RESEARCH
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Associations between Diet, the Gut Microbiome, and Short-Chain Fatty Acid Production among Older Caribbean Latino Adults

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ABSTRACT

Background Caribbean Latino adults have disproportionately high prevalence of chronic disease; however, underlying mechanisms are unknown. Unique gut microbiome profiles and relation to dietary quality may underlie health disparities.

Objectives To examine the dietary quality of an underrepresented group of Caribbean Latino older adults with high prevalence of chronic disease; characterize gut microbiome profiles in this cohort; determine associations between dietary quality, gut microbiome composition, and short-chain fatty acid (SCFA) production; examine associations of clinical factors (body mass index, type 2 diabetes [T2D] status, and laxative use) with gut microbiome composition.

Design The study design was cross-sectional.

Participants/setting Recruitment and interviews occurred at the Senior Center in Lawrence, MA, from September 2016-September 2017. A total of 20 adults aged ≥50 years, self-identified of Caribbean Latino origin, without use of antibiotics in 6 months or intestinal surgery were included in the study.

Exposure and outcome measures Diet was assessed by two, 24-hour recalls and dietary quality was calculated using the Healthy Eating Index 2015 and the Mediterranean Diet Score. The gut microbiome was assessed by *16S rRNA* sequencing and fecal SCFA content. Anthropometrics (ie, weight and height) were measured by a trained interviewer, and self-reported laxative use, and other self-report health outcomes (ie, T2D status) were assessed by questionnaire.

Statistical analyses Faith Phylogenetic Diversity (alpha diversity) and unique fraction metric, or UniFrac (beta diversity) and nonphylogenetic metrics, including Shannon diversity index (alpha diversity) were calculated. Spearman correlations and group comparisons using Kruskal-Wallis test between alpha diversity indexes and nutrient intakes were calculated. Patterns in the microbiome were estimated using a partitioning around medoids with estimation of number of clusters, with optimum average silhouette width. Log odds were calculated to compare predefined nutrients and diet score components between microbiome clusters using multivariable logistic regression, controlling for age and sex. Pearson correlation was used to relate SCFA fecal content to individual nutrients and diet indexes. Final models were additionally adjusted for laxative use. Differences in lifestyle factors by gut microbiome cluster were tested by Fisher's exact test.

Results Generally, there was poor alignment of participant's diets to either the Mediterranean Diet score or Healthy Eating Index 2015. Range in the Healthy Eating Index 2015 was 36 to 90, where only 5% (n=1) of the sample showed high adherence to the Dietary Guidelines for Americans. Mediterranean Diet scores suggested low conformance with a Mediterranean eating pattern (score range=2 to 8, where 45% scored ≤3 [poor adherence]). The gut microbiome separated into two clusters by difference in a single bacterial taxon: *Prevotella copri (P copri)* (permutational multivariate analysis of variance [PERMANOVA] R^2=0.576, ADONIS function P=0.001). Significantly lower *P copri* abundance was observed in cluster 1 compared with cluster 2 (Mann-Whitney P<0.0001). Samples in the *P copri* dominated cluster 2 showed significantly lower alpha diversity compared with *P copri* depleted cluster 1 (Shannon diversity index P=0.01). Individuals in the *P copri* dominated cluster showed a trend toward higher 18:3 α-linolenic fatty acid intakes (P=0.09). Percentage of energy from total fat intake was significantly, positively correlated with fecal acetate (r=0.46; P=0.04), butyrate (r=0.50; P=0.03) and propionate (r=0.52; P=0.02). Associations between dietary intake and composition of the gut microbiome were attenuated by self-report recent laxative use.

Individuals with T2D exhibited a significantly greater abundance of the Enterobacteriales (P=0.01) and a trend toward lower fecal content of butyric acid compared to subjects without T2D (P=0.08). Significant beta diversity differences were observed by weight (Mantel P<0.003) and body mass index (Mantel P<0.07).

Conclusions Two unique microbiome profiles, identified by abundance of *P copri*, were identified among Caribbean Latino adults. Microbiome profiles and SCFA content were associated with diet, T2D, and lifestyle. Further research is needed to determine the role of *P copri* and SCFA production in the risk for chronic disease and associated lifestyle predictors.

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CARIBBEAN ORIGIN HISPANICS OR CARIBBEAN Latinos adults have disproportionately high prevalence of chronic disease.[1,2] It has been suggested that a major contributing factor to the high rates of chronic disease among Caribbean Latino adults may be poor dietary quality.[3,4] Typically, diets of acculturated Caribbean Latino adults are low in whole grains and high in refined grains and corn oil.[5] Caribbean Latinos are among the fastest growing segments of the US population[6] and are underrepresented in health research. Thus, determining potential dietary and lifestyle influences of chronic disease, specific to Caribbean Latino culture, is imperative to better elucidate underlying mechanisms and to develop culturally appropriate interventions.

Diet is a modifiable, noninvasive, inexpensive lifestyle change that is commonly used to prevent and treat chronic diseases.[7] The influence of diet on chronic disease outcomes may be partially due to influence on the gut microbiome and downstream effects against inflammation,[8,9] the main comorbidity of most chronic diseases. Particularly, colonic bacteria degrade fibers from the diet and as a result of such degradation short chain fatty acids (SCFAs) are produced.[10] SCFAs regulate the expression of cytokines (tumor necrosis factor alpha, interleukin [IL] 2, IL-6, and IL-10), eicosanoids and chemokines (eg, monocyte chemoattractant protein-1 and cytokine-induced neutrophil chemoattractant-2 by acting on macrophages and endothelial cells.[11,12] High levels of microbial-derived SCFAs then promote a hyporesponsive immunological environment to commensal bacteria through the downregulation of those proinflammatory effectors, aiding homeostasis maintenance.[9,13]

Dietary changes have been shown to alter the composition of the gut microbiome both short and long term,[14,15] supporting the role of diet as a powerful intervention target to prevent and treat these chronic disease conditions. Among adults, varying dietary patterns have been shown to predict microbiota profiles. For example, individuals consuming animal-based diets show a reduction of *Firmicutes* compared with individuals on plant-based diets.[15] Not surprisingly, the gut microbiome composition and function, specifically SCFA production, differs by ethnicity, where culture shapes different dietary habits.[16-18] In addition, lifestyle choices, such as laxative use, can directly affect the gut microbiome[19] and bias diet-microbiome interactions. Therefore, it is important to assess microbiome composition and its function, as it relates to diet and lifestyle, across different ethnic cohorts. However, few studies have described how diet is linked to microbiome profiles among Caribbean Latinos with high prevalence of chronic disease (eg, diabetes, obesity, and cardiovascular disease[1]), and how these profiles may relate to dietary and lifestyle-based cultural differences. It is known that patients with such chronic diseases exhibit an altered microbiome or dysbiosis, yet most of the studies only include non-Hispanic white patients.[20,21] Therefore, the main objectives of this study were to examine the dietary quality of an underrepresented group of Caribbean Latino older adults with high prevalence of chronic disease; characterize gut microbiome profiles in this cohort; determine associations between dietary quality, gut microbiome composition, and SCFA production; examine associations of clinical factors (eg, body mass index [BMI], type 2 diabetes [T2D] status, and laxative use) with gut microbiome composition.

RESEARCH SNAPSHOT

Research Questions: How do the diets of acculturated Caribbean Latino adults line up with dietary quality indexes? How are the unique gut microbiome profiles of Caribbean Latino adults characterized? What dietary predictors are related to gut microbiome profiles and short-chain fatty acid production among Caribbean Latino adults? How does the gut microbiome differ between groups with varying clinical factors (eg, body mass index, type 2 diabetes status, and laxative use).

Key Findings: Most acculturated Caribbean Latino adults in this small cohort demonstrate low dietary quality scores. This cross-sectional pilot study demonstrated two unique gut microbiome profiles distinguished by abundance of *Prevotella copri*. Dietary determinants of the gut microbial clusters and of fecal short-chain fatty acid concentrations included 18:3 α-linolenic acid and percentage of energy from total fat. Associations were attenuated with recent laxative use. Individuals with type 2 diabetes exhibited a significantly greater abundance of the Enterobacteriales and lower fecal content of butyric acid. Individuals with obesity exhibited a higher abundance of *Coprococcus* compared with individuals with a healthy body mass index.

METHODS

Description of Study Participants

A total of 30 Caribbean Latino adults aged ≥50 years were recruited through flyers and events at the Lawrence Senior

Center in Lawrence, MA, from September 2016 through September 2017. The Lawrence Senior Center serves more than 5,000 adults aged ≥50 years each year, providing health and social services and referrals to health and social programs within the community. Approximately 75% of adults that attend the Senior Center are Hispanic, primarily of Caribbean origin. Participants were eligible if they self-identified as being of Caribbean origin and were aged at least 50 years. Exclusion criteria included antibiotic medication use during the past 6 months and self-reported intestinal surgery or diagnosis of irritable bowel disease or other disease of the lower intestine. After telephone screening, 10 participants were excluded due to use of antibiotic medications within the past 6 months. A total of 20 individuals were enrolled in the study. Participants were asked to complete two in-person interviews with a trained, bilingual community interviewer within a 1-week period. All study protocols were approved by the institutional review board at the University of Massachusetts Lowell (no. 16-116-MAN-XPD). Participants provided written informed consent.

