# Physical activity and diet associations with the gut microbiota in the Coronary Artery Risk Development in Young Adults (CARDIA) study

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A B S T R A C T

**Background:** Gut microbiota may influence metabolic pathways related to chronic health conditions. Evidence for physical activity and diet influences on gut microbial composition exists, but data from diverse population-based cohort studies are limited.
**Objectives:** We hypothesized that gut microbial diversity and genera are associated with physical activity and diet quality.
**Methods:** Data were from 537 participants in the Coronary Artery Risk Development in Young Adults (CARDIA) Study, a prospective cohort, who attended the year 30 follow-up examination (2015–2016; aged 47–61 y; 45% Black race/55% White race; 45% men/55% women). The 16S ribosomal RNA marker gene was sequenced from stool DNA, and genus-level taxonomy was assigned. Within-person microbial diversity (α-diversity) was assessed with Shannon diversity index and richness scores; between-person diversity (β-diversity) measures were generated with principal coordinates analysis (PCoA). Current and long-term physical activity and diet quality measures were derived from data collected over 30 y of follow-up. Multivariable-adjusted regression analysis controlled for: sociodemographic variables (age, race, sex, education, and field center), other health behaviors (smoking, alcohol consumption, and medication use), and adjusted for multiple comparisons with the false discovery rate (<0.20).
**Results:** Based on PCoA β-diversity, participants' microbial community compositions differed significantly ($P < 0.001$), with respect to both current and long-term physical activity and diet quality. α-Diversity was associated only with current physical activity (positively) in multivariable-adjusted analysis. Multiple genera ($n = 45$) were associated with physical activity and fewer with diet ($n = 5$), including positive associations with *Lachnospiraceae UCG-001* and *Ruminococc*aceae *Incertae Sedis* with both behaviors.
**Conclusions:** Physical activity and diet quality were associated with gut microbial composition among 537 participants in the CARDIA study. Multiple genera were associated with physical activity. Physical activity and diet quality were associated with genera consistent with pathways related to inflammation and short-chain fatty acid production.

*Keywords*: gut microbiota, microbiome, physical activity, diet, health behaviors, epidemiology, cohort

## Introduction

A growing body of literature supports a role for the gut microbiota in a range of cardiometabolic outcomes, including through pathways involving systemic inflammation and metabolite production [1–4]. Animal and human data indicate that physical activity and diet influence gut microbial composition and function [5–13], suggesting that microbiota-related health effects may be modifiable. Nevertheless, the type and duration of physical activity and dietary habits necessary for substantial changes to the gut microbiome are not known. Intervention studies of physical activity and diet have documented behavior-associated changes in the gut microbiota [5–7, 14–20], although translation of these findings to population-based

*Abbreviations used:* CARDIA, Coronary Artery Risk Development in Young Adults; FDR, false discovery rate; PCoA, principal coordinate analysis; PERMANOVA, permutational multivariate analysis of variance; SCFA, short-chain fatty acid.

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samples has been limited, and follow-up after intervention periods have been inconsistent.

Cross-sectional, observational studies have also illustrated associations between the gut microbiota and physical activity and diet [8, 14, 15, 21–28]. However, many publications reflect targeted sample recruitment [14, 21–23], such as professional athletes, from which it is difficult to generalize. Among population-based observational studies, there has been a paucity of samples with sociodemographic diversity, including race/-ethnicity, and a frequent lack of covariate adjustment for other health behaviors, such as smoking [29, 30]. Furthermore, studies generally have not included both short- and long-term measures of physical activity and diet, which may be distinctly relevant for gut microbial community composition.

To address these gaps in the literature, we investigated associations between gut microbial composition and physical activity and diet in the Coronary Artery Risk Development in Young Adults (CARDIA) Study, a prospective cohort of self-reported Black and White US adults. At the year 30 follow-up examination (2015–2016), fecal samples were collected from a sample of CARDIA participants and gut microbial compositional measures were generated for analysis. Over 30 y of follow-up, CARDIA has collected data on an extensive set of covariates, including sociodemographic indicators and health behaviors. Physical activity and diet have been repeatedly assessed using standardized and validated protocols, allowing study of both current and longer-term behavioral exposures. We hypothesize that microbial diversity will be positively associated with physical activity and dietary quality scores and that genera with higher physical activity and better dietary quality scores will be associated with beneficial metabolic products.

## Methods

### Study participants

The CARDIA study was designed to study the evolution of cardiovascular disease risk beginning in young adulthood [31]. At baseline, in 1985–1986, 5115 Black and White adults, aged 18–30 y, were enrolled from 4 US urban centers: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. There have been 8 follow-up examinations at years 2, 5, 7, 10, 15, 20, 25, and 30, with retention of the majority of surviving cohort members (91%, 86%, 81%, 79%, 74%, 72%, 72%, and 71%, respectively).

