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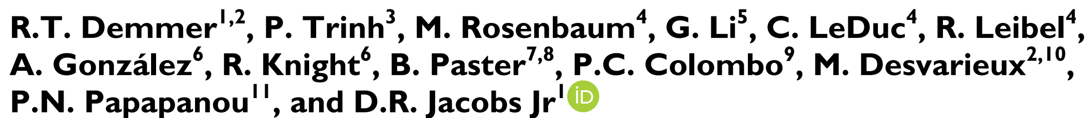
Subgingival Microbiota and Longitudinal Glucose Change: The Oral Infections, Glucose Intolerance and Insulin Resistance Study (ORIGINS)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Abstract

Microbial communities along mucosal surfaces throughout the digestive tract are hypothesized as risk factors for impaired glucose regulation and the development of clinical cardiometabolic disease. We investigated whether baseline measures of subgingival microbiota predicted fasting plasma glucose (FPG) longitudinally. The Oral Infections, Glucose Intolerance and Insulin Resistance Study (ORIGINS) enrolled 230 diabetes-free adults (77% female) aged 20 to 55 y (mean ± SD, 34 ± 10 y) from whom baseline subgingival plaque and longitudinal FPG were measured. DNA was extracted from subgingival plaque, and V3 to V4 regions of the 16S rRNA gene were sequenced. FPG was measured at baseline and again at 2 y; glucose change was defined as follow-up minus baseline. Multivariable linear models regressed 2-y glucose change onto baseline measures of community diversity and abundances of 369 individual taxa. A microbial dysbiosis index (MDI) summarizing top individual taxa associated with glucose change was calculated and used in regression models. Models were adjusted for age, sex, race/ethnicity, education, smoking status, body mass index, and baseline glucose levels. Statistical significance was based on the false discovery rate (FDR; <0.05) or a Bonferroni-corrected P value of 1×10^{-4}, derived from the initial 369 hypothesis tests for specific taxa. Mean 2-y FPG change was 1.5 ± 8 mg/dL. Baseline levels of 9 taxa predicted FPG change (all FDR <0.05), among which *Stomatobaculum* sp oral taxon 097 and *Atopobium* spp predicted greater FPG change, while *Leptotrichia* sp oral taxon 498 predicted lesser FPG change (all 3 P values, Bonferroni significant). The MDI explained 6% of variation in longitudinal glucose change ($P < 0.001$), and baseline glucose levels explained 10% of variation ($P < 0.0001$). FPG change values ± SE in the third versus first tertile of the MDI were 4.5 ± 0.9 versus 1.6 ± 0.9 ($P < 1 \times 10^{-4}$). Subgingival microbiota predict 2-y glucose change among diabetes-free men and women.

Keywords: microbiome, diabetes risk, periodontal, epidemiology, impaired glucose regulation, periodontitis

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A supplemental appendix to this article is available online.

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Introduction

The global prevalence of type 2 diabetes mellitus increased between 1980 and 2014 by >100% among men and by 50% among women. It is estimated that >422 million adults are living with diabetes in the world as of 2014 (NCD Risk Factor Collaboration 2016).

A growing body of literature suggests that microbial communities along the digestive tract may contribute to diabetogenesis (Qin et al. 2012; Wang and Jia 2016). Proposed biologically plausible hypotheses suggesting a diabetes-promoting role of microbial dysbiosis include indirect effects through energy balance and adiposity as well as through insulin resistance, secondary to a chronic systemic inflammatory response to the digestive tract microbiota (Demmer et al. 2017).

Prior studies found gut and oral microbial communities to be altered among people with diabetes versus healthy controls, although these case-control studies are limited due to the inability to infer temporality (Arumugam et al. 2011; Lalla and Papapanou 2011; Qin et al. 2012; Karlsson et al. 2013; Zhou et al. 2013; Wang and Jia 2016; Ganesan et al. 2017). Therefore, is it unknown whether features of the diabetes phenotype (e.g., medical management, behavioral changes after diagnosis, or hyperglycemia) might influence the microbiota or if microbial community shifts precede (and possibly contribute to) diabetes. To address this limitation, important insights into the temporality of associations have arisen by studying the relationship between microbes and preclinical biomarkers of diabetes risk among diabetes-free individuals and/or using longitudinal designs. Le Chatelier et al. (2013) found the gut microbiome to be associated with adverse metabolic profiles cross-sectionally and longitudinally among diabetes-free individuals. Additionally, among initially diabetes-free populations, clinical measures of gingival inflammation (known to be linked to altered subgingival microbial communities) have been linked to prediabetes (Arora et al. 2014) and insulin resistance (Demmer et al. 2012) cross-sectionally, as well as incident diabetes (Demmer et al. 2008; Winning et al. 2017) and hemoglobin A1c change (Demmer, Desvarieux, et al. 2010) longitudinally. More recently, the subgingival microbiome has been reported to be associated with inflammation, insulin resistance (Demmer et al. 2012), and prediabetes (Demmer et al. 2015) prevalence cross-sectionally.