Medical History and Anthropometric Assessment

Participants were asked to complete two visits to the Senior Center. During the first visit, the trained, bilingual community research assistant obtained information on sociodemographic characteristics, health, and health behaviors through interviewer-administered questionnaires. Specifically, participants provided information on age, sex, educational attainment, marital status, household income, and migration history. Participants self-reported being diagnosed with a list of chronic health conditions and use of prescription and over-the-counter medications. Smoking was assessed through questionnaire, adopted from the Framingham Heart Study.[22] Smoking status was categorized as current cigarette smoker (smoked regularly during the past year), former smoker, or never smoked. Packs per day were ascertained in current smokers. Alcohol intake was assessed from questions on usual alcohol consumption, adopted from the Boston Puerto Rican Health Study food frequency questionnaire.[23] Height (in centimeters) and weight (in kilograms) were measured in duplicate using the Detecto 439 Eye Level Beam Physician Scale W/Height Rod after asking participants to remove their shoes and any outer layers of clothing. An average of the two measures was used. Physical activity was assessed by the validated and reliable Community Healthy Activities Model Program for Seniors (CHAMPS) survey for older adults.[24]

Fecal Sample Collection Methods

Participants were provided with detailed instructions for self-collection of a fecal sample at home using OMNIgene Gut Kit (OMNIgene•GUT DNA genotek). In brief, participants were provided with gloves, a stool collection hat, one OMNIgene Gut Kit, printed instructions, zip-top bags, and a plastic container with a tight seal. After production, samples were refrigerated at home until collected at the next visit (receipt of the sample at the Senior Center within 2 days of production was strongly encouraged; however, samples produced and brought to the Senior Center within 5 days following the first visit were accepted due to high prevalence of constipation in this sample of older adults). During the second visit at the Senior Center (within 1 week of the baseline visit), participants returned their fecal sample. Samples were kept at −80°C until processing.

Dietary Quality Assessment

Participants completed an interviewer administered 24-hour dietary recall at their first visit to obtain detailed information on food and beverage consumption, as described.[23] Briefly, 24-hour dietary recalls were collected using the US Department of Agriculture Automated Multiple Pass Method[25] in conjunction with the University of Minnesota Nutrition Data System for Research software version 2017.[26] This method collects detailed information on all foods and beverages consumed from midnight to midnight on the day before the interview. Participants were shown food models to help in estimating portion size. Participants completed a second interviewer administered 24-hour dietary recall at their second visit. Average daily nutrient intakes and food groups were calculated using Nutrition Data System for Research version 2017.[26]

Diet quality was calculated by two methods: the 2015 Healthy Eating Index (HEI-2015) and the Mediterranean Diet (MD) score. The HEI-2015 was chosen because it includes dietary components such as refined grains and added sugars. The HEI-2015 was characterized as described by Krebs-Smith and colleagues[27] and assesses dietary quality following the most recent Dietary Guidelines for Americans 2015-2020 (DGA). The population ratio method[28] was used to compute the 13 HEI-2015 components: total fruits, whole fruits, total vegetables (includes beans and peas), greens and beans (also includes beans and peas), dairy, total protein foods (also includes beans and peas), seafood and plant proteins (nuts, seeds, soy products and legumes), fatty acids (ratio of polyunsaturated and monounsaturated fatty acids to saturated fatty acids), refined grains, whole grains, sodium, added sugars, and saturated fats. Six food groups were assigned values from zero to five and seven were assigned values from zero to 10, for a total maximum score of 100. Higher values represent closer adherence to the dietary guideline recommendations.

The HEI-2015 includes beans and/or legumes in four dietary components (total vegetables, greens and beans, total protein foods, and seafood and plant proteins).[27] For this reason the total HEI-2015 score for Caribbean Latino adults may be inflated as Hispanic subgroups have been shown to eat significantly greater proportions of beans compared with non-Hispanic populations.[29] To provide additional scientific rigor, the MD score was calculated as developed by Trichopoulou and colleagues,[30] which contains one separate category for legume intake. Further, the original MD score was modified to include whole grains rather than total grains, allowing more appropriate assessment of dietary patterns for this population.[31] The MD score included nine components scored using the energy-adjusted, sex-specific population median, including vegetables, fruit, whole grains, nuts and legumes, meat, fish, dairy, a ratio of monounsaturated fatty acids to saturated fatty acids, and alcohol. Energy-adjustment was completed using the residual method. Scores of zero were given to those consuming below the median for

healthful dietary components and a score of one for above the median. Subsequently, a score of zero was given to those consuming above the median for unhealthy components and one for those consuming below the median. A total score was calculated as zero to nine with higher values indicating better adherence to an MD pattern.

Microbial Sequencing and Taxonomy Assignation

Microbial sequencing was performed at the Center for Microbiome Research at UMass Medical School, Worcester, MA. DNA from fecal samples was isolated using the DNeasy PowerSoil kit (Qiagen) following the manufacturer recommendation. Barcoded Illumina adaptor-containing primers 515F and 806R were used to amplify the *16S rRNA* variable region 4 by polymerase chain reaction. Libraries were then sequenced in the MiSeq platform (Illumina) using the 2×250 bp paired-end protocol yielding pair-end reads that nearly overlap.[32-34] The read pairs were demultiplexed based on the unique barcodes, and were merged using USEARCH v10[35] (parameters: -fastq_mergepairs -fastq_maxdiffs 10 -fastq_pctid 80). The 16S rRNA gene sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm (using the parameters: -cluster_otus -minsize 2).[36] OTU centroid sequences were classified using the SINTAX algorithm and the RDP training set database version rdp_16s_v16 (parameter: -sintax).[37,38] Reads were mapped to OTUs using USEARCH (parameter: -otutab) and the final table was subjected to feature and sample filtering before analysis. The filters applied were removal of OTUs with a total read count across all samples of <10 reads (<0.005% of total read data), removal of OTUs present in <2 samples, and removal of OTUs that failed to classify with at least 80% confidence to a taxonomic order—the classification filter removes OTUs that are frequently of mitochondrial and/or chloroplast origin. Sequence data is deposited under NCBI BioProject (PRJNA579996).

SFCA Analysis

Frozen aliquots of fecal samples were sent to the Victoria Genome British Columbia Proteomics Centre and analyzed for SCFA content using ultra-performance liquid chromatography/multiple reaction monitoring mass spectrometry methods.[39] Concisely, approximately 200 mg of each sample were precisely weighed, and 20 mL 50% aqueous acetonitrile were added, followed by vortex mixing for 5 minutes to extract the SCFAs. The samples were vortex mixed at 3,000 rpm for 1 minute, followed by sonication in an ice-water batch for 2 minutes before centrifugal clarification at 5°C and 15,000 rpm for 10 minutes in an Eppendorf 5420R centrifuge. Then, 100 μL supernatants were used for chemical derivatization using a pair of 12C6/13C6-3-nitrophenylhydrazine derivatization followed by ultra-performance liquid chromatography/multiple reaction monitoring mass spectrometry quantitation with negative-ion detection on an Agilent 1290 ultra-high-performance liquid chromatography system coupled to a Sciex 4000 QTRAP mass spectrometry instrument. After the SCFA analysis, the leftover material of each sample was transferred to another test tube and was lyophilized. The dry mass of each sample was weighed and recorded. Concentrations of SCFAs in the samples were calculated by interpolating the calibration curves of 10 individual SCFAs with the analyte-to-internal standard peak area ratios measured from the sample solutions. Major microbial produced SCFAs acetic acid, butyric acid, and propionic acid are presented as nanomoles per gram dry fecal mass.

Data from a comparison study of different methods of fecal collection indicates that for the most predominant SCFA (butyric acid and propionic acid), the OMNIgene GUT kit has high concordance (all intraclass correlations ≥0.82) with the immediate freezing method (traditionally gold standard method for collecting fecal samples).[40] For acetic acid, the intraclass correlation was lower, but still acceptable (0.64, 95% CI, 0.34–0.93). More importantly, biologically plausible correlations were observed between bacterial genera and the predominant SCFA in fecal samples. Most correlations were reproduced with immediate freezing and OMNIgene GUT, and the correlation coefficients were similar across these collection methods. Therefore, the current study estimated fecal SCFA content from samples collected with the OMNIgene GUT kit.

