AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

Host-Microbial Interactions | Research Article

# Association of intestinal bacteria with immune activation in a cohort of healthy adults

**Niknaz Riazati,[1] Mary E. Kable,[2,3] Charles B. Stephensen[2,3]**

**AUTHOR AFFILIATIONS** See affiliation list on p. 12.

**ABSTRACT** Interactions among intestinal bacteria and the immune system contribute to the maintenance of a functional intestinal barrier in healthy individuals, and possibly to systemic immune activity. We hypothesized that intestinal bacteria would be associated with systemic biomarkers of innate and adaptive immune responses in healthy adults. 79 immune function markers were subjected to factor analysis resulting in 17 Immune Factors (IFs), each composed of 2–10 immune variables. Bacterial taxa from stool samples were identified at the family and genus levels by 16S rRNA amplicon sequence analysis and their read counts and relative abundances were utilized in a multiple linear regression model to identify microbial taxa associated with the IFs. A total of 10 significant associations were identified between bacterial taxa and IFs. The family *Rikenellaceae* showed a positive association with innate IF5 (including 5 chemokines, 2 cytokines, 2 adhesion molecules, and the macrophage metabolite neopterin) and a negative association with adaptive IF4 (including T-cells with activation marker HLA-DR). Additionally, *Pseudomonadaceae* and its genus *Pseudomonas* showed a negative relationship with innate IF5, and adaptive IF13 (including T-cell cytokines IL-10, IL-17, and IFN-γ) was negatively associated with *Butyrivibrio* and positively associated with *Slackia*. These associations suggest ongoing interactions between gut bacteria and the systemic immune system in healthy adults. The association of these taxa with the IFs may result from specific microbial-immune system interactions that play a role in maintenance of a healthy barrier integrity in our cohort of healthy adults.

**IMPORTANCE** Chronic inflammation may develop over time in healthy adults as a result of a variety of factors, such as poor diet directly affecting the composition of the intestinal microbiome, or by causing obesity, which may also affect the intestinal microbiome. These effects may trigger the activation of an immune response that could eventually lead to an inflammation-related disease, such as colon cancer. Before disease develops it may be possible to identify subclinical inflammation or immune activation attributable to specific intestinal bacteria normally found in the gut that could result in future adverse health impacts. In the present study, we examined a group of healthy men and women across a wide age range with and without obesity to determine which bacteria were associated with particular types of immune activation to identify potential preclinical markers of inflammatory disease risk. Several associations were found that may help develop dietary interventions to lower disease risk.

**KEYWORDS** Immune Factors, innate immune response, adaptive immune response, intestinal bacteria, host-microbe interactions, linear regression model, healthy adults, observational study

Intestinal bacteria interact with the mucosal immune system of the intestine and can contribute to maintenance of healthy barrier integrity. Intestinal bacteria may modulate immune responses in both mucosal and systemic immune tissues either via

**Editor** Laxmi Yeruva, USDA-ARS Arkansas Children's Nutrition Center, Little Rock, Arkansas, USA

Address correspondence to Charles B. Stephensen, charles.stephensen@usda.gov.

The authors declare no conflict of interest.

See the funding table on p. 13.

**Received** 13 March 2023
**Accepted** 13 August 2023
**Published** 11 October 2023

direct interaction with the intestinal epithelial barrier or by the secretion of bioactive small molecules (e.g., short-chain fatty acids or other metabolites). One example of common host-microbe interaction in the intestine is the recognition of microbial-associated molecular patterns via pattern-recognition receptors. Antigen-specific activation of memory T and B lymphocytes may also occur (1–4). In this study, our goal is to identify microbial taxa modulating innate and adaptive components of the systemic immune response in a cohort of healthy adults. We hypothesized that intestinal bacteria would be associated with function markers of innate and adaptive immune responses in healthy adults.

We used a uniquely large and diverse set of immune biomarkers measured in a population of clinically healthy adults. We applied factor analysis to these immune biomarkers to develop a smaller set of composite variables containing multiple specific measures of immune function that might be useful in identifying associations with types of immune activity (e.g., T-cell activation or blood levels of innate immune cells or inflammatory mediators). 17 Immune Factors (IFs) were defined, including 8 innate IFs and 9 adaptive IFs. The obtained IFs were tested for association with intestinal bacteria at the family and genus levels. Our study identified microbial taxa significantly associated with innate and adaptive immune responses.

## MATERIALS AND METHODS

### Study design

Healthy adults were recruited into a Nutritional Phenotyping Study conducted at the USDA Western Human Nutrition Research Center (*ClinicalTrials.gov*: NCT02367287) as previously described (5, 6). Data on race and ethnicity of the participants were presented previously (5). Data used for the analysis of the association of microbial taxa with immune biomarkers in this observational study is from 355 females and males categorized into the three age categories (18–34, 35–49 and 50–65 yr) and three BMI categories (18.5–24, 25–29 and 30–44 kg/m$^2$) (Table 1). A stool sample for microbial sequencing was collected by each participant, and to measure immune function, fasting blood was collected at the center after a 12-h overnight fast following consumption of a standard meal the evening before. Ethical approval for this study was received from the Institutional Review Board of the University of California, Davis, USA, as previously described (5, 6).

**TABLE 1** Demographic characteristics of healthy adult participants ($n$ = 355)

| | CMV-IgG+[a] | CMV-IgG-[b] | BMI (kg$m^{-2}$) | | | Participants, $n$ | %Total |
|---|---|---|---|---|---|---|---|
| **Age, yr** | | | **18–24** | **25–29** | **30–44** | **355** | **100** |
| **Men** | | | | | | | |
| 18–34 | 26 | 31 | 27 | 25 | 5 | 57 | 16.1 |
| 35–49 | 38 | 23 | 28 | 26 | 7 | 61 | 17.2 |
| 50–66 | 26 | 25 | 16 | 21 | 14 | 51 | 14.4 |
| All | 90 | 79 | 71 | 72 | 26 | 169 | 47.6 |
| **Women** | | | | | | | |
| 18–34 | 28 | 37 | 29 | 26 | 10 | 65 | 18.3 |
| 35–49 | 25 | 33 | 15 | 19 | 24 | 58 | 16.3 |
| 50–66 | 33 | 30 | 20 | 13 | 30 | 63 | 17.7 |
| All | 86 | 100 | 64 | 58 | 64 | 186 | 52.4 |
| %Total | 49.6% | 50.4% | 38.0 | 36.6 | 25.4 | 100 | |

[a]CMV-IgG positive.
[b]CMV-IgG negative.

## Immune function markers

79 immune variables were measured from 362 healthy, fasting adults as described previously (7). The immune variables include soluble immune biomarkers measured in plasma and supernatants of cultured peripheral blood mononuclear cells (PBMC), and cellular markers measured using flow cytometry and a complete blood count (CBC). The immune markers are defined using the following categories: effector/memory T-cells and activation levels ($n$ = 24), other lymphocytes including Th cells, NK, NK T-cells, and B cells ($n$ = 11), PBMC cytokines ($n$ = 8), CBC ($n$ = 6), innate cell activation ($n$ = 11), and plasma markers ($n$ = 19) (Table S1).

## CMV antibody test

A history of infection with cytomegalovirus (CMV) may expand memory/effector T-cells and increase the level of the CD4 and CD8 T-cell subsets (8, 9). For this reason, we controlled the regression analysis of the association of microbial taxa with IFs for the CMV infection status. The study participants were assessed for a history of infection with CMV by measuring the CMV IgG level as described previously (7).

## Bacterial 16S rRNA gene sequence analysis

Amplification and sequencing of the 16S rRNA V4–V5 region from bacterial DNA extracted from stool was performed by the Dalhousie University Integrated Microbiome Resource using primers 515F, GTGYCAGCMGCCGCGGTAA, and 926R, CCGYCAATTYMTT TRAGTT (10–12) as previously described (13). Sequences were analyzed using Qiime2 version 2019.10 (14) also as previously described (13). Except for two samples with 5,211 and 9,860 sequence counts, all samples included in the analysis had at least 10,000 sequence counts after quality filtering.

## Statistical analysis

All statistical analyses were conducted in SAS 9.4 (SAS Institute, Cary, North Carolina, USA) other than the adjustment of microbial read counts which was performed using R software (v 3.6.3) (15).

