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Nutritional Correlates of Human Oral Microbiome

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Abstract

Background—Despite many potential effects of the oral microbiome on oral and systemic health, scant information is available regarding the associations between diet and the oral microbiome.

Methods—Oral rinse DNA samples from 182 participants in a population-based case–control study for colorectal cancer were used to amplify a V3–V4 region of bacterial 16S rRNA gene. The amplicons were sequenced using Illumina MiSeq paired end chemistry on 2 runs, yielding approximately 33 million filtered reads that were assigned to bacterial classes. Relative abundances of each class and family as well microbial diversity/richness indices were correlated with selected dietary intakes from a food frequency questionnaire.

Results—Saturated fatty acids (SFAs) and vitamin C intakes were consistently correlated with alpha (within-subjects) diversity indexes in both richness and diversity. SFA intake was positively correlated with relative abundance of betaproteobacteria and fusobacteria. Vitamin C and other vitamins with correlated intakes—for example, the B vitamins and vitamin E—exhibited positive correlations with fusobacteria class, its family Leptotrichiaceae and a clostridia family Lachnospiraceae. In addition, glycemic load was positively correlated with Lactobacillaceae abundance.

Conclusion—The observed associations in this study were modest. However, the results suggest that the effects of diets are likely to be habitat specific, and observations from the gut microbiome are not transferrable to the oral microbiome. Further studies are warranted, incorporating a range of host biomarkers, such as cytohistological, molecular, or biochemical measurements, in order to address biological consequences of these dietary intakes in human oral health.

Keywords

oral microbiome; diet; fat; vitamins; glycemic load

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INTRODUCTION

The human oral microbiome includes more than 2000 bacterial taxa, including a large number of opportunistic pathogens [1,2], and it is considered to be the second most diverse community following stool [2,3]. However, unlike microbiomes in other anatomic sites where symbiosis is generally maintained, the oral microbiome causes disease in a majority of humans throughout their lifetime [1]. Humans have coevolved with their microbes over thousands of years, but this relationship is now being dramatically affected by changes in the environment and societal norms [4]. In fact, investigations on ancient calcified dental plaques have revealed the evolution of the oral microbiome, shifting to a disease-associated cariogenic configuration through the transition from hunter–gatherer to farming lifestyles and during the Industrial Revolution [5]. Caries and periodontitis are 2 major dental diseases caused by oral bacteria, but they are not exactly infectious diseases in the classical sense because they are not contagious and result from a complex interaction between commensal microbiota and the host and host lifestyle [2].

Oral bacteria have been recognized as etiological agents for other oral conditions, such as alveolar osteitis, tonsillitis, and osteomyelitis, and are also linked to certain systemic diseases, such as aspiration pneumonia and cardiovascular diseases [6,7]. Furthermore, several lines of evidence support their involvement in pathogenesis of head and neck cancer. Specifically, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* have been postulated to activate the beta-catenin and metalloprotease 9 pathways, leading to carcinogenesis through their virulence factors; that is, FadA and gingipain [8]. Oral bacteria also possess enzymatic activities that produce a range of potentially carcinogenic or anti-carcinogenic metabolites [9], such as acetaldehyde [10], nitrite [11], hydrogen sulfide [12], and short-chain fatty acids [2].

Given this assortment of potential health effects of oral microbiome, an increase in knowledge concerning how host lifestyle factors influence human oral microbial composition is likely useful in devising new methods for preventing the linked diseases. Despite global roles of diet in oral microbiome evolution [5] and the vital roles of host diet in gut microbiome [9], relatively scant information has been available regarding the associations between diet and oral microbiome, most of which have been limited to culturable bacteria. This may be partly due to the fact that the primary substrates for oral bacterial growth are endogenous nutrients variously provided by saliva, tissue excludes, crevicular fluids, degenerating host cells, or other bacterial metabolites [2,13,14], not directly derived from the food ingested. Yet, dietary intake is an important factor that influences these endogenous nutritional environments through systemic circulation and thus warrants further investigation using advanced sequencing technology to obtain more complete information about the oral microbial community.

MATERIALS AND METHODS

Study Design

This study was designed as secondary analyses of biological specimens and epidemiologic data collected for published studies described elsewhere [15,16]. The study was approved by

the Wayne State University Human Investigation Committee. A total of 1335 cases (41.7%) and 1682 controls (59.4%) consented to the study, and 1205 cases and 1547 controls remained eligible after completion of the study. The subjects were interviewed over the telephone using structured questionnaires regarding their usual diet and other risk factors for colorectal cancer for the time period preceding cancer diagnosis (approximately 2 years prior to the interview). A validated semiquantitative food frequency questionnaire (FFQ), Block 98.2 (Block Dietary Data Systems, Berkeley, CA), was used to estimate daily nutrient (including individual fatty acid groups) intake. Energy-adjusted nutrient intake was calculated by means of the residual method described by Willett and Stampfer [17]. Total vitamin and mineral intakes were computed as the sum of energy-adjusted dietary intake and intake from supplements. Healthy Eating Index score was calculated as described originally based on 10 food/nutrient items [18].

