at risk for developing non-communicable diseases (NCDs) like obesity, diabetes, and cardiovascular disease (CVD) later in life.[1, 6-10] These findings reinforce the importance of gut microbiota in understanding the mechanisms through which the environment influences human health, especially the progression and onset of chronic diseases.

### Microbiome and Obesity

Many studies have observed correlations with diet, obesity, and the abundance of microbes capable of efficiently digesting both plant-based foods and animal-derived products. One such study by De Filippo et al. (2010) compared the fecal microbiota of children from Burkina Faso (BF) to children of Italy (EU), who consume a more westernized diet that is high in animal protein, fat, and sugar. The researchers discovered that BF children had a greater abundance of Bacteroidetes, a class of bacteria capable of efficiently digesting complex carbohydrates which ferment into SCFAs and confer protection from inflammation and colonic disease; whereas the EU children had a depletion of Bacteroidetes and enrichment of Firmicutes, which are better suited for digesting simple sugars and animal-derived products but are limited in their ability to ferment carbohydrates into SCFAs in the gut.[8] Based on these findings, De Filippo et al. (2010) concluded that the greater abundance of Firmicutes in the EU children, most likely resulting from their westernized diet, may predispose this population to obesity and metabolic disease in the future. These differences highlight the importance of not only diet, but also geography, in the composition of microbial communities among populations with varying genetic, geographical, and cultural origins.

Research comparing the microbiota of twins has also revealed the impact of shared early-environment exposures on the gut microbiome.[11, 12] In a study by Turnbaugh et al. (2009) conducted in Missouri, the fecal microbiota of 31 monozygotic and 23 dizygotic twins and their mothers were genotyped. Researchers discovered that while each individual's gut microbiota varied, individuals from the same family shared a wide array of microbial genotypes and had a more similar microbial community than unrelated individuals.[11] Based on these findings, the researchers suggested that, at the gene level, there exists a core microbiome that plays an important functional role in humans and deviations from this core may lead to altered

physiological states, such as obesity.[11, 12] Furthermore, when comparing obese and lean twins, obesity was associated with a higher abundance of Actinobacteria and lower abundance of Bacteroidetes.[11] As demonstrated in both human and mouse studies,[11-15] Actinobacteria has shown to be more efficient at extracting energy from food. Moreover, these microbes are often abundant in the gut of overweight and obese individuals.[13] Together, these findings support the notion that obesity is associated with reduced microbiota composition and diversity, and further demonstrates the influence that diet and gut microbiota have on the development of NCDs, like obesity, in humans.

Maternal Obesity and Infant Health

The Developmental Origins of Health and Disease (DOHaD) framework suggests that negative early life environments are followed by an adult physiology that is more susceptible to poor health outcomes.[16] The relationship between maternal and infant health provides a unique opportunity to understand the DOHaD framework in practice because it explains how disruptions in early life environments, such as nutritional input or maternal metabolic status, can directly influence adult health. One example is the "thrifty phenotype hypothesis", which was proposed in an attempt to explain the associations between poor fetal and infant growth and increased risk for type 2 diabetes and metabolic syndrome in adulthood.[17] As several supporting studies have demonstrated,[17-20] the quantity and quality of nutrition the fetus receives from the mother greatly influences fetal development, such that deficiencies during this crucial developmental period shape functional and metabolic abnormalities seen later in life.[18] In this manner, nutrition deficits compromise fetal health and result in poor health outcomes in adulthood, as the DOHaD framework would predict.

The relationship between maternal and infant health can be further assessed when viewed through the lens of obesity. During pregnancy, metabolic changes occur to prepare the mother's body for the energetic demands of lactation, such as increasing adipose tissue that promotes insulin resistance.[21, 22] For women who are obese, these metabolic changes are more likely to lead to the development of gestational diabetes mellitus (GDM).[22, 23] GDM during pregnancy increases fuel availability for the fetus,[22] but at a great cost to both mother and child. For GDM women, this series of metabolic changes most often results in the development of type 2 diabetes in the first decade after delivery[22]; and for the developing fetus, increased risk for stillbirth, obesity, cardiovascular disease, and/or glucose intolerance is common.[22, 23] Furthermore, maternal obesity is an increased risk factor for childhood obesity. [23] Understanding the mechanisms by which offspring respond to environmental stimulus, such as nutrition or maternal obesity, can help identify targeted approaches to improve health outcomes for humans and inform future public health research in maternal and infant health.

