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Subgingival Host-Microbial Interactions in Hyperglycemic Individuals

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Abstract

Type 2 diabetes mellitus (T2DM) is an established risk factor for periodontitis, yet its contribution to creating host-bacterial disequilibrium in the subgingival crevice is poorly understood. The present investigation aimed to quantify the impact of hyperglycemia on host-bacterial interactions in established periodontitis and to map shifts in these dynamics following mechanical nonsurgical therapy. Seventeen T2DM and 17 non-T2DM subjects with generalized severe chronic periodontitis were recruited along with 20 periodontally healthy individuals. Subjects with periodontitis were treated with scaling and root planing (SRP). Samples of subgingival biofilm and gingival crevicular fluid were collected at baseline and at 1-, 3-, and 6 mo postoperatively. Correlations were generated between 13.7 million 16S ribosomal DNA sequences and 8 immune mediators. Intermicrobial and host-microbial interactions were modeled using differential network analysis. Periodontal health was characterized by a sparse interbacterial and highly connected cytokine-bacterial network, while both normoglycemics and T2DM subjects with periodontitis demonstrated robust congeneric and intergeneric hubs but significantly fewer cytokine-bacterial connections. Following SRP, the cytokine-bacterial edges demonstrated a 2-fold increase 1 mo postoperatively and a 10-fold increase at 6 mo in normoglycemics. In hyperglycemics, there was a doubling at 1 mo but no further changes thereafter. These shifts accompanied an increasingly sparse interbacterial network. In normoglycemics, the nodes anchored by interleukin (IL)–4, IL-6, and IL-10 demonstrated greatest rewiring, while in hyperglycemics, IL-1β, IL-6, INF-γ, and IL-17 exhibited progressive rewiring. Thus, the present investigation points to a breakdown in host-bacterial mutualism in periodontitis, with interbacterial interactions rather than host-bacterial interactions primarily determining community assembly. Hyperglycemia further exacerbates this uncoupled mutualism. Our data also demonstrate that while nonsurgical therapy might not consistently alter microbial abundances or lower proinflammatory molecules, it "reboots" the interaction between the immunoinflammatory system and the newly colonizing microbiome, restoring a role for the immune system in determining bacterial colonization. However, this outcome is lower and delayed in hyperglycemics.

Keywords: periodontitis, microbiome, diabetes, interactome, cytokine, 16S

Introduction

Ever since bacteria were identified as the primary etiological agents of certain human diseases (e.g., cholera and typhoid), our view of infectious diseases has tended to be pathogen-centric (Dethlefsen et al. 2007). However, humans and the microbiome they host form a holobiont, and bacterial colonization of mucosal and integumentary surfaces is integral to human development (Warinner et al. 2014). Bacteria have coevolved with their human hosts for millions of years, participating in an intricately orchestrated interaction with the mammalian immune system (Barroso-Batista et al. 2015). Many polymicrobial diseases result from a breakdown of this host-bacterial mutualism rather than acquisition of pathogens, the so-called damage-response framework (Casadevall and Pirofski 2000). This is especially true of periodontal diseases. The subgingival sulcus hosts a polymicrobial community that is temporally stable in the absence of major environmental perturbations (Kumar et al. 2006) and exists in a state of dynamic equilibrium with the host immunoinflammatory system (Bartold and Van Dyke 2013). In this sense, it is a balanced ecosystem. As with all ecosystems, several factors can affect this balance, either by influencing the microbiome or host immune pathways or both. One such factor is hyperglycemia, a common manifestation of type 2 diabetes mellitus (T2DM).

Worldwide, 8.5% of individuals older than 18 y have T2DM. Periodontitis is considered the sixth complication of

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A supplemental appendix to this article is available online.

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diabetes (Löe 1993). While early investigations attributed this risk to the effects of hyperglycemia on immunoinflammatory pathways (Lalla and Papapanou 2011), recent investigations report that hyperglycemia has a similarly high impact on the subgingival microbiome (Casarin et al. 2013; Ganesan et al. 2017). Taken together, these studies point to an unraveled host-bacterial interactome in diabetics.

Interactions between the immunoinflammatory system and the bacterial ecosystem can be studied at many biomolecular levels: gene-gene, gene-protein, protein-RNA, protein-protein, and so on. In the present investigation, we aimed to study the host-microbiome interaction (interactome), using metataxonomics, to map bacterial recolonization following nonsurgical therapy, a well-validated set of pro- and anti-inflammatory cytokines to measure inflammatory burden, and graph theory to examine correlations between the 2 compartments.