The study participants provided one of the following types of biospecimens: (1) peripheral blood through a home phlebotomy service, (2) buccal cells collected by commercial mouthwash liquid, or (3) archived (grossly normal) tissue blocks. The oral rinse samples were collected by 30-second swishing with a commercial mouthwash liquid containing 15% alcohol. The participants were instructed not to brush teeth, rinse mouth, eat, or drink for at least 1 hour before collection. DNA was isolated with the Gentra Autopure system and then stored at −80°C until analysis at the Wayne State University Applied Genomics Technology Center. Blood samples were obtained from 71% of the controls, and the rest provided mouthwash samples. Among cases, phlebotomy accounted for 66%, followed by mouthwash (27%) and tissue blocks (7%). From the participants who provided oral rinse samples, we randomly selected 192 samples for this exploratory study, oversampling smokers, after excluding those with insufficient residual volume. To minimize potential effects of immunosuppression from the disease and treatment, colorectal cancer cases were further limited to those who did not have distant metastasis or did not receive chemotherapy.

Sequencing

Amplification and sequencing of the 16S ribosomal RNA gene was achieved following the Illumina 16S Metagenomic Sequencing Library Preparation protocol, using S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 primer pair [19]. A 464 bp segment of the V3 and V4 regions of the 16S gene was amplified and the product was run on an Agilent Tape Station (Agilent Technology, Santa Clara, CA) to confirm the predicted size. Amplified products were purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN) to remove excess primers and primer dimers and indexed using the 96-sample Nextera XT Index Kit (Illumina, San Diego, CA), giving each sample a unique identifier with dual 8-base adapters. The indexed products were isolated with AMPure XP beads and the size was checked again with the Tape Station to con-firm indexing success. Samples were quantified fluorometrically using Invitrogen’s Qubit 2.0, diluted to a 4 nM concentration, and then pooled. The library pool was then denatured and diluted to a 20 pM concentration and a PhiX control was added. The pool was sequenced on an Illumina MiSeq using paired 300 bp reads with MiSeq v3 reagents and the data were analyzed using the MiSeq Reporter software.

Bioinformatic Analysis

Data were analyzed using the Basespace high throughput sequence analysis suite accessed through the Illumina online portal [20]. 16S Metagenomics analysis uses DNA from amplicon sequencing of prokaryotic 16S small subunit rRNA genes with the high version of RDP naïve Bayes algorithm [21]. FASTQ sequences were uploaded to Basespace and the 16S metagenomics application was executed. Sequences were paired and diversity analysis for the Shannon index and unique species was generated. 16S Metagenomics was referenced against the Illumina curated version of Greengenes (May 2013). After assembling, full length sequences from paired ends were referenced against the Illumina curated version of Greengenes database (May 2013) at a 97% identity level. We also used the Quantitative Insights for Molecular Ecology (Qiime) suite of programs [22] to compute several α and β diversity indices, including Chao1, Shannon, and Simpson indexes, after rarefying samples at 10,851 reads, and to perform principal coordinate analysis based on weighted Unifrac distance matrices [23]. Xming and Emperor Plot [24] were used to visualize principal coordinate analysis plots.

Statistical Analysis

Basespace summary text files reporting number of reads for each identified taxonomic unit were aggregated for all samples from 2 separate runs. We removed 2 samples that failed to generate 20,000 classifiable reads and 8 with no valid dietary data, leaving 182 analytical samples. Analyses were restricted to bacterial groups that represented an average of at least 1% of total reads classifiable at each taxonomic level and focused on nutritional components known to be present in saliva, to be required for bacterial growth, and/or to possess antimicrobial properties. The associations of relative abundance of each taxonomic unit (number of reads for each unit/number of total classifiable reads) with dietary intake were first screened by partial Spearman's rank correlation coefficients with selected covariates at class and family levels. Dietary intakes that showed correlations with more than one bacterial group were further analyzed by a negative binomial model as an extension of Poisson regression for count data, to deal with overdispersion [25], with adjustment of selected covariates. In these analyses, dietary intake was grouped into quartiles based on distributions of all samples combined and the effect of dietary intake was estimated for the highest compared to the lowest quartile intake. Zero-inflated models were also employed when the count was 0 for more than 5% of the samples, which produced the parameter estimates for both counts and zero proportion. To ease interpretation for the latter estimate, we presented its reverse term (powered −1) as an estimate for the presence (non-zero). In both analyses the total number of reads classifiable at each taxonomic level was used as an offset variable. Analysis of variance and linear regression were used to compute covariate adjusted means of microbial richness and diversity indices from QIIME analyses according to quartile levels of dietary intake as well as to test linear treads in the adjusted means according to quartile levels (scored 0–3). Two covariates were included in analyses for individual bacterial groups, experimental batch (run 1 vs 2), advanced age (defined as 65 years or older, the approximate median of the whole study population), and experimental batch only for overall microbial diversity/richness analyses, based on preliminary analyses. Because case status, cigarette smoking, and alcohol use had modest effects on frequency of

some bacterial groups only [26], these variables were not included as covariates. All statistical analyses were performed using SAS Ver 9 (SAS, Cary, NC).

