Volume 9:1-13

UJEMI+

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

The gut microbiome belonging to Colombian adults of younger generations has shifted to a more Westernized composition compared to older generations

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SUMMARY Communities undergoing Westernization experience changes to their lifestyle and diet. These changes are associated with decreased levels of activity, increased levels of obesity, increased risk for cardiometabolic disease, and changes to the microbiome composition. Obese individuals tend to have reduced levels of diversity and an increased abundance of potentially pathogenic bacteria. Additionally, aging has been associated with increased levels of diversity, which can be linked to changes in lifestyle. In this study, we examined the impact of obesity, defined by waist circumference and body fat percentage, on the gut microbiome of individuals from communities undergoing Westernization. Our study also explored the relationship between gut microbiome composition and obesity across multiple generations. Within our dataset, there was no significant difference in alpha diversity between obese and healthy individuals but there was a significant difference in diversity between two age groups, 18-28 and 38-48. Taxonomic analysis revealed that the age group 18-28 was defined by taxa *Bifidobacterium* and *Bacteroides* which are marker taxa for a Westernized microbiome, and the age group 38-48 was defined by greater abundance of *Prevotella,* a marker taxon for a non-Westernized diet. Taken together, our results indicate that our defined obesity metrics do not have an impact on the gut microbiome of individuals from communities in the midst of Westernization. Our results instead highlight the possibility that younger generations may have been more affected by Westernization than older generations. Our study has the potential to add to our growing understanding of Westernization and its varying impacts across generations.

INTRODUCTION

Westernization is when a population undergoes an epidemiological and nutritional transition that is characterized by changes in diet (such as shifting from traditional recipes to more processed foods), reduced physical activity and an increased prevalence of non-communicable diseases (1). These lifestyle changes associated with Westernization have the potential to alter the gut microbiome. Westernization has been linked with compositional changes to the microbiome including reduced alpha diversity and increased abundance of potentially pathogenic microbes (1).

The association between gut microbiota composition and lifestyle choices has been vastly studied among communities throughout the world. In one study, researchers found that the microbiome of the Hadza hunter-gatherer community had increased microbial richness and diversity compared to an urbanized Italian community (2). Furthermore, this study highlighted gut microbiome differences between sexes, which could further define how lifestyle, in terms of division of labour, can impact the microbiome composition (2).

Published Online: September 2023

Citation: Bodykevich, Ibtisam, Mao. 2023. The gut microbiome belonging to Colombian adults of younger generations has shifted to a more Westernized composition compared to older generations. UJEMI+ 9:1-13

Editor: Lauren Pugsley, University of British Columbia

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Additionally, recent meta-analyses have identified marker taxa that are associated with microbiomes of non-Westernized and Westernized individuals. Non-Westernized microbiomes have increased diversity and are rich in fibre-degrading bacteria such as *Prevotella* and *Treponema* (1, 3, 4). Westernized microbiomes are associated with bacteria that benefit host health by preventing colonization of foreign pathogens and enhance the immune response such as *Bifidobacterium, Bacteroides,* and *Barnesiella* (1, 5, 6, 7). While these studies provide context into the make-up of these two types of microbiomes, they both represent static diet and lifestyle, and give no insight into developing and changing diets and lifestyles. In fact, there has been little research into populations who are in the middle of dietary and lifestyle changes such as Westernization (1). Individuals living in Colombia can serve as a model for populations who are in the midst of Westernization. These individuals remain traditional in their diets composed of complex carbohydrates, but their lifestyles resemble those of a Westernized culture including rapid economic growth, reduced levels of activity, and increased levels of obesity and cardiometabolic disease (8).

Using Colombians as their model, de la Cuesta-Zuluaga et al. characterized the microbiota of 441 adults aged 18-62 through 16S rRNA gene sequencing and determined that a gut microbiome in the midst of Westernization is linked to obesity and cardiometabolic disease (1). These researchers defined the Colombian gut microbiota as containing taxa from both hunter-gatherer and industrialized communities. They also identified five consortia of microorganisms that were associated with metabolic pathways that could explain the association between microbiota and host health. In their study, de la Cuesta-Zuluaga et al. used body mass index as their main measurement of obesity. Body mass index is not an ideal measurement for obesity as it does not take into account bone and muscle mass, both of which are denser than fat (9, 10). Additionally, de la Cuesta-Zuluaga et al. did not sufficiently address the differences in microbiome composition between age groups as they split individuals into two groups; 18-40 and 41-62 (1). This presents a limitation of their study, as these two groups encompass large age ranges that could include vastly different lifestyle choices and diets.