## Factor analysis

We used factor analysis as a method of data reduction to explain the large number of immune variables by a few factors through grouping correlated variables together. To optimize the effect estimates, the immune variables with missing values were imputed prior to factor analysis by the multiple imputation procedure using PROC MI. The number of missing variables in the data set is shown in Table S2. To achieve a normal distribution, immune variables were normalized using rank-based normal transformation. Factor analysis was applied to the rank-normalized immune variables using PROC FACTOR and the method of Principal, and a correlation matrix was computed for all the immune variables included (Table S3). The correlation matrix includes correlation coefficients between the immune variables and IFs. We used the sum of the squared factor loadings (coefficients) also known as the eigenvalues as the factor extraction method and we used eigenvalues > 1 as the extraction threshold, leaving 17 IFs. Orthogonal rotation was applied to the component matrix using the varimax method, in which the factors are assumed to act independently and not correlated. Factor loadings included positive factor loadings indicating a positive correlation between the factor and raw data, and negative factor loadings indicating a negative correlation. Factor scores (the load coefficient scores) obtained for each participant were used as dependent variables in multiple linear regression analysis. The scree plot showing the relationship between eigenvalues and IFs (Fig. 1) and the heatmap showing immune variable loadings on the IFs (Fig. 2) were created in GraphPad Prism V9.5.0.

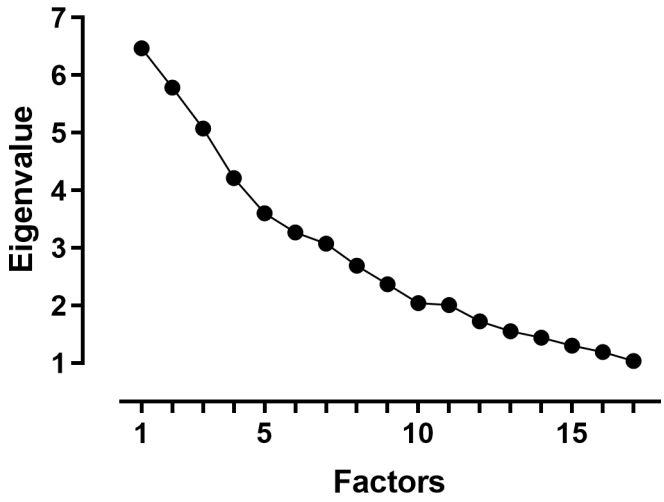


**FIG 1** Scree plot showing eigenvalues against 17 factors. The plot shows how much variance in the data, in terms of eigenvalues, is explained by each factor.

## Microbial taxa

16S rRNA sequencing libraries for microbial taxa were available from 355 participants. Microbial taxa found in 5% or fewer of the stool samples were filtered out from the data set. Taxa that were found in fewer than 25% of the subjects were represented as binary variables by the absence-presence scale where absence is indicated by "0" and presence is indicated by "1." The remaining taxa (found in ≥25% of the subjects) were analyzed as continuous variables. Our data set is composed of 26 families and 50 genera as continuous variables and 9 families and 25 genera as binary variables. Sequence counts attributed to microbial taxa were adjusted using two different approaches. In the first approach, total sum scaling (the number of sequence counts attributed to each taxon divided by the total number of sequence counts within each sample) was used to adjust for variation in sample library size. The resulting percent abundance values were then rank transformed to achieve a normal distribution. In the second approach, sequence counts were adjusted for variation in library size with the DESeq2 package (v 1.26.0) (16) in R (v 3.6.3) (15). The DESeq2-adjusted sequence counts for continuous variables were transformed using the log function in SAS returning natural logarithm values (with pseudo-counts of one added to all input data with zero read counts prior to log transformation so that zero counts would be handled by the normalization scheme explicitly). In addition, prior to modeling, all the DESeq-2-adjusted counts (continuous and binary variables) were standardized using PROC Standard in SAS. Only associations that were significant by linear regression analysis using both approaches for normalization of sequence counts are reported in the body of this manuscript.

## Multiple linear regression analysis

### *Association of microbial taxa with Immune Factors*

Associations of microbial taxa at the family and genus levels with IFs were assessed using generalized linear regression model by the PROC GLM method in SAS. The models to describe associations with the 17 IFs as dependent variables included microbial taxa as independent variables, and categorical variables used as covariates including the three age categories, three BMI categories, and sex. The model was also controlled for the CMV IgG antibody status (positive/negative) as it was correlated with some immune markers of our study, primarily with different T-cell measures.

To minimize the effect of technical variation on the linear regression model, the GLM procedure was implemented on the microbial taxa using the two abundance scales, i.e., relative abundances and sequence counts, and two normalization methods, i.e.,