Data are from a microbiome ancillary study conducted at the year 30 follow-up examination (2015–2016) among a subset of CARDIA participants (aged 48–60 y, $n = 537$, 45% self-reported Black race/55% White race, 55% female/45% male). Participants were excluded if they reported being pregnant at the time of the exam, taking antibiotics within the past month, having diagnosed inflammatory bowel disease, or experiencing gastrointestinal illness within the past week on the screening questionnaire. Among the 537 individuals who met the eligibility criteria and completed the microbiome study component, participants were excluded from multivariable-adjusted analysis of current behaviors if they were missing relevant exposure or covariate data ($n =$ 45 were excluded for missing dietary data, $n = 5$ for missing physical activity data, $n = 7$ for missing smoking data, and $n = 6$ for missing alcohol consumption data, resulting in 480 participants with complete health behavior data at year 30. There were no missing data for analysis of lifetime behavioral measures, as we used available reports from 30 y of follow-up, and $n = 537$ were available for lifetime analysis. The approach for deriving the sample size in the fully adjusted linear regression model is summarized in Supplemental Figure 1. CARDIA was approved by institutional review boards of each field center, and study participants provided written informed consent for both the CARDIA core examination and the microbiome study.

### Measurement of physical activity, diet, and covariates

Standardized questionnaires were used to obtain sociodemographic and health behavioral data at the CARDIA field centers during study examinations. Participants self-reported their race and gender at baseline, as well as age and educational attainment at each examination. At each examination, the interviewer-administered CARDIA physical activity questionnaire queried the participant's past-year engagement (frequency and intensity) in 13 activities. From these data, a total activity score (exercise units) was calculated as previously described [32]. We note that 300 CARDIA exercise units is approximately equal to meeting current physical activity guidelines [33].

At years 0, 7, and 20, CARDIA used an interviewer-administered dietary history to comprehensively assess past-month food and beverage consumption and the use of dietary supplements [34]. The dietary history was not administered at year 30, but microbiome participants completed a 23-item frequency-based instrument [35] in their home at the time of sample collection, reporting their typical frequency of consumption of major food/food groups (further detailed in Supplemental Methods). A diet quality score was derived as previously described [36].

All CARDIA participants were asked to self-report medication use. We derived a medication use score by summing the number of medications participants reported taking. Participants in the microbiome ancillary study were additionally asked to report their use of antibiotics, prebiotics (fiber substitutes), or probiotics over the past 6 mo.

For lifetime exposure analysis, imputations of exam-specific data assuming linear change between available measurements were conducted in R (v 3.5.1) with the '*zoo*' package (v 1.8-9) [37], and time-weighted averages were generated for physical activity and diet using the full set of data, reported and imputed, over follow-up. For the time-varying behavioral covariates, smoking and alcohol consumption, we created current and lifetime measures for adjusted models. At baseline, participants reported their current smoking status and past smoking history, including age of initiation and number of cigarettes smoked per day. Updated smoking data were collected at each follow-up examination. Lifetime smoking was modeled as pack-years, as previously described (one pack = 20 cigarettes) [38]. At each examination, participants reported the amount and frequency of beer, wine, and spirits consumption, from which daily consumption of alcohol in milliliters (mL) was calculated. Lifetime alcohol consumption was modeled as time-weighted mL-days.

### Microbiome data collection, sequencing, and data processing

Standard protocols were followed for collection and processing of stool samples [39, 40], as previously described [41]. Participants completed the stool collection in their home and

shipped their sample to the Nutrition Research Institute at the University of North Carolina, Chapel Hill, where samples were stored at -80°C until processing. DNA was extracted from 0.2 grams of stool using the MoBio PowerSoil kit for 6 batches. The V3-V4 hypervariable regions of the 16S ribosomal RNA marker gene (Primers: 341F/785R) were amplified and sequenced, in random order, using the Illumina MiSeq platform (2 × 300), over 6 sequencing runs. Sequence data were processed with DADA2, which integrates tools for quality control, read error correction, and taxonomic assignment [42]. Samples were sequenced to a median sample depth of 101,177 (interquartile range: 84,051–115,831) reads. The DADA2-formatted Silva database (silva_nr_v138_train_set.fa.gz) was used to assign taxonomy [43]. Full Silva taxonomic classifications can be found in Supplemental Table 15. We note that assignments of *Incertae Sedis* refer to genera for which hierarchical classification is unknown [44].

Microbiota diversity among participants was derived in R with the 'vegan' package (v 2.5-6) [45]. Raw counts were used to calculate richness (read count-normalized number of distinct genera) and Shannon diversity index, an integrated measure of richness and distributional evenness [46]. Both richness and Shannon diversity were standardized (mean = 0; standard deviation = 1) for analyses with health behaviors. For assessing β-diversity, genera were log-normalized by applying following equation, $log_{10}\left[\left(\frac{RC}{n}\right)\left(\frac{x}{N}\right)+1\right]$, with $RC$ representing the total raw genus count per participant, $n$ as the total counts of genera per participant, $x$ as the total of all genera and participants, and $N$ as the total number of participants [41]. Principal coordinates analysis (PCoA) plots were generated based on Bray-Curtis dissimilarity [47]. For regression analysis of log-normalized genera, we restricted analysis to genera that were present in at least 25% of participants to test associations with the most prevalent genera and to limit the influence of rare assignments that may lead to spurious findings [41].