Bioinformatics and Statistical Analyses

Descriptive statistics for the population were calculated as mean±standard deviation or % for all variables. Log odds were calculated to compare predefined nutrients (associated with the gut microbiome in the literature[41]) and diet score components between the two microbiome clusters using multivariable logistic regression. Proc logistic was used with SAS version 9.4,[42] controlling for age and sex in each model. Fecal SCFA content (micrograms per gram) were log transformed for normality. Pearson correlation was used to test the relation between each fecal SCFA with each dietary exposure (continuous). Differences in the number of laxative users by gut microbiome cluster were tested by Fisher's exact test. Means for continuous variables by group, cluster, or disease outcome were calculated using proc means and frequencies by group, cluster or disease outcome were calculated using proc freq. Statistical analyses were performed using SAS software version 9.4.[42]

Statistical analyses of the microbiome were performed in QIIME2 2018.4 and the R package Phyloseq v1.19.1.[43] Phylogenetic metrics (Faith Phylogenetic Diversity [Faith PD][44] (alpha diversity) and unique fraction metric [UniFrac][45] (beta diversity) and nonphylogenetic metrics (Shannon diversity index [alpha diversity]) were calculated.[46,47] Spearman correlations (for continuous variables) and group comparisons using Kruskal-Wallis test (for categorical variables) between alpha diversity indexes and nutrient intakes were calculated. The R package cluster v1.4-1 was used to estimate patterns in the microbiome using a partitioning around medoids with estimation of number of clusters (PAMK function), with optimum average silhouette width.[48,49] The distance between samples was measured by weighted UniFrac distances.[45] Statistical significance of weighted UniFrac distances comparing samples by either: cluster, predefined nutrient variables or diet scores, was assessed using PERMANOVA and Mantel tests.[50] Bacterial taxon/taxa driving the differences in alpha and beta diversity by specific nutrients were identified using gneiss,[51] an analysis approach that applies the concept of balance trees to

compositional data to identify microbial subcommunities that respond to environmental variables; in this case, nutrient intakes. Here, the log ratio abundances of subcommunities within the microbiome are considered to indicate taxa whose abundances change relative to other taxa in response to the nutrient variable. Determinant bacterial species were identified by robust algorithms such as analysis of composition of microbiomes (ANCOM)[52] and Gneiss[51] incorporated in QIIME2. In exploratory analyses, the package DESeq2[43,53] was used to perform differential abundance testing of the microbiome by self-reported diabetes status. Statistical tests of $P<0.05$ are considered significant. Due to the low sample size (n=20), P values <0.1 are reported as trends.

RESULTS

Description of the cohort can be found in Table 1. Briefly, 100% of the sample self-reported cardiovascular disease and 45% self-reported T2D. Generally, there was poor alignment of participant's diets to either the MD or the DGA, as assessed by the MD score and HEI-2015. Range in the HEI-2015 was 36 to 90, where 5% (n=1) of the sample received a HEI-2015 grading[27] of A (high adherence to the DGA), 0% score of B, 45% (n=9) score of C, 25% (n=5) score of D, and 25% a score of F (n=5) (very poor adherence to the DGA). MD scores suggested low conformance with a Mediterranean eating pattern, score range: two to eight, where only 5% of the sample received a score of eight (moderate-high adherence) and 45% scored ≤3

Table 1. Descriptive characteristics of Caribbean Latino adults from Lawrence, MA (n=20), September 2016 to September 2017

Characteristic	Result
	n (%)
Women	14 (70)
Body mass index categories[a]	
Healthy	5 (25)
Overweight	8 (40)
Obese class I	5 (25)
Obese class II	2 (10)
Smoking status, current (%)	2 (10)
Self-reported type 2 diabetes, yes (%)	9 (45)
Self-reported cardiovascular disease, yes (%)	20 (100)
Place of birth	
Dominican Republic	15 (75)
Puerto Rico	2 (10)
Other Caribbean Island	3 (15)
Education	
Fifth-eighth grade	5 (25)
Ninth-12th grade or GED	1 (5)
Some college or bachelor's degree	3 (15)
Some graduate school	11 (55)
Laxative use in the past 30 d	
Yes	4 (20)
No	15 (75)
Don't know	1 (5)
	mean±standard deviation
Age (y)	62.7±8.1 (range: 51-76)
Height (cm)	161.5±12.2

(*continued on next page*)

Table 1. Descriptive characteristics of Caribbean Latino adults from Lawrence, MA (n=20), September 2016 to September 2017 (*continued*)

Characteristic	Result
Weight (kg)	75.2±13.4
Body mass index (kg/m^2)	28.9±4.9
Short-chain fatty acid fecal content[b] **(μg/g)**	
Acetate	2,164±4,137
Butyrate	901±1,678
Propionate	513±637
Physical activity (MET hours per week)	14±13
Total energy intake (kcal/d)	1,651±635
% energy from carbohydrates	50.3±8.9
% energy from fat	28.7±6.1
% energy from protein	19.6±5.3
Dietary protein (g/d)	77±28
Dietary carbohydrate (g/d)	214±95
Dietary total fiber (g/d)	20±9
Dietary soluble fiber (g/d)	7±3
Dietary insoluble fiber (g/d)	14±7
Dietary pectins (g/d)	4±3
Total sugars (g/d)	84±52
Total fat (g/d)	55±27
Healthy Eating Index-2015[c]	67±12
Mediterranean Diet score[d]	4±2
Alcohol intake (g/d)	4±10

[a]BMI categories: healthy 18.5 to 24.9, overweight 25.0 to 29.9, obese class I 30.0 to 34.9, obese class II 35.0 to 39.9.
[b]Not all fatty acids present in feces are listed. The three short chain fatty acids (SCFA) hypothesized to differ by diet quality are shown. SCFA are shown as μg/g. To convert μg/g acetic acid to nmol/g, multiply μg/g by 60.051^-1e-3. To convert μg/g butyrtic acid to nmol/g, multiply μg/g by 74.079^-1e-3. To convert μg/g propionic acid to nmol/g, multiply μg/g by 88.1051^-1e-3.
[c]Maximum score=100.
[d]0 to 9.

Table 2. Nutrients and dietary index components significantly correlated with alpha diversity (Shannon diversity index and Faith Phylogenetic Diversity) among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016-September 2017

	Shannon diversity index		Faith PD	
Dietary (continuous) variables	Spearman correlation	*P value*[a]	Spearman correlation	*P value*[a]
Total PUFA[b] (g/d)	−0.564	**0.01**	−0.478	**0.03**
Calories from PUFA	−0.550	**0.01**	−0.502	**0.02**
18:2 Total linoleic acid (g/d)	−0.555	**0.01**	−0.526	**0.02**
Total dietary fiber (g/d)	−0.490	**0.03**	−0.424	0.06
Insoluble dietary fiber (g/d)	−0.480	**0.03**	−0.555	**0.01**
Pectins (g/d)	−0.460	**0.04**	−0.048	0.84
Vegetable protein (g/d)	−0.275	0.24	−0.559	**0.01**
Healthy Eating Index-2015 components				
Sodium	−0.556	**0.01**	−0.488	**0.03**
Total Healthy Eating Index-2015 score	−0.559	**0.01**	−0.184	0.44

	Shannon index group significance	Faith PD group significance
	Kruskal-Wallis	Kruskal-Wallis
Dietary (categorical) variables	H test *P value*	H test *P value*
Mediterranean Diet components		
Vegetable	0.07	**0.01**

[a]Values in boldface type indicates significant *P values* <0.05, whereas underlined values represent trending *P values* <0.1.
[b]PUFA=polyunsaturated fatty acids.

(poor adherence). Due to limited representation of persons with diets reflecting high conformance to either the MD or the DGA, examining differences in the gut microbiome between high and low adherence groups was not possible. Therefore, all associations with diet are presented as linear relations with dietary nutrients and/or diet scores.

Associations of Individual Dietary Nutrients, Diet Scores, and Nutrient Variables with Gut Microbiome Diversity

Significant associations between individual nutrient variables and gut microbiome Shannon's alpha diversity index,[46,47] which weights both microbial community richness (observed OTUs) and evenness (equitability), were observed. Particularly, intakes of polyunsaturated fatty acids, 18:2 total linoleic acid, total dietary fiber, insoluble dietary fiber, and dietary pectins were significantly negatively associated with alpha diversity ($P<0.05$) (see Table 2 and Table 3, available at www.jandonline.org). Similarly, significant negative associations between diet quality index scores and microbiome alpha diversity were observed for HEI-2015 components, including sodium and the total HEI-2015 score ($P<0.05$) (Table 2).

Nutrient variables were correlated with faith phylogenetic diversity or Faith PD,[54] which is another measure of alpha diversity that is based on phylogeny or species relatedness (sum of branch lengths). Similar results were obtained to that of the Shannon alpha diversity index, except that pectins and the total HEI-2015 score did not show significant negative correlation with alpha diversity measured by Faith PD (Table 2). In addition, intakes of vegetable protein and total vegetables (a component of the MD score) were negatively associated with Faith PD alpha diversity ($P<0.05$) (Table 2). This suggests that specific phylogenetic lineages may be influenced by intakes of vegetable protein and vegetables.