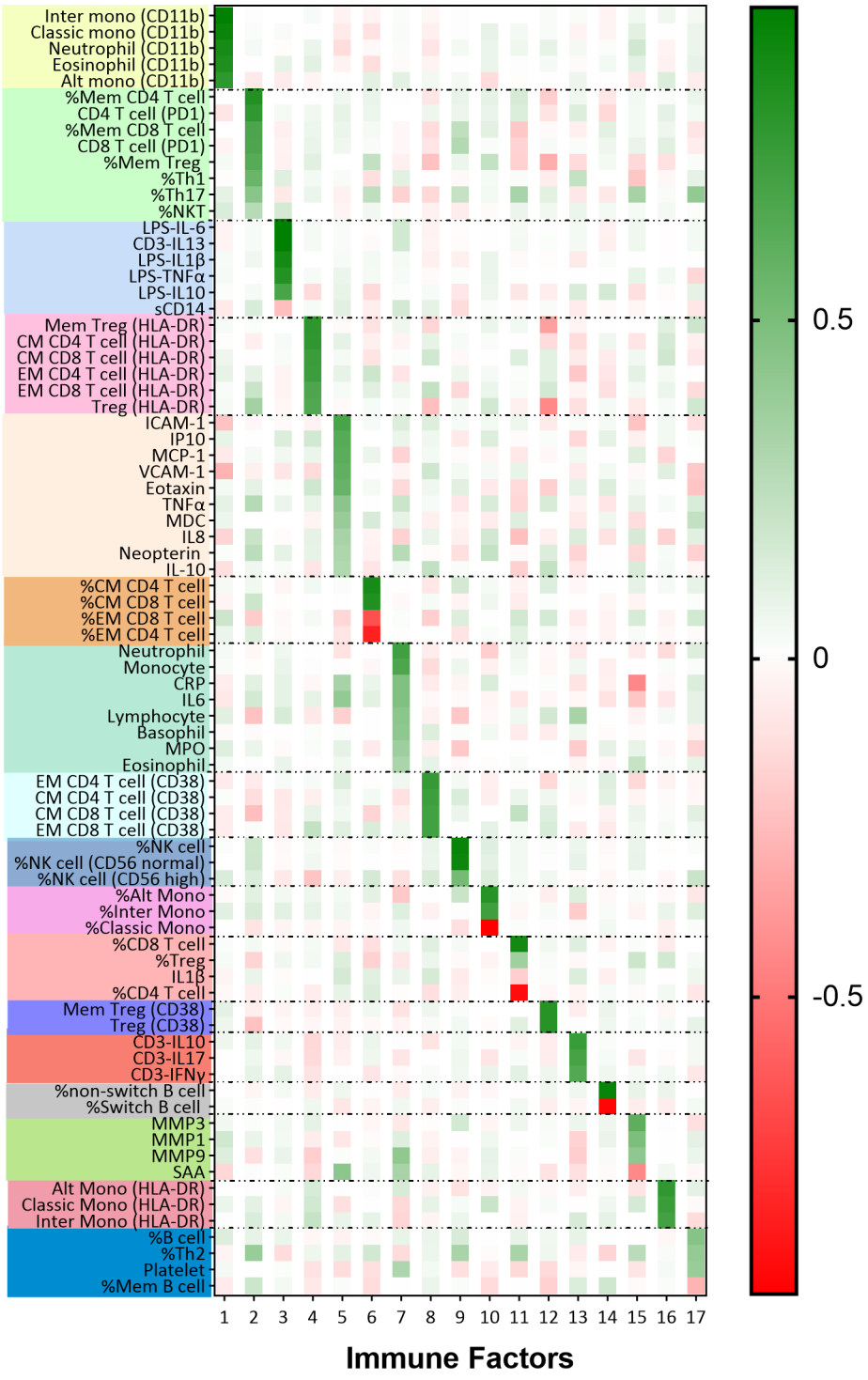


**FIG 2** Heat map of immune variable loadings for the 17 Immune Factors from 362 study participants. Inter Mono, intermediate monocyte; Alt Mono, alternate monocyte; Mem, memory; NKT, natural killer T-cells; Th, helper T-cell; LPS, lipopolysaccharide; LPS-IL, LPS-stimulated Interleukin secretion by PBMC culture; CD3-IL, Interleukin secretion by T-cells in PBMC culture; TNF-α, tumor necrosis factor-alpha; sCD14, soluble CD14; CM, central memory; EM, effector memory; ICAM/VCAM, intracellular/vascular cell adhesion molecule; IP-10, interferon gamma-induced protein-10; MCP-1, monocyte chemoattractant protein-1; MDC, macrophage-derived chemokine; CRP, C-reactive protein; MPO, myeloperoxidase; NK, natural killer cells; SAA, serum amyloid A.

normalization by rank and natural logarithm as were explained above. Where bacteria were evaluated by sequence counts, taxa with sequence counts below the threshold of 25% were subjected to multiple regression analysis for presence/absence. To account for multiple comparisons, Hochberg adjusted *P*-values were computed. Statistical significance was set at $P < 0.05$.

The results of the analyses by the relative abundance and sequence count scales were compared. Reported in this paper are results from the analysis of microbial taxa by sequence counts and represent significant associations overlapping across the two approaches (Tables 2 and 3). All the significant associations found by each of the two analyses are presented separately in Tables S6 and S7. To compare regression coefficients between the two analyses, standardized regression coefficients were calculated.

To calculate the standardized regression coefficients, prior to the modeling, the immune data set was standardized by rank-normalization and also by factor analysis producing standardized data by default. Where taxa were evaluated using relative abundance, data were standardized using rank transformation. Where taxa were evaluated using read counts, data were standardized using PROC Standard in SAS so that we would be able to compare regression coefficients between microbial taxa below and above the threshold of 25% (binary and continuous variables).

The standardized regression coefficients of the associations of microbial taxa with IFs represent the effect size of the associations according to the conventionally used values of 0.1–0.3 for small effect, 0.3–0.5 for moderate effect, and 0.5–1 for large effect (17–19).

The unstandardized regression coefficients for the significant associations are summarized in Table S8. GraphPad Prism v9.5.0 was used for the graphical representation of relationship of the IFs with microbial taxa present in <25% of the subjects (violin plots). Associations of microbial taxa present in ≥25% of the subjects with IFs were plotted using SAS (scatter plots).

For the 4 families and 3 genera that had significant associations with one or more IFs, a secondary, *post hoc* regression analysis was performed with all 79 immune variables in a regression model adjusted for sex, age, and BMI categories, and CMV infection status to identify further significant associations that might help in interpreting the primary findings with the IFs. A Benjamini-Hochberg (BH) *P*-value of <0.10 was used for this secondary analysis. Results are shown in Tables S11 and S12.

## RESULTS

### Characteristics of study participants

The study data set including the data of the immune function and microbial sequencing were collected from 355 clinically healthy adults recruited into the cross-sectional Nutritional Phenotyping Study. The participants were men and women enrolled into the sampling bins of the three age and BMI categories. The participants were also grouped into the two categories of CMV seronegative and seropositive IgG status. Measurement of the plasma IgG level confirmed that 176 (49.6%) individuals were CMV seropositive which means that they had a history of CMV infection, and 179 (50.4%) individuals were seronegative. The participants' characteristics are summarized in Table 1.

### Immune Factors

Factor analysis was carried out on 79 immune biomarkers from 362 healthy adults. The variance in the original data set of immune variables is distributed among the retained IFs with the first IF explaining more variation than the other IFs (Fig. 1). 17 IFs were extracted which jointly explained 86.6% of the variance in the data. Extracted components for each factor are shown in Fig. 2 and the related explained variances after the orthogonal rotation are reported in Table S4. 8 of the IFs are comprised primarily of innate immune system biomarkers and 9 factors are comprised primarily of adaptive immune system biomarkers (Table S5).

### Association of microbial taxa with Immune Factors

35 bacterial families were examined for associations with the 17 IFs. 4 families were found to have significant associations with 4 different IFs with small effect sizes (Table 2; Fig. 3 and 4). *Pseudomonadaceae* with a range of relative abundance between 0 and 0.3% had a negative association with IF5, an unknown family of the order *ML615J-28* with a range of relative abundance between 0 and 0.9% had a negative association with IF15, family *Muribaculaceae* with a range of relative abundance between 0 and 12.6% had a negative association with IF16, and *Rikenellaceae* with a range of relative abundance between 0 and 5.2% had a negative association with IF4 and a positive association with IF5.

Independently from the family-level analysis, 75 genera were examined for associations with the 17 IFs. 4 genera were found to have significant associations with 3 different IFs with small effect sizes (Table 3; Fig. 5 and 6), including genera from two families (*Pseudomonadaceae* and *Rikenellaceae*) already identified as having associations with IF4 and IF5. *Pseudomonas*, the only genus present in the family *Pseudomonadaceae* in our data set, with a range of relative abundance between 0 and 0.3% had a negative association with IF5, similar to the association seen with *Pseudomonadaceae* at the family level, and an unknown genus of the family *Rikenellaceae* with a range of relative abundance between 0 and 4.4% had a negative association with IF4 as well as a positive association with IF5, as was seen with *Rikenellaceae* at the family level. In addition, the genus *Butyrivibrio* (family *Lachnospiraceae*) with a range of relative abundance between 0 and 4% had a negative association with IF13, while the genus *Slackia* (family *Coriobacteriaceae*) with a range of relative abundance between 0 and 0.3% had a positive association with the same factor, IF13.

Associations of these taxa at the family and genus level with the individual components of the identified IFs are summarized in Tables S9 and S10. From 25% to 67% of the constituent variables within each factor were also significantly associated with the same taxa. In addition, we conducted a secondary, *post hoc* analysis of these taxa with all 79 immune variables to identify variables from other IFs that might be significantly associated with these taxa, as described in Methods. Only two additional immune variables were identified in this manner: blood lymphocyte concentration was negatively associated with *Rikenellaceae* (Table S11), and percent intermediate monocytes were negatively associated with *Slackia* (Table S12).

## DISCUSSION

Weak associations (i.e., standardized regression coefficients < 0.3) identified among specific microbial taxa and IFs in healthy adult human gut microbial communities suggest that even in healthy humans *Rikenellaceae* might be associated with innate immune activation and *Slackia* with adaptive immune activation. On the other hand, *Pseudomonas*, *Muribaculaceae*, and *Butyrivibrio* were correlated with dampened innate immunity, monocyte activation and T-cell activation, respectively, in our data set.

The positive association of *Rikenellaceae,* a family in the phylum *Bacteroidetes*, with innate IF5 (particularly the constituent variables ICAM-1, VCAM-1, and MCP-1) and its negative association with both adaptive IF4 (indicating T-cell activation) and with blood lymphocyte concentration, indicates that this taxon is associated with both increased innate immune response (e.g., vascular adhesion molecules and chemokines

**TABLE 2** Microbial families associated with IFs with adjusted *P*-values < 0.05

| Family | Immune Factor | Factor characteristics | Effect size | Standardized β | Std err | Adjusted *P* |
|---|---|---|---|---|---|---|
| *Rikenellaceae* | IF5 | Innate: inflammation | Small | 0.165 | 0.048 | 0.01 |
| | IF4 | Adaptive: T-cell activation | Small | 0.164 | 0.050 | 0.02 |
| *Pseudomonadaceae*[a] | IF5 | Innate: inflammation | Small | 0.162 | 0.048 | 0.01 |
| *Muribaculaceae*[a] | IF16 | Innate: Monocyte activation | Small | 0.149 | 0.050 | 0.049 |
| *Unknown family*[a] (Order *ML615J-28*) | IF15 | Innate: MMPs | Small | 0.124 | 0.041 | 0.049 |

[a] indicates microbial families with read counts in <25% of the subjects which were used as binary variables.