## Statistical analysis

Study participant characteristics were compared across quartiles of lifetime dietary pattern scores and physical activity (exercise units) using chi-square for categorical characteristics, Kruskal-Wallis for means of continuous variables, and Mood's test for medians of continuous variables. Using multivariable-adjusted regression, we analyzed associations between health behaviors and 3 standard microbial measures: within-person α-diversity, between-person β-diversity, and individual genera. Microbial measures were set as dependent variables, and health behaviors and confounders were set as independent variables. Primary analysis was at the genus level, which reflects the most refined taxonomic view available from our data. Physical activity and dietary quality were modeled as continuous and in quartiles. Continuous physical activity was log-transformed, to account for non-linearity, for statistical analysis.

We conducted 3 levels of multivariable adjustments in our analysis. In Model 1, we adjusted for sequencing run to account for batch effect. In subsequent analysis, we added (Model 2) sociodemographic variables: age (continuous), educational attainment in years (continuous), field center (Birmingham/Chicago/Minneapolis/Oakland), race (Black/White), and sex (male/female), and, in Model 3, smoking and alcohol consumption. In analysis of current physical activity and diet, smoking was modeled as never, former, and current categories; in analysis of lifetime measures, smoking was modeled as categorical: never smokers, and among ever smokers, below and above the median pack-years, an approach previously applied [48]. Similarly, in both current and lifetime analyses for alcohol consumption, categories were modeled as: abstainers, and among drinkers, below and above the median (mL/day).

α-Diversity measures were included as a participant-level measure (richness, Shannon) in linear regression models, with $P < 0.05$ the threshold for statistical significance. For β-diversity, model significance was assessed with the permutational multivariate analysis of variance (PERMANOVA) test (1000 permutations), at $P < 0.05$. For visual display of PCoA plots, centroids of health behavior categories were overlaid onto the first PCoA 2 axes, although we note that the PERMANOVA test indicates significance through permutations for differences between microbial dissimilarity and the health behavior in the full multidimensional space. For genus-specific regressions, separate regressions were run for each log-normalized genus [41], accounting for multiple comparisons with the Benjamini-Hochberg false discovery rate (FDR) [49]. Genus-specific associations with FDR <0.20 were considered significant.

In addition to our primary analysis, sensitivity analyses were conducted to evaluate the robustness of results when controlling for medication use, a potential confounder. We tested all associations with a fourth model, Model 4, in which medication use was added to Model 3 as a categorical variable: no medications used ($n = 186$), 1–2 medications used ($n = 127$), ≥ 3 medications used ($n = 167$). A range of medications were reported by CARDIA participants, including cholesterol-lowering (e.g., statins), antihypertensives (e.g., β-blockers), and diabetes (e.g., metformin). In addition, we examined specific medications or supplements that may be particularly relevant for the gut microbiota, including metformin ($n = 33$), past-year antibiotics ($n = 81$), and use of pre- or probiotics ($n = 99$). We also tested the sensitivity of genus-specific results based on a different rare taxa restriction, excluding only genera with prevalence under 10% [50]. Because of differences in the diet assessment methodology between years 20 and 30 of follow-up, we conducted analysis with year 20 as the last measure for both current and lifetime measures.

# Results

Participant characteristics according to lifetime health behaviors are presented in Table 1; see Supplemental Table 1 for characteristics with respect to current health behavior measures. Compared with men, women had lower mean physical activity scores and higher mean diet quality scores. Compared with White participants, Black participants had lower mean physical activity and diet quality scores. Physical activity and diet quality were positively associated.

## Between- and within-person microbial diversity analysis

For both current and lifetime measures of physical activity and diet quality, between-person microbial diversity was statistically significant in multivariable-adjusted PCoA analysis (PERMANOVA $P < 0.001$) (Figure 1). These data show that levels of physical activity and diet quality were distinguished

**TABLE 1**
Characteristics[1] of CARDIA (Coronary Artery Risk Development in Young Adults) microbiome study participants according to health behaviors categories[2]: CARDIA Year 30, $n = 537$[3]