Maternal Influence on Infant Gut Microbiome

There is much evidence to support that maternal-offspring exchanges of microbiota shape the natural colonization and maturation of the infant gut.[24] For example, studies investigating the association of delivery mode and infant gut microbiota showed that vaginally delivered infants acquired communities resembling the mother's vaginal communities (e.g. Lactobacillus, Prevotella); whereas infants born via Cesarean section (C-section) fostered communities similar to those found on mother's skin (e.g. Staphylococcus, Corynebacterium). [24-26] As these studies show, an infant's first onslaught of microbiota is entirely dependent on their birth environment. Additionally, the microbiota of infants born to mothers who consumed a high-fat diet during

pregnancy were more likely to display gut dysbiosis that favored increased selection pressures against commensal species like Bacteroides, which may suggest a heightened risk for developing obesity in adulthood.[27] Furthermore, antibiotics during pregnancy are associated with a substantial loss in microbiota diversity and increased risk of obesity and asthma among infants.[28-30] Breastfeeding practices have also been shown to play a critical and beneficial role in the establishment of the infant immune system through the maternal transfer of commensal microbiota.[31, 32] Although far from conclusive, the current body of evidence points to an association between early life environments and increased risk for NCDs, like obesity, likely mediated through microbiota. Together these findings highlight the importance of maternal-offspring exchanges of microbiota in shaping infant physiology and immune function and emphasize the important role lifestyle factors such as delivery mode, diet, antibiotic use, and breastfeeding play in influencing the composition and diversity of microbial communities in infants.

Objective

The objective of this study was to investigate the correlation between maternal obesity and the infant gut microbiota. It was designed to test the hypothesis that infants born from obese mothers have different gut microbiotas than those born from non-obese mothers (e.g. reduced diversity), which may impact the health of the infant during later stages of their life.

Public Health Relevance

Results of this research could have major implications for the field of public health, especially in regard to maternal and infant health. To begin, analyzing microbiota data from

184 infants and their mothers allows us to assess how the gut microbiota of biologically related
185 individuals compare. It also provides insight into the influence of maternal obesity on the
186 development of the infants' microbiome. Furthermore, comparing microbial communities in
187 infants across maternal BMI groups contributes to the growing volume of epigenetic data
188 surrounding environmental influence on human biology. If certain microbiota is found to be
189 significantly associated with obesity, health care providers and the public can take responsible
190 steps to reduce maternal and infants' risk. Identifying possible risk factors is an important first
191 step toward establishing treatment and prevention for obesity.
192
193 **Methods**
194 Study Population, Sample Collection, and Study Design
195 This study was conducted on a cohort of Filipino women and their newborn children
196 taking part in the Cebu Longitudinal Health and Nutrition Survey (CLHNS). The CLHNS is a
197 prospective cohort study of women who were born between May 1, 1983 and April 30, 1984 in
198 rural and urban municipalities in Cebu, Philippines[33]. These women (known as "index child" or
199 IC), their mothers and their children (known as "index child's child" or ICC), caretakers, and
200 other household members have participated in survey data collection at various timepoints
201 throughout the study, resulting in a comprehensive dataset detailing information on diet,
202 environment, socioeconomic status, and physiology[34-37].
203 Fecal samples from pregnant and non-pregnant "index children" of the cohort – now
204 adult women aged 36 and 37 – were collected from November 2017 through February 2020,
205 resulting in a total of 106 distinct samples. When these pregnant women gave birth (n=53), infant
206 fecal samples were taken both at two weeks of age and six months of age for a total of 93 ICC

samples. All samples were stored in 95% ethanol within six hours of production and shipped from Cebu, Philippines over the course of three years to the Amato laboratory at Northwestern University and were stored at -80°C until processing for DNA extraction.

Clinical information collected on the "index children" included: reproductive status (pregnant, non-pregnant); age; height; weight; smoking status (smoker, non-smoker); BMI; total kcal intake; triceps skinfold; and geography (rural, urban). Clinical information collected on the infants (ICC) born to these "index children" included: sex, height, weight, and geography.