Since correlation in a cross-sectional study does not imply causation, we used a longitudinal study design to establish a timeline of the perturbation. Since the logistics of examining individuals prior to and following the onset of both periodontitis and diabetes are extremely challenging, we mapped shifts in host-bacterial dynamics during resolution of periodontal disease.

Materials and Methods

Subject Selection and Groups

Study was approved by the Ethical Committee at The Paulista University (UNIP), Brazil (Protocol Number: 303.729) and conducted in accordance with approved guidelines. This study was performed in accordance with STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for human observational investigations. Seventeen T2DM and 17 non-T2DM patients diagnosed with chronic periodontitis were recruited, and informed consent was obtained. An additional group of 20 periodontally healthy, American Society of Anesthesiologists 1 (ASA1) individuals were recruited for cross-sectional sampling. Inclusion criteria were as follows: ≥15 natural teeth, clinical attachment loss (CAL) in >30% of sites, probing depth (PPD) ≥5 mm on ≥9 teeth with bleeding on probing, and PPD ≥7 mm on at least 2 of these teeth. T2DM was defined as glycated hemoglobin (HbA1c) concentration of >8 (American Diabetes Association 2015) for at least 5 y and non-T2DM as HbA1c <6. Exclusion criteria included pregnancy; lactation; cardiovascular, pulmonary, liver, or cerebral diseases; requirement for antibiotics prior to periodontal examination; antibiotic use within the previous 3 mo; long-term anti-inflammatory treatment; and periodontal therapy within 6 mo. Detailed methods are available as appendix material.

Clinical Examination

Periodontal condition was assessed by a calibrated examiner (intraclass correlation = 89% for PPD). Plaque index (PI; Ainamo and Bay 1975), bleeding score (Mühlemann and Son 1971), PPD, and CAL were recorded at 6 sites per tooth.

Sample Collection

Subgingival plaque was collected using paper points from 5 noncontiguous sites with PPD >5 mm and CAL >6 mm. Teeth presenting furcation lesions, endodontic pathology, and/or extensive crown destruction were excluded from sampling. Gingival crevicular fluid (GCF) was collected using filter paper strips (Periopaper; Oraflow). Fluid volume was measured with a calibrated device (Periotron 8000; Oraflow).

Periodontal Treatment

All individuals with periodontitis received scaling and root planing, extractions, provisional restorations, and oral hygiene instructions as needed, followed by monthly periodontal maintenance. Change in clinical parameters are shown in Appendix Table 1.

Inflammatory Analysis

Levels of interferon (IFN)–γ; interleukin (IL)–10, IL-17, IL-1β, IL-4, IL-6, and IL-8; and tumor necrosis factor (TNF)–α in GCF were determined using the High Sensitivity Human Cytokine-08plex (Millipore Corporation) at Luminex/MAGpix platform (Luminex Corporation).

Microbial Analysis

Bacterial DNA was isolated and sequenced as previously described (Kumar et al. 2011) on the HiSeq 2500 system (Illumina). Analyses were conducted using QIIME (Caporaso et al. 2010) and PhyloToAST (Dabdoub, Fellows, et al. 2016). Sequences are deposited in Sequence Read Archive under ID PRJNA598720.

Comparative Metataxonomics

Phylogenetic (UniFrac) and nonphylogenetic (Bray-Curtis, Jaccard) distances were used to estimate beta-diversity. Linear regression models were used to correct for sequencing depth (*phyloseq* in R). Principal coordinate analysis (PCoA) was performed on distance matrices and significance of clustering interrogated using Adonis with 999 permutations. DESeq2 was used to identify differential abundances (Love et al. 2014). *P* values were adjusted for multiple testing ($P < 0.05$, false discovery rate [FDR]–adjusted Wald test). A common core microbiome was identified when species were present in at least 80% of patients in each group using get_core_ids.py script (https://github.com/akshayparopkari/kadambari/blob/master/python/get_core_ids.py).

FDR-corrected significance and overlapping pairwise Spearman's ρ and Kendall τ correlations were used to create graph structures using Networkx. Gephi was used to visualize and label the network graphs (https://github.com/akshayparopkari/kadambari/blob/master/python/correlation.py). A correlation value of ≥0.75 and a *P* value <0.05 were used. PiCRUSt