| Weighted lifetime physical activity | | | | | |
|---|---|---|---|---|---|
| *n* | Q1<br>130 | Q2<br>130 | Q3<br>139 | Q4<br>138 | *P*[4] |
| Physical activity (exercise units) | 143 [96.5, 175] | 270 [235, 299] | 398 [360, 440] | 622 [558, 791] | $<2.20 \times 10^{-16}$ |
| Age (y) | 55.4 (3.51) | 55.3 (3.69) | 55.2 (3.63) | 55.2 (3.29) | 0.883 |
| Female, % | 80.8 | 59.2 | 41.0 | 35.5 | $1.64 \times 10^{-14}$ |
| Black race, % | 55.4 | 50.0 | 43.2 | 30.4 | $2.76 \times 10^{-4}$ |
| Education attained (y) | 15.4 (2.55) | 15.9 (2.58) | 16.0 (2.55) | 16.2 (2.56) | 0.0943 |
| Diet quality score | -0.408 (0.732) | -0.0742 (0.786) | 0.0393 (0.813) | 0.341 (0.863) | $7.63 \times 10^{-12}$ |
| Smoked, % ever | 44.6 | 45.4 | 43.1 | 39.2 | 0.315 |
| Consumed alcohol, % ever | 71.5 | 80.8 | 90.7 | 95.7 | $5.29 \times 10^{-9}$ |
| Weighted lifetime diet - Year 30 | | | | | |
| *n* | Q1<br>136 | Q2<br>132 | Q3<br>133 | Q4<br>136 | *P* |
| Diet quality score | -1.06 (0.326) | -0.348 (0.165) | 0.251 (0.179) | 1.08 (0.404) | $<2.20 \times 10^{-16}$ |
| Age (y) | 53.9 (3.46) | 54.8 (3.86) | 55.5 (3.21) | 56.9 (2.81) | $2.40 \times 10^{-11}$ |
| Female, % | 43.4 | 50.8 | 51.1 | 69.1 | $2.02 \times 10^{-4}$ |
| Black race, % | 77.9 | 55.3 | 30.8 | 14.0 | $<2.20 \times 10^{-16}$ |
| Education attained (y) | 14.6 (2.43) | 15.5 (2.49) | 16.1 (2.38) | 17.4 (2.10) | $<2.20 \times 10^{-16}$ |
| Physical activity (exercise units) | 268 [162, 384] | 285 [190, 406] | 367 [225, 519] | 442 [300, 603] | $3.19 \times 10^{-7}$ |
| Smoked, % ever | 42.6 | 47.0 | 39.9 | 42.6 | 0.0178 |
| Consumed alcohol, % ever | 75.8 | 87.9 | 87.2 | 89.0 | 0.0121 |

[1] Means (standard deviation) or median [interquartile range] unless otherwise noted.
[2] 537 participants in the microbiome study. Variable-specific totals may be lower because of missing data.
[3] Diet quality score was standardized (mean = 0, standard deviation = 1). Weighted scores include diet year 30.
[4] *P* values from chi-square for frequencies (%), Kruskal-Wallis for means, and the Mood's Median test for medians.

with respect to this multivariate measure, a global indicator of gut microbial composition based on genera abundance.

Current physical activity was positively associated with α-diversity measures, Shannon diversity and richness, in multivariable-adjusted analysis (Model 3, Supplemental Table 2). Lifetime physical activity, as well as both current and lifetime diet, were associated with α-diversity measures in semiadjusted analysis (Models 1 and 2).

## Genus-specific analysis

Regression results for genus-specific analysis were sensitive to multivariable-adjustment (Supplemental Tables 3–14). In regression analysis adjusted for one demographic covariate at a time, we note that we found that that adjustment for sex or race generally yielded the largest changes in estimates.

### *Physical activity*

Forty-five [45] genera were significantly associated with current physical activity at FDR <0.20 in multivariable-adjusted analysis (Figure 2); 43 genera were associated with current physical activity modeled continuously, 15 of which were also associated when physical activity was modeled as lowest versus highest quartiles. As compared with current physical activity, we observed fewer genera associations for lifetime physical activity (Figure 2). Four genera were associated, positively, with all measures of physical activity: *Agathobacter, Butyricicoccus, GCA-900066575, and Lachnospiraceae NK4A136 Group*.

### *Diet quality*

Few genera were significantly associated (FDR <0.20) with diet quality in Model 3 (Figure 3), all observed in analyses that modeled diet quality as quartiles, comparing the fourth to the first quartile. Four genera were associated with the lifetime measure of diet quality, 2 negatively (*Anaerotruncus* and *Ruminococcaceae Incertae Sedis*) and 2 positively (*Lachnospiraceae FCS020 Group* and *Lachnospiraceae UCG-001*). One genus, *Ruminococcus,* was associated, positively, with current diet as quartiles, comparing the fourth to first quartile.

### *Sensitivity analysis*

Results were not materially affected by adjustment for use of medications, past-year antibiotics, or pre-/probiotics. Statistical significance was maintained for β-diversity (PERMANOVA $P < 0.001$). α-Diversity results did not change after these further adjustments, with current physical activity the only health behavior associated with α-diversity (positively). Genus-specific associations were also largely unchanged, with 37 genera significantly associated with continuous current physical activity at FDR <0.20 (Supplemental Table 5), and 7 with current physical activity modeled as quartiles (Supplemental Table 6). Some associations between genera and diet were attenuated on further adjustment for medications (Supplemental Tables 3–6), although some previously nonsignificant associations strengthened and achieved significance (Supplemental Table 8) or remained consistent with Model 3 adjustments (Supplemental Tables 9, 12–14).