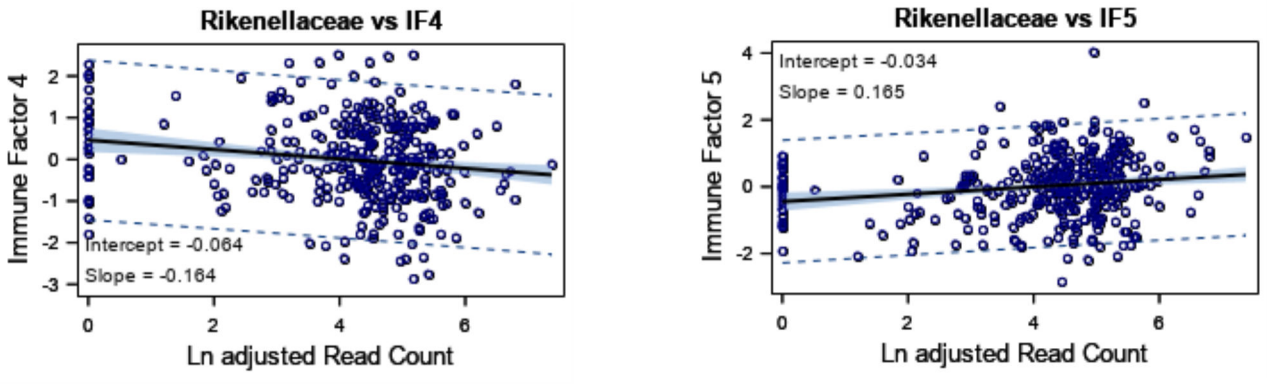


**FIG 3** Linear regression plots showing association of family *Rikenellaceae* with the adaptive IF4 and innate IF5 with the best fit line, shaded area representing 95% confidence intervals for the linear regression model, and dotted lines representing 95% prediction limit. The read counts were adjusted for varying sequencing depths by the median normalization method in DESeq2 and normalized by the log ratio based on natural logs (Ln) for varying distribution across the subjects.

affecting monocyte and other leukocyte trafficking) and decreased T-cell activation and lower circulating lymphocyte concentrations. The association of this taxon with reduced activation level of T-cells or lower lymphocyte concentrations has not been previously reported and should be assessed further by experimental studies. Previous observations regarding the association of *Rikenellaceae* with innate immune response have been mixed. For example, the positive association of *Rikenellaceae* with low BMI, indicating higher abundance in lean subjects (20, 21), negative association with high-fat diet (22), triglyceride (TG), and low-density lipoprotein levels (LDL) (23), and positive association with the reduction of visceral adipose tissue possibly through the production of acetate and propionate (24, 25) have been documented by human and animal studies, suggesting an association with healthier metabolic outcome and low inflammation. However, studies in mice examining diet-induced obesity have shown an association between weight gain and increased abundance of *Rikenella*, a genus in the family *Rikenellaceae*. The same studies demonstrated that *Rikenella* was positively associated with levels of TG and LDL, plasma and aortic mRNA levels of inflammatory

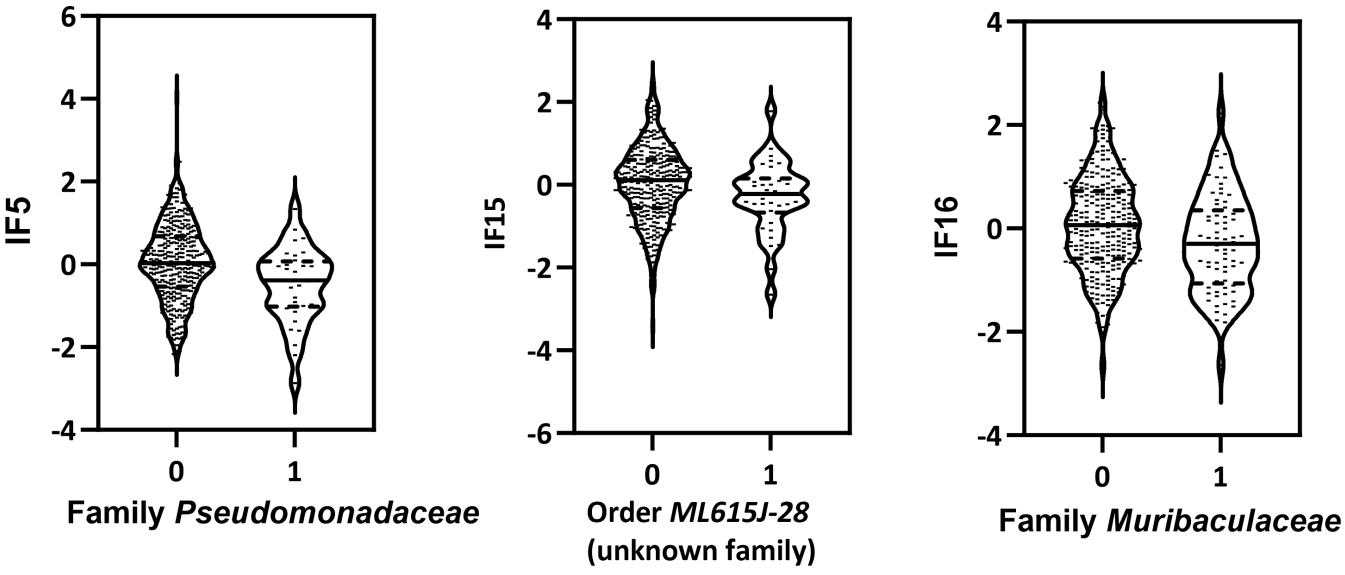


**FIG 4** Violin plots demonstrating the distribution of IFs by microbial families with read counts in <25% of the subjects. "0" indicates absence and "1" indicates the presence of the microbial taxa in 355 subjects. Median values are annotated with full lines, and lower and upper quartiles with dashed lines.

**TABLE 3** Microbial genera associated with IFs with adjusted *P*-values < 0.05

| Genus | Immune Factor | Factor characteristics | Effect size | Standardized β | Std err | Adjusted *P* |
|---|---|---|---|---|---|---|
| *Slackia*[a] (Family *Coriobacteriaceae*) | IF13 | Adaptive: T-cell cytokine | Small | 0.202 | 0.047 | 0.0004 |
| *Butyrivibrio*[a] (Family *Lachnospiraceae*) | IF13 | Adaptive: T-cell cytokine | Small | 0.163 | 0.047 | 0.01 |
| *Pseudomonas*[a] (Family *Pseudomonadaceae*) | IF5 | Innate: inflammation | Small | 0.162 | 0.048 | 0.01 |
| *Unknown genus* (Family *Rikenellaceae*) | IF4 | Adaptive : T-cell activation | Small | 0.152 | 0.050 | 0.04 |
| | IF5 | Innate: inflammation | Small | 0.150 | 0.048 | 0.03 |

[a]Indicates microbial genera with read counts in <25% of the subjects which were used as binary variables.

cytokines IL-1β and IL-6, atherosclerotic lesion in the aortic area, and macrophage infiltration within the lesions (26–28). These latter findings are consistent with the positive association of *Rikenellaceae* with markers of inflammation in IF5 including VCAM-1, ICAM-1, and MCP-1 found by this study, indicating that this taxon might be associated with enhanced migration and adhesion of innate (and other) immune cells to the site of inflammation. However, future studies are required to determine whether the increased abundance of *Rikenellaceae* is a consequence or cause of modulations of immune activity in subjects with higher BMI.

The negative association of the family *Muribaculaceae*, another family in the phylum *Bacteroidetes*, with IF16 including monocytes expressing HLA-DR suggests that this family might be associated with suppressed monocytes activation and inflammation. Association of this taxon, which is positively associated with short-chain fatty acids such as butyrate and propionate (29–33), with reduced intestinal inflammation has been demonstrated in an obesity mouse model fed with resistant starch (34). Moreover, the abundance of *Muribaculaceae* was shown to decrease in a mouse model of colitis, and in a variety of inflammatory diseases in humans such as obesity and irritable bowel syndrome (35–38), suggesting a possible association of this taxon with reduced inflammation.