Waist circumference and body fat percentage have both been gaining traction as strong indicators of cardiometabolic risk and are directly associated with adiposity, more so than BMI (11, 12, 13, 14). Previous literature has shown an overall decreased diversity in the gut microbiome of obese individuals (15, 16). Therefore, we decided to utilize waist circumference and body fat percentage as proxies for obesity. One study reported an association between higher waist circumference and lower alpha diversity and identified a correlation between *Oscillospira* and leanness (13). Furthermore, body fat was negatively correlated with *Bacteroidetes*, but positively correlated with *Firmicutes*, and the relative abundance of *Enterobacteriaceae* was increased in individuals with higher body fat percentage (14). Another study found that body fat percentage and waist circumference correlated negatively with the *Bacteroidetes* taxa among a sample of Italian adults, while *Firmicutes* taxa were positively correlated with body fat (14).

In addition to waist circumference and body fat percentage, aging has been associated with a reduction in commensal bacteria, but an increase in microbial diversity (17, 18). This change in the microbiome composition can be associated with the different lifestyle and diet choices we make as we age, which have been shown to have an impact on the gut microbiota (19). For example, younger generations tend to display higher levels of stress compared to older generations, and stress has the potential to alter the gut microbiota by reducing the amount of beneficial *Lactobacillus* (19, 20). Additionally, exercise and diet are capable of causing shifts in the gut microbiome, as professional athletes have increased diversity in their microbiome which can be linked to their increased levels of exercise and associated diet (19). We theorized that individuals who are ten years apart could display differences in their lifestyle choices that would have an impact on our defined obesity metrics, and therefore the gut microbiome diversity.

Previous literature shows associations between obesity metrics and lower gut microbiome diversity, and that gut microbiome diversity increases as an individual ages (15, 16, 17, 18). In this study, we explored whether these relationships can be identified using Colombians as a model, and whether younger or older generations of Colombians show stronger degrees of correlation between gut microbiome diversity and obesity metrics. We hypothesized that

waist circumference and body fat percentage will be negatively correlated with gut microbiome diversity and that this will be more pronounced in younger generations.

METHODS AND MATERIALS

Dataset and Metadata. The original dataset by de la Cuesta-Zuluaga et al. was generated by collecting 16S rRNA sequences from faecal samples of 441 Colombian adults ages 18-62. The participants were from the largest urban cities in Colombia. Individuals were of healthy, overweight, and obese BMI, non-pregnant, not taking antibiotics or antiparasitics, and were not diagnosed with cancer, neurodegenerative or gastrointestinal diseases. The metadata created by de la Cuesta-Zuluaga et al. included obesity metrics such as waist circumference and body fat percentage of each participant.

Data processing in RStudio. Using RStudio version 4.2.1, the metadata was manipulated to categorize participants into age groups 18-28, 28-38, 38-48, 48-58, and 58-68, and binned by obesity metrics. The cutoffs for healthy and obese waist circumference and body fat percentage were determined according to the British Heart Foundation and the Heart & Stroke Foundation (21, 22). An obese waist circumference was defined as greater than 90 cm for males and greater than 84 cm for women. An obese body fat percentage was defined as greater than 25.8% for men and greater than 37.1% for women. Using the set cutoffs, men and women were categorized as obese if they had both an unhealthy waist circumference and body fat percentage, and the rest were categorized as healthy. The revised metadata was used for all further analyses in this study.

Data processing and diversity metrics in QIIME2. 16S rRNA sequences from the Colombia dataset were demultiplexed using QIIME2 (23). Sequence quality control was performed with the Dividive Amplicon Denoising Algorithm 2 (DADA2) method, setting a truncation length of 250 nucleotides, the max sequence length for retaining sufficient sequence quality (24). From the sequence quality control metrics, a feature table of all the amplicon sequencing variants (ASVs) that appeared in each sample, and a table of representative sequences were generated. The feature table was rarefied to a sampling depth of 25772 because this depth retained the maximum number of features (53.08%) and samples (72.11%). The representative sequences were processed into a taxonomy table using the Silva 138-99 classifier and subsequently used to produce a rooted and unrooted phylogenetic tree using the sequence-based MAFFT alignment tool from QIIME2. The metadata created in the previous subsection was imported to QIIME2 and alpha and beta diversity metrics were calculated based on healthy or obese individuals. Upon visualizing the diversity plots, further analyses were performed in RStudio.