Associations between dietary variables and beta diversity were measured. Beta diversity captures the similarities between microbial communities. Specific nutrient variables as well as diet quality index scores and their components were tested for associations with two measures of beta diversity: phylogenetic weighted or unweighted UniFrac method.[45,47] Overall, two dietary variables 18:3 α-linolenic acid and total sugars, and a component of the HEI-2015 (whole grains) were significantly positively associated with beta diversity ($P<0.05$) (see Table 4 and Table 5, available at www.jandonline.org). In addition, trends toward statistical positive associations were detected with microbiome beta diversity and total energy intake, 18:3 total linolenic acid, total n-3 fatty acids, total carbohydrate intake, soluble dietary fiber, and pectins ($P<0.1$) (see Table 4 and Table 5 available at www.jandonline.org). Similarly, components of the HEI-2015 (greens and beans, and seafood and plant proteins) and MD score components (vegetables and whole grains) also trended toward a positive correlation with microbiome beta diversity ($P<0.1$) (see Table 4 and Table 5, available at www.jandonline.org). No significant associations were observed for total

Table 4. Nutrients and dietary indexes components significantly related to beta diversity weighted and unweighted unique fraction metric (UniFrac) distances among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016 to September 2017

	Weighted UniFrac		Unweighted UniFrac	
Dietary (continuous) variables	Spearman correlation[a]	*P value*[b]	Spearman correlation[a]	*P value*[b]
Total energy intake (kcal/day)	0.14	0.08	−0.08	0.44
18:3 Total linolenic acid (g/d)	0.19	0.06	−0.15	0.27
18:3 Alpha-linolenic acid (g/d)	0.19	**0.04**	−0.15	0.26
Total n-3 fatty acid (g/d)	0.15	0.08	−0.18	0.16
Total carbohydrate (g/d)	0.15	0.07	−0.01	0.94
Total sugars (g/d)	0.21	**0.03**	0.08	0.52
Soluble dietary fiber (g/d)	0.02	0.79	0.22	0.07
Pectins (g/d)	0.16	0.09	0.02	0.91
Healthy Eating Index-2015 components				
Greens and beans	0.04	0.68	−0.21	0.08
Seafood and plant proteins	0.21	0.05	−0.06	0.69
Whole grains	−0.01	0.97	0.25	**0.03**

	Weighted UniFrac		Unweighted UniFrac	
Dietary (categorical) variables	Pseudo-F[c]	*P value*	Pseudo-F[c]	*P value*
Mediterranean Diet components				
Vegetables	1.22	0.24	1.44	0.07
Whole grains	2.75	0.09	1.01	0.46

[a]Mantel test results
[b]Values in boldface type indicate significant *P values* <0.05 and underlined values represent trending *P values* <0.1.
[c]Permutational multivariate analysis of variance test results.

HEI-2015 or total MD score and beta diversity (see Table 5, available at www.jandonline.org).

Overall, a significant correlation with log ratio abundances of specific sub-communities and several nutrient variables were observed and are presented in Table 6 (geiss, false discovery rate [FDR] corrected coefficient $P<0.05$). Significant, positive correlations between the abundance of *P copri* (OTU 456) and higher consumption of total n-3 fatty acids, 18:3 total linolenic acid, and 18:3 α-linolenic acid were observed (Table 6). A significant positive correlation was observed between the abundance of Enterobacteriaceae (OTU 121) and intake of pectins (Table 6). Other taxa that significantly differed by soluble fiber (*Parabacteroides gordonii,* OTU 221) showed a positive trend with soluble fiber intake (Table 6**).** Comparatively, a significant negative correlation was observed between the abundance of Clostridiales (OTU 196) and HEI-2015 total score, but not with Clostridiales (OTU 116), although significant by gneiss analysis (Table 6).

Differences in Dietary Intakes by Gut Microbiome Clusters

Patterns in the microbiome were determined using a partitioning around medoids with estimation of number of clusters (PAMK), to find the optimal number of clusters. As shown in Figure 1 (available at www.jandonline.org), samples were divided into two clusters, arbitrarily called cluster 1 (n=14, weighted Unifrac distance, silhouette score 0.46) and cluster 2 (n=6, weighted Unifrac distance, silhouette score 0.65). Discriminant bacterial taxa between the two clusters were identified with gneiss and also by an analysis of composition of microbiomes, or ANCOM. Both analyses identified a single bacterial taxon classified as *P copri* to discriminate between the two clusters (W>200, out of the 230-genus level OTUs analyzed in ANCOM). Significantly lower *P copri* abundance was observed in "cluster 1" compared with "cluster 2" (Mann-Whitney $P<0.0001$) (Figure 2A). When examining microbiome diversity measures, samples in cluster 2, the *P copri* dominated cluster, showed significantly lower alpha diversity compared to cluster 1 (Figure 2B) (Shannon diversity index, $P=0.01$). Using a nonmetric multidimensional scaling of weighted UniFrac distances, a clear separation of samples into the two clusters was confirmed (Figure 2C) (PERMANOVA $R^2= 0.576$; ADONIS $P=0.001$).

The bacterial taxon *P copri*, is the same bacterial taxon found to be positively correlated with 18:3 α-linolenic acid intake. A trend toward a significant difference in 18:3

Table 6. Bacterial taxa ratios that significantly differ by nutrient intakes among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016 to September 2017

Variable[a]	Determinant OTU[b]	gneiss, FDR[c] corrected *P value*	Spearman correlation	*P value*
Total n-3 fatty acids	*Prevotella copri* (OTU 456)	0.01	0.45	0.04
18:3 Total linolenic acid	*Prevotella copri* (OTU 456)	0.01	0.48	0.03
18:3 Alpha-linolenic acid	*Prevotella copri* (OTU 456)	0.01	0.47	0.03
Pectins	Enterobacteriaceae (OTU 121)	0.02	0.53	0.01
Soluble fiber	*Parabacteroides gordonii (OTU 221)*	0.01	0.80	0.05
HEI-2015[d] total score	Clostridiales (OTU 196)	0.006	−0.56	0.01
	Clostridiales (OTU 116)	0.006	−0.25	0.29

[a]Nutrient value was assessed from two, standardized 24-hour dietary recall interviews.
[b]OTU=Operational Taxonomic Unit, assessed by 16s RNA sequencing from human stool.
[c]FDR = false discovery rate.
[d]HEI-2015=Healthy Eating Index 2015.

α-linolenic acid intakes between clusters was observed (likelihood ratio 6.3; P=0.09), where individuals with samples in *cluster 1* presented lower mean 18:3 α-linolenic acid intakes (0.8±0.4 mg/day) compared with individuals with samples in cluster 2, the *P copri* dominated cluster, (1.6±0.9 mg/day). Thus, the differential abundance of *P copri* between clusters may be influenced by greater 18:3 α-linolenic acid intakes. No significant differences between clusters were observed with any other individual nutrients (P value range=0.10 to 0.42), HEI-2015 total score (P=0.30), total MD score (P=0.37), or diet score components (P value range=0.10 to 0.95), following adjustment for age and sex (data not shown).

Associations of Diet with SCFA Fecal Content

Correlations between individual fecal SCFA and dietary components are shown in Table 7 (available at www.jandonline.org). Percentage of calories from fat was positively correlated with fecal content of acetate, butyrate and propionate (r=0.46, P=0.04; r=0.50, P=0.03; and r=0.52, P=0.02, respectively). Total MD score trended toward negative correlation with fecal acetate and butyrate (r=−0.40, P=0.08 and r=−0.40, P=0.08, respectively). Total HEI-2015 score trended toward positive correlations with acetate and propionate (r=0.42, P=0.06 and r=0.42, P=0.07, respectively). No other nutrients were correlated with fecal SCFA content Table 7 (available at www.jandonline.org).

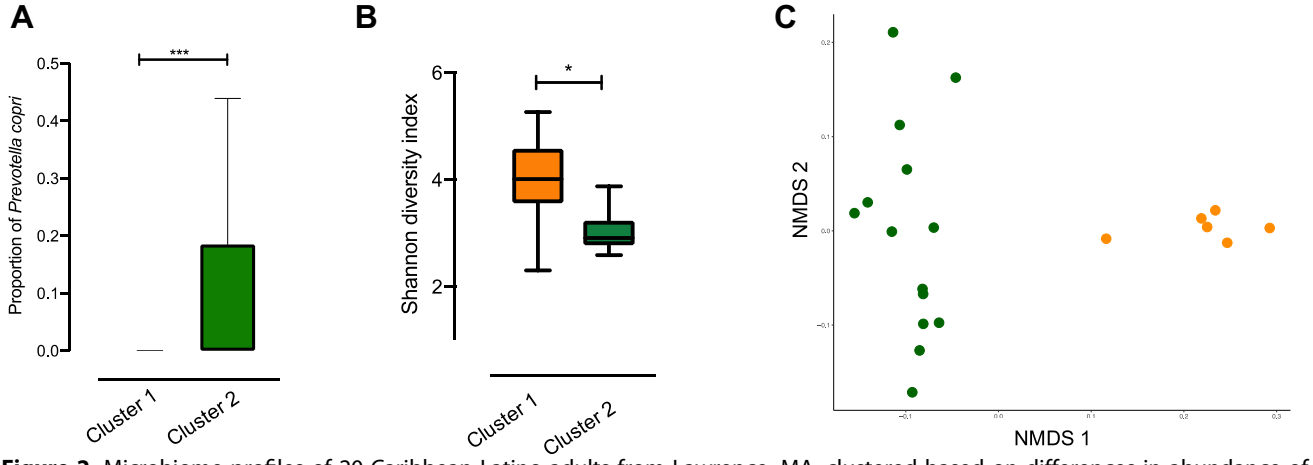

Figure 2. Microbiome profiles of 20 Caribbean Latino adults from Lawrence, MA, clustered based on differences in abundance of *Prevotella copri (P copri)*. Panel A: Boxplot of the proportion of *P copri* present in the samples belonging to each cluster: *P copri* depleted cluster 1; and *P copri* dominated cluster 2. Panel B: Boxplot of the median alpha diversity index (Shannon diversity index) of the microbiome of participants in the *P copri* depleted cluster 1 and on the *P copri* dominated cluster 2. Panel C: Nonmetric multidimensional scaling (NMDS) representing the ordering relationships of bacterial communities based on the weighted unique fraction metric (UniFrac) distances (phylogenetic beta diversity metric). The NMDS visualization shows clear clustering of *P copri* depleted samples (*orange*) separated from the *P copri* dominated samples (*green*). Each coordinate—NMDS 1 and NMDS 2—represents the two dimensions in which the samples are ordinated based on their the weighted UniFrac distances. *P<0.05. ***P<0.001.