Negative associations of both *Pseudomonadaceae* and *Pseudomonas* with innate IF5 may suggest that *Pseudomonas* is associated with dampened systemic inflammation, which seems counterintuitive based on the known role of *Pseudomonas* as a pathogen. Some *Pseudomonas* species are opportunistic pathogens which cause infections in immunocompromised and hospitalized patients, including individuals with cystic fibrosis, cancer, and diabetic wounds (39). However, previous research shows that secondary metabolites of *Pseudomonas* such as indole, amino acids, and peptides have significant biological activity, including antiinflammatory activity. For example, *in vitro*

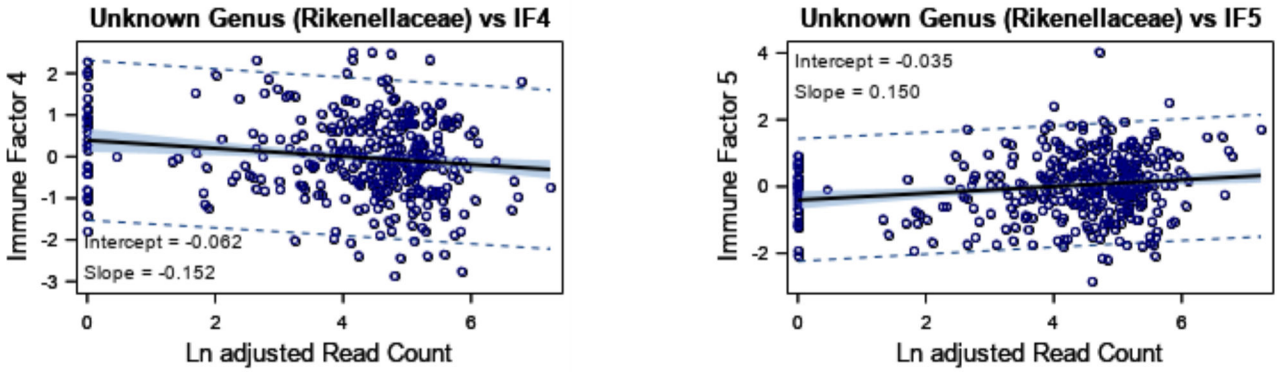


**FIG 5** Linear regression plots showing the association of an unknown genus of the family *Rikenellaceae* with the adaptive IF4 and innate IF5 with the best fit line, shaded area representing 95% confidence intervals for the linear regression model, and dotted lines representing 95% prediction limit. The read counts were adjusted for varying sequencing depths by the median normalization method in DESeq2 and normalized by the log ratio based on natural logs (Ln) for varying distribution across the subjects.

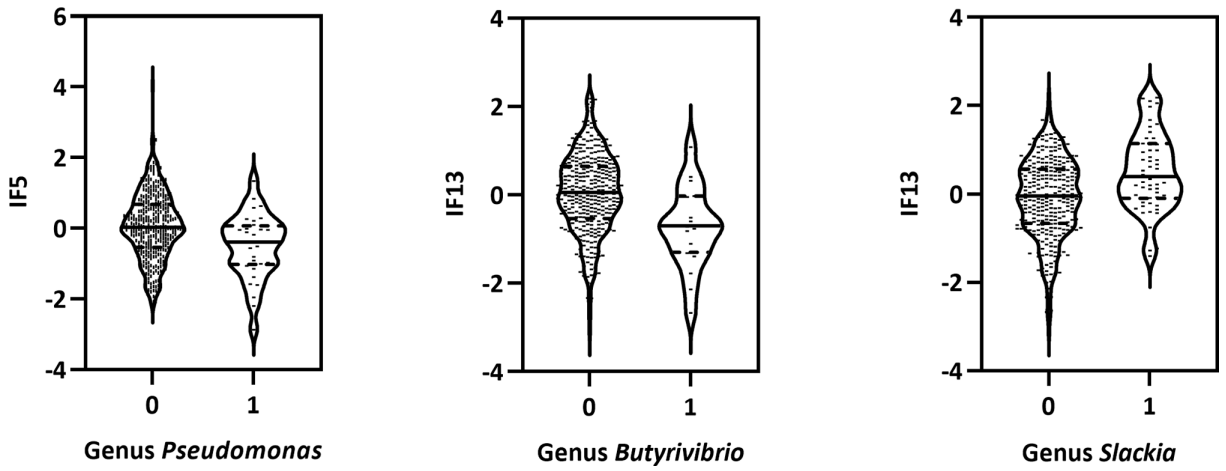


**FIG 6** Violin plots demonstrating the distribution of IFs by microbial genera with read counts in <25% of the subjects. "0" indicates absence and "1" indicates the presence of the microbial taxa in 355 subjects. Median values are annotated with full lines, and lower and upper quartiles with dashed lines.

studies of macrophage cell lines showed that supernatants from *P. aeroginosa* culture broth significantly reduced the release of TNF-$\alpha$, one of the components of IF5, and its mRNA expression level and inhibited the LPS-induced polarization of macrophages (40, 41). In addition, the study by Khan et al. (41) reported a decreased plasma level of TNF-α with oral administration of the bacterial supernatant as well as a proline-based cyclic dipeptide, a compound identified in the bacterial supernatant, to rats followed by LPS injection (41). These findings are consistent with the negative association of this microbial taxon with IF5 and the possibility that the *Pseudomonas* species found in these healthy volunteers may dampen inflammation, though this observational study does not assess causality. In addition, no previous human study has been found to validate this association. On the other hand, *Pseudomonas* rarely causes gastrointestinal infections in healthy individuals. A small human study showed that oral administration of live cultures of *P. aeroginosa* had no clinical symptoms in healthy individuals (42, 43). The reason is not known. However, detection in the stool was transient unless antibiotics were administered prior to inoculation, suggesting that in most healthy individuals, *Pseudomonas* ingested with food does not colonize the gastrointestinal tract. *Pseudomonas* species are found in milk and vegetable products (44). It is therefore possible that the detection of higher amounts of *Pseudomonas* in this study is due to the consumption of foods more likely to contain *Pseudomonas*. We speculate that higher consumption of minimally processed vegetables and milk products may be associated with a healthier diet in our study volunteers. Therefore, we suggest that *Pseudomonas* may be acting as a marker of a healthy dietary pattern, though this speculation does not have a precedent in the literature.

The negative association of an unknown family of the order *ML615J-28* with IF15 (inflammation markers; the MMPs) is supported by studies showing that the abundance of this taxon is reduced in patients with disease-associated chronic inflammatory states such as the one in obesity (37, 38, 45). In addition, the abundance of this taxon was shown to be increased in lean-BMI individuals and it was found by another study to be negatively associated with BMI and multiple disease biomarkers in blood such as LDL, triglycerides, and uric acid (46, 47).

Positive association of genus *Slackia* (member of family *Coriobacteriaceae*) with adaptive IF13, which includes production of the T-cell cytokines IL-10, IFN-γ, and IL-17 has not been reported previously. However, the family *Coriobacteriaceae* and genus *Slackia* have been associated with increased gut permeability and inflammation (48–53) which could cause increased activation and development of effector/memory T-cells producing these effector cytokines. Secondary analysis of individual immune

variables which identified a negative association between *Slackia* and the percentage of intermediate monocytes is also a novel finding and should be assessed by experimental studies.