Diversity Metrics and Statistical Analysis in RStudio. The following R packages were used for diversity analysis: *tidyverse, ape, phyloseq* (25, 26, 27). A phyloseq object was generated with the exported metadata, feature table, taxonomy table, and phylogenetic tree from QIIME. Low abundance reads, which are classified as rare ASVs that have <0.005-0.01% of total reads, were removed from the phyloseq. These are removed from the dataset as they are unlikely true biological events, they are often not biologically informative, and they have the potential to negatively affect downstream analyses. Additionally, samples with less than 100 reads were removed from the phyloseq because these sample reads are likely of poor quality. Samples were rarefied to a sampling depth of 25772 for the same reason as mentioned in the previous subsection. For the Shannon diversity box plots, samples were compared based on age, obesity, or combined age and obesity. The combined age and obesity category classified each individual based on their age group and their obesity. A Kruskal-Wallis test was performed to determine the statistical significance between Shannon diversity of different age groups, obese vs. healthy, and between combined age and obesity groups. Beta diversity was assessed using the Bray Curtis, Weighted Unifrac, and Unweighted Unifrac methods, and visualized using principal coordinates analysis (PCoA) plots.

Taxonomic Analysis. The following R packages were used in addition to the previously mentioned packages for taxonomic analyses: *microbiome, ggVennDiagram, indicspecies, DESeq2* (28, 29, 30, 31). Core microbiome, indicator species, and differential expression sequence (DESeq2) analyses were performed on two age groups, 18-28 and 38-48 as these two age groups were found to have significantly different alpha diversities. For core microbiome analysis, ASVs from genera that were present in 80% or more of the samples were visualized using a Venn diagram. For indicator species analysis, ASVs needed to be significantly ($p<0.01$) more abundant in one age group compared to the other. To visualize the DESeq results, a volcano plot and bar plot were generated to visualize the significantly increased or decreased ASVs in the 38-48 age group compared to the 18-28 age group. As not all ASVs had bacterial identifications, the unidentified ASVs were searched against the National Center for Biotechnology Information (NCBI) database using the Nucleotide Basic Local Alignment Search Tool (BLASTn).

RESULTS

Alpha diversity of age group 38-48 is significantly higher than that of age group 18-28, though beta diversity remains similar. We found that the gut microbiomes of the 38-48 age group had a significantly higher alpha diversity, which is the diversity within each sample, than the 18-28 age group with p-value <0.05 (Fig. 1A). According to the Shannon