Data are currently not available informing the association between oral (or gut) microbiota and longitudinal glucose change among a diabetes-free population. Data from longitudinal designs reduces the opportunity for reverse causality bias and informs the potential for either early screening or interventions on the microbiome to reduce disease risk prior to the onset of prediabetes or diabetes.

In this report, we examine the association between measures of the subgingival microbiota and 2-y longitudinal glucose change among a cohort of diabetes-free individuals. We hypothesized that features of the subgingival microbiota would predict longitudinal glucose change after accounting for known metabolic risk factors.

Methods

ORIGINS is a human observational cohort study investigating subgingival microbial community composition as a risk factor for impaired glucose metabolism (Demmer et al. 2015; Demmer et al. 2017). From February 2011 to May 2013, 300 men and women were enrolled. Inclusion criteria were as follows: 1) age 20 to 55 y; 2) no diabetes mellitus (T1 or T2) based on participant self-report of no previously diagnosed disease, hemoglobin A1c values <6.5%, and fasting plasma glucose <126 mg/dL; and 3) no history of myocardial infarction, congestive heart failure, stroke, or chronic inflammatory conditions based on participant self-report. Among the enrolled 300 participants, 281 had baseline subgingival bacterial data collection available, among which 230 (82% follow-up) had a follow-up glucose measurement 2 y after baseline (Appendix Fig. 1). The Columbia University and University of Minnesota institutional review boards approved the protocol. All participants provided informed consent. This article conforms to the STROBE guidelines.

Periodontal Examination

Dental examiners conducted full-mouth periodontal examinations as previously described (Demmer et al. 2015).

Subgingival Plaque Collection and Bacterial Assessments

A total of 281 subgingival plaque samples (1 sample per participant) were collected from the second-most posterior tooth (excluding third molars) in the lower right quadrant with sterile curettes, after removal of supragingival plaque. The samples were suspended in 300 µL of TE buffer (50mM Tris, 1mM EDTA; pH 7.6), and microbial DNA was extracted with the MasterPure Gram Positive DNA Purification Kit (Epicentre).

16S rRNA Sequencing and Taxonomic Classification

Sequencing of the 16S rRNA gene was conducted per the HOMINGS methodology (Human Oral Microbiome Identification Using Next Generation Sequencing), designed specifically for oral taxa and to generate species-level information. A modified protocol based on the work of Caporaso et al. (2011) was used (Gomes et al. 2015): 16S rDNA (50 ng) was amplified with 341F/806R universal primers (V3 to V4 region), and polymerase chain reaction products were purified with AMPure beads; 100 ng of each library was pooled, gel purified, and quantified with a bioanalyzer; and 12 pM of the library mixture was spiked with 20% PhiX and run on a MiSeq (Illumina) platform. The 16S data curation pipeline is outlined in the Appendix Materials. Overall, 18,531,931 sequences were generated for final analysis (median, 75,977 sequences per sample).

Sequence reads were taxonomically classified with 2 approaches. First, a customized BLAST program (ProbeSeq for

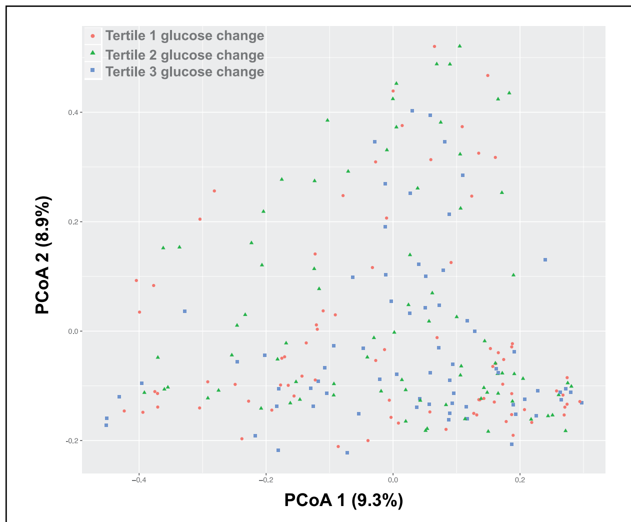

Figure 1. Two principal coordinates are plotted, as derived from the Bray-Curtis measure of subgingival plaque beta-diversity. Plot symbols refer to tertile of glucose change. The ranges of glucose change (time 2 – time 1) observed in each tertile are as follows: tertile 1 (red circle) = −27 to −2 mg/dL, tertile 2 (green triangle) = −2 to 4 mg/dL, tertile 3 (blue square) = 4 to 33 mg/dL. *P* value derived from an Adonis test (not significant).

HOMINGS) blasted the 16S rRNA reads against species-specific, 16S rRNA–based oligonucleotide "probes" (Mougeot et al. 2017). Second, we utilized a recently published bioinformatics workflow that utilizes DADA2 to identify "exact sequence variants," and we assigned taxonomy with the Silva Projects (version 128; Callahan et al. 2016; Callahan et al. 2017).

Laboratory Measures

Blood was collected following an overnight fast. Plasma glucose, serum lipids, and hemoglobin A1c from whole blood were measured at baseline with standard methods (Demmer et al. 2015; Demmer et al. 2017).