On the other hand, *Butyrivibrio*, a genus in the family *Lachnospiraceae*, was negatively associated with adaptive IF13. Multiple studies have shown that butyrate-producing *Butyrivibrio* is associated with gut health. For example, a report using a mouse model of amyotrophic lateral sclerosis showed that the reduction in the relative abundance of *Butyrivibrio* was associated with a significant reduction in the expression level of tight junction protein zonula occludens-1 (ZO-1), increased intestinal permeability, and increased intestinal and plasma levels of the inflammatory cytokine IL-17 (54). Also, studies have shown that administration of *Butyrivibrio* to mice can decrease intestinal damage and inflammation caused by *Campylobacter*-induced enterocolitis or chemical damage (55–57). It is possible that induction of Treg cells by butyrate (58, 59) could account for such protection. Butyrate may also dampen local innate immune activation and support enterocyte growth and survival as additional protective mechanisms (60). Thus, the negative association of *Butyrivibrio* with adaptive IF13 could be related to higher Treg activity dampening the development of memory CD4 and CD8 T-cells that would produce these cytokines. However, in our secondary, *post hoc* analyses after identification of this association of IF13 with *Butyrivibrio*, we did not observe a positive association of *Butyrivibrio* with Tregs which would have supported a role for Tregs in our observations, nor did we observe a negative association with innate immune variables. However, in support of the idea that *Butyrivibrio* may be protective against tissue damage in the intestine (or elsewhere), our secondary analysis did find a marginally significant ($P = 0.072$ after adjustment for multiple comparison) negative association of *Butyrivibrio* with plasma matrix metalloproteinase (MMP) −3 (stromelysin-1). MMPs in plasma are used as indicators of tissue damage and MMP-3 is known to be increased in a human model of small bowel ischemia–reperfusion injury in conjunction with enterocyte apoptosis (61). This association indirectly supports the hypothesis that higher *Butyrivibrio* levels in the gut may protect against intestinal epithelial damage. Though the mechanism behind this possible protection is unknown it may involve dampened T-cell activity, as suggested by the negative association with IF13.

## Strengths and limitations

One strength of our study is that we used a broad range of immune markers to identify associations of both the innate and adaptive immune systems with intestinal bacteria. A second strength is that we examined these associations in a population of healthy adults, perhaps allowing identification of less robust associations than have previously been identified in other populations including both healthy individuals and those with diagnosed, immune-mediated diseases. A third strength is that we were careful to identify statistically significant associations using adjustment for multiple comparisons as well as reproducible associations with bacterial taxa by highlighting associations found using two different approaches to quantifying bacteria (i.e., relative abundance and DESeq2-adjusted sequence counts).

A limitation of our study is that its cross-sectional design does not allow conclusions to be drawn about the cause-effect association between the microbial taxa and IFs. Therefore, future experimental studies in human and animal models are required to further examine the causality of these associations. However, the associations of microbial taxa with IFs identified in our cohort of healthy adults may be indicative of the potential preclinical markers of inflammatory diseases and may represent potential targets of dietary intervention to lower the disease risk. A second potential limitation of our study is that we retained low-abundance microbial taxa (i.e., <1% relative abundance in all study participants) that might not reach a threshold to trigger immune activation. However, previous work has shown that even small numbers of certain bacteria can affect the immune function (62, 63), thus, we included these taxa in our analysis. Although our findings, particularly those on the low abundant microbial taxa identified

in this study including *Pseudomonadaceae, Pseudomonas, ML615-J28,* and *Slackia* should be interpreted with caution, the identified associations between these taxa and immune responses provide hypothesis-generating results to support the design of future studies examining the relationships between commensal microbes and immune response in healthy adults.

## Conclusion

In conclusion, the associations identified in this study between the microbial taxa and innate and adaptive IFs suggest that there are multiple, ongoing interactions between these taxa and the immune system. These associations may contribute to low level, chronic immune activation, but may also contribute to the maintenance of an intact intestinal barrier and lower systemic inflammation in our group of healthy individuals without acute or chronic disease. Some of these associations may have been more difficult to identify in more diverse study populations, containing both healthy individuals and those with diagnosed inflammatory diseases.

## ACKNOWLEDGMENTS

The authors would like to thank Janet Peerson for providing expert advice in statistical analysis. We thank Yuriko Adkins, Ph.D., Tammy Freytag, Ph.D., and Xiaowen Jiang for the measurement of immune biomarkers. We thank Lacey Baldiviez, Ph.D., Eduardo Cervantes, and Yasmine Bouzid for their work with study volunteers. We thank Dr. Ellen Bonnel for her contributions to coordinating and managing the study, including her general oversight of the day-to-day study operations. We thank Dr. Leslie Woodhouse and the WHNRC Bioanalytical Support Lab staff including Debra Standridge, Connor Osato and Joseph Domek, and the Physiology Support Lab staff, including Justin Waller, for their work on the study.

This work was supported with funding from a USDA Agricultural Research Service (Project numbers 2032-51530-026-000-D, 2032-51530-025-00-D, 2032–53000–001-00-D, and 2032-51530-022-00-D) as well as the National Center for Advancing Translational Sciences (NCATS), and National Institutes of Health (NIH) through a grant, UL1 TR000002.

C.B.S., M.E.K., and N.R. conceived the study, designed scientific objectives, and provided editorial and conceptual input to the final version of the manuscript. M.E.K. generated 16S rRNA datasets from human fecal samples. N.R. performed the statistical analysis and the literature review and composed the manuscript. All authors approved the submitted version of the manuscript.

We have no conflict of interest to declare.

## AUTHOR AFFILIATIONS

[1]Graduate Group of Molecular, Cellular, and Integrative Physiology, University of California, Davis, California, USA
[2]Agricultural Research Service, Western Human Nutrition Research Center, USDA, Immunity and Disease Prevention Unit, Davis, California, USA
[3]Department of Nutrition, University of California, Davis, California, USA

## AUTHOR ORCIDs

Niknaz Riazati http://orcid.org/0000-0002-0721-4912
Mary E. Kable http://orcid.org/0000-0002-5485-6539
Charles B. Stephensen http://orcid.org/0000-0002-2117-2237

## FUNDING

| Funder | Grant(s) | Author(s) |
|---|---|---|
| USDA \| Agricultural Research Service (ARS) | 2032-51530-026-000-D | Charles B. Stephensen |

## AUTHOR CONTRIBUTIONS

Niknaz Riazati, Data curation, Formal analysis, Writing – original draft | Charles B. Stephensen, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review and editing.

## DATA AVAILABILITY

The 16S rRNA amplicon sequencing data is publicly available from the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) under accession number PRJEB53463.

## ETHICS APPROVAL

The studies involving human participants were reviewed and approved by University of California Davis Institutional Review Board under identification number 691654. The patients/participants provided their written informed consent to participate in this study.

## ADDITIONAL FILES

The following material is available online.

### Supplemental Material

**Supplemental Material (Spectrum01027-23-s0001.xlsx).** Supplemental Tables and Figures.

## REFERENCES

1. Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. 2020. Gut microbiota and immune system interactions. Microorganisms 8:1587. https://doi.org/10.3390/microorganisms8101587
2. Zheng D, Liwinski T, Elinav E. 2020. Interaction between microbiota and immunity in health and disease. Cell Res 30:492–506. https://doi.org/10.1038/s41422-020-0332-7
3. Ghosh S, Whitley CS, Haribabu B, Jala VR. 2021. Regulation of intestinal barrier function by microbial metabolites. Cell Mol Gastroenterol Hepatol 11:1463–1482. https://doi.org/10.1016/j.jcmgh.2021.02.007
4. Belkaid Y, Hand TW. 2014. Role of the microbiota in immunity and inflammation. Cell 157:121–141. https://doi.org/10.1016/j.cell.2014.03.011
5. Dimitratos SM, Hercules M, Stephensen CB, Cervantes E, Laugero KD. 2021. Association between physiological stress load and diet quality patterns differs between male and female adults. Physiol Behav 240:113538. https://doi.org/10.1016/j.physbeh.2021.113538
6. Baldiviez LM, Keim NL, Laugero KD, Hwang DH, Huang L, Woodhouse LR, Burnett DJ, Zerofsky MS, Bonnel EL, Allen LH, Newman JW, Stephensen CB. 2017. Design and implementation of a cross-sectional nutritional phenotyping study in healthy US adults. BMC Nutr 3:79. https://doi.org/10.1186/s40795-017-0197-4
7. Riazati N, Kable ME, Newman JW, Adkins Y, Freytag T, Jiang X, Stephensen CB. 2022. Associations of microbial and indoleamine-2,3-dioxygenase-derived tryptophan metabolites with immune activation in healthy adults. Front Immunol 13:917966. https://doi.org/10.3389/fimmu.2022.917966
8. Semmes EC, Hurst JH, Walsh KM, Permar SR. 2020. Cytomegalovirus as an immunomodulator across the lifespan. Curr Opin Virol 44:112–120. https://doi.org/10.1016/j.coviro.2020.07.013
9. Chidrawar S, Khan N, Wei W, McLarnon A, Smith N, Nayak L, Moss P. 2009. Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals. Clin Exp Immunol 155:423–432. https://doi.org/10.1111/j.1365-2249.2008.03785.x
10. Parada AE, Needham DM, Fuhrman JA. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18:1403–1414. https://doi.org/10.1111/1462-2920.13023
11. Comeau AM, Douglas GM, Langille MGI. 2017. Microbiome helper: a custom and streamlined Workflow for microbiome research. mSystems 2:e00127-16. https://doi.org/10.1128/mSystems.00127-16
12. Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R. 2016. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems 1:e00009-15. https://doi.org/10.1128/mSystems.00009-15
13. Kable ME, Chin EL, Storms D, Lemay DG, Stephensen CB. 2022. Tree-based analysis of dietary diversity captures associations between fiber intake and gut microbiota composition in a healthy US adult cohort. J Nutr 152:779–788. https://doi.org/10.1093/jn/nxab430
14. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez

AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0252-6

15. R Core Team. 2021. R: a language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria. Available from: www.R-project.org/
16. Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8
17. Nieminen P, Lehtiniemi H, Vähäkangas K, Huusko A, Rautio A. 2013. Standardised regression coefficient as an effect size index in summarising findings in epidemiological studies. Epidemiol Biostat Public Health 10. https://doi.org/10.2427/8854
18. Rothwell JC, Julious SA, Cooper CL. 2018. A study of target effect sizes in randomised controlled trials published in the health technology assessment journal. Trials 19:544. https://doi.org/10.1186/s13063-018-2886-y
19. Cohen J. 1988. Statistical power analysis for the behavioral sciences. 2nd ed. Lawrence Earlbaum Associates, Hillsdale, NJ.
20. Fu J, Bonder MJ, Cenit MC, Tigchelaar EF, Maatman A, Dekens JAM, Brandsma E, Marczynska J, Imhann F, Weersma RK, Franke L, Poon TW, Xavier RJ, Gevers D, Hofker MH, Wijmenga C, Zhernakova A. 2015. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. Circ Res 117:817–824. https://doi.org/10.1161/CIRCRESAHA.115.306807
21. Oki K, Toyama M, Banno T, Chonan O, Benno Y, Watanabe K. 2016. Comprehensive analysis of the fecal microbiota of healthy Japanese adults reveals a new bacterial lineage associated with a phenotype characterized by a high frequency of bowel movements and a lean body type. BMC Microbiol 16:284. https://doi.org/10.1186/s12866-016-0898-x
22. Moughaizel M, Dagher E, Jablaoui A, Thorin C, Rhimi M, Desfontis J-C, Mallem Y. 2022. Long-term high-fructose high-fat diet feeding elicits insulin resistance, exacerbates dyslipidemia and induces gut microbiota dysbiosis in WHHL rabbits. PLoS One 17:e0264215. https://doi.org/10.1371/journal.pone.0264215
23. Liu Q, Li Y, Song X, Wang J, He Z, Zhu J, Chen H, Yuan J, Zhang X, Jiang H, Zhang S, Ruan B. 2020. Both gut microbiota and cytokines act to atherosclerosis in ApoE-/- mice. Microb Pathog 138:103827. https://doi.org/10.1016/j.micpath.2019.103827
24. Tavella T, Rampelli S, Guidarelli G, Bazzocchi A, Gasperini C, Pujos-Guillot E, Comte B, Barone M, Biagi E, Candela M, Nicoletti C, Kadi F, Battista G, Salvioli S, O'Toole PW, Franceschi C, Brigidi P, Turroni S, Santoro A. 2021. Elevated gut microbiome abundance of *Christensenellaceae*, *Porphyromonadaceae* and *Rikenellaceae* is associated with reduced visceral adipose tissue and healthier metabolic profile in Italian elderly. Gut Microbes 13:1–19. https://doi.org/10.1080/19490976.2021.1880221
25. Lu Y, Fan C, Li P, Lu Y, Chang X, Qi K. 2016. Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating G protein-coupled receptors and gut microbiota. Sci Rep 6:37589. https://doi.org/10.1038/srep37589
26. Wang A, Guan B, Shao C, Zhao L, Li Q, Hao H, Gao Z, Chen K, Hou Y, Xu H. 2022. Qing-Xin-Jie-Yu granule alleviates atherosclerosis by reshaping gut microbiota and metabolic homeostasis of ApoE-/- mice. Phytomedicine 103:154220. https://doi.org/10.1016/j.phymed.2022.154220
27. Centner AM, Khalili L, Ukhanov V, Kadyan S, Nagpal R, Salazar G. 2023. The role of phytochemicals and gut microbiome in atherosclerosis in preclinical mouse models. Nutrients 15:1212. https://doi.org/10.3390/nu15051212
28. Dong Y, Cheng H, Liu Y, Xue M, Liang H. 2019. Red yeast rice ameliorates high-fat diet-induced atherosclerosis in ApoE-/- mice in association with improved inflammation and altered gut microbiota composition. Food Funct 10:3880–3889. https://doi.org/10.1039/c9fo00583h
29. Smith BJ, Miller RA, Schmidt TM, Suen G. 2021. Muribaculaceae genomes assembled from metagenomes suggest genetic drivers of differential response to acarbose treatment in mice. mSphere 6:e0085121. https://doi.org/10.1128/msphere.00851-21
30. Ormerod KL, Wood DLA, Lachner N, Gellatly SL, Daly JN, Parsons JD, Dal'Molin CGO, Palfreyman RW, Nielsen LK, Cooper MA, Morrison M, Hansbro PM, Hugenholtz P. 2016. Genomic characterization of the uncultured bacteroidales family S24-7 inhabiting the guts of homeothermic animals. Microbiome 4:36. https://doi.org/10.1186/s40168-016-0181-2
31. Wang B, Kong Q, Li X, Zhao J, Zhang H, Chen W, Wang G. 2020. A high-fat diet increases gut microbiota biodiversity and energy expenditure due to nutrient difference. Nutrients 12:3197. https://doi.org/10.3390/nu12103197
32. Monk JM, Lepp D, Wu W, Pauls KP, Robinson LE, Power KA. 2017. Navy and black bean supplementation primes the colonic mucosal microenvironment to improve gut health. J Nutr Biochem 49:89–100. https://doi.org/10.1016/j.jnutbio.2017.08.002
33. Cao W, Chin Y, Chen X, Mi Y, Xue C, Wang Y, Tang Q. 2020. The role of gut microbiota in the resistance to obesity in mice fed a high fat diet. Int J Food Sci Nutr 71:453–463. https://doi.org/10.1080/09637486.2019.1686608
34. Barouei J, Bendiks Z, Martinic A, Mishchuk D, Heeney D, Hsieh Y-H, Kieffer D, Zaragoza J, Martin R, Slupsky C, Marco ML. 2017. Microbiota, metabolome, and immune alterations in obese mice fed a high-fat diet containing type 2 resistant starch. Mol Nutr Food Res 61. https://doi.org/10.1002/mnfr.201700184
35. Zhang F, Zhou Y, Chen H, Jiang H, Zhou F, Lv B, Xu M. 2022. Curcumin alleviates DSS-induced anxiety-like behaviors via the microbial-brain-gut axis. Oxid Med Cell Longev 2022:6244757. https://doi.org/10.1155/2022/6244757
36. Rooks MG, Veiga P, Wardwell-Scott LH, Tickle T, Segata N, Michaud M, Gallini CA, Beal C, van Hylckama-Vlieg JET, Ballal SA, Morgan XC, Glickman JN, Gevers D, Huttenhower C, Garrett WS. 2014. Gut microbiome composition and function in experimental colitis during active disease and treatment-induced remission. ISME J 8:1403–1417. https://doi.org/10.1038/ismej.2014.3
37. Siddiqui R, Makhlouf Z, Alharbi AM, Alfahemi H, Khan NA. 2022. The gut microbiome and female health. Biology (Basel) 11:1683. https://doi.org/10.3390/biology11111683
38. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Leber B, Horvath A, Pieber TR, Gorkiewicz G, Stadlbauer V, Obermayer-Pietsch B. 2017. Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study. PLoS One 12:e0168390. https://doi.org/10.1371/journal.pone.0168390
39. Diggle SP, Whiteley M. 2020. Microbe profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Microbiology (Reading) 166:30–33. https://doi.org/10.1099/mic.0.000860
40. Xiao J, Thwe AA, Liu T, Gong D, Lin W, Shang C, Lu ZJ. 2021. Anti-inflammatory effects of an extract from *Pseudomonas aeruginosa* and its purified product 1-hydroxyphenazine on RAW264.7 cells. Curr Microbiol 78:2762–2773. https://doi.org/10.1007/s00284-021-02544-3
41. Khan R, Basha A, Goverdhanam R, Rao PC, Tanemura Y, Fujimoto Y, Begum AS. 2015. Attenuation of TNF-α secretion by l-proline-based cyclic dipeptides produced by culture broth of *Pseudomonas aeruginosa*. Bioorg Med Chem Lett 25:5756–5761. https://doi.org/10.1016/j.bmcl.2015.10.075
42. von Klitzing E, Ekmekciu I, Bereswill S, Heimesaat MM. 2017. Acute Ileitis facilitates infection with multidrug resistant *Pseudomonas aeruginosa* in human microbiota-associated mice. Gut Pathog 9. https://doi.org/10.1186/s13099-017-0154-4
43. Buck AC, Cooke EM. 1969. The fate of ingested *Pseudomonas aeruginosa* in normal persons. J Med Microbiol 2:521–525. https://doi.org/10.1099/00222615-2-4-521