Ethics

The original CLHNS study protocols were approved by the University of Pennsylvania IRB, and written informed consent was obtained from all study participants. Consent forms, patient surveys, fecal sample collection, and related materials for this specific sample collection effort were approved by the Northwestern University Institutional Review Board (IRB), approval number STU00205714. Data analysis of the fecal samples was considered exempt under Northwestern IRB, as all samples were de-identified.

Fecal DNA Extraction

DNA was extracted from ethanol-preserved fecal samples using the DNeasy Powersoil Pro Kit protocol (Qiagen Inc, Valencia, CA, USA) at Northwestern University, Evanston, Illinois according to the manufacturer's instructions and without modification. For each sample, 0.25g of fecal matter was added to PowerBead tubes. The homogenate was pelleted and incubated at 65°C with 60 µl of Solution C1, vortexed at maximum speed, and centrifuged at 10,000 g. The resulting supernatant was transferred to a clean 2 ml collection tube containing 250 µl of

Solution C2, vortexed, incubated at 4°C, and centrifuged at 10,000 g. This process was repeated with the supernatant from the prior step using 200 µl of Solution C3. Then, 750 µl of the supernatant was transferred to a clean 2 ml collection tube containing 1.2 ml of Solution C4 and briefly vortexed. In order to bind the DNA to a spin column filter, 675 µl of the supernatant was added to the spin column, centrifuged at 10,000 g, and the remaining flow through discarded. This process was repeated three times before the column was rinsed with 500 µl of Solution C5 and centrifuged twice at 10,000 g. Finally, the spin filter was placed into a clean 2 ml collection tube and 100 µl of Solution C6 was added to the filter membrane and centrifuged. The eluted DNA was stored at -80°C until use in PCR.

PCR and Microbiome Sequencing

Using a modified version of the Earth Microbiome Project protocol[38, 39] and the 515Fa/926R primer set,[40] a two-step PCR was used to amplify the V4 region of the 16S rRNA gene in order to generate amplicon data describing microbial communities. Amplicons were barcoded and pooled in equimolar concentrations for sequencing on Illumina MiSeq V3 (Illumina, San Diego, CA, USA) by the University of Illinois Chicago (UIC) Sequencing Core under the supervision of Dr. Stefan Green at a depth of at least 20,000 sequences per sample

Bioinformatic Analysis using QIIME2

Raw sequence data were quality-filtered, paired-end sequences were joined, and amplicon sequence variants (ASVs) were denoised using the QIIME2 v2019.4 wrapper for DADA2.[41] A total of 2,612,351 reads with an average of 11,508 reads per sample were generated. We then assigned taxonomy using a Naive Bayes classifier trained on the Greengenes

13_8 99% OTU database using the full 16S rRNA gene sequence lengths. Reads mapping to chloroplast and mitochondria sequences were removed. We calculated the Shannon and Faith's Phylogenetic diversity measures, as well as unweighted and weighted UniFrac distance matrices describing pairwise similarity between samples, using the core-metrics-phylogenetic command, rarefying the data to 7450 reads per sample to ensure maximum sample retainment.

Statistical Analysis using R

The resulting sequence data and clinical information were analyzed using the statistical programming software R with the goal of testing the significance of the association of infant gut microbiota with maternal obesity. Maternal pre-pregnancy BMI's were categorized according to the World Health Organization (WHO) guidelines (underweight [<18.5 $kg/m^2$], normal weight [18.5–24.9 $kg/m^2$], overweight [25.0–29.9 $kg/m^2$], and obesity [≥30.0 $kg/m^2$]). However, due to the relatively low prevalence of obesity among this sample, we decided to group overweight and obese individuals (BMI≥25.0 $kg/m^2$) for a total of three maternal BMI categories.

To test for differences in the gut microbiome composition of infants born to obese and non-obese mothers, we ran a permutational analysis of variance (PERMANOVA) with both the unweighted UniFrac and weighted UniFrac distances with 5000 permutations using the adonis function of the vegan package for R, which is used for analyzing the diversity of ecological communities.[42] This included testing the effect of multiple variables on overall gut microbiome composition, including maternal BMI status (underweight, normal, overweight/obese), geography (rural/urban), maternal energy intake (kcal/day), maternal smoking status (smoker/non-smoker), and maternal triceps skinfold measurements. We ran each variable individually in the model to determine significance. Variables whose association had a

significance of .1 or below were included in the final model. Analysis of the variables maternal smoking status, maternal total energy intake, and maternal triceps skin fold measurement revealed no association with infant gut microbiome composition, so these variables were not included in the final model. However, we did control for geography (p=.09).