Changing the rare taxa exclusion criteria altered the number of genera available for study: $n = 95$ with filtering at 25% to $n = 132$ with filtering at 10%. However, results for α-diversity were consistent across the 2 approaches, and the genera most strongly associated with physical activity or diet quality were generally robust to filtering. In additional sensitivity analysis, we used the year 20 dietary history as the most recent measure of diet, in both current and lifetime analyses, given the larger amount of

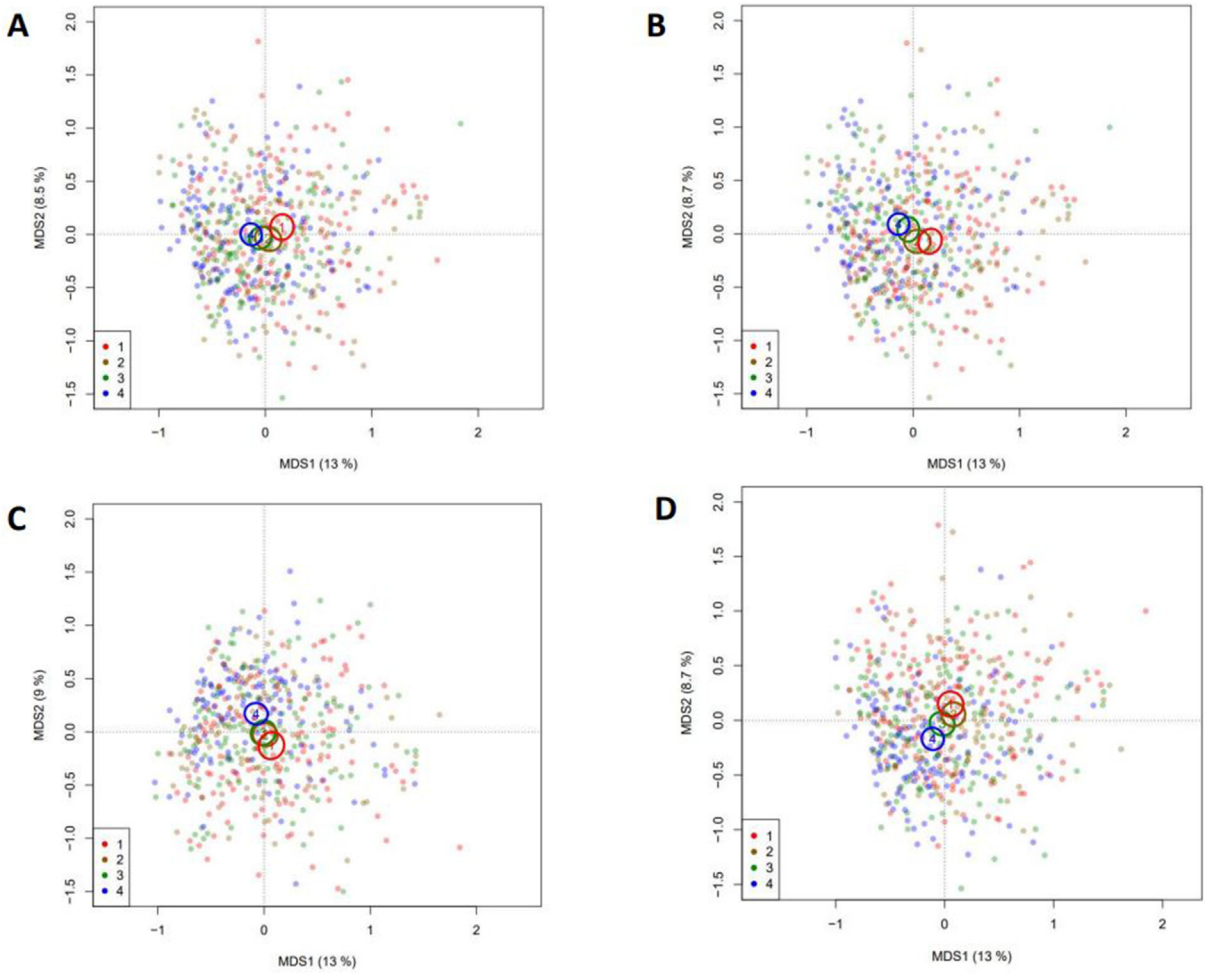


**FIGURE 1.** β-Diversity plotted using PCoA biplots based on the Bray-Curtis dissimilarity matrix between quartiles of physical activity (lifetime and current) and diet (lifetime and current) engagement among study participants. Centroids indicate quartiles (95% confidence interval for the mean location of each population group) in the adjusted Model 3 of demographics and health behaviors and are significantly different between the groups per $P < 0.001$ in the permutational multivariate analysis of variance test. (A) Associations in quartiles of current physical activity against microbial diversity; (B) Associations in quartiles of lifetime physical activity; (C) Associations in quartiles of current diet with year 30 measures; (D) Associations in quartiles of lifetime diet. MDS: Multi-Dimensional Scaling.

information provided in the dietary history, as compared with the brief year 30 survey. In these analyses, *Anaerotruncus* was negatively associated with current (year 20) and lifetime (years 0, 7, 20) measures of diet quality, and *Ruminococcaceae Incertae Sedis* was negatively associated with lifetime diet quality (Supplemental Tables 9, **10**, **13**, and **14**). Several associations from our primary analysis were no longer observed when we excluded the year 30 diet data. We note that, for some findings, results varied depending on whether diet quality was modeled continuously or as quartiles.