Risk Factors

Risk factors were measured in space provided by a Clinical Translational Science Award. Systolic and diastolic blood pressures were measured in triplicate, and the last 2 measurements were averaged. Body mass index (BMI) was calculated as kg/m^2. Questionnaires assessed information on the following: age, sex, race/ethnicity (non-Hispanic Black, non-Hispanic White, Hispanic, other), educational level (high school completion, college or vocational training, advanced degrees), cigarette smoking (current, former, or never smoking and duration/intensity of smoking), and leisure time physical activity (categorized into 4 categories; Thai et al. 2014).

Statistical Analysis

All analyses were performed with R (version 3.4.2), SAS (version 9.4), QIIME2, or gneiss. Alpha diversity was defined on the Silva-defined taxonomies with the Shannon index. Analysis of beta-diversity utilized a principal coordinates analysis of the Bray-Curtis dissimilarity index, and an Adonis test informed whether baseline microbial community membership differed according to glucose change.

Taxon-level analyses were conducted with the HOMINGS-derived taxonomies as follows. Serial inverse Poisson regressions of the absolute taxon abundance (i.e., raw read counts from next-generation sequencing) were performed. A generalized linear model was fit under the Poisson distribution regressing absolute levels of each taxon on glucose change, with adjustments for age, sex, education, race/ethnicity, cigarette smoking, BMI, and baseline glucose; library sizes were used as an offset. A dispersion parameter was included to assess overdispersion of the response. Taxa detected in <10% of participants were filtered, leaving 369 taxa for analysis. Sensitivity analyses are described in the Appendix Materials. The false discovery rate (FDR) was used to correct for multiple-hypothesis testing. We also consider Bonferroni-adjusted *P* values of 1.4×10^{-4} based on an $\alpha = 0.05$ and 369 statistical tests for our primary analysis.

Subsequent to the inverse Poisson regression analyses, we conducted additional sensitivity analyses (see Appendix Materials) to select top taxa associated with glucose change that were then used to develop a microbial dysbiosis index (MDI). The MDI was calculated by taking the log of total abundance of taxa positively associated with glucose change over total abundance of taxa inversely associated with glucose change, as done previously (Gevers et al. 2014). Multivariable linear regression models were then used to regress glucose change on tertiles of the MDI. Stepwise linear regression modeling was used to determine the most parsimonious set of variables that maximally explained glucose change.

We also utilized the balance tree approach to analyzing compositional microbiota data (Morton et al. 2017), and microbial community network analyses were performed to visualize the co-occurrence or co-exclusion network of organisms. These methods are described in detail in the Appendix Materials.

Results

General Characteristics of the Cohort

General characteristics are presented in the Table. The mean ± SD age of participants at baseline was 35 ± 10 y (78% female, 49% Hispanic, 21% White, 19% Black, and 11% other). Nearly 70% of participants had a college degree and only 4% had lower than a high school education; 91% of participants were not current smokers and 79% were never smokers. Participants lost to follow-up were 3 y younger than followed participants, but otherwise there were no appreciable differences (Appendix Table 1). Moderate and severe periodontitis was present in 51% and 7% of participants, respectively. Among sites from which subgingival plaque was sampled, 95% had a probing depth ≤3 mm, and the remaining 5% (11 sites) had a probing depth of 4 mm. More broadly, in the full mouth, the extent of periodontal disease was modest, as expected in this younger population without clinical cardiometabolic disease.

Mean ± SD baseline and follow-up glucose levels were 85.2 ± 7.6 and 86.7 ± 9.2 mg/dL, respectively, yielding a mean 2-y FPG change of 1.5 ± 8.0 mg/dL. Baseline glucose was

Table. Baseline Characteristics among All Participants and according to Tertiles of the Microbial Dysbiosis Index: ORIGINS, 2011 to 2015.