Differences in gut microbial diversity between the maternal BMI groups were assessed using a one-way analysis of variance (ANOVA) by means of the Shannon diversity index, which accounts for both abundance and evenness of the microbial taxa present.[43] We tested for differences in the relative proportions of specific microbial taxa using a linear discriminant analysis (LDA) of effect size (LEfSe) method[44] at the sequence variant, genus, family and phylum levels on the web-based genome analysis tool Galaxy[45] using an effect size of 2.0.

## Results

### Sample Characteristics

Characteristics of the 53 pregnant women and 93 infant samples with complete clinical data are noted in **Table 1**. As shown, the median maternal BMI was 21.0 $kg/m^2$, which is considered normal according to WHO standards. There is also a low prevalence of overweight and obese individuals among this population, with only six women (11.3%) falling into this category.

### Microbiome Composition and Diversity

Maternal BMI was not significantly associated with overall infant gut microbiome composition. **Table 2** presents comparisons of infant microbiome composition across categorical maternal BMI groups (i.e., underweight, normal, overweight/obese). Comparisons were not

299 statistically significant (p=.28), and the association with maternal BMI only slightly increases
300 when controlling for geography (p=.25; **Table 3**). These findings suggest the gut microbiota of
301 infants born to mothers of different physical nutritional status (i.e., underweight, normal, or
302 overweight/obese) are not significantly different.
303 There were no statistically significant differences in infant gut microbiota diversity
304 between the maternal BMI groups (**Table 4;** $F_2 = 0.778$). Together these findings suggest that the
305 overall composition and diversity of gut microbiota of children born to mothers with varying
306 BMIs in this Filipino cohort do not significantly differ.
307
308 Relative Abundance of Microbial Taxa
309 We found differentially abundant microbial taxa between infant groups with mothers
310 whose BMIs were categorized as underweight and overweight/obese. At the genus, family, and
311 phylum levels, there was one taxon enriched in each group (**Figure 1**), but at the strain/ASV
312 level there were two taxa enriched in each group, which are identified in **Table 5** below. These
313 findings suggest that infants born to mothers with normal BMIs were not significantly different
314 from the other two groups. Further, at the genus, family, and phylum levels, infants born to
315 mothers with overweight/obese BMIs had a greater abundance of Bacteroidetes in their gut
316 microbiota; whereas infants born to mothers with underweight BMIs had a greater abundance of
317 Proteobacteria.
318
319 **Discussion**
320 Our findings reveal that infant gut microbiota composition and diversity across maternal
321 BMI groups (i.e., underweight, normal, overweight/obese) in this Filipino cohort did not

significantly differ (see Table 3-4). Therefore, these findings do not support the hypothesis that infants born from obese mothers have different gut microbiota than infants born from non-obese mothers. Similar studies regarding this topic have reached conflicting conclusions.[46-50] For example, a study by Sugino et al. (2019) observed no difference in alpha diversity of infant gut microbiota by maternal pre-pregnancy BMI category. However, Galley et al. (2014) found higher alpha diversity and lower beta diversity in children of obese mothers when compared to children with non-obese mothers, and Singh et al. (2020) observed that maternal overweight and obesity were associated with greater infant gut microbiota diversity. Results of our study were likely influenced by the low prevalence of obesity among this population (see Table 1), which may have limited our ability to detect an effect of maternal BMI on infant gut microbiota composition and diversity. Another factor to consider is the widely documented finding that Asian populations have a relatively higher percent body fat and lower BMIs.[51-54] Therefore, measuring obesity by an excess of body fat and not an excess of weight would result in reduced cut-off points for obesity in Asian populations[52] and likely would have influenced these results.