## Discussion

In this population-based study of CARDIA participants, we tested associations between 2 modifiable health behaviors, physical activity and diet quality, with respect to 3 standard measures of gut microbial composition: β-diversity, α-diversity, and genera abundance. A multivariate measure of community composition, β-diversity, distinguished participants with respect to current and lifetime physical activity and diet quality. In addition to this high-level finding, we observed several genus-specific associations with physical activity and, to a lesser extent, dietary quality in multiple comparisons-adjusted analysis. These results were robust to adjustment for a range of potential confounders, including sociodemographics, other health behaviors, and medication use. Associations for specific genera were consistent with postulated pathways through which the gut microbiota may impact health. Our findings are consistent with the hypothesis that higher physical activity and diet quality may influence the gut microbial composition in ways that are beneficial to health.

We observed multiple associations between microbial measures and physical activity, and these results are consistent with results from other studies. The positive association between alpha-diversity and physical activity has been observed in both observational and exercise intervention studies [14, 21, 51]. Several genera (*Agathobacter, CAG-56*, *GCA-900066575*, *Lachnospiraceae UCG-001*, *Lachnospiraceae UCG-004*, *Lachnospiraceae UCG-010*, and *Roseburia*) that were positively associated with physical activity in CARDIA derive from family *Lachnospiraceae*, which was positively associated with step counts among older, community-dwelling men [25]. As in our data, *Roseburia* was