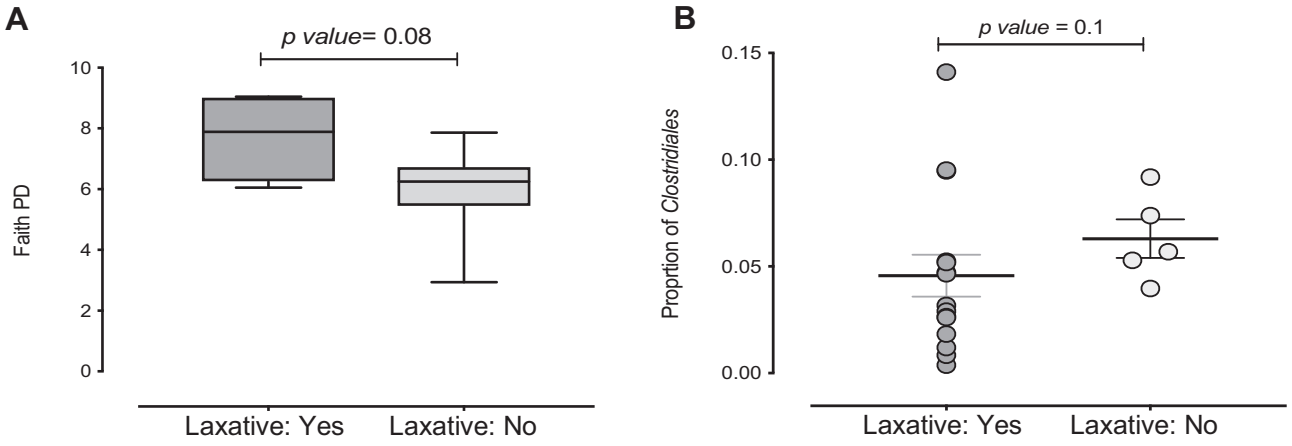

Figure 3. Microbial differences of the gut microbiome among 20 Caribbean Latino adults from Lawrence, MA, self-reporting using laxatives or not using laxatives (Laxative: Yes and Laxative: No, respectively), September 2016 to September 2017. Panel A: Boxplot of the Faith Phylogenetic Diversity (PD) index measuring alpha diversity of the microbiome of Caribbean Latino adults participating in the study grouped by those who self-reported using a laxative (*dark gray*) and those who reported not using laxatives (*light gray*). Panel B: Different abundance of Clostridiales OTU distinguished between microbiomes from Caribbean Latino adults self-reporting using laxative and (*dark gray*) those who reported not using laxatives (*light gray*).

Associations of Laxative Use, BMI, and Self-Reported Diabetes with the Gut Microbiome

In this study, 20% of the participants (n=4) self-reported use of laxatives in last 30 days. The microbiome of these participants showed a trend to higher alpha diversity compared with participants with no self-reported laxative use (Faith PD, Kruskal Wallis, P=0.08) (Figure 3A). In addition, individuals who self-reported laxative use exhibited significantly different beta diversity from those that did not report use of laxatives (Pairwise PERMANOVA P=0.039, data not shown). A single bacterial taxon classified as order Clostridiales was identified as a discriminant OTU between individuals with and without laxative use (of the 230-genus level OTUs analyzed in ANCOM, W=96 were considered significant). A trend toward higher abundance (~1.3-fold) of the Clostridiales OTU in individuals reporting no laxative use is shown in Figure 3B. A trend toward differences in the number of laxative users between gut microbiome clusters was observed (Pearson χ^2 4.82, Exact CI 0.64 to 733; P=0.06], where 50% (n=3) of individuals with samples in the *P copri* dominated cluster reported laxative use compared with 7% (n=1) individuals with samples in cluster 1.

Significant beta diversity differences were observed by weight (Mantel P<0.003) and BMI (Mantel P<0.07). Specifically, obese subjects exhibited a higher abundance of *Coprococcus* compared with individuals with a healthy BMI (ANCOM, of the 230-genus level OTUs analyzed in ANCOM, considered significance is reported for W=55).

In this cohort of Caribbean Latino adults, 45% of adults self-reported diabetes (n=9). Individuals reporting diabetes exhibited a trend toward less alpha diversity when compared with participants not reporting diabetes (Figure 4A) (Faith

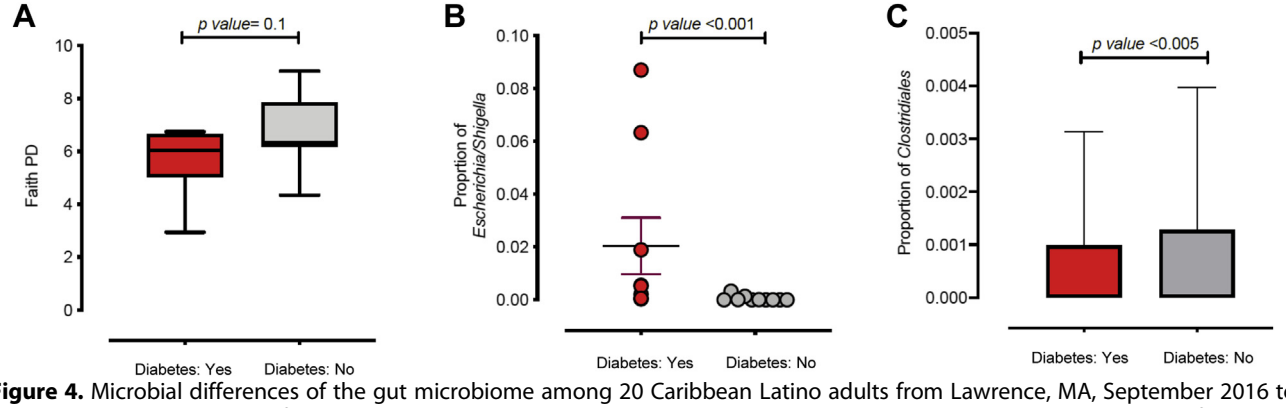

Figure 4. Microbial differences of the gut microbiome among 20 Caribbean Latino adults from Lawrence, MA, September 2016 to September 2017 between self-reported type 2 diabetes status (Diabetes: Yes and Diabetes: No, respectively). A) Boxplot of the Faith Phylogenetic Diversity (PD) index measuring alpha diversity of the gut microbiome among Caribbean Latino adults participating in the study grouped by those who self-reported type 2 diabetes status (*red*) or no diabetes status (*gray*). Different abundance of *Escherichia/Shigella* (Panel B) and Clostridiales OTUs (Panel C) distinguished between microbiomes from Caribbean Latino adults self-reporting type 2 diabetes (*red*) or no type 2 diabetes (*gray*).

PD, Kruskal Wallis P=0.1). No significant differences in beta diversity by self-reported diabetes status were observed (pairwise PERMANOVA P>0.05, data not shown). However, at the order level, a bacterial taxon classified as Enterobacteriales was distinguished between individuals self-reporting diabetes vs those not reporting the disease (ANCOM of the 14-order level OTUs analyzed in ANCOM considered significance is reported for W>2, and DeSeq2 adjusted P=0.01). Further, a single bacterial taxon classified *Escherichia/Shigella* was increased (~1.5-fold) in subjects with self-reported diabetes (Mann-Whitney test P= 0.0008) (Figure 4B). Participants reporting diabetes were not exclusive to either of the microbiome clusters previously identified. The gneiss analysis also identified several OTUs classified as Clostridiales to distinguish between individuals with or without diabetes (gneiss, FDR corrected P=0.04). The proportion of Clostridiales was observed to be significantly lower in patients self-reporting diabetes compared with participants reporting no diabetes (Mann-Whitney test P=0.003) (Figure 4C). A trend toward lower fecal butyric acid content in individuals with T2D was observed (567±542 μg/g) compared with individuals without T2D (1,174±2,220 μg/g; P=0.08). Mean fecal content of acetic acid and propionic acid among individuals with T2D (1,668±2,741 and 492±465 μg/g, respectively) compared with individuals without T2D (2,571±5,110 and 530±773 μg/g, respectively) were not statistically significantly different (P=0.12 and P=0.10, respectively).