		Mean ± SD or %			
	All (*N* = 230)	Tertile 1 (*n* = 76)	Tertile 2 (*n* = 77)	Tertile 3 (High) (*n* = 77)	*P* Value
Age, y	34.8 ± 10.0	35.3 ± 10.5	33.6 ± 8.6	35.6 ± 10.9	0.42
Female	78	86	72	75	0.13
Race/ethnicity					0.07
Hispanic	49	55	45	47	
Non-Hispanic White	21	13	31	18	
Black	19	16	16	25	
Other	11	16	8	10	
Education					0.65
<College	34	34	30	36	
4-y college	44	43	42	46	
>Bachelor degree	23	22	29	28	
Smoking status					0.24
Never	79	76	73	87	
Former	13	15	17	7	
Current	9	9	10	7	
Pack-years of smoking	1.1 ± 4.1	1.5 ± 4.8	0.9 ± 3.4	0.9 ± 3.8	0.67
Activity level					0.46
None	31	33	25	33	
Low	14	11	17	15	
Moderate	14	13	11	19	
High	41	43	47	33	
Body mass index					
kg/m^2	27.3 ± 6.3	27.3 ± 6.1	26.4 ± 5.9	28.4 ± 6.8	0.13
Normal	43	38	52	38	
Overweight	33	37	26	35	
Obese	25	25	22	27	
Family history of diabetes	54	54	51	56	0.86
Blood pressure, mm Hg					
Systolic	117.6 ± 12.3	117.2 ± 12.1	115.4 ± 11.8	119.1 ± 13.8	0.14
Diastolic	74.9 ± 9.7	72.5 ± 12.1	75.2 ± 9.3	74.7 ± 12.2	0.47
Cholesterol, mg/dL					
LDL	97.6 ± 28.1	98.7 ± 30.0	96.9 ± 26.7	97.3 ± 27.7	0.92
HDL	59.8 ± 16.9	60.4 ± 18.7	61.6 ± 16.7	57.4 ± 15.2	0.29
Triglycerides, mg/dL	78.2 ± 47.6	74.1 ± 40.2	76.3 ± 36.7	84.3 ± 61.8	0.38
Tumor necrosis factor α, pg/mL	2.0 ± 2.3	2.2 ± 2.8	2.1 ± 2.7	1.7 ± 1.1	0.37
Interleukin 6, pg/mL	1.8 ± 6.4	2.9 ± 10.0	1.0 ± 0.7	1.7 ± 4.8	0.16
C-reactive protein, mg/L	3.6 ± 9.3	4.7 ± 13.8	2.2 ± 2.7	3.9 ± 7.8	0.23
Adiponectin, ng/mL	9,376.3 ± 4,941.5	9,219.7 ± 4,173.6	9,482.5 ± 5,456.3	9,424.7 ± 5,155.4	0.94
Mean probing depth, mm	2.3 ± 0.3	2.4 ± 0.3	2.3 ± 0.2	2.4 ± 0.3	0.54
Sites with probing depth, %					
≥4	6.4 ± 0.4	6.8 ± 0.7	5.4 ± 0.7	6.9 ± 0.7	0.31
≥5	1.0 ± 0.2	1.5 ± 0.2	0.5 ± 0.2	1.0 ± 0.2	0.08
Attachment loss, mm	1.6 ± 0.6	1.6 ± 0.7	1.5 ± 0.5	1.6 ± 0.7	0.37
Sites with attachment loss, %					
≥3	17.0 ± 0.8	18 ± 1.5	15 ± 1.5	18 ± 1.5	0.31
≥4	5.8 ± 0.6	6 ± 1.0	5 ± 1.0	6 ± 1.0	0.39
Periodontal BOP, % (sites/mouth)	50 ± 0.2	46 ± 0.3	42 ± 0.2	49 ± 0.2	0.22

BOP, bleeding on probing; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ORIGINS, Oral Infections Glucose Intolerance and Insulin Resistance Study.

positively correlated with follow-up glucose (r = 0.56, P < 0.0001) and inversely correlated with glucose change (r = −0.36, P < 0.0001). Nonmicrobial baseline predictors of glucose change are presented in Appendix Table 2. In multivariable models, age and BMI were marginally associated with short-term glucose change, and baseline glucose levels were statistically significant predictors of 2-y glucose change.

A 5-y higher baseline age or a 5-unit higher BMI was associated with 0.75-mg/dL (P = 0.10) and 0.78-mg/dL (P = 0.09) greater glucose change, respectively, while a 1-mg/dL higher level of baseline glucose was associated with a 0.41-mg/dL lower glucose change (P < 0.0001). Mean glucose change among participants with none/mild, moderate, and severe periodontitis was 1.1, 1.6, and 1.1 mg/dL, respectively (P = 0.88).