RESULTS

Daily Dietary Intake Profiles of the Study Subjects

Table 1 presents daily dietary intake profiles (median and quartile range) of the study subjects estimated by the FFQ. The median total calorie intake (~2000) and median Healthy Eating Index (63) are consistent with those of the original study population [16,27]. Median percentage calories from fat was 37.5% (data not shown) with 29 g of saturated fat acids (SFAs), indicating a typical American high-fat diet. Otherwise the subjects were nutritionally replete in terms of micronutrient intakes.

Correlations between Dietary Intake and Microbial Abundance, Diversity, and Richness

The results of nonparametric Spearman correlations (Table 2) showed that SFA and vitamin C were rather consistently correlated with alpha (within subjects) diversity indexes in both richness (Chao and number of operational taxonomic units) and diversity (whole-tree and Shannon). On the individual class level, SFA intake was positively correlated with relative abundance of betaproteobacteria and fusobacteria, but SFA associations with bacilli varied with families, being positive with Gemellaceae and negative with Streptococcaceae. Vitamin C and other vitamins with correlated intakes—for example, B vitamins and vitamin E—exhibited positive correlations with fusobacteria class, its family Leptotrichiaceae, and a clostridia family Lachnospiraceae. A few other dietary items showed correlations with some bacterial groups. Sodium intake was inversely correlated with relative abundance of clostridia class, glycemic load was positively correlated with Lacto-bacillaceae relative abundance, and omega-3 polyunsatu-rated fatty acid (PUFA) was positively associated with Leptotrichiaceae and Lachnospiraceae families. Although we did not collect information about probiotic supplement use, we did ask about yogurt intake. In this study population, 38% reported intake of yogurt at least once a week, of whom 14% consumed yogurt daily. Partial Spearman correlation coefficients were calculated for the frequency of intake, adjusting for other basic covariates, with the relative abundance of the Lactobacillaceae family, and we did not find any significant correlation ($r = 0.03$, $p = 0.65$). It is possible that regular yogurt users were too few to see a correlation, and sampling timing after ingestion is likely to have altered detection probability of transient food-borne lactobacilli.

Effects of Dietary Intakes on Relative Bacterial Abundance at the Family Level

We also quantitatively assessed the effects of these dietary intakes with relative bacterial abundance at the family level using negative binomial regression, adjusted for experimental batch and advanced age, estimating the effect of a change in intake from the lowest to the highest quartile (Table 3). We found that the presence of Neisseriaceae, a dominant family of Betaproteobacteria, increased almost 3-fold with an increase in intakes of SFAs and vitamins B, C, and E but decreased to the same degree with an increase in glycemic load. Vitamin C intake had more pronounced effects on the presence of Lepto-trichiaceae and Lachnospiraceae, with estimated effect of 5.94 (95% confidence interval [CI], 1.65–21.46) and 5.58 (95% CI, 1.21–25.74), respectively. On the other hand, the presence of

Gemellaceae was increased by vitamins B1 and E by almost 6 times (6.67, 95% CI, 1.16–38.17; 6.91, 95% CI, 1.21–11.72, respectively). We also found that relative abundance of Lacto-bacillaceae increased by 3 times (3.22, 95% CI, 1.11–9.33) with increasing glycemic load.

Mean Microbial Diversity/Richness Indices according to SFA and Vitamin C Intake

Mean microbial diversity and richness indexes, according to quartile levels of SFA and vitamin C intake, are presented in Table 4. Adjusted for experimental batch and the other nutrient (vitamin C or SFA), all indexes in both diversity and richness increased progressively with quartile levels of SFA intake ($p < 0.05$). Similar linear trends occurred with levels of vitamin C intake, but the associations with richness indexes, Chao, and number of operational taxonomic units were less apparent, with p-values of 0.105 and 0.056, respectively. On the other hand, the associations with diversity indexes were stronger, with p-values of 0.027 and 0.006, respectively.

DISCUSSION

To our knowledge, this exploratory study is the first to report associations between a comprehensive dietary profile and human oral microbiome using high-throughput 16S rRNA metagenomic sequencing. Associations of specific nutrient intakes with microbial community diversity and richness were present, suggestive of an impact of diet on oral microbiome. Specifically, we report that SFA and vitamin C intakes were consistently correlated with alpha (within subjects) diversity indexes of human oral microbiome. SFA intake was positively correlated with relative abundance of Betaproteobacteria and Fusobacteria, whereas vitamin C exhibited positive correlations with abundance in fusobacteria class and Leptotrichiaceae and Lachnospiraceae families. In addition, glycemic load was positively correlated with Lactobacillaceae abundance. However, we recognize that the observed associations were rather modest. If we apply Bonferroni adjustment for multiple comparisons, none of the associations, except that between n-3PUFA and Lachnospiraceae, remains statistically significant. However, as discussed later, Bonferroni's method may over-correct in this type of study. Recent studies, employing other types of high-throughput platforms—that is, microarray and pyrosequencing—also concluded that diet has little influence on salivary bacterial profiles, based on an FFQ [28] and based on a broad classification (omnivore, vegetarian, and vegan) [29].