DISCUSSION

To our knowledge, this is the first study to describe the gut microbiome composition and its relation to diet and laxative use among older Caribbean Latino older adults, a population with high prevalence of chronic illness.[1] Two distinct microbiome profiles were identified, unique among Caribbean Latino adults, the largest Hispanic subgroup in the United States, second to Mexican Americans.[55,56] The current study links dietary intakes, specifically n-3 fatty acids, sodium HEI-2015 component, and percentage of calories from fat with the gut microbiome and SCFA fecal content. Results of this pilot study suggest larger diet–gut microbiome studies among Caribbean Latinos are needed to evaluate the influence of fatty acids and sodium on the gut microbiome and their potential influence on chronic illness.

Two microbiome clusters were identified among this cohort of Caribbean Latino older adults. Particularly, the two microbiome clusters identified differed by their abundance of *P copri*. These data, in combination with other literature, support differences in predominant bacterial taxa in gut communities by ethnicity. For example, two enterotypes dominated by *Bacteroides* or *Prevotella,* have been distinguished in non-Hispanic white adults in the United States,[57] whereas healthy individuals from Europe exhibited three distinct enterotype dominated by either *Bacteroides, Prevotella,* or *Ruminococcus.*[58,59] A study comparing the gut microbiome of a Hispanic community living in the United States and Human Microbiome Project subjects (mainly non-Hispanic white adults from the United States) reported an increased abundance of *Prevotella* in the Hispanic cohort,[60] supporting results of the current study.

Differences between microbiome clusters among different ethnic groups has been attributed to differences in diet. For example, *Prevotella* enterotypes have been associated with long-term diets consisting of higher proportions of carbohydrates (grains, fruits, legumes, and dairy).[17,57,61,62] In the current study, individuals with samples belonging to the *P copri* dominated cluster showed a trend toward significance with consumption of 18:3 α-linolenic acid, a fatty acid predominantly found in vegetable oils. The relation of *P copri* to health has been recently debated. Although healthy individuals consuming a plant-rich diet seem to harbor high abundance of *Prevotella,*[63] strains of *Prevotella* have also been associated with increased prevalence of arthritis,[64] specifically early-onset arthritis.[65] More recently, the Human Microbiome Project reported that shifts on the gut microbiome of healthy adults is mainly due to repeated expansion and relaxation cycles in *P copri* abundance over time.[66] In the current study, individuals in the *P copri* dominated cluster exhibited overall lower gut microbial diversity. Three out of the four participants reporting laxative use, belonged to the *P copri* dominated cluster and altogether laxative users exhibited higher diversity than the participants with no reported laxative use. Thus, more studies are needed to further elucidate the role of *Prevotella* and laxative use in health and disease among Caribbean Latino-origin adults.

Laxative use was a strong determinant of identification in the *P copri* dominated cluster and was associated with reduction of butyrate-producer Clostridiales. Laxative use also attenuated the relation between diet and the gut microbiome. In a large Dutch-Belgian population, drug use (including osmotic laxatives) had the largest explanatory power on microbiota composition (10% of community variation).[19] Moreover, in mice, osmotic laxative treatment caused a long-lasting perturbation to the gut microbiota.[67] Approximately 20% of the US population report prevalence of constipation and of that subgroup, 40% regularly use laxatives to alleviate constipation.[68] Therefore, further research is warranted to understand patterns in, and reasons behind, the use of laxatives among Caribbean Latino adults and to elucidate the influence of laxative use on the gut microbiome and overall long-term health, especially among aging adults with high prevalence of constipation.

Despite the small sample size of the current study, significant correlations with diet and specific members of the gut microbiome were observed. Of note, n-3 fatty acids, 18:3 α-linolenic acid, and 18:3 total linolenic acid were consistently associated with the composition of the microbiome. There are several studies describing the health benefits of consuming diets rich in n-3 fatty acids, 18:3 α-linolenic acid, and 18:2 total linoleic acid for glycemic control and insulin sensitivity.[69-73] In addition, there is some evidence suggesting that the effect of n-3 fatty acids on the gut microbiota may be key on the influence of n-3 fatty acids on clinical parameters.[74-77] Thus, results from the current study highlight the relevance of specific members of the gut microbiota, such as *P copri*, whose abundance positively correlated with fatty-acid intake, and this fraction of the microbiota could serve as a biomarker of health. In this respect, due to cultural-specific intakes of fatty acids among Latino adults (high in corn and vegetable oils),[78] further understanding of the influence of specific fatty acids on the gut microbiome within this population are necessary to develop future dietary

interventions that may alter the ratio of the differing n-type fatty acids and evaluate their influence on the gut.

Other dietary variables consistently associated with microbiome diversity (alpha or beta) were dietary fibers, whole grains (HEI-2015 component), and vegetables (MD score component). These fiber-rich foods and nutrients are known to produce microbiota shifts favoring the abundance of certain bacterial species associated with health.[79,80] However, in the current study dietary fiber was negatively related to alpha diversity. This may be explained by the high percentage of participants with T2D. In previous work, patients with diabetes consuming a high fiber diet resulted in an overall decrease of alpha diversity.[81] Because the current study is cross-sectional, the negative correlation may be explained by reverse causation, where individuals with T2D are now consuming more fiber following diagnosis, and the presence of disease overcomes this dietary shift.

Dietary habits are complex and are usually simplified by using dietary indices that summarize dietary variance in a single measure and offer a means of controlling for diet in microbiota studies. In the current study, the HEI-2015 and MD scores were used to assess overall dietary quality and to link diet quality with the gut microbiome. Overall, a negative correlation between HEI-2015, alpha diversity, and the abundance of Clostridiales was observed. In a previous study, investigators reported that HEI-2015 was the best summary dietary measure to capture microbiome variance among individuals.[82] However, contrary to what was reported in the aforementioned study, HEI-2015 was negatively correlated to alpha diversity in the current cohort, perhaps due to the low variation in HEI-2015 score with the majority of the current sample with poor dietary quality. Thus, careful consideration of a dietary scoring system by ethnicity might be required when analyzing diet-dependent changes of the microbiome.

The relation of the gut microbiome with diabetes among a Latino community within the United States has been reported elsewhere.[83] Members of the Enterobacteriales order are among the most commonly overgrown symbionts in many conditions involving inflammation, including diabetes[84,85] as well as inflammatory bowel diseases,[86,87] obesity,[88-90] colorectal cancer,[91] and celiac disease.[92,93] Although future studies confirming diabetes status by standard methods are necessary, the current study adds to the growing literature pointing at the relevance of Enterobacteriales as contributing factor to inflammation and prevalence of diabetes. In contrast, maintenance of immune homeostasis in the gut has been attributed to the expansion of CD4+ T regulatory cells promoted by indigenous commensal members of Clostridiales order.[94,95] Studies report that patients with T2D exhibited a moderate intestinal dysbiosis characterized especially by a decrease in the number of Clostridiales bacteria that produce butyrate (*Roseburia intestinalis* and *Faecalibacterium prausnitzii*).[96,97] Similarly, the current study found that members of the bacterial taxa belonging to the Clostridiales order were decreased in Caribbean Latino adults with self-reported diabetes. Moreover, decreased abundance of Clostridiales was also associated with participants self-reporting laxative use despite having higher alpha diversity compared with participants with no recent laxative use. More studies on the influence of laxatives on the microbiome are warranted.

The current study has many strengths and some limitations. The main limitation of the current study is the small sample size. However, despite the sample size of 20 adults, results demonstrate significant associations between the gut microbiome composition and function, diet, and lifestyle factors. Moreover, analysis of SCFA composition provided a closer look at metabolites produced by the bacteria in the gut. In addition, diabetes status was self-reported; thus, these results are exploratory in nature and should be interpreted as such. Strengths include the standardized collection of dietary data (average intake over two 24-hour recall days, collected by prompted, validated methods[25]).

CONCLUSIONS

The current study describes microbiome profiles unique to an ethnic community of aging adults with disproportionately high rates of chronic disease that have been underrepresented in this area of research. This cohort represents a population of adults with chronic disease that may benefit from diet and lifestyle intervention to treat disease and potentially lower risk of mortality. These data suggest that dietary intakes, T2D status and lifestyle factors may be important predictors of gut microbiome profiles among Caribbean Latino older adults. Therefore, the current research can be used in support of prospective, larger cohorts to further understand diet–lifestyle interactions with the gut microbiome among adults at high risk for chronic disease.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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AUTHOR CONTRIBUTIONS

Conceptualization was handled by A. Maldonado-Contreras, S. E. Noel, M. Velez, and K. M. Mangano. Data curation was handled by A. Maldonado-Contreras, S. E. Noel, and K. M. Mangano. Formal analysis was conducted by A. Maldonado-Contreras, S. E. Noel, D. Ward, and K. M. Mangano. Funding acquisition was handled by A. Maldonado-Contreras, S. E. Noel, and K. M. Mangano. Methodology was handled by M. Velez. Supervision was handled by S. E. Noel and K. M. Mangano. The original draft was written by A. Maldonado-Contreras, S. E. Noel, and K. M. Mangano, whereas review and editing was handled by D. Ward.