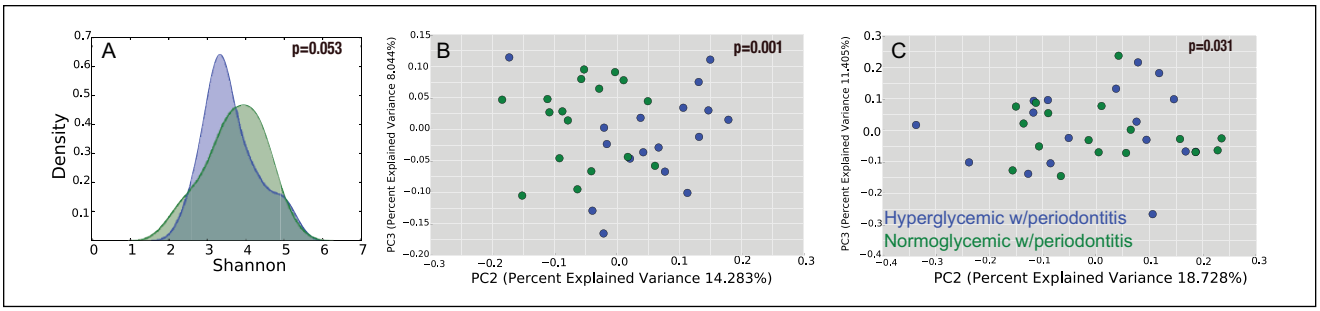

Figure 1. Alpha and beta-diversity characteristics of the subgingival microbiome of hyperglycemics and normoglycemics with periodontitis. (**A**) Density curves of alpha diversity (ACE). The peak indicates the median values for each group, and the x-axis shows the data range. Principal coordinate analysis (PCoA) plot of (**B**) unweighted and (**C**) weighted UniFrac distances. While hyperglycemics did not differ from normoglycemics in alpha diversity ($P > 0.05$, Kruskal-Wallis), hyperglycemics were colonized by bacteria belonging to different lineages as compared to normoglycemics ($P = 0.031$ and $P = 0.001$, Adonis test of weighted and unweighted UniFrac distances).

(Langille et al. 2013) was used to estimate the functions encoded by the species in each cluster.

Differential Network Analysis

Networks were inducted into a differential network analysis using the Dynet and Diffany applications in Cytoscape (Goenawan et al. 2016; Van Landeghem et al. 2016). The baseline network was the central reference to which the 1-, 3-, and 6-mo networks were compared. In addition, the 6-mo network was used as reference for comparison with periodontal health. A D-n score was computed for each node and used to measure the extent of "rewiring." Rewiring was defined as changes in the network neighbors as well as the changes in abundances of interactions (edge weights).

Results

Consistent with previous reports linking hyperglycemia to shifts in the oral microbiome (Casarin et al. 2013; Ganesan et al. 2017), we found that although species richness between hyper- and normoglycemic individuals with periodontitis was not different ($P = 0.067$, Wilcoxon test, Fig. 1A), hyperglycemics were colonized by bacteria belonging to different lineages ($P = 0.031$ and $P = 0.001$, Adonis test of weighted and unweighted UniFrac distances, Fig. 1B, C). Expanding on previous reports, we found that the disease-associated microbiome in both normoglycemics and hyperglycemics is highly idiosyncratic (only 21.6% of species were present in ≥80% of normoglycemics and 18.7% of species in ≥80% of hyperglycemics; Appendix Table 2). Species belonging to 17 genera representing 23% of the entire microbiome were significantly more abundant in normoglycemics, and species belonging to 7 genera accounting for 25% of the microbiome were more abundant in hyperglycemics (Appendix Table 2), indicating that these differences could not be simply attributable to fluctuations in the "rare biosphere" but are differences within the numerical dominant members of the community.

Since bacterial survival in an ecosystem is driven by nutritional and metabolic interactions, we combined network graph theory with predictive metagenomic analysis (PiCRUST) to examine co-occurrence patterns and identify important community members. As no framework exists for defining health, we began by interrogating interbacterial interrelationships in health. We found a sparse network in the healthy periodontal microbiome (Fig. 2A). However, periodontitis in nondiabetics was associated with a highly modular network with 179 nodes and 187 edges and 3 congeneric hubs (Fig. 2B), indicating that even though the disease is microbially heterogeneous, within each individual, important co-occurrence relationships exist between members of the same order. PiCRUST analysis revealed that fermentation, lipopolysaccharide biosynthesis, and type III secretion systems exhibited the greatest betweenness centrality and degree centrality in normoglycemic subjects, suggesting that the disease environment selects for species with nutritional and metabolic similarities. We found that hyperglycemia imposes a further selection drive, as evidenced by twice the amount of edges and 8 large intergeneric and congeneric modules in diabetics with periodontitis (Fig. 2C), anchored by a shared functionality for sulfate reduction, invasion and intracellular resistance, metal efflux pumps, and antibiotic resistance.