The Human Microbiome Project has revealed that each body microhabitat is distinct, maintaining a unique ecosystem [2,3]. Changes in the diversity of microbes within a given body habitat, which can be defined as number, abundance, and distinct types of organisms, have been linked to several human diseases [2,3]. In some ecosystems, such as the gut, high biodiversity is associated with a healthy status, whereas low biodiversity is linked to pathological conditions such as obesity and inflammatory bowel disease [2,3]. On the contrary, in other ecosystems such as the vagina, high diversity is directly associated with illness such as vaginosis [30]. Compared to the gut microbiome, the oral microbiome has lower diversity [2,3], suggesting that symbiosis is maintained by a relatively limited community membership. Thus far, it has been controversial whether orodental diseases are

linked to reduced or increased microbial biodiversity. Some investigators suggest that changes in microbial diversity depend on the complexity of microorganisms involved in a particular condition, either simple infection, such as periimplantitis (reduced) or polymicrobial origin; for example, periodontitis (increased) [31]. Periodontal diseases have been recognized to result from an increase in the complexity and volume of biofilms located in gingival crevice [32]. Microbial richness indexes have also been reported to be higher in oral squamous cell carcinoma tissue than in normal mucosa [33]. On the other hand, reduced community diversity occurred as caries progressed in cavitated teeth [34]. In this study, contrary to the gut microbiome whose diversity correlates with a healthy diet index [35], we observed no indication that healthy diets (measured as Healthy Eating Index) altered oral microbiome composition.

Dietary carbohydrates have long been acknowledged to have a major influence on microbial ecology, because dietary sugars provide readily available substrates for the oral microorganisms, which depend on carbohydrates for energy sources [13]. Accordingly, the influence of sugar content in the diet has been studied more extensively than any other nutritional factors [13,36]. Specifically, individuals who frequently ingest high levels of carbohydrates harbor a greater abundance of acidogenic and aciduric bacteria, particularly lactobacilli and *Streptococcus mutans*, in their oral cavities [2,13,14]. Acid products from these bacteria are in fact considered to be the primary cause of dental caries [2,34]. Although our dietary questionnaire did not provide a direct estimate of sucrose intake, the present 16S rRNA-based metagenomic survey was able to confirm a close relationship between total glycemic load (as glucose) and relative abundance of Lactobacillaceae family.

Dietary fats are not digested in the oral cavity, but saliva is known to contain various lipids, including fatty acids, cholesterol, and triglycerides [37–39] at concentrations much lower than those in serum or plasma. Some of these lipids may originate from host sebaceous glands in the oral cavity [37], but groups of oral bacteria are also capable of synthesizing short-and long-chain fatty acids [2,14,40]. Though these fatty acids are an essential carbon source for certain oral bacteria [2,14], some of these fatty acids inhibit growth of various oral bacteria *in vitro* when they are added to the culture medium [40,41]. Furthermore, dietary fats can alter food texture, resulting in longer retention time [42] in the mouth but less adherence on the teeth [13]. Yet, except for the data from studies concerning fat/lipids and periodontitis risk, direct evidence to support potential influence of dietary fats on oral microbe compositions is lacking. Studies have reported that salivary lipid contents are higher in caries-susceptible than in caries-resistant subjects [39] and that bacterially synthesized lipid contents increase with dental plaque maturation [39]. A comparison between generalized chronic periodontitis with gingivitis showed significantly higher plasma triglycerides and PUFA levels in the former patient groups but provided no data on SFA [43]. The dietary n-6/n-3 PUFA ratio has been reported to be predictive of periodontal disease events [44], and frequent intake of fatty foods in overweight individuals has been associated with periodontitis risk [45]. Furthermore, a low-fat/high-fiber diet intervention has been shown to improve periodontal disease indexes [46]. In our study, all fatty acids displayed similar trends with diversity indexes and with individual bacterial groups, but SFA yielded the most consistent observation being positively related to increased diversity and

richness, and thus more complex microbial community, which are inclusive of various pathogens.

The role of micronutrients in oral health has been well established, because certain minerals, such as calcium, fluorine, and phosphorus, are known to be important in maintaining structural integrity of the teeth [47]. Several metals, possibly of dietary origin, are present in saliva as well [48,49], with fairly high concentrations of Fe and Zn. Although elevated salivary Zn and Cu levels have been observed in individuals with dental caries [50,51], this could more likely reflect host response via induction of antioxidant enzymes in which Zn and Cu are required. In this study we found little association of these minerals/metals with microbial diversity or abundances of individual bacteria.