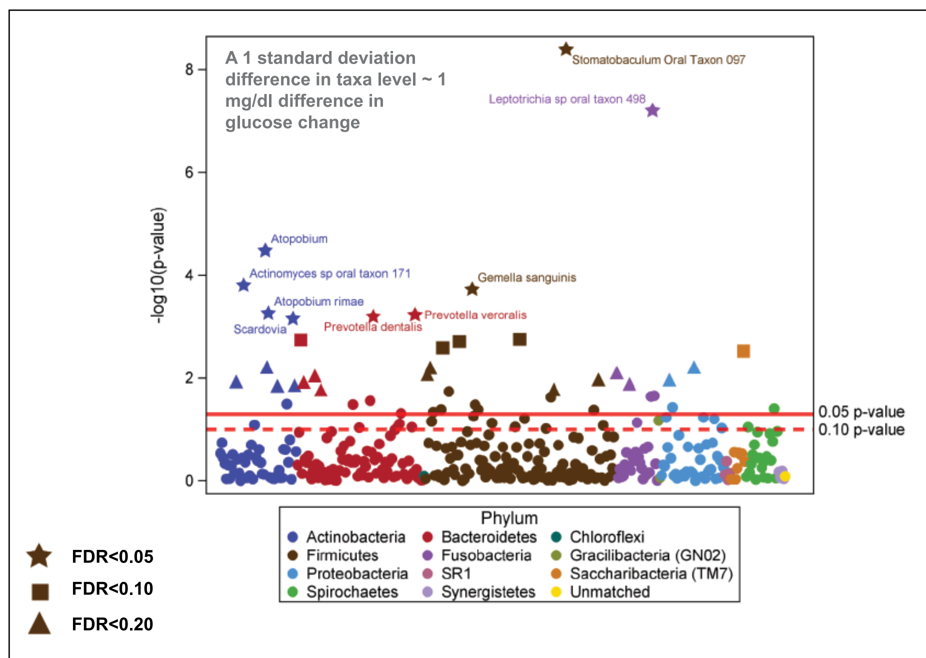

Figure 2. Manhattan plot summarizes multivariable adjusted association between baseline subgingival taxa and 2-y fasting glucose change in the Oral Infections Glucose Intolerance and Insulin Resistance Study (ORIGINS). Results adjusted for age, sex, race/ethnicity, education, smoking status, body mass index, and baseline glucose levels. The following taxa were significant after Bonferroni correction: *Stomatobaculum* sp oral taxon 097 (positive association), *Leptotrichia* sp oral taxon 498 (inverse association), *Atopobium* genus probe (positive association), *Actinomyces* sp oral taxon 171 (inverse association). FDR, false discovery rate.

Mean ± SD values of the Shannon index were 3.2 ± 0.9. Neither measures of α-diversity (Appendix Figs. 2, 3) or β-diversity (Fig. 1) were associated with glucose change. To explore these community differences at a finer resolution, we examined the associations between individual taxon levels and glucose change, and results are summarized in Figure 2 and Appendix Table 3. We observed 44 taxa to be associated with glucose change with a $P < 0.05$, among which higher levels of 27 taxa were associated with greater glucose change while higher levels of 17 taxa were associated with less glucose change. Among the aforementioned 44 taxa, 3 taxa (*Stomatobaculum* sp oral taxon 097, *Leptotrichia* sp oral taxon 498, *Atopobium* genus probe) had a Bonferroni-adjusted P value <0.05, and an additional 6 had an FDR <0.05 (*Actinomyces* sp oral taxon 171, *Gemella sanguinis, Atopobium rimae, Prevotella veroralis, Prevotella dentalis, Scardovia* genus probe). Among the top taxa, a 1-SD difference in taxa levels was generally associated with an approximate 1-mg/dL 2-y change in glucose. Results were consistent when sensitivity analyses were conducted with different thresholds of taxon prevalence to remove low-prevalence taxa. When taxa were filtered prior to regression analysis at a threshold of 20% prevalence, we observed 29 statistically significant discoveries with $P < 0.05$ (11 with FDR <0.05 and 5 with Bonferroni-adjusted $P < 0.05$); using a 30% filtering threshold, we observed 21 statistically significant discoveries with $P < 0.05$ (9 with FDR <0.05 and 5 with Bonferroni-adjusted $P < 0.05$).

The community network analysis shown in Figure 3a visualizes the interrelation of a set of top taxa and the relationship between taxa levels and glucose change. Among the top taxa, about half are positively associated with longitudinal glucose change, while the remaining are inversely related to glucose change. The strongest positive CCREPE value was 0.55, for *Atopobium* genus probe and *Atopobiu rimae* ($P = 1.20 \times 10^{-26}$), while the strongest negative CCREPE value was −0.14, for *Gemella sanguinis* and *Treponema* sp oral taxon 270 ($P < 0.001$). The detection prevalence and mean relative abundance values for taxa visualized in Figure 3a are summarized in Figure 4.

Higher levels of the MDI were strongly associated with higher levels of glucose change after multivariable adjustment. Figure 3b shows that the mean ± SE multivariable-adjusted glucose change observed in the highest versus lowest tertile of the MDI was 4.6 ± 1.6 versus 0.04 ± 1.1 mg/dL, respectively ($P < 3.3 \times 10^{-7}$). In a stepwise linear regression modeling approach with variables described in Appendix Table 2 as well as the MDI, 3 variables provided explanation of glucose change variation: 1) baseline glucose levels explained 10% ($P < 0.0001$) of variation in longitudinal glucose change; 2) the MDI explained an additional 6% ($P < 0.0001$) of variation; and 3) smoking history explained 3% of variation ($P = 0.005$).

Balance tree analyses demonstrated Bonferroni-adjusted statistically significant relationships between subgingival microbial communities and longitudinal glucose change. Appendix Figure 4 shows the scatter plot between the top microbial community balance variable (derived from the fifth internal node of the bifurcating tree described in the methods) and 2-y glucose change. The balance variable for each participant represents the ratio of taxa that were generally more abundant versus less abundant in participants who experienced high glucose change; as the ratio increases, it reflects a relative (but not necessarily absolute) increase in "high glucose" versus "low glucose" taxa. After multivariable linear regression, a 1-unit increase in this balance variable was associated with a 1-mg/dL 2-y glucose increase ($P = 2.4 \times 10^{-9}$). Mean ± SE 2-y glucose change values across tertiles of this balance variable were −1.9 ± 0.9, 0.8 ± 0.9, and 5.2 ± 0.9 mg/dL ($P < 0.0001$). Similarly, in separate multivariable analyses, a 1-unit increase in either the first or third internal nodes was associated with a 1.7-mg/dL ($P = 3.0 \times 10^{-7}$) and 1.4-mg/dL ($P = 1.8 \times 10^{-5}$) greater glucose change, respectively.