The results of this study are important for research investigating the epigenetic inheritance of microbial communities because it questions the transmissibility of the microbiome and the degree and route in which microbial communities are conferred from mother to child. Prior research has established that factors such as obesity,[55-57] antibiotics,[57-58] and mode of delivery[58-59] influence the microbiome. Therefore, in our study, one would expect to see differences in the gut microbiota of the infants separated by maternal BMI categories. Since these differences were not found, this suggests that transmission of maternal gut microbiota to children may be less than we think or there may be other routes of vertical microbial transmission that influence infant microbiome development that were not considered here.[60]

Nevertheless, while our findings do not support a largescale effect of maternal BMI on the infant gut microbiome, there were a few individual taxa that are affected by maternal BMI status (e.g., overweight/obese and underweight) (see Table 5). For example, while the bacteria *Sutterella* was identified in both maternal BMI categories, the underweight BMI group had a greater relative abundance of *Desulfovibrio*, and the overweight/obese BMI group had a greater relative abundance of *Prevotella copri*. These patterns could have health implications for infants. For example, *Desulfovibrio* is a genus of sulfate-reducing bacteria[61] belonging to the phyla *Proteobacteria* that has been associated with human infections like ulcerative colitis, inflammatory bowel disease, and bacteremia.[62-66] However, the true incidence of infection by this bacterium may be underreported due to its slow growth and challenging culture and identification.[62] Conversely, *P. copri* is a genus of bacteria belonging to the phylum *Bacteroidetes* with conflicting accounts of associations with human health.[67-71] For instance, *P. copri* has been associated with gut dysbiosis in individuals with rheumatoid arthritis,[68-69] while other studies have associated *P. copri* with improved glucose and insulin tolerance in individuals who consume a high-fiber diet. [70] Furthermore, *P. copri* is more prevalent in non-Westernized populations, suggesting that differences in diet may be driving reduced prevalence in Westernized populations.[71] Therefore, it is possible that these taxa have important effects on host physiology. However, whether or not differences in the relative abundances of *Desulfovibrio* and *P. copri* in our population result in distinct health outcomes remains to be seen.

Despite these findings, our study could not determine whether maternal BMI is influencing these infant gut taxa directly or whether some other associated but unmeasured factor was driving the minor association that was observed. For instance, differences in breast milk composition due to maternal diet may influence the diversity and composition of the infant gut

368 microbiome in breastfed infants.[72-74] Therefore, additional research is necessary to elucidate the
369 correlation between maternal obesity and the infant gut microbiota and whether feeding
370 practices, like breastfeeding and bottle feeding, drive this association.
371 Findings from this research may have public health implications. To begin, our study did
372 not reveal correlations with maternal obesity and infant gut microbiome composition and
373 diversity, nor did it identify microbiota that is significantly associated with obesity. Therefore,
374 more work is needed to determine the risk that microbiota pose to the development of obesity in
375 humans and what steps healthcare providers and the public will need to take in order to reduce
376 this risk. Additionally, more research is needed to understand the microbial mechanisms for
377 environmental effects on health, diet and obesity and how this contributes to the progression and
378 onset of chronic disease in humans. Future work investigating the impact of early-environment
379 exposures on health will need to consider the DOHaD framework in order to identify targeted
380 approaches to improve health outcomes for humans and inform future public health research in
381 maternal and infant health.
382 It is important to note that our study had several limitations. First, maternal BMI was
383 based on data from 2005; therefore, the relationship between current maternal BMI and infant
384 gut microbiome composition and diversity may be different than reported here. Moreover,
385 maternal BMI was analyzed as a categorical rather than continuous variable, and some maternal
386 BMI information was missing. Future analyses may wish to consider other measures of adiposity
387 that were not investigated here. Additionally, BMI cutoffs were based on Westernized standards
388 and were not ethnic-specific,[53-54] which may have influenced the true prevalence of overweight
389 and obesity among this population. Lastly, the rarefaction depth of the sequence data can
390 produce a systemic bias because rare taxa are less likely to be detected at lower sequencing

depths, and statistical power is reduced when samples with low read depth are eliminated from the data set. While we did maintain a rarefaction depth of 7450 reads in order to maintain the greatest number of samples, the exclusion of samples (n=5) with less than 7450 reads reduced the sample size and may impact the generalizability of these data.