Human saliva contains most of the known vitamins, with vitamin C in the largest quantity [52]. Among the micronutrients, the most consistent trend was observed for vitamin C, but the association was not in the direction that we anticipated, indicating the potential growth-stimulating effects of vitamin C for certain bacteria. The associations were similar to those observed with SFA, although these 2 intakes were not positively correlated. Although vitamin C may be systemically beneficial to health, it is an acid with the potential for weakening the enamel layer. Two studies in children have reported that vitamin C supplement and fruit juice/syrup consumption were associated with increased risk of caries [53,54].

We acknowledge several limitations in this exploratory study. First, there is significant likelihood that the associations observed in this study were chance findings due to multiple comparisons, although we restricted the analysis to a limited number of nutritional parameters that may have growth-stimulating or antimicrobial effects as discussed above. Traditionally, nutritional epidemiology does not enforce adjustment for multiple comparisons, partly because intakes of individual nutrients are closely correlated with each other, traveling in the same foods and meal ingredients. The relative abundance of some bacterial families may also be interrelated, especially if they are derived from same classes. Bonferroni adjustment indeed reduced most of the association to statistically nonsignificant levels, but it may also have introduced overadjustment. Second, investigators have recognized that the estimated intakes from FFQs, such as the one used in this study, are subject to substantial error that can profoundly attenuate diet–disease associations [55]. Thus, the overall null associations in the present study are possibly due to insufficient statistical power, in view of the attenuation of the associations from measurement errors as well as the fact that the beta (between-subjects) diversity of salivary microbiome was the lowest, whereas the alpha (within-subjects) diversity was one of the highest [3]. In addition, though the effects of diet on gut micro-biome have been primarily examined through global change or manipulations of diets [56], our dietary information was from residents in one U.S. region of a narrow age range. Thus, the extent of variability in diets was limited. Moreover, it has been well documented that the choice of the V regions of bacterial 16S rRNA gene significantly influences the results [57–59]. We used primers for combined regions including the V4 that reportedly can assign sequences down to the genus level with good accuracy [57]. Though we acknowledge that a choice of any region would not be perfect in quantifying the relative abundance of bacteria accurately within individuals, this

should not interfere with interindividual comparisons, which was the primary interest of this study. Finally, because the parent study was designed to address medical questions, information was not collected for oral hygiene practices or oral and dental disease histories, which are likely to be critical determinants of oral microbiome. Strengths of the study include a population (non-clinic)-based study design and sequence depth much greater than earlier studies, coverage of microbiome from global oral locations, and quantitative analyses beyond graphic presentation typically used in metagenomic studies.

An important conclusion from this study is that observations from gut microbiome are not transferrable to oral microbiome. In fact, we were not able to replicate the associations between colorectal cancer and suspected oral pathogens using these oral microbiome data [26]. Nevertheless, using high-throughput 16S rRNA metagenomic technology, the present study confirmed the association between higher intake of digestible carbohydrate and Lactobacillaceae abundance, which has been known for decades as an etiological factor for dental caries. In addition, the study provided a few intriguing observations that warrant follow-up investigations; that is, potential effects of SFA and vitamin C on oral microbiome composition. It is noteworthy that these 2 nutrients interact synergistically in producing carcinogenic nitrosamines [60], which is known to be catalyzed by oral bacterial enzymatic activities [61]. Further studies are necessary, incorporating a range of host biomarkers, such as cytohistological, molecular, or biochemical measurements, as well as a more robust quantitative technique, such as quantitative polymerase chain reaction, on a selected number of bacteria, in order to address the biological consequences of these dietary intakes in oral health.

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Table 1

Median, 25% and 75% Percentiles of Estimated Dietary Intake of Interest in 182 Subjects

Dietary Intake	25th Percentile	Median	75th Percentile
Calorie (kcal)	1454.5	2008.1	2545.6
H_Eat Index	56.0	63.0	72.0
PctSweet (%)	8.6	13.9	20.5
Glycemic load (g)	83.3	117.7	149.7
Sodium (mg)	2773.0	3050.2	3372.5
Fiber (g)	15.5	18.6	23.4
SFA (g)	25.3	28.9	32.3
n3PUFA (g)	1.7	2.0	2.5
n6PUFA (g)	17.1	20.1	24.2
Carotene (mcg)	3160.5	4476.3	6414.3
Quercetin (mg)	4.6	6.6	10.6
Iron (mg)	15.0	26.0	32.9
Zinc (mg)	12.2	21.7	27.8
Phosphorus (mg)	1241.2	1365.8	1557.9
Magnesium (mg)	329.7	411.6	466.4
Calcium (mg)	761.3	950.6	1494.2
Vitamin B1 (mg)	1.7	2.8	3.2
Vitamin B2 (mg)	2.1	3.2	3.7
Vitamin B6 (mg)	2.0	3.4	4.1
Vitamin B12 (mcg)	4.9	8.7	10.7
Niacin (mg)	24.7	39.6	45.3
Folate (mcg)	536.2	1002.8	1219.6
Vitamin C (mg)	129.0	185.3	287.1
Vitamin D (IU)	168.3	452.5	559.4
Vitamin E (aTE)	12.6	30.0	39.2

H_Eat Index = Heathy Eating Index, PctSweet = percentage of calories from sweets and deserts, SFA = saturated fatty acids, PUFA= polyunsaturated fatty acids, aTE = alpha-tocopherol equivalent.