Table 3. Complete analysis of nutrients, dietary index components, and participant characteristics in relation to alpha diversity (Shannon diversity index and Faith Phylogenetic Diversity) among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016 to September 2017

	Shannon diversity index		Faith PD	
Continuous variables	**Spearman correlation**	***P value*[a]**	**Spearman correlation**	***P value*[a]**
Anthropometric variables				
Age	−0.03	0.92	−0.34	0.14
Body mass index	−0.01	0.99	−0.25	0.29
Weight (kg)	0.03	0.89	−0.31	0.19
Dietary variables				
Total energy intake (kcal/d)	−0.31	0.19	−0.36	0.13
Total protein (g/d)	−0.19	0.43	−0.32	0.17
Animal protein (g/d)	−0.06	0.80	0.05	0.82
Vegetable protein (g/d)	−0.27	0.24	−0.56	**0.01**
Total carbohydrate (g/d)	−0.33	0.15	−0.20	0.41
Total sugars (g/d)	−0.32	0.17	−0.06	0.79
Total dietary fiber (g/d)	−0.49	**0.03**	−0.42	0.06
Soluble dietary fiber (g/d)	−0.35	0.13	−0.28	0.24
Insoluble dietary fiber (g/d)	−0.48	**0.03**	−0.56	**0.01**
Pectins (g/d)	−0.46	**0.04**	−0.05	0.84
Total fat (g/d)	−0.40	0.08	−0.39	0.09
Total MUFA[b] (g/d)	−0.26	0.27	−0.37	0.10
Total PUFA[c] (g/d)	−0.56	**0.01**	−0.48	**0.03**
Total n-3 fatty acid (g/d)	−0.27	0.24	−0.32	0.17
18:2 total linoleic acid (g/d)	−0.55	**0.01**	−0.53	**0.02**
18:3 total linolenic acid (g/d)	−0.33	0.15	−0.33	0.16
18:3 alpha-linolenic acid (g/d)	−0.32	0.16	−0.34	0.14
20:4 arachidonic acid (g/d)	−0.26	0.28	−0.02	0.94
20:5 eicosapentaenoic acid (g/d)	0.23	0.32	−0.02	0.95
22:5 docosapentaenoic acid (g/d)	0.31	0.18	0.22	0.36
22:6 docosahexaenoic acid (g/d)	0.41	0.07	0.18	0.45
Calories from carbohydrate	−0.20	0.39	0.01	0.99
Calories from fat	−0.01	0.98	−0.09	0.72
Calories from protein	0.29	0.21	0.12	0.62
Calories from alcohol	−0.13	0.59	0.01	0.95
Calories from MUFA	0.08	0.73	−0.23	0.33
Calories from PUFA	−0.55	**0.01**	−0.50	**0.02**
Calories from saturated fat	0.16	0.49	0.07	0.78
Healthy Eating Index-2015 components				
Added sugars	−0.10	0.67	−0.11	0.64
Dairy	−0.07	0.77	0.26	0.27
Fatty acids	−0.05	0.83	−0.20	0.39
Greens and beans	−0.22	0.36	−0.42	0.07
Refined grains	−0.30	0.20	0.05	0.83
Saturated fats	−0.13	0.59	−0.09	0.71

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Table 3. Complete analysis of nutrients, dietary index components, and participant characteristics in relation to alpha diversity (Shannon diversity index and Faith Phylogenetic Diversity) among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016 to September 2017 (*continued*)

	Shannon diversity index		Faith PD	
Continuous variables	**Spearman correlation**	***P value*[a]**	**Spearman correlation**	***P value*[a]**
Seafood and plant proteins	0.17	0.47	−0.43	0.06
Sodium	−0.56	**0.01**	−0.49	**0.03**
Total fruits	−0.31	0.18	0.33	0.15
Total protein foods	−0.08	0.73	−0.38	0.09
Total vegetables	0.13	0.60	0.14	0.57
Whole fruits	−0.21	0.35	0.32	0.17
Whole grains	−0.43	0.06	−0.33	0.15
Total Healthy Eating Index-2015 score	−0.56	**0.01**	−0.18	0.44
Mediterranean Diet score	−0.24	0.31	−0.29	0.22

	Shannon index	Faith PD
Categorical variables	**Kruskal-Wallis H test *P value***	**Kruskal-Wallis H test *P value***
Sex	0.93	0.68
Type 2 diabetes status (y/n)	0.68	0.18
Mediterranean Diet score components		
Alcohol	0.64	0.57
Dairy	0.76	0.94
Fish	0.94	0.76
Fruit	0.08	0.54
Meat	0.55	0.59
Nuts and legumes	0.88	0.94
Ratio of MUFA to saturated fatty acids	0.76	0.94
Vegetable	0.07	**0.01**
Whole grain	0.36	0.82

[a]Values in boldface type indicates significant *P* values <0.05, whereas underlined values represent trending *P* values <0.1.
[b]MUFA=monounsaturated fatty acids.
[c]PUFA=polyunsaturated fatty acids.

Table 5. Complete analysis of nutrients and dietary index components in relation to beta diversity weighted and unweighted unique fraction metric (UniFrac) distances among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016 to September 2017

	Weighted UniFrac		Unweighted UniFrac	
Dietary (continuous) variables	**Spearman correlation[a]**	***P value*[b]**	**Spearman correlation[a]**	***P value*[b]**
Total energy intake (kcal/d)	0.14	0.08	−0.08	0.44
Total protein (g/d)	0.11	0.22	−0.10	0.42
Animal protein (g/d)	0.08	0.43	−0.13	0.37
Vegetable protein (g/d)	−0.07	0.49	0.10	0.47
Total carbohydrate (g/d)	0.15	0.07	−0.01	0.94
Total sugars (g/d)	0.21	**0.03**	0.08	0.52
Total dietary fiber (g/d)	0.09	0.31	0.09	0.48
Insoluble dietary fiber (g/d)	0.05	0.65	0.03	0.82
Soluble dietary fiber (g/d)	0.02	0.79	0.22	0.07
Pectins (g/d)	0.16	0.09	0.02	0.91
Total fat (g/d)	0.08	0.47	−0.09	0.53
Total MUFA[c] (g/d)	0.02	0.86	−0.01	0.53
Total PUFA[d] (g/d)	0.12	0.17	−0.01	0.96
Total n-3 fatty acids (g/d)	0.15	0.08	−0.18	0.16
18:2 total linoleic acid (g/d)	0.11	0.26	0.02	0.91
18:3 total linolenic acid (g/d)	0.19	0.06	−0.15	0.27
18:3 alpha-linolenic acid (g/d)	0.19	**0.04**	−0.15	0.26
20:4 arachidonic acid (g/d)	0.01	0.88	−0.20	0.12
20:5 eicosapentaenoic acid (g/d)	−0.07	0.52	−0.17	0.18
22:5 docosapentaenoic acid (g/d)	−0.05	0.61	−0.13	0.38
22:6 docosahexaenoic acid (g/d)	−0.08	0.42	−0.19	0.15
Calories from carbohydrate	−0.12	0.17	−0.05	0.66
Calories from protein	0.02	0.81	−0.07	0.56
Calories from fat	−0.05	0.57	0.09	0.50
Calories from MUFA	−0.13	0.17	0.04	0.76
Calories from PUFA	0.10	0.19	0.09	0.40
Calories from saturated fat	−0.07	0.47	−0.07	0.53
Calories from alcohol	0.01	0.93	−0.01	0.95
Healthy Eating Index-2015 components				
Added sugars	0.09	0.41	0.01	0.95
Dairy	−0.09	0.19	-0.11	0.16
Fatty acids	−0.06	0.39	−0.06	0.46
Greens and beans	0.04	0.68	−0.21	0.08
Refined grains	−0.01	0.93	−0.14	0.28
Saturated fats	−0.01	0.88	−0.03	0.76
Seafood and plant proteins	0.21	0.05	−0.06	0.69
Sodium	−0.04	0.64	0.07	0.42
Total fruits	0.06	0.49	−0.12	0.24
Total protein foods	0.13	0.29	0.01	0.97
Vegetables	0.08	0.26	−0.05	0.62

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Table 5. Complete analysis of nutrients and dietary index components in relation to beta diversity weighted and unweighted unique fraction metric (UniFrac) distances among Caribbean Latino adults (n=20) from Lawrence, MA, September 2016 to September 2017 (*continued*)

	Weighted UniFrac		Unweighted UniFrac	
Dietary (continuous) variables	**Spearman correlation[a]**	***P value*[b]**	**Spearman correlation[a]**	***P value*[b]**
Whole fruits	−0.12	0.20	−0.21	0.14
Whole grains	−0.01	0.97	0.25	**0.03**
Total Healthy Eating Index-2015 score	−0.01	0.98	−0.07	0.62
Mediterranean Diet score	−0.05	0.53	0.06	0.53

	Weighted UniFrac		Unweighted UniFrac	
Dietary (categorical) variables	**pseudo-F[e]**	***P value***	**pseudo-F[e]**	***P value***
Mediterranean Diet score components				
Alcohol	1.50	0.23	0.64	0.94
Dairy	0.28	0.79	0.74	0.80
Fish	0.09	0.97	0.90	0.59
Fruit	1.52	0.21	0.99	0.46
Meat	0.41	0.72	0.58	0.95
Nuts and legumes	0.18	0.90	0.73	0.83
Ratio of MUFA to saturated fatty acids	0.28	0.81	0.74	0.84
Vegetable	1.22	0.24	1.44	0.07
Whole grain	2.75	0.09	1.01	0.46

[a]Mantel test results.
[b]Values in boldface type are significant at $P<0.05$, whereas underlined values represent trending *P values* <0.1.
[c]MUFA=monounsaturated fatty acids.
[d]PUFA=polyunsaturated fatty acids.
[e]Permutational multivariate analysis of variance test results.