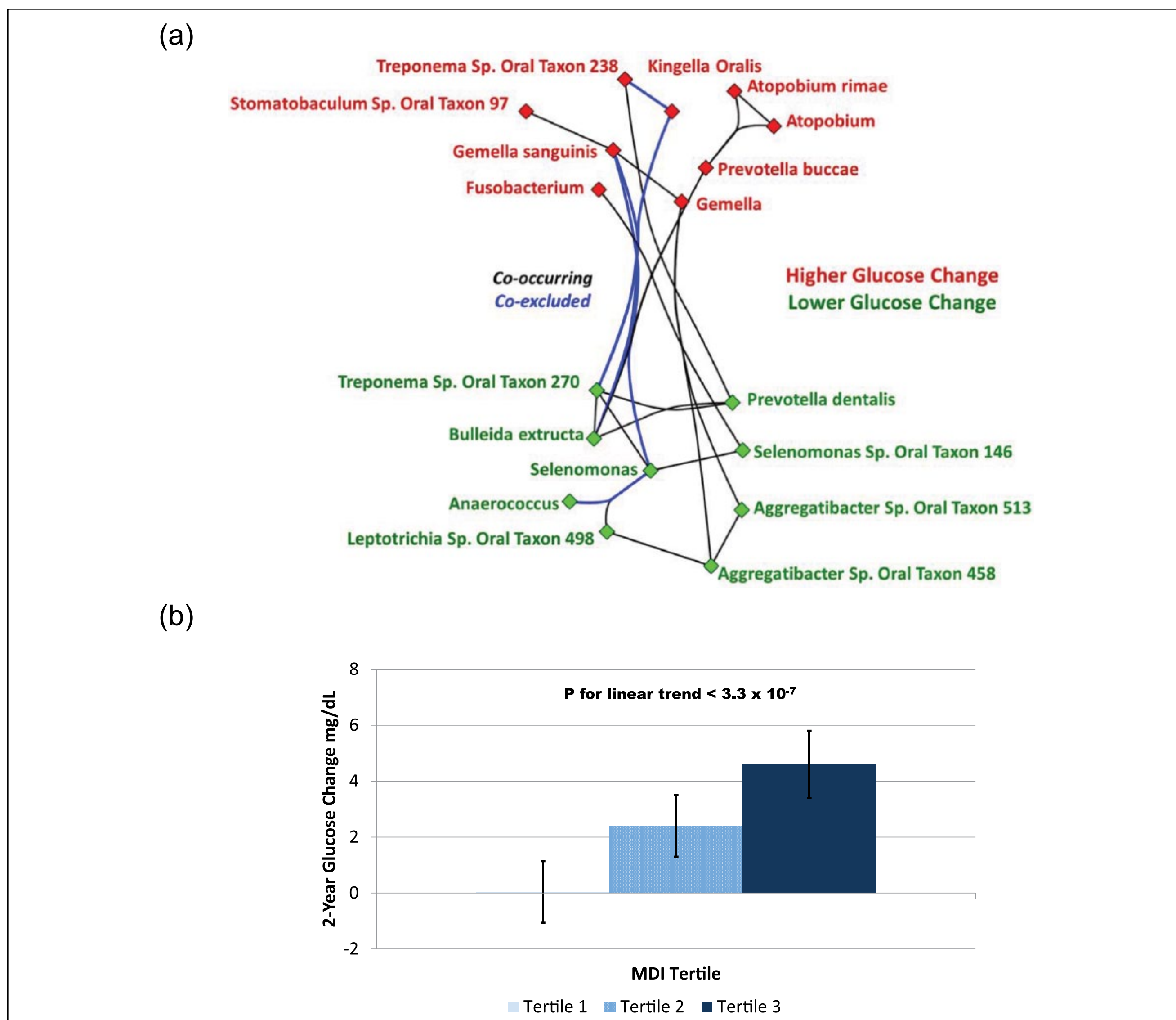

Figure 3. A small subset of taxa predict glucose change. (**a**) Microbial co-occurrence network. To enhance visibility and interpretation, taxa visualized were selected per their having all of the following: 1) a false discovery rate <0.2 in inverse Poisson regression models, 2) a *P* value <0.05 in standard linear regression models where individual taxa were predictors of longitudinal glucose change, and 3) at least 1 CCREPE correlation <−0.10 or >0.15. The nodes represent taxa associated with glucose change. Higher levels of red taxa are associated with greater glucose change, while higher levels of green taxa are associated with lesser glucose change. Blue edges represent inverse CCREPE correlations among bacteria, and black edges represent positive CCREPE correlations among bacteria. (**b**) Mean ± SE adjusted levels of 2-y fasting glucose change according to tertiles of the microbial dysbiosis index (MDI), adjusted for age, sex, race/ethnicity, education, smoking status, body mass index, and baseline glucose levels. The MDI is a ratio of taxa observed to be positively versus inversely associated with glucose change, as summarized in Figure 3a. Five taxa names were shortened from their full names on the HOMINGS platform to simplify presentation: *Fusobacterium* = *Fusobacterium* genus probe 1; specifically recognizes *F. naviforme* and *F. nucleatum. Selenomonas* = *Selenomonas* genus probe 2; specifically recognizes *S. sputigena* and sp oral taxon 143. *Gemella* = *Gemella* genus probe; specifically recognizes *G. asaccharolytica, G. bergeri, G. cuniculi, G. haemolysans, G. morbillorum, G. palaticanis*, and *G. sanguinis. Atopobium* = *Atopobium* genus probe; specifically recognizes *A. fossor, A. minutum, A. parvulum, A. rimae*, and sp oral taxon 199. *Anaerococcus* = *Anaerococcus* genus probe 2; specifically recognizes *A. hydrogenalis, A. lactolyticus, A. octavius, A. prevotii, A. tetradius*, and *A. vaginalis*.