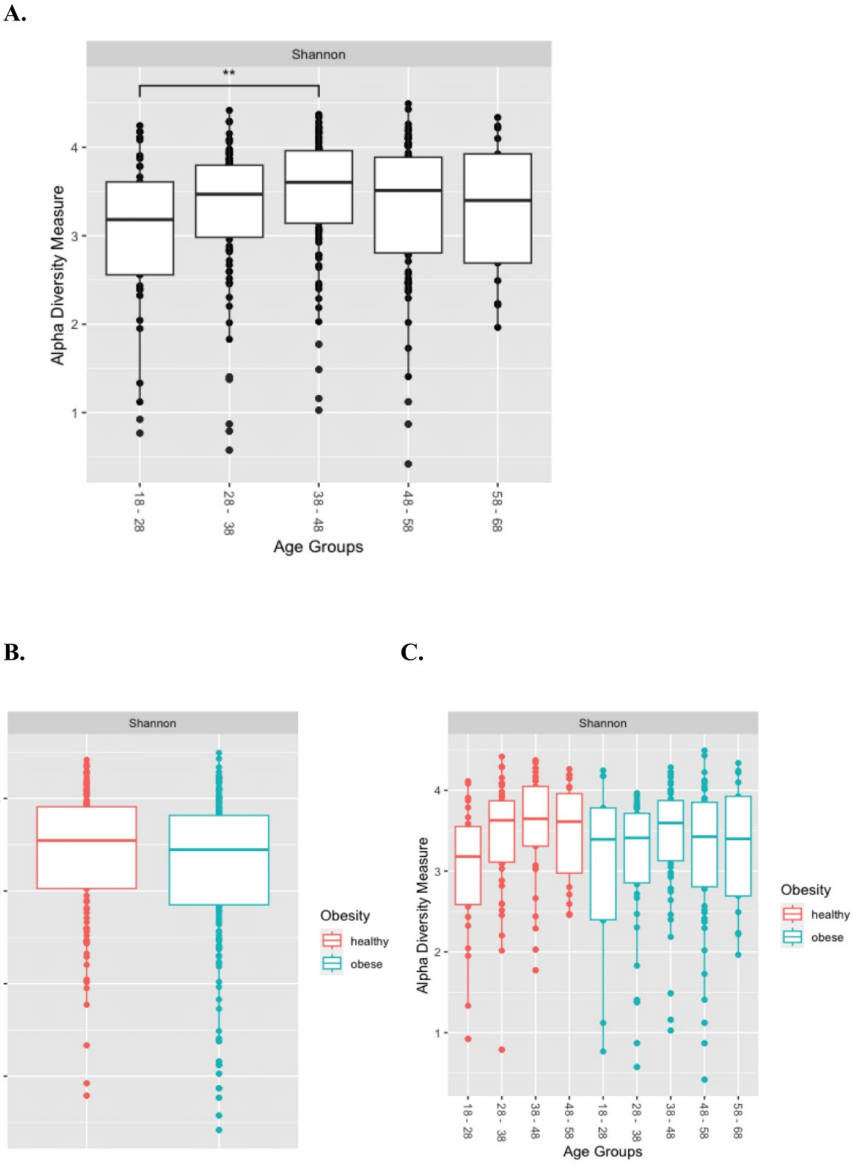

FIG. 1 Shannon diversity of age group 38-48 is significantly higher than the 18-28 age group. A. Shannon diversity plot of age groups (separated by decades). Significance was determined with the Kruskal-Wallis test. A *p*-value of < 0.05 is designated by two asterisks (**). B. Shannon diversity plot of healthy and obese individuals as deemed by both waist circumference and body fat percentage. C. Shannon diversity plot of age groups separated into healthy and obese based on both waist circumference and body fat percentage.

index that was used to analyze the diversity between age groups, the 38-48 age group potentially has more diversity in terms of richness and/or evenness within each individual than the 18-28 age group. Although there was a significant difference when only the age variable was examined, the alpha diversity of healthy and obese individuals was similar, showing that obesity as defined by waist circumference and body fat percentage did not have a significant effect on the gut microbiome diversity of the sample (Fig. 1B). When age and obesity were combined, no significant differences in alpha diversity were found between any groups, indicating that obesity is a potential confounding variable. Taxonomic analysis of the 18-28 and 38–48 age groups indicated that the younger adults may have a more Westernized gut microbiome than the older adults (Fig. 2-3). The findings of this study suggest that obesity does not have a significant effect on gut microbiome diversity (Fig. 1B-C). Beta diversity metrics including Bray-Curtis, Weighted and Unweighted Unifrac showed similar results, indicating that the difference in gut microbiome between the 18-28 and 38-48 age groups seems to be restricted to their alpha diversity (Supp. Fig. S1).

Core microbiome analysis reveals multiple unique ASVs in each age group. Core microbiome analysis revealed the two age groups 18-28 and 38-48 shared 72% of their microbiome (Fig. 2). The results also revealed 3 unique ASVs associated with the age group 18-28 and 4 unique ASVs associated with the age group 38-48 (Fig. 2). The unique ASVs identified by core microbiome analysis were searched in the NCBI database using BLASTn to determine their taxonomy (Table 1). Notably, two of the species unique to the 18-28 age group, *Phocaeicola vulgatus* (also known as *Bacteroides vulgatus*) and *Bifidobacterium adolescentis*, are marker taxa for a Westernized diet (1, 33).

TABLE. 1 Marker taxa of westernized microbiome identified in the 18-28 age group. Unique ASVs from the core microbiome analysis in Figure 2 were searched in the NCBI database using BLASTn to identify their taxonomy.

Species	
18 - 28 Age Group	**38 - 48 Age Group**
Phocaeicola vulgatus	*Fusicateniacter saccharivorans*
Bifidobacterium adolescentis	*Oscillospiraceae* sp.
Faecalibacterium prausnitzii	*Ruminococcus faecis*
	Coprococcus comes

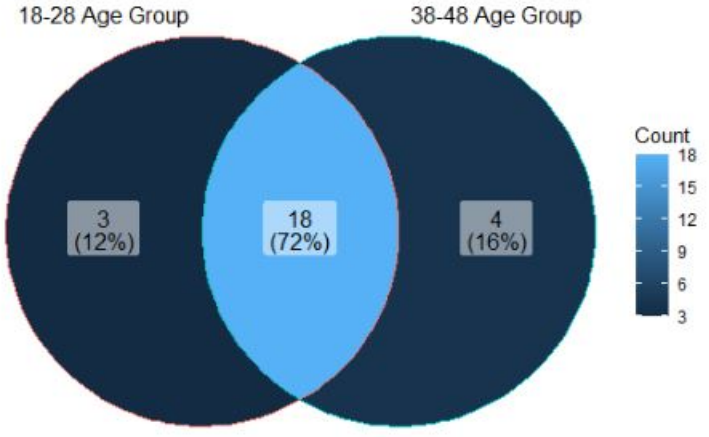

FIG. 2 Three and four unique members of the 18-28 and 38-48-year-old microbiomes, respectively. An abundance threshold of 0 and a prevalence of 0.8 was used. The marker taxa of each group are detailed in Table 1.

***Oscillospiraceae* is strongly associated with the 38-48 age group.** Indicator species analysis showed that two families, *Pentoniphilcaea* and *Lachnospiraceae,* are strongly associated with the age group 18-28, and one family, *Oscillospiraceae,* is strongly associated with the age group 38-48 (Table 2). Indicator species were required to have a p-value less than 0.01.