Table 7. Correlations between dietary components, dietary indexes, and short-chain fatty acid (SCFA) fecal content among Caribbean Latino adults from Lawrence, MA, September 2016 to September 2017

Dietary variable	Fecal SCFA[a]	Pearson correlation	*P value*[b]
Total energy (kcal/d)	Acetate	−0.23	0.33
	Butyrate	−0.24	0.30
	Propionate	−0.19	0.43
Total carbohydrate (g/d)	Acetate	−0.25	0.29
	Butyrate	−0.31	0.18
	Propionate	−0.26	0.27
% of calories from carbohydrate	Acetate	−0.23	0.32
	Butyrate	−0.33	0.15
	Propionate	−0.33	0.14
Total protein (g/d)	Acetate	−0.28	0.22
	Butyrate	−0.24	0.30
	Propionate	−0.19	0.41
% of calories from protein	Acetate	−0.07	0.32
	Butyrate	−0.02	0.93
	Propionate	−0.06	0.79
Total fat (g/d)	Acetate	−0.05	0.82
	Butyrate	−0.05	0.82
	Propionate	−0.01	0.97
% of calories from fat	Acetate	0.46	**0.04**
	Butyrate	0.50	**0.03**
	Propionate	0.52	**0.02**
Total monounsaturated fatty acids (g/d)	Acetate	0.01	0.96
	Butyrate	0.01	0.97
	Propionate	0.06	0.79
Total polyunsaturated fatty acids (g/d)	Acetate	−0.03	0.89
	Butyrate	−0.02	0.93
	Propionate	0.06	0.79
n-3 Fatty acids (g/d)	Acetate	−0.02	0.94
	Butyrate	0.07	0.77
	Propionate	0.01	0.96
18:2 Total linoleic acid	Acetate	−0.03	0.89
	Butyrate	−0.03	0.89
	Propionate	0.06	0.79
18:3 Total linolenic acid	Acetate	−0.01	0.98
	Butyrate	0.08	0.75
	Propionate	0.05	0.83
18:3 Alpha-linolenic acid	Acetate	−0.01	0.99
	Butyrate	0.08	0.74
	Propionate	0.05	0.83
Total dietary fiber (g/d)	Acetate	−0.09	0.70
	Butyrate	−0.15	0.52
	Propionate	−0.06	0.79
Insoluble fiber (g/d)	Acetate	−0.04	0.88
	Butyrate	−0.08	0.72
	Propionate	0.01	0.97
Soluble fiber (g/d)	Acetate	−0.20	0.40
	Butyrate	−0.27	0.24
	Propionate	−0.22	0.34
Pectins (g/d)	Acetate	0.13	0.57
	Butyrate	0.03	0.89
	Propionate	0.01	0.56
Total sugar (g/d)	Acetate	−0.16	0.50
	Butyrate	−0.29	0.22
	Propionate	−0.24	0.31
MDS[c] total score	Acetate	−0.40	0.08
	Butyrate	−0.40	0.08
	Propionate	−0.28	0.23
HEI[d]-2015 total score	Acetate	0.42	0.06
	Butyrate	0.24	0.31
	Propionate	0.42	0.07

[a]SCFA measured in μg/g, log transformed for normality.
[b]Values in boldface type are significant at $P<0.05$, whereas underlined values represent trending *P values* <0.1.
[c]MDS=Mediterranean Diet Score.
[d]HEI=Healthy Eating Index 2015.

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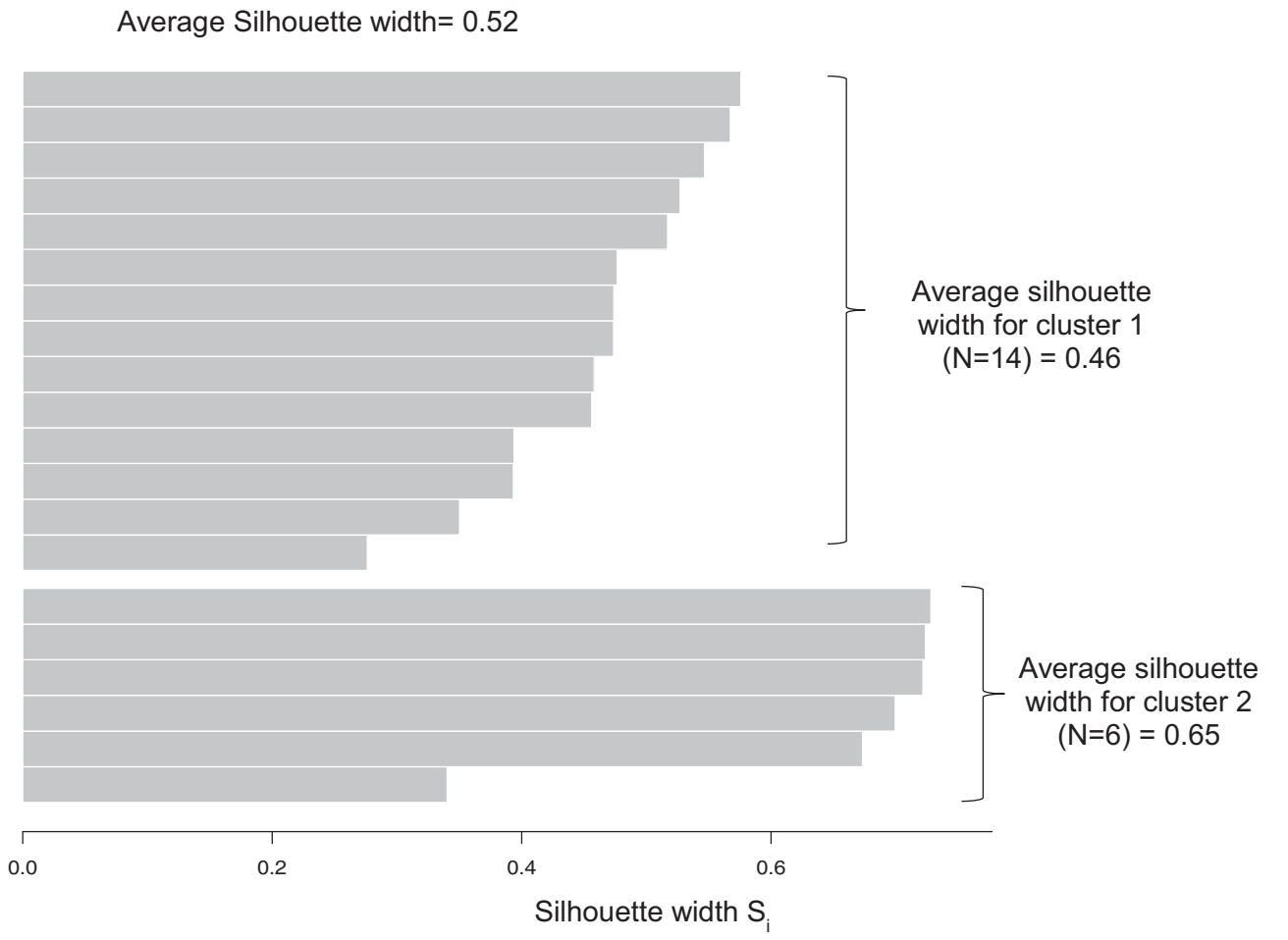

Figure 1. Cluster analysis of the gut microbiome among 20, older Caribbean Latino adults. Two distinct microbiome clusters were identified using the silhouette width method, a measure of similarity among microbiome communities or comparison to other clusters. The silhouette width ranges from −1 to +1, where a high value indicates that the microbiome of an individual matches to its own cluster and poorly matches to neighboring clusters. Each bar in the figure represents the silhouette width of an individual sample. Fecal samples were collected from September 2016 to September 2017 from participants recruited at the Senior Center and surrounding community in Lawrence, MA.