Table 2

Spearman Partial Correlation Coefficients of Selected Dietary Intake with Microbial Diversity/Richness and with Relative Bacterial Abundance at Class and Family Levels

	Diversity/Richness*			Classes#								Families#												
Dietary intake	PD_whole_tree	Chao	observed_otus	Shannon	Bacilli	Clostridia	Actinobacteria	Bacteroidia	Betaproteobacteria	Gammaproteobacteria	Fusobacteria	Streptococcaceae	Veillonellaceae	Prevotellaceae	Micrococcaceae	Pasteurellaceae	Neisseriaceae	Gemellaceae	Actinomycetaceae	Fusobacteriaceae	Carnobacteriaceae	Leptotrichiaceae	Porphyromonadaceae	Lactobacillaceae	Lachnospiraceae
(Mean)	(37.14)	(827.7)	(422.5)	(4.914)	(47.8%)	(13.4%)	(12.8%)	(12.3%)	(4.0%)	(3.9%)	(3.7%)	(40.5%)	(10.9%)	(9.4%)	(7.7%)	(3.9%)	(3.8%)	(3.7%)	(3.2%)	(1.9%)	(1.9%)	(1.9%)	(1.6%)	(1.3%)	(1.2%)
Calorie	0.072	0.029	0.023	0.070	−0.023	0.145	−0.042	0.083	−0.152	−0.038	−0.041	−0.091	0.108	0.119	−0.025	−0.025	−0.154	−0.041	−0.028	−0.020	−0.082	−0.039	−0.065	0.182	0.074
H_Eat Index	−0.054	−0.079	−0.070	−0.095	0.048	−0.073	0.005	−0.093	0.011	0.082	−0.079	0.110	−0.027	−0.072	0.108	0.065	0.012	−0.025	−0.100	−0.059	−0.001	−0.045	−0.047	−0.091	−0.095
PctSweet	−0.068	−0.013	−0.032	−0.032	0.043	0.075	−0.005	−0.010	−0.136	−0.098	−0.098	0.037	0.120	−0.034	0.003	−0.088	−0.135	−0.048	−0.056	−0.098	0.009	−0.102	−0.057	0.117	−0.146
Glycemic load	0.010	−0.021	−0.028	0.027	0.029	0.140	−0.011	0.031	−0.196	−0.037	−0.091	−0.021	0.134	0.066	0.018	−0.039	−0.193	−0.091	−0.054	−0.049	−0.081	−0.091	−0.086	0.205	0.019
Sodium	0.018	−0.005	0.005	0.003	0.014	−0.156	0.005	−0.011	0.077	0.142	0.028	−0.014	−0.151	−0.006	0.062	0.136	0.076	0.117	−0.077	0.080	0.018	0.025	0.045	0.000	−0.020
Fiber	0.032	0.000	0.019	−0.022	−0.008	−0.096	−0.066	−0.021	0.117	0.093	−0.009	0.022	−0.108	−0.022	0.017	0.099	0.110	0.059	−0.054	−0.019	0.048	0.028	0.005	−0.152	−0.034
SFA	0.141	0.166	0.156	0.169	−0.099	−0.043	−0.056	0.098	0.202	0.077	0.141	−0.170	−0.086	0.044	−0.132	0.085	0.196	0.152	0.060	0.098	0.052	0.150	0.140	−0.006	0.088
n3PUFA	0.063	0.025	0.033	0.071	−0.080	−0.028	−0.038	0.024	0.048	−0.018	0.138	−0.098	−0.114	0.010	−0.108	−0.038	0.042	0.071	0.083	0.077	−0.065	0.161	0.050	0.019	0.202
n6PUFA	0.063	0.029	0.040	0.072	−0.054	0.066	−0.029	0.031	−0.017	−0.025	0.100	−0.090	−0.005	0.043	−0.101	−0.040	−0.026	0.009	0.100	0.113	−0.044	0.058	0.006	0.061	0.217
Carotene	0.068	0.060	0.084	0.080	−0.029	−0.108	−0.061	−0.087	0.122	0.108	0.004	−0.030	−0.099	−0.095	0.000	0.127	0.108	0.015	−0.080	0.004	0.050	0.018	−0.022	−0.090	−0.077