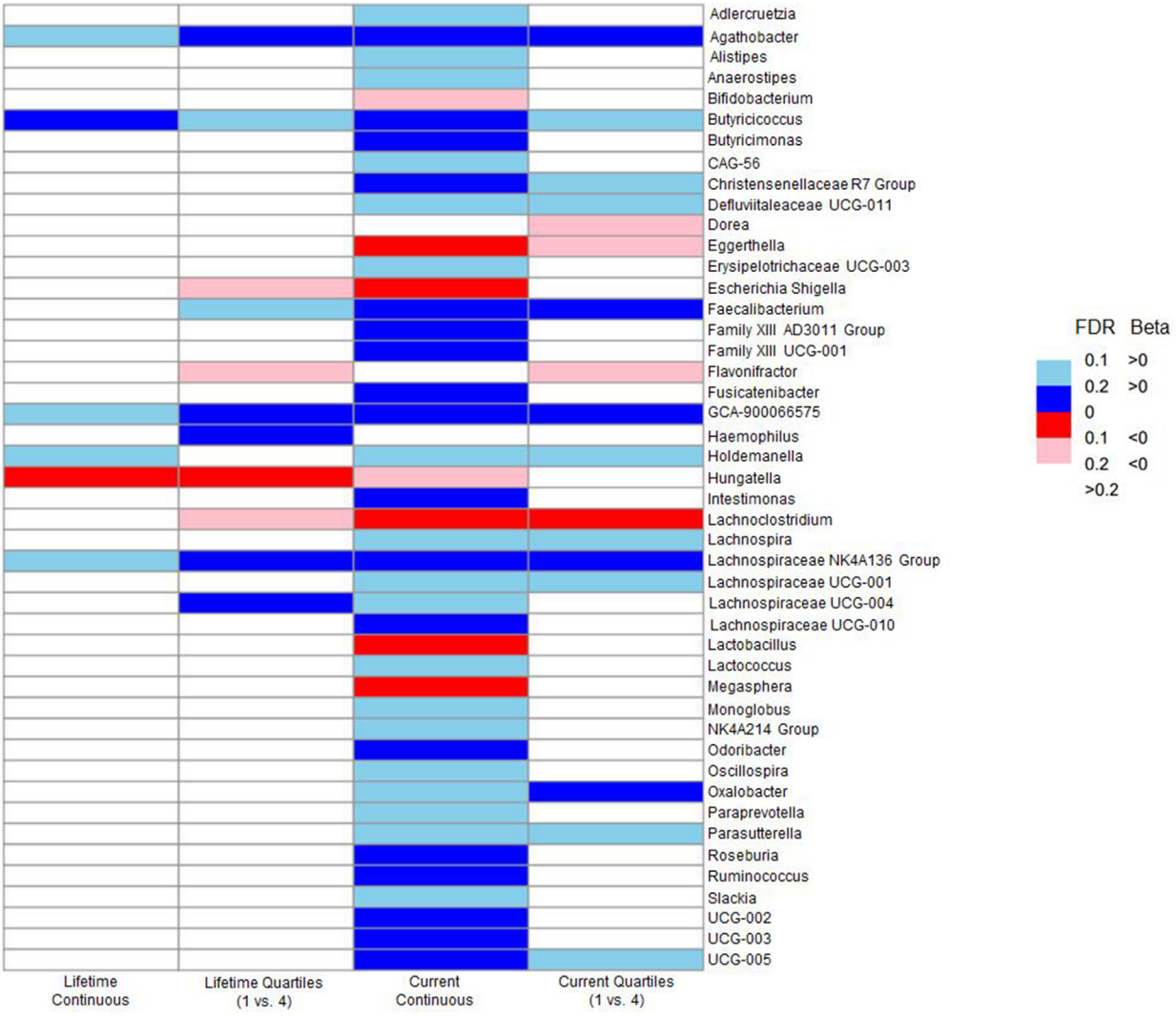


**FIGURE 2.** Genera associated with physical activity (lifetime and current) with at least one association at false discovery rate (FDR) <0.20. Genera present among 25% of the total sample ($n = 537$) are included. FDR values for positive and negative associations between physical activity measures and individual genera are listed for genera with at least one adjusted significant association in the respective direction at FDR <0.20. The direction of association is noted as blue for positive associations from the beta value of the linear regression equation of the multivariable adjusted models. Only the FDR values from the Model 3 multivariate regression model are shown. Model 3 adjusts for: sequencing run, age, sex, race, clinical field center, education, diet, smoking, and alcohol. All FDR values were rounded up.

positively associated with physical activity in a cross-sectional study of premenopausal women [27], *Lactobacillus* was inversely associated with physical activity in a study comparing elite rugby players with low body mass index controls [14], and *Faecalibacterium* had a higher relative abundance among athletes, compared with sedentary individuals [14, 21, 23]. Similarly, exercise interventions have shown mean increases in *Roseburia* and *Faecalibacterium* after a period of aerobic exercise

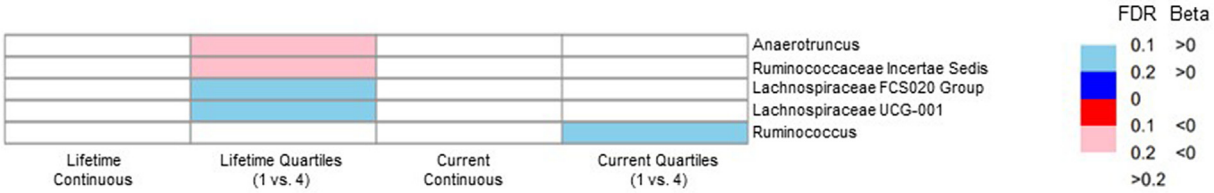


**FIGURE 3.** Genera associated with diet variables (lifetime and current) with at least one association at false discovery rate (FDR) <0.20. Genera present among 25% of the total sample ($n = 537$) are included. FDR values for positive and negative associations between diet measures and individual genera are listed for genera with at least one adjusted significant association in the respective direction at FDR <0.20. The direction of association is noted as blue for positive associations from the beta value of the linear regression equation of the multivariable adjusted models. Only the FDR values from the Model 3 multivariate regression model are shown. Model 3 adjusts for: sequencing run, age, sex, race, clinical field center, education, physical activity, smoking, and alcohol. All FDR values were rounded up.

training; these findings have been observed in a range of samples, including healthy men, adolescents, and physically inactive older women [7, 15, 16].

Many studies support a role for dietary factors on the gut microbiota [29, 30], and we were surprised by the relatively stronger evidence for associations with physical activity than with diet. These findings may reflect our use of an abbreviated dietary instrument that assessed frequency of food group consumption, but lacked quantitative estimates, in the microbiome ancillary study at year 30. The lack of detailed, quantitative dietary information may have limited our ability to detect diet–microbiome associations. Intervention studies have illustrated significant diet-related shifts in gut microbial composition [5, 6, 17–20], as well as functional changes, such as the production of microbiota-dependent metabolites [6, 18]. These studies provide strong empirical support for dietary effects on the gut microbiota, at least in the short term. Findings from observational studies have tended to be weaker than those from intervention studies, perhaps because of more limited variability in observed as compared with allocated diets as well as potential measurement error in self-reported diet [5, 6]. Despite these differences, several diet–microbiome findings appear robust across designs. For example, we observed positive associations between genera within family *Lachnospiraceae* (*Lachnospiraceae FCS020 Group*, *Lachnospiraceae UCG-001*) and diet quality in CARDIA is consistent with results from dietary interventions in which the relative abundance of genera within family *Lachnospiraceae* increased following time on a plant-based diet, as compared with time on an animal-based diet [6] and in response to allocation to a Mediterranean diet [18]. Similarly, family *Lachnospiraceae* was positively associated with dietary quality in the Multiethnic Cohort [52] and Osteoporotic Fractures in Men study [26].