Table 2. *Oscillospiraceae* is strongly associated with individuals aged 38-48. Indicator Species Analysis of 18-28 and 38-48 age groups. Significance threshold was set at with p-value < 0.01 indicated with three asterisks (***).

Family	p-value
18 - 28 Age Group	
Pentoniphilcaea (Peptoniphilus)	0.005 ***
Lachnospiraceae	0.005 ***
38 - 48 Age Group	
Oscillospiraceae (Acetivibrio)	0.005 ***

The 38-48 age group has 41 ASVs that have significantly different expressions compared to the 18-28 age group. DESeq analysis showed that 41 ASVs were significantly increased or decreased in expression in the 38-48 age group relative to the 18-28 age group (Fig. 3A). ASVs that were significantly upregulated were increased in expression by 2-6 fold in the 38-48 age group, and the ASVs that were significantly downregulated were decreased in expression by 2-2.5 fold in the 38-48 age group relative to the 18-28 age group (Fig. 3A). The significant ASVs were searched in the NCBI database using BLASTn to identify which taxa were present in greater abundance in the 38-48 age group (Fig. 3B). Overall, the DESeq results show more increased expression of ASVs in the 38-48 age group relative to the 18-28 age group.

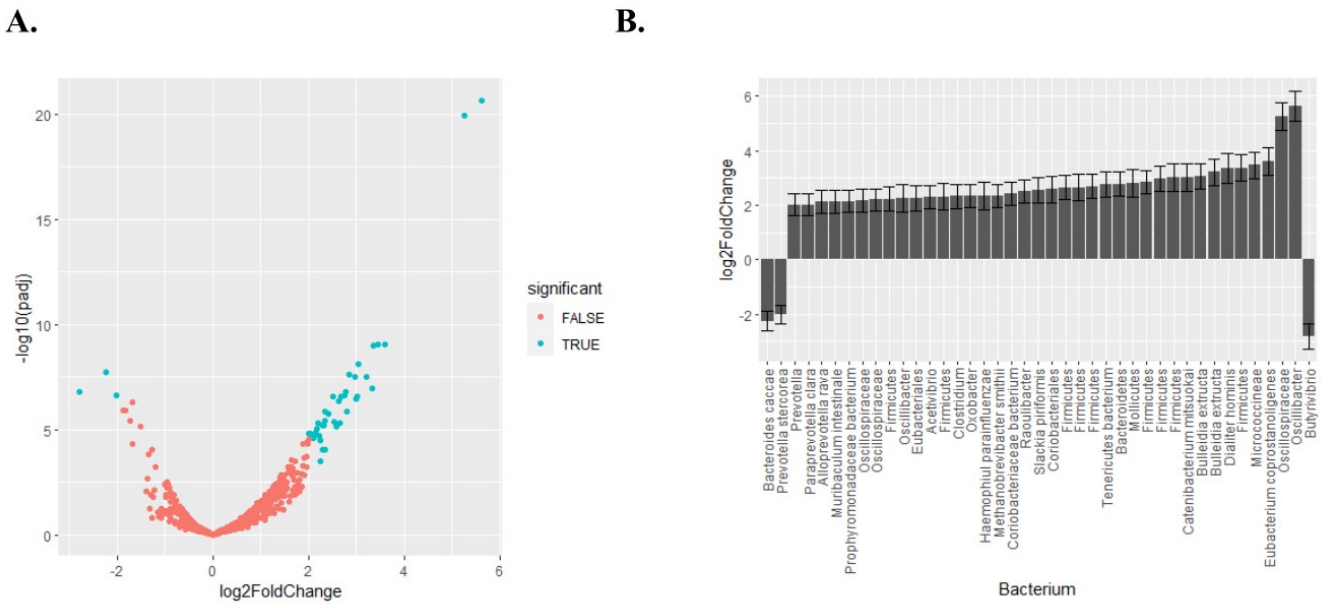

FIG. 3 Most taxa increase between ages 38-48 compared to 18-28, including *Oscillospiraceae and Firmicutes*. DESeq analysis of age group 38-48 compared to age group 18-28. A. Differential gene expression volcano plot of ASVs in the age group 38-48 compared to the 18-28 age group. Significant fold changes are indicated by the blue points, p-value < 0.05. B. Taxa bar plot set at genus level of taxa significantly increased and decreased in expression in the 38-48 age group relative to the 18-28 age group. ASVs without defined genus were identified using NCBI BLASTn.