44. Kumar H, Franzetti L, Kaushal A, Kumar D. 2019. *Pseudomonas fluorescens*: a potential food spoiler and challenges and advances in its detection. Ann Microbiol 69:873–883. https://doi.org/10.1007/s13213-019-01501-7
45. Zhou L, Ni Z, Cheng W, Yu J, Sun S, Zhai D, Yu C, Cai Z. 2020. Characteristic gut microbiota and predicted metabolic functions in women with PCOS. Endocr Connect 9:63–73. https://doi.org/10.1530/EC-19-0522
46. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. 2014. Human genetics shape the gut microbiome. Cell 159:789–799. https://doi.org/10.1016/j.cell.2014.09.053
47. Manor O, Dai CL, Kornilov SA, Smith B, Price ND, Lovejoy JC, Gibbons SM, Magis AT. 2020. Health and disease markers correlate with gut microbiome composition across thousands of people. Nat Commun 11:5206. https://doi.org/10.1038/s41467-020-18871-1
48. Margiotta E, Caldiroli L, Callegari ML, Miragoli F, Zanoni F, Armelloni S, Rizzo V, Messa P, Vettoretti S. 2021. Association of sarcopenia and gut microbiota composition in older patients with advanced chronic kidney disease, investigation of the interactions with uremic toxins, inflammation and oxidative stress. Toxins (Basel) 13:472. https://doi.org/10.3390/toxins13070472
49. Bonder MJ, Tigchelaar EF, Cai X, Trynka G, Cenit MC, Hrdlickova B, Zhong H, Vatanen T, Gevers D, Wijmenga C, Wang Y, Zhernakova A. 2016. The influence of a short-term gluten-free diet on the human gut microbiome. Genome Med 8:45. https://doi.org/10.1186/s13073-016-0295-y
50. Chen J, Wright K, Davis JM, Jeraldo P, Marietta EV, Murray J, Nelson H, Matteson EL, Taneja V. 2016. An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. Genome Med 8:43. https://doi.org/10.1186/s13073-016-0299-7
51. Romo-Vaquero M, Cortés-Martín A, Loria-Kohen V, Ramírez-de-Molina A, García-Mantrana I, Collado MC, Espín JC, Selma MV. 2019. Deciphering the human gut microbiome of urolithin metabotypes: association with enterotypes and potential cardiometabolic health implications. Mol Nutr Food Res 63:e1800958. https://doi.org/10.1002/mnfr.201800958
52. Gomez-Arango LF, Barrett HL, Wilkinson SA, Callaway LK, McIntyre HD, Morrison M, Dekker Nitert M. 2018. Low dietary fiber intake increases collinsella abundance in the gut microbiota of overweight and obese pregnant women. Gut Microbes 9:189–201. https://doi.org/10.1080/19490976.2017.1406584
53. Andriulli A, Bevilacqua A, Palmieri O, Latiano A, Fontana R, Gioffreda D, Castellana S, Mazza T, Panza A, Menzaghi C, Grandone E, di Mauro L, Decina I, Tricarico M, Musaico D, Mäki M, Isola J, Popp A, Taavela J, Petruzzi L, Sinigaglia M, Rosaria Corbo M, Lamacchia C. 2022. Healthy and pro-inflammatory gut ecology plays a crucial role in the digestion and tolerance of a novel gluten friendly bread in celiac subjects: a randomized, double blind, placebo control *in vivo* study. Food Funct 13:1299–1315. https://doi.org/10.1039/d1fo00490e
54. Wu S, Yi J, Zhang Y-G, Zhou J, Sun J. 2015. Leaky intestine and impaired microbiome in an amyotrophic lateral sclerosis mouse model. Physiol Rep 3:e12356. https://doi.org/10.14814/phy2.12356
55. Ohkawara S, Furuya H, Nagashima K, Asanuma N, Hino T. 2007. Effect of oral administration of *Butyrivibrio fibrisolvens* MDT-1, a gastrointestinal bacterium, on 3-methylcholanthrene-induced tumor in mice. Nutr Cancer 59:92–98. https://doi.org/10.1080/01635580701397608
56. Ohkawara S, Furuya H, Nagashima K, Asanuma N, Hino T. 2009. Effect of oral administration of *Butyrivibrio fibrisolvens* MDT-1 on experimental enterocolitis in mice. Clin Vaccine Immunol 16:291–291. https://doi.org/10.1128/CVI.00400-08
57. Ohkawara S, Furuya H, Nagashima K, Asanuma N, Hino T. 2006. Effect of oral administration of *Butyrivibrio fibrisolvens* MDT-1 on experimental enterocolitis in mice. Clin Vaccine Immunol 13:1231–1236. https://doi.org/10.1128/CVI.00267-06
58. Asarat M, Apostolopoulos V, Vasiljevic T, Donkor O. 2016. Short-chain fatty acids regulate cytokines and TH17/treg cells in human peripheral blood mononuclear cells *in vitro*. Immunol Invest 45:205–222. https://doi.org/10.3109/08820139.2015.1122613
59. Jacob N, Jaiswal S, Maheshwari D, Nallabelli N, Khatri N, Bhatia A, Bal A, Malik V, Verma S, Kumar R, Sachdeva N. 2020. Butyrate induced tregs are capable of migration from the GALT to the pancreas to restore immunological tolerance during type-1 diabetes. Sci Rep 10:19120. https://doi.org/10.1038/s41598-020-76109-y
60. Zhang M, Wang Y, Zhao X, Liu C, Wang B, Zhou J. 2021. Mechanistic basis and preliminary practice of butyric acid and butyrate sodium to mitigate gut inflammatory diseases: a comprehensive review. Nutr Res 95:1–18. https://doi.org/10.1016/j.nutres.2021.08.007
61. Di Sabatino A, Brunetti L, Biancheri P, Ciccocioppo R, Guerci M, Casella C, Vidali F, MacDonald TT, Benazzo M, Corazza GR. 2013. Mucosal changes induced by ischemia-reperfusion injury in a jejunal loop transplanted in oropharynx. Intern Emerg Med 8:317–325. https://doi.org/10.1007/s11739-011-0615-6
62. de Cena JA, Zhang J, Deng D, Damé-Teixeira N, Do T. 2021. Low-abundant microorganisms: the human microbiome's dark matter, a scoping review. Front Cell Infect Microbiol 11:689197. https://doi.org/10.3389/fcimb.2021.689197
63. Pust M-M, Tümmler B. 2022. Bacterial low-abundant taxa are key determinants of a healthy airway metagenome in the early years of human life. Comput Struct Biotechnol J 20:175–186. https://doi.org/10.1016/j.csbj.2021.12.008