Our findings are consistent with biologic pathways postulated to influence chronic disease risk and may contribute to our understanding of a mediating role for the gut microbiota in diet and physical activity effects on chronic disease risk. However, the complexity of the gut microbial community and the potential for differential metabolic activity subgenus favors a cautious interpretation. For example, consider our findings related to several genera within family *Lachnospiraceae*, which were positively associated with physical activity and diet quality. Members of family *Lachnospiraceae* have been shown to be involved in the fermentation of nondigestible polysaccharides to short-chain fatty acids (SCFAs) [53], which may play a role in mitigating inflammation. On the other hand, SCFAs have also been associated with overweight and glycemic dysregulation, perhaps through their contribution of excess energy.

Physical activity and diet may influence gut microbial composition through systemic and gastrointestinal pathways. Systemically, both diet and physical activity influence energy balance, with attendant effects on body composition; profiles of gut microbiota have been associated with body fat type and distribution patterns [54, 55]. Compelling data also support a role for colonic transit time. Colonic transit time appears to decrease with greater physical activity [24, 56] and with higher fiber consumption [57] and increase with protein catabolism [58]. Gut microbial compositional differences have been observed with respect to colonic transit times, with lower transit times positively associated with α-diversity [59]. Longer colonic transit time has been associated with lower fecal SCFAs, likely reflecting both typical dietary consumption of nondigestible polysaccharides as well as gut microbial composition and function [60].

Our study has many strengths. CARDIA is an established population-based cohort of sociodemographically diverse Black and White participants with high participant retention. Over 30 y of follow-up, the CARDIA study has collected comprehensive covariate data, including sociodemographic variables, health behaviors, and medical histories using standardized and validated assessments. These data allow us to examine both current and long-term measures of physical activity and diet. Our study included rigorous covariate adjustment, which has not been consistently employed in observational studies of the microbiome. We note that genus-specific associations were sensitive to these adjustments, particularly sex, race, and other health behaviors (e.g., smoking, alcohol consumption). The CARDIA microbiome subsample was overall similar to the larger CARDIA cohort.

Several limitations merit mention. Our cross-sectional data did not permit assessment of temporality, and it is possible that associations may reflect microbial influences on behaviors [61]. Physical activity and diet were self-reported, and both are known to be susceptible to measurement error [62, 63]. However, we expect that our microbiome data, like other biological measures, have error that is independent from self-reports of diet and physical activity, decreasing the potential for correlated errors that may increase the potential for bias. Within-person variability in microbial composition has been documented, and our use of a single sample may influence the reproducibility of some findings, particularly genus-specific associations [64]. However, it is reasonable that extraneous variability due to within-person variability is likely random. In addition, we note that studies indicate that between-person variability exceeds within-person variability [65], supporting our ability to detect between-person differences from a single sample. Our sample size may have been insufficient for robust detection of some genus-specific associations, particularly at more stringent FDR thresholds; we considered our sample too small to test for stratum-specific differences, such as by race or sex. Finally, the field of the microbiome is still young, and there is a lack of consensus on valid and reproducible methodology, and researchers have documented large extraneous variability related to sample collection and DNA extraction [66, 67]. Our methods were consistent with those of other contemporaneous cohort efforts [68], but we acknowledge the potential lack of comparability across studies due to protocol differences.

Our characterization of microbial diversity and taxonomic composition was based on relative genera abundance estimates, derived from 16S ribosomal RNA sequence data. This approach does not allow ascertainment of functional potential, such as metabolic pathways or gene families, or refined taxonomic assignment (subgenus). Future work with whole metagenomic sequences will allow the derivation of a richer and more biologically relevant set of measures. We elected not to use existing algorithms, such as PICRUSt2, to approximate metabolic pathways [69], although there remains debate about the accuracy of such approaches. With respect to taxonomic resolution, we cannot, with these data, distinguish subgenus associations. We know that large diversity can exist at lower levels of taxonomy [70, 71].

We report associations between gut microbial composition and 2 major health behaviors, physical activity frequency and dietary intake, in a demographically diverse cohort of middle-

aged Black and White adults. The strongest associations remained statistically significant after adjusting for sociodemographic variables and health behaviors that are potential confounders. The results of this study support the possibility that modifiable behaviors, such as diet and physical activity, may influence the gut microbiota in ways that impact cardiometabolic risk factors.

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## Author disclosures

The authors report no conflicts of interest.

## Data Availability

CARDIA data are available upon reasonable request from the CARDIA Coordinating Center. CARDIA investigators are eager to collaborate with investigators interested in using CARDIA data. Please see the CARDIA website (https://www.cardia.dopm.uab.edu) for publications policies and for a list of CARDIA investigators. CARDIA data are also publicly available on the NIH-supported BioLINCC and dbGaP platforms.

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The authors' responsibilities were as follows—KAM and LJL designed the project; AM and AL conceptualized the study idea and conducted analyses; HL assisted with analyses and CJ assisted with expanding on analysis ideas. AM and KAM drafted the manuscript. All authors critically reviewed and revised the manuscript, and all authors read and approved the final manuscript.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2022.12.019.

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