DISCUSSION

This study originally sought to explore the correlations between obesity (defined by waist circumference and body fat percentage measurements) and gut microbiome diversity amongst a population of Colombian adults. This study also sought to determine if these correlations could be different among various age groups of adults (ages 18-28, 38-48, 48-58, 58-68). We found that the gut microbiomes of the 18-28 age group have a significantly lower alpha diversity than the 38-48 age group (Fig. 1A). When obesity was observed alone, as well as when age group and obesity were looked at together, no significant effects were observed

(Fig. 1C), demonstrating that obesity is a key confounding variable in our findings. Taxonomic analysis of the 18-28 and 38–48 age groups indicated that the younger adults may have a more Westernized gut microbiome than the older adults (Fig. 2-3).

The 18-28 age group has a less diverse microbiome than the 38-48 age group. This study found differences in the gut microbiome diversity of individuals in two different age groups, with the middle-aged adult group showing significantly higher alpha diversity than the young adult group (Fig. 1A). This is similar to the findings of previous literature that indicate that gut microbiome diversity seems to gradually increase as individuals get older (17, 32). However, there has also been research stating that elderly individuals tended to show decreased microbial diversity (18). As the 38-48 group could be considered middle-aged or just under-middle-aged, our results agree with previous literature as the older age group showed higher gut microbial diversity than the 18-28 age group.

Additionally, the findings of this study suggest that obesity does not have a significant effect on gut microbiome diversity (Fig. 1B-C), which does not agree with previous literature. The original researchers of our metadata found that there were significant associations between gut microbiota and obesity, specifically between the five abundant OTUs they referred to as co-abundance groups with BMI and waist circumference (1). Other researchers have found that obesity is associated with decreased gut microbiome diversity, with obesity metrics used including BMI and anthropometric measurements (13, 15). Our results could be different from the literature due to the presence of potential confounding variables such as diet, and calorie intake, as well as the way that we defined our obesity metrics.

The 18-28 age group has more key marker taxa for a Westernized microbiome than the 38-48 age group.

Bacteroides and *Bifidobacterium* are marker taxa for a Westernized diet (1, 33). The association of *Phocaeicola vulgatus* (also known as *Bacteroides vulgatus*) and *Bifidobacterium adolescentis* with the 18-28 age group indicates that this generation has a more Westernized microbiome compared to the 38-48 age group (Table 1). Additionally, *Bacteroides* are significantly downregulated in the 38-48 age group microbiome compared to the 18-28 age group, which supports that the younger generation has a more Westernized microbiome (Fig. 3). The literature surrounding the function of *Bacteroides* bacteria within the human gut microbiome is contradicting, as many studies have found *Bacteroides* to benefit gut health by metabolizing polysaccharides and providing nutrients and vitamins to both the host and other resident microbes (34). However, numerous other studies have shown the potentially pathogenic behaviour of *Bacteroides* within the gut microbiome, specifically that of *P. vulgatus* (35). Specifically, one study identified that *P. vulgatus* may be associated with the pathogenesis of Crohn's disease, while another study found an increased abundance of *P. vulgatus* in patients with inflammatory bowel disease (36, 37). While neither study identified the mechanism of pathogenesis of *P. vulgatus*, both results indicate its nature as an opportunistic pathogen. This finding supports the claim that the 18-28 age group has a more Westernized microbiome because, in addition to the occurrence of the two marker taxa, Westernized microbiomes are associated with increased levels of potentially pathogenic bacteria (1).

The association of *Bifidobacterium* with the younger age group is supported by the literature, as studies have shown a negative correlation between *Bifidobacterium* abundance and aging (38, 39). *Bifidobacterium adolescentis* is a key producer of gamma-aminobutyric acid (GABA), a natural antidepressant, and one study identified a positive correlation between *B. adolescentis* abundance and mental disorders like anxiety and depression (40, 41). Numerous studies have identified younger generations as having higher occurrences of anxiety and depression, significantly more so in the age of technology and social media (42, 43).

In addition to the 18-28 age group being associated with two marker taxa of Westernized microbiomes, the 38-48 age group showed an increase in the abundance of *Prevotella*. *Prevotella* species are fibre-degrading microbes that are marker taxa for a non-Westernized microbiome (1, 3). As fibre is a major component of the traditional Colombian diet, it makes sense that we see an increased abundance of the *Prevotella* species within the 38-48 age group. This result indicates that the diet of the 38-48 age group potentially remains more traditional and relatively less Westernized. In addition to the increased abundance of

Prevotella, the 38-48 age group also had increased expression of *Alloprevotella* and *Paraprevotella* additional genera of *Prevotellaceae*. While these two genera are not marker taxa for a non-Westernized microbiome, *Alloprevotella* is also associated with fibre degradation, and *Paraprevotella* has been linked with enzymatic function for degrading insoluble fibres (44, 45). Altogether, the greater abundance of *Prevoellaceae* genera indicates that the microbiome of the 38-48 age group resembles a non-Westernized composition, and this may be a result of a traditional diet.