## Conclusion

In conclusion, the effect of maternal BMI on the infant gut microbiome does not appear to be substantial in this sample of Filipino mothers and infants. While some individual taxa do appear to be impacted by maternal BMI status, the mechanisms through which this occurs remains unclear. Future studies examining the relationship between maternal diet and components of breast milk on the infant gut microbiome may offer insight into the mechanisms by which maternal obesity influences specific infant gut taxon abundances. Finally, utilization of ethnic-specific BMI cut-offs and alternate measures of adiposity may provide further insight into the effect that maternal obesity has on infant gut microbiota composition and diversity.

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## Tables and Figures

**Table 1.** Sample characteristics of participants.

| Variable | Pregnant Mothers (n=53) | Infants 2 weeks (n=45) | Infants 6 months (n=48) |
|---|---|---|---|
| **Female, *n* (%)** | 53 (100.0) | - | - |
| **Height (cm), mean ± SD** | 150.3 ± 0.8 | - | - |
| **Weight (kg), mean ± SD** | 47.6 ± 1.7 | - | - |
| **BMI (kg/m2), mean ± SD** | 21.0 ± 0.6 | - | - |
| **Overweight/obese, *n* (%)** | 6 (11.3) | - | - |
| **Rural, *n* (%)** | 16 (30.2) | 12 (26.7) | 12 (25.0) |
| **Smoker, *n* (%)** | 5 (9.4) | - | - |
| **Total Energy Intake (kcal), mean ± SD** | 1428.9 ± 101.5 | - | - |
| **Triceps Skinfold (cm), mean ± SD** | 21.5 ± 0.9 | - | - |

**Missing data:** height, n=5 (9.4%); weight, n=10 (18.9%); BMI, n=7 (13.2%); smoker, n=1 (1.9%); total energy intake, n=5 (9.4%); triceps skinfold, n=5 (9.4%).

**Table 2.** Results of PERMANOVA comparing infant gut microbiota composition across maternal BMI groups.

| | Df | F value | p-value |
|---|---|---|---|
| **Body Mass Index (BMI)** | 2 | 1.1062 | 0.2791 |

**Table 3.** Results of PERMANOVA comparing infant gut microbiota composition across maternal BMI groups while controlling for geography.

| | Df | F value | p-value |
|---|---|---|---|
| **Geography** | 1 | 1.53 | 0.09 |
| **BMI*Geography** | 2 | 1.14 | 0.25 |

**Table 4.** Results of ANOVA comparing infant gut microbiota diversity across maternal BMI groups.

| | Df | F value | p-value |
|---|---|---|---|
| **Shannon Diversity + BMI** | 2 | 0.78 | 0.46 |

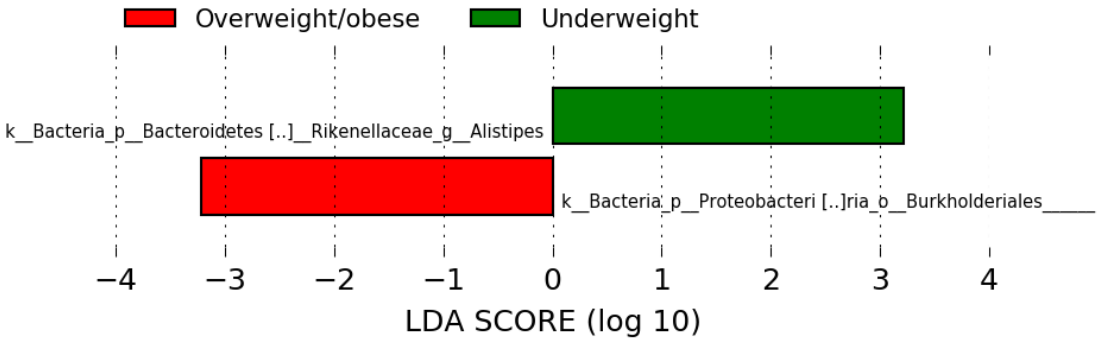


**Table 5.** Taxonomy of strains identified in LEfSe analysis.

| Identifier | Strain | BMI |
|---|---|---|
| e9d60f0f9c98313da6c1231888323fcc | k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__ | Overweight/obese |

| | | |
|---|---|---|
| a5c97a8b67924053479422a1c31a4d86 | k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri | Overweight/obese |
| f_4570bafaf63effb4ee8758eaf834ca31 | k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__ | Underweight |
| f_78289f1e6bc690928839f6aa73c6d334 | k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Sutterella; s__ | Underweight |