Discussion

We observed that a small subset of subgingival bacteria predicted changes in fasting glucose during 2 y of longitudinal follow-up among a sample of diabetes-free young adults. A microbial score summarizing the ratio of taxa positively versus inversely associated with glucose change was a stronger predictor of future glucose change than any single taxon by ~4-fold, which is consistent with the notion of a pathogenic microbial community as discussed previously (Demmer, Papapanou, et al. 2010; Berezow and Darveau 2011). Participants in the highest versus lowest tertile of the microbial score realized 2-y glucose increases that were 4 mg/dL higher after multivariable adjustment. Microbial variables were

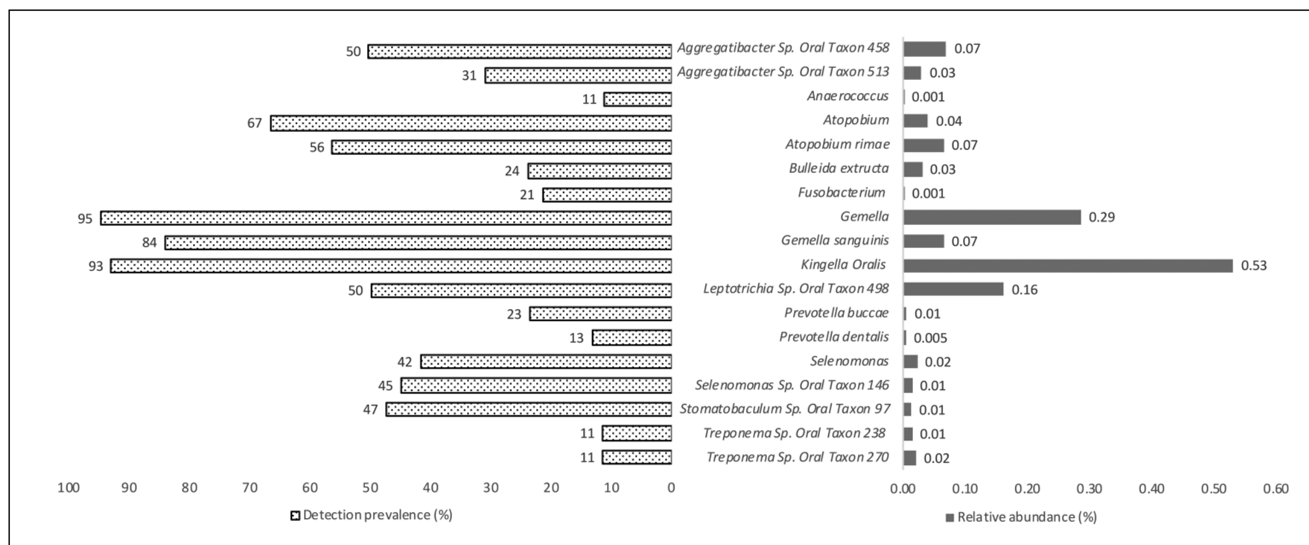

Figure 4. Detection prevalence and mean relative abundance values for top taxa associated with fasting glucose change. Five taxa names were shortened from their full names on the HOMINGS platform to simplify presentation: *Fusobacterium* = *Fusobacterium* genus probe 1; specifically recognizes *F. naviforme* and *F. nucleatum. Selenomonas* = *Selenomonas* genus probe 2; specifically recognizes *S. sputigena* and sp oral taxon 143. *Gemella* = *Gemella* genus probe; specifically recognizes *G. asaccharolytica, G. bergeri, G. cuniculi, G. haemolysans, G. morbillorum, G. palaticanis*, and *G. sanguinis. Atopobium* = *Atopobium* genus probe; specifically recognizes *A. fossor, A. minutum, A. parvulum, A. rimae*, and sp oral taxon 199. *Anaerococcus* = *Anaerococcus* genus probe 2; specifically recognizes *A. hydrogenalis, A. lactolyticus, A. octavius, A. prevotii, A. tetradius*, and *A. vaginalis*.

among the strongest predictors of longitudinal glucose change, outperforming age and BMI in this young, generally healthy population.