The 18-28 age group microbiome is composed of bacteria associated with Westernized diets. As previously mentioned, Bifidobacterium is associated with the 18-28 age group. A potential explanation for this is linked to the GABA-producing function of the species. Another potential explanation for the association of Bifidobacterium with the 18-28 age group is due to increased dairy intake. Several studies have highlighted a positive correlation between dairy intake and Bifidobacterium abundance (46). It is noted that a Western-style diet consists of increased consumption of animal proteins, including dairy products (47).

From our indicator species analysis, we identified *Peptoniphilus* and *Lachnospiraceae* as being associated with the 18-28 age group. These findings are not supported by the literature as there seems to be a lack of research conducted on the relationship between age and the abundance of these two taxa in the gut microbiome. However, the literature does support their association with diet (48, 49, 50). *Peptoniphilus* is a butyrate-producing genus where its high abundance is associated with a diet of high red meat consumption (51). Similar to *Peptoniphilus, Lachnospiraceae* is a family of bacteria that also produces butyrate (52). Several studies have demonstrated the correlation between *Lachnospiraceae* and a high-fat diet (49, 50, 51). The increased consumption of red meat and a high-fat diet is a result of Westernization (53).

We found a significant increase in *Firmicutes* in the 38-48 age group relative to the 18-28 age group as shown by DESeq analysis. Members of *Firmicutes* are excellent at degrading fibre (54). A previous study on obese/overweight individuals that increased fibre in their diet by consuming wheat bran found increases in *Firmicutes* (55). Another study, this one exploring the effects of barley, also found increases in *Firmicutes* (55). Societies in Latin American countries have traditionally had dietary patterns that include high consumption of foods such as roots, tubers and cereal, i.e., foods that are high in fibre (56). However, in recent decades, changes in industrialization and the availability of resources have affected the lifestyles and eating patterns of individuals, including a decrease in diets rich in fibre (56). Although, it should be noted that *Lachnospiraceae*, found in abundance in the 18-28 age group, is a family within *Firmicutes*. While this highlights a potential weakness within our analysis, a possible explanation may be that the 38-48 age group has overall greater abundances of the *Firmicutes* families, while the 18-28 age group only has a higher abundance of the one specific *Firmicutes* family, *Lachnospiraceae*. Nevertheless, further studies could explore the exact composition of *Firmicutes* in each age group to identify significant differences in composition.

The overall increase in *Firmicutes* we observed in the older generation of Colombian adults may be due to a higher presence of fibre-rich foods in their diets relative to the young adult group. It is possible that the older age group not only consumes more fibre within their diet but also consumes a wider variety of fibre sources which could potentially influence the composition of the *Firmicutes* we see in the 38-48 age group. One study found that over a ten-year period, whole grain, fruit, and vegetable consumption decreased throughout the Colombian population, and individuals younger than 40 had an overall worse diet quality than older individuals (57). Overall, this study supports our data and the possibility that younger generations of Colombians are experiencing greater Westernization than the older generations, however further analysis into nutritional content between age groups in this dataset should be done. Together, the taxonomic analysis of this study indicates that the 18-28 age group is experiencing stronger effects of Westernization than the 38-48 age group, and this change is potentially occurring in their diet.

***Oscillospiraceae* is strongly associated with the 38-48 age group.** *Oscillospirecea* is a family of bacteria that was significantly upregulated in the age group 38-48. *Oscillospirecea* was also identified as a core member and an indicator species for the age group 38-48 (Table

1). The occurrence of *Oscillospirecea* family in all three taxonomic analyses indicates that it is strongly associated with the 38-48 age group. *Oscillibacter* is a genus of *Oscillospirecea that* was identified as being significantly upregulated in the 38-48 age group. *Oscillibacter* is capable of enhancing the differentiation of IL-10-producing Tregs and producing valerate (58). Valerate is the conjugate base to valeric acid, which may be a potent inhibitor of histone deacetylase (HDAC) (59). The presence of high HDAC proteins has been linked to the prevalence of diseases such as cancer, colitis, and cardiovascular disease (59). Another study showed that the inhibition of HDAC using valeric acid had anti-cancer effects on liver and breast cancer (60). While cancer can occur at any age, most diagnoses of liver and breast cancer occur after the age of 50 (61, 62). It is possible that as we age, our gut microbiome composition is shifted towards bacteria that have roles in protecting us from diseases and illnesses that are associated with older age. To support this claim, further analyses should look specifically at the abundance of bacteria associated with health benefits, and how they may increase with age.