Quercetin	0.024	0.037	0.037	0.025	−0.021	−0.035	−0.049	−0.014	0.062	0.099	−0.046	−0.016	−0.040	0.001	0.035	0.107	0.063	0.057	−0.046	−0.005	0.171	−0.060	−0.063	−0.114	−0.111
Iron	0.017	0.007	0.017	0.027	0.064	−0.011	0.015	−0.065	0.061	0.058	0.068	0.053	−0.003	−0.041	0.060	0.068	0.057	0.091	−0.008	0.017	0.057	0.089	−0.013	−0.046	0.076
Zinc	0.089	0.080	0.089	0.095	−0.016	−0.002	−0.037	−0.029	0.102	0.039	0.130	−0.021	−0.028	−0.022	−0.022	0.066	0.097	0.094	−0.014	0.084	0.053	0.140	0.057	−0.060	0.119
Phosphorus	0.028	0.012	0.025	−0.029	−0.004	−0.050	−0.114	−0.001	0.094	0.074	−0.090	−0.037	−0.037	−0.001	−0.040	0.076	0.092	0.026	−0.124	−0.081	−0.049	−0.058	0.010	0.016	−0.071
Magnesium	0.025	0.004	0.012	0.008	−0.068	0.018	−0.026	−0.015	0.090	0.059	0.047	−0.050	−0.005	0.011	−0.011	0.084	0.089	0.022	0.033	0.004	0.030	0.054	−0.029	−0.103	0.095
Calcium	0.031	0.027	0.029	−0.019	−0.024	−0.089	−0.011	−0.048	0.133	0.118	0.042	−0.007	−0.065	−0.025	0.007	0.113	0.137	0.114	−0.003	0.014	0.019	0.064	−0.031	−0.140	0.017
Vitamin B1	0.107	0.101	0.108	0.142	−0.088	0.103	−0.082	0.085	0.072	0.069	0.173	−0.072	0.069	0.124	−0.085	0.081	0.068	0.087	0.058	0.106	0.002	0.164	0.025	−0.052	0.192
Vitamin B2	0.105	0.107	0.117	0.127	−0.052	0.057	−0.098	0.083	0.100	0.071	0.131	−0.061	0.028	0.107	−0.080	0.086	0.095	0.119	0.010	0.060	0.030	0.143	0.031	−0.051	0.150
Vitamin B6	0.092	0.060	0.081	0.127	−0.033	0.028	−0.087	0.031	0.111	0.079	0.154	−0.031	−0.017	0.056	−0.070	0.100	0.106	0.121	0.042	0.108	0.083	0.134	0.028	−0.086	0.165
Vitamin B12	0.100	0.092	0.109	0.124	−0.050	0.027	−0.077	0.046	0.126	0.065	0.143	−0.064	−0.012	0.059	−0.072	0.093	0.118	0.093	0.007	0.085	0.017	0.143	0.061	−0.038	0.148
Niacin	0.022	0.004	0.021	0.044	0.026	0.070	−0.108	−0.022	0.045	0.006	0.065	0.031	0.026	0.009	−0.096	0.026	0.035	0.071	0.011	0.037	0.000	0.047	−0.009	0.011	0.106
Folate	0.061	0.058	0.069	0.087	−0.037	0.054	−0.063	0.029	0.066	0.073	0.125	−0.038	0.036	0.061	−0.047	0.084	0.064	0.097	0.000	0.087	0.055	0.132	0.015	−0.077	0.129
Vitamin C	0.150	0.118	0.141	0.164	−0.164	0.089	−0.027	0.076	0.116	0.083	0.187	−0.109	0.048	0.096	−0.037	0.090	0.111	0.031	0.069	0.214	0.045	0.176	0.112	−0.146	0.251
Vitamin D	0.041	0.049	0.051	0.051	−0.003	0.031	−0.005	0.015	0.037	0.022	0.110	−0.034	0.028	0.041	−0.021	0.037	0.029	0.089	0.076	0.020	0.034	0.137	−0.030	−0.041	0.146
Vitamin E	0.069	0.043	0.065	0.119	−0.023	0.008	−0.037	−0.022	0.110	0.028	0.164	−0.043	−0.028	−0.024	−0.050	0.046	0.104	0.100	0.041	0.129	0.089	0.143	0.070	−0.084	0.160