Our observations are consistent with the broader hypothesis that microbial communities along the digestive tract might be involved in the early natural history of diabetes development (Wang and Jia 2016). Previous studies reported notable differences in gut taxa between individuals with and without diabetes (Arumugam et al. 2011; Qin et al. 2012; Karlsson et al. 2013). Accordingly, Le Chatelier et al. (2013) reported that low gut bacterial richness correlated with metabolic parameters in a cross-sectional analysis. However, these prior findings were limited by use of cross-sectional study designs.

In this study, increased levels of *Leptotrichia* sp oral taxon 498 were related to less glucose change. Although we might have expected the opposite relationship because prior studies show *Leptotrichia* spp to be overly abundant in periodontal disease (Lourenco et al. 2014), other studies have found *Leptotrichia* spp to be more abundant among healthy controls versus patients with diabetes (Zhou et al. 2013) or with rheumatoid arthritis (Zhang et al. 2015), which is consistent with our current findings. Furthermore, a probe targeting levels of *Atopobium* genus was currently related to greater glucose change, and others similarly reported *Atopobium* spp to be overly abundant in rheumatoid arthritis (Zhang et al. 2015) and diabetes (Sato et al. 2014). The finding that a probe recognizing *Fusobacterium nucleatum* was associated with greater glucose changes is consistent with prior research linking *F. nucleatum* to gingival inflammation (Socransky and Haffajee 2005). More research is necessary to inform the biological plausibility of other top taxa observed to be associated with glucose change.

Notably, we did not observe established periodontal pathogens, such as *P. gingivalis, A. actinomycetemcomitans, T. forsythia*, or *T. denticola*, to be related to glucose change. This might be due to the fact that there was minimal periodontal disease present in sites from which subgingival plaque was sampled, and prior research suggests that these taxa are likely to demonstrate meaningful shifts in abundance at later stages of periodontitis (Socransky and Haffajee 2005). Accordingly, periodontitis was not associated with glucose change in this population. This is potentially due to the fact that there was limited severity or extent of periodontal disease in the full mouth in our study population. Consequently, the lack of gradient in clinical disease potentially limits the ability of clinical measures to predict glucose change.

The difference in glucose change of 4 mg/dL when comparing the third versus first tertile of the MDI is clinically meaningful. The Diabetes Prevention Program Research Group demonstrated that metformin reduced fasting glucose by ~5 mg/dL at 2 and 4 y (Knowler et al. 2002). A prior study among healthy participants reported a 12-mg/dL difference in FPG to be associated with an 82% increase in diabetes risk over 5 y (Tirosh et al. 2005). Our current results demonstrate that features of the oral microbiome predict a 4-mg/dL greater increase in glucose during 2 y of follow-up. Whether this pattern is linear over time is unknown.

Important limitations of our current approach exist. Presently, due to the resource-intensive nature of sampling, processing, and sequencing subgingival plaque samples, we have analyzed microbial data obtained from 1 subgingival plaque sample per participant, which misses or underrepresents potentially important low-abundance taxa and/or taxa

that are transient over time. However, this measurement error is likely to bias findings toward the null. Our use of 16S rRNA target gene sequencing, while providing a comprehensive assessment of the taxa, cannot address microbial community functional capacity, thereby limiting mechanistic inference. Due to known limitations of analyzing compositional 16S data, we cannot make inference about how changes in the absolute abundance of any specific taxa relates to our outcomes. Rather, our findings suggest that imbalance in microbial subcommunities are related to longitudinal glucose change and the precise nature of this imbalance (or "dysbiosis") requires further study.

It is possible that poorly measured or unmeasured/unknown confounders also obscured our results; however, the most prominent confounder in previous studies of periodontal disease and systemic outcomes has been smoking, and our multivariable models demonstrated that smoking adjustments had minimal impact on findings and the majority of participants in ORIGINS did not smoke. Diet is another potential confounder, although there are no data to our knowledge detailing the role of diet in shaping the subgingival microbiome; therefore, the importance of this limitation is unknown.

As prior research has shown that the diabetes phenotype can influence the oral microbiota (Longo et al. 2018; Sabharwal et al. 2019), our current results are based on a longitudinal study design among a younger cohort of participants free of diabetes mellitus, which is an important strength as it minimizes the potential for reverse causality or confounding to bias our findings.

We report oral microbiota profiles to be predictive of 2-y fasting glucose change among a sample of diabetes-free participants. These findings do not prove causality and require replication in other populations. Additional studies that can sample the oral microbiota more broadly and address oral microbial community function will be important for informing potential causal mechanisms. The current findings, if confirmed, suggest potential value for objectively measured microbiota-based risk scores in early screening and prevention of type 2 diabetes mellitus.

Author Contributions

R.T. Demmer, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; P. Trinh, A. González, contributed to data analysis and interpretation, critically revised the manuscript; M. Rosenbaum, M. Desvarieux, P.N. Papapanou, D.R. Jacobs Jr, contributed to design, critically revised the manuscript; G. Li, R. Knight, contributed to data interpretation, critically revised the manuscript; C. LeDuc, R. Leibel, B. Paster, P.C. Colombo, contributed to data acquisition, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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