Limitations One profound limitation of our study is the number of dietary confounding variables that can affect the gut microbiota (63, 64, 65). de la Cuesta-Zuluaga et al did not control for factors such as caloric intake, diet composition (ratio of macronutrient intake), and duration between meals (i.e., intermittent fasting) which have been shown by numerous studies to have a significant impact on the gut microbiota (63, 64, 65, 66, 67). Sbierski-Kind et al. studied the effects of an 8-week-calorie-restricted diet on the gut microbiome and found the overall alpha diversity post-diet was higher compared to pre-diet (66). In contrast to a limited diet, a Westernized diet which is high in fatty acids and simple carbohydrates was reported to decrease gut microbiota diversity (3). In addition to the amount of fats and sugars consumed, the type of diet (plant-based, keto, Mediterranean), as well as the time of food intake (intermittent fasting, 5:2), can also influence the abundance of certain species as revealed by previous papers (64, 67). Furthermore, exercise has also been shown to alter the gut microbiome by several studies (68, 69, 70). Lastly, since an individual had to be obese in both body fat percentage and waist circumference in order to be classified in the obese group, this may be a potential reason why we see a divergence from the literature. As we did not account for numerous confounding variables and we classified our individuals using different obesity categories, our study does not completely align with the available literature and the applicability of our results is limited.

Conclusions Waist circumference and body fat percentage do not seem to play a significant role in the gut microbiome diversity of the individuals in this Colombian adult sample. No significant difference was found when individuals were categorized for both age group and obesity. However, a significant difference in alpha diversity was found between the 18-28 age group and the 38-48 age group, with the older age group showing higher microbial diversity. Among bacteria that are unique to each age group, *Oscillospiraceae* appeared (for the 38-48 age group) throughout taxonomic analysis including core microbiome, ISA and DESeq. Furthermore, two out of the three core members in age group 18-28 are marker taxa for a Westernized microbiome as noted by de la Cuesta-Zuluaga et al. (71). Though the findings of this study must be taken with caution due to the presence of potential confounding variables such as obesity and diet, it provides a novel perspective on the gut microbiome diversity of two different age groups of adults in Colombia, indicating that young adults in Colombia could have a more Westernized gut microbiome than adults closer to middle age.

Future Directions Although we were able to identify some significant differences in the gut microbiome composition between the two age groups, our obesity results did not align with the literature available. A potential explanation for this could be the aforementioned method of how we classified individuals as obese, i.e. had to be obese in both body fat percentage and waist circumference. To demonstrate the robustness and replicability of our results, future studies can look into repeating our analyses for each condition separately, and not combining body fat percentage and waist circumference. Furthermore, repeating this study in a different

population that is also in the middle of Westernization but has a different culture, different climate, and different diets will show the replicability of our results.

Our study identified multiple bacteria associated with either age groups 18-28 or 38-48. To strengthen our findings, future studies could repeat the core microbiome, indicator species, and DESeq analyses in the older age groups to confirm that the species from the 38-48 age group are even more enriched and that the key marker taxa of Westernization found in the 18-28 age group are represented at lower abundances in the older generations. Additionally, looking at the abundances of these key marker taxa in the generations between our selected 18-28 and 38-48 to see if there is a gradual decrease in Westernized marker taxa, or a gradual increase in key non-Westernized taxa, would strengthen our conclusions. Some of the highlighted bacteria in the older generation were associated with benefits for host health. Further literature research into the human microbiome composition could be done to identify bacteria that are associated with host health and immune benefits. A further study could then observe the changes in the abundance of these bacteria throughout the generations to see if older generations have higher abundances of bacteria that support the immune system.

Additionally, our study identified marker taxa within the younger generation that was associated with a Westernized microbiome. Based on the literature, younger generations tend to exhibit a diet that is unbalanced, has less nutritional value, and resembles a Westernized diet more so than the older generations. It is possible that the process of Westernization is having an impact on the younger generation's microbiome through their diet. Further analysis of the nutritional content of each generation's diet may provide insight into the Westernization of younger generations' microbiomes.

ACKNOWLEDGEMENTS

This study was funded by the Department of Microbiology and Immunology at the University of British Columbia. We would like to extend our deepest gratitude to Dr. Evelyn Sun, Dr. Melissa Chen, Bretta McCall, and the rest of the MICB 475 teaching team for their generous guidance, support and feedback provided throughout the course of this study. We would also like to thank researchers de la Cuesta-Zuluaga et al. for providing the essential metadata used in this study. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

CONTRIBUTIONS

Lanyin wrote the materials and methods and study limitations sections, as well as contributed to writing the discussion.
Adriana generated the figures and figure captions, as well as wrote the results and conclusions, and contributed to the discussion.
Grace wrote the abstract, introduction, and future directions, as well as contributed to the discussion. Also generated the reference list.
All co-authors proofread and contributed edits and revisions to the final product.

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