[a]Bacteria groups are limited to those representing at least average 1% in the study subjects and the mean percentage (%) of each group is presented in the parentheses. Gray shaded cells: With white figures: postive correlation, dark $p < 0.01$, medium $0.01 \le p < 0.05$, light $0.05 \le p < 0.10$; with black figures: negative correlation, dark $p < 0.01$, medium $0.01 \le p < 0.05$; light $0.05 \le p < 0.10$.

*Adjusted for experimental batch, #adjusted for experimental batch and age (≥65 vs < 65 years) of subjects.

H_Eat Index = Heathy Eating Index, PctSweet = percentage of calories from sweets and deserts, SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids.

Table 3

Effects of Selected Dietary Intake and 95% Confidence Intervals (Parentheses) on Relative Abundance of Bacterial Familes Based on Negative Binomial Models[a]

Bacterial Groups	Glycemic Load	Sodium	SFA	n3PUFA	Vitamin B1	Vitamin C	Vitamin E
Streptococcaceae	0.99 (0.88–1.11)	1.02 (0.91–1.14)	0.90 (0.81–1.01)	0.91 (0.81–1.01)	0.98 (0.87–1.10)	0.93 (0.83–1.05)	0.97 (0.87–1.09)
Veillonellaceae	1.20 (0.94–1.53)	0.81(0.64–1.04)	0.84 (0.66–1.07)	0.96 (0.75–1.23)	1.00 (0.79–1.28)	1.10 (0.86–1.41)	0.92 (0.72–1.18)
Neisseriaceae[b]	0.86 (0.58–1.29)	0.90 (0.59–1.37)	1.37 (0.92–2.06)	1.03 (0.67–1.58)	0.77 (0.52–1.16)	0.73 (0.49–1.09)	0.93 (0.63–1.38)
	0.34 (0.13–0.89)	1.85 (0.73–4.69)	3.31(1.28–8.55)	1.23 (0.49–3.07)	2.78 (1.08–7.03)	3.58 (1.38–9.32)	2.84 (1.10–7.38)
Gemellaceae[b]	0.94 (0.71–1.23)	1.28 (0.98–1.69)	1.28 (0.97–1.67)	1.08 (0.81–1.45)	0.94 (0.72–1.24)	0.84 (0.64–1.11)	0.98 (0.74–1.29)
	0.67 (0.14–3.15)	0.62 (0.13–2.91)	2.49 (0.53–11.72)	0.70 (0.15–3.22)	6.67 (1.16–38.17)	3.81 (0.75–19.27)	6.91(1.21–11.72)
Fusobacteriaceae[b]	0.88 (0.62–1.23)	0.93 (0.66–1.30)	1.08 (0.79–1.49)	1.21 (0.86–1.70)	1.17 (0.85–1.60)	1.26 (0.93–1.72)	1.18 (0.85–1.62)
	0.79 (0.24–2.56)	3.40 (0.98–11.79)	1.82 (0.55–6.07)	0.95 (0.29–3.06)	1.23 (0.38–3.97)	2.10 (0.63–7.02)	1.04 (0.32–3.41)
Leptotrichiaceae[b]	0.69 (0.48–1.00)	0.89 (0.63–1.28)	1.13 (0.79–1.61)	1.41 (0.96–2.06)	1.45 (1.02–2.08)	0.92 (0.65–1.29)	1.30 (0.89–1.90)
	0.94 (0.30–2.96)	1.47 (0.46–4.63)	2.35 (0.73–7.59)	1.65 (0.52–5.24)	3.02 (0.92–9.95)	5.94 (1.65–21.46)	2.15 (0.64–6.87)
Lactobacillaceae[b]	3.22 (1.11–9.33)	0.99 (0.35–2.79)	1.37 (0.58–3.25)	1.09 (0.49–2.41)	0.45 (0.18–1.10)	0.42 (0.18–0.99)	0.76 (0.28–2.05)
	2.34 (0.98–5.60)	1.32 (0.56–3.10)	0.92 (0.40–2.15)	1.01 (0.43–2.35)	0.74 (0.32–1.73)	0.78 (0.33–1.82)	0.66 (0.28–1.57)
Lachnospiraceae[b]	1.18 (0.86–1.62)	0.89 (0.66–1.20)	1.25 (0.92–1.70)	1.88 (1.41–2.52)	1.52 (1.12–2.06)	1.40 (1.03–1.88)	1.58 (1.17–2.14)
	0.71 (0.18–2.75)	0.95 (0.25–3.64)	1.87 (0.27–7.47)	1.91 (0.48–7.58)	1.91 (0.48–7.62)	5.58 (1.21–25.74)	2.09 (0.55–7.99)

[a]Effects were expressed as times increase in the relative bacterial count by increase in dietary intake from the lowest to the highest quartile

PctSweet = Percentage of calories from sweets and deserts, SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids.

[b]Based on a zero-inflated model: upper cell indicates effect on the relative counts beyond 0; lower indicates effect on the prevalence (non-zero) of a given bacterial group.

Table 4

Diversity and Richness Indices According to Saturated Fatty Acid and Vitamin C Intake[a]

Nutritional Intake	Levels	PD_whole_tree	*p*-value*	Chao	*p*-value*	observed_otus	*p*-value*	Shannon	*p*-value*
Saturated fatty acids	<25.32 (g)	34.4	—	740.7	—	386.6	—	4.72	—
	25.33–28.80	38.0	0.091	864.7	0.038	435.3	0.054	4.94	0.117
	28.81–32.0	36.9	0.236	819.8	0.187	420.9	0.177	4.93	0.145
	32.1+	39.3	0.024	885.6	0.017	447.1	0.019	5.07	0.014
	p-Value for trend	0.046		0.042		0.040		0.021	
Vitamin C	<129.4 (mg)	35.3	—	781.0	—	399.0	—	4.78	—
	129.4–185.2	35.9	0.781	807.6	0.658	415.7	0.511	4.80	0.868
	185.3–294.4	37.5	0.314	851.4	0.250	428.6	0.252	4.93	0.263
	294.5+	39.8	0.038	870.9	0.140	446.7	0.064	5.14	0.010
	p-Value for trend	0.027		0.105		0.056		0.006	

[a]Each index was adjusted for experimental batch and the other nutrient intake (saturated fatty acids or vitamin C).

*_p_-values for the difference from the first group.