Interactions of advanced glycation end products with their receptors (AGE-RAGE interactions) transform macrophages into hyperactive cells that produce proinflammatory cytokines (Taylor et al. 2013). Therefore, we measured the concentrations of 8 well-described inflammatory immune mediators. We found lower levels of INF-γ ($P = 0.015$), IL-4 ($P < 0.001$), and IL-10 ($P = 0.001$) in diabetics when compared to normoglycemics (Appendix Table 2).

Analysis of cytokine-bacterial interactions revealed distinct network topologies in the 3 groups (Fig. 2D–F). The periodontally healthy network was characterized by 234 statistically significant polygeneric-cytokine connections ($P < 0.05$, Spearman's ρ). The network of normoglycemics with periodontitis contained 100 edges, of which 58% had negative correlations, while the hyperglycemic periodontitis interactome demonstrated 2.5-fold lower correlations and fewer negative correlations. Moreover, in hyperglycemics, 40% of the hubs were anchored by species belonging to *Treponema,*

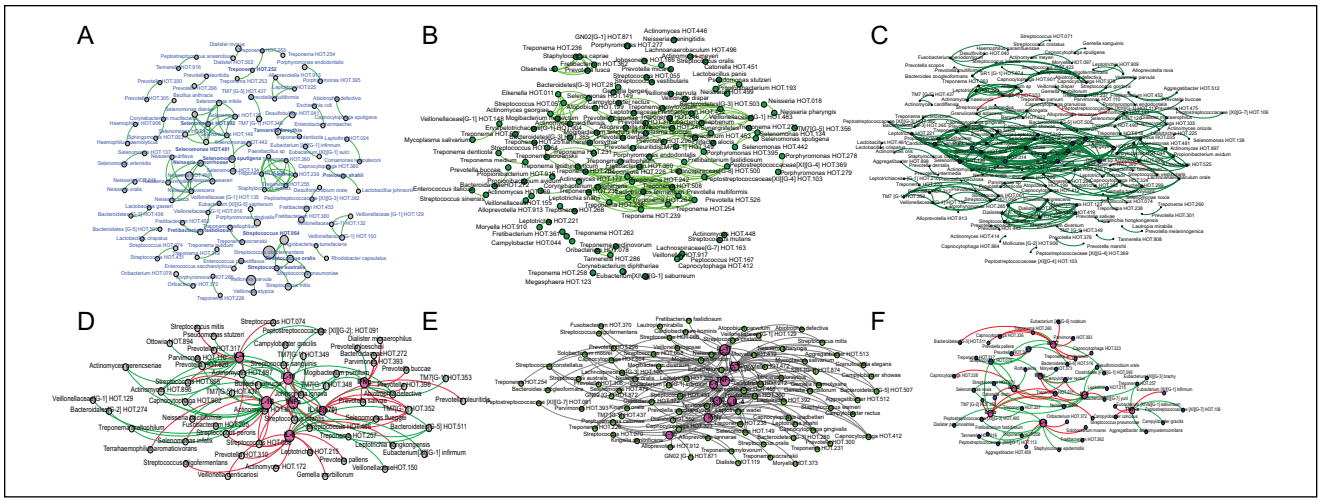

Figure 2. Interbacterial and host-bacterial interactions in hyperglycemia and health. (**A–C**) Co-occurrence networks between bacterial species in periodontally healthy normoglycemics, normoglycemics with periodontitis, and hyperglycemics with periodontitis, respectively. (**D–F**) Network graphs between bacterial species and cytokines in each group. Each network graph contains nodes (circles) and edges (lines). Nodes represent cytokines and/or bacterial species, and edges represent Spearman's ρ. Edges are colored green for positive correlation and red for negative correlation. Only significant correlations ($P < 0.05$, *t* test) with a coefficient of at least 0.80 are shown. This figure is also available in a larger size in the Appendix.

Streptococcus, Campylobacter, and *Capnocytophaga* and clustered on INF-γ, IL-4, and IL-10.

Together, the cross-sectional data suggest that the health-compatible ecosystem is governed more by host-bacterial interactions rather than interbacterial interactions. Disease, on the other hand, presented as an uncoupled host-bacterial interactome, with bacteria that share similar nutritional and metabolic requirements emerging as the main drivers of community architecture. This was exacerbated when hyperglycemia was superimposed on the disease-associated dysbiosis.

To capture the dynamics of host-bacterial interactions, we performed nonsurgical periodontal therapy on these individuals and followed them over a 6-mo period. Normoglycemics demonstrated a continuous and significant increase in clinical attachment levels as well as a decrease in probing pocket depths ($P < 0.0001$, Dunn's method for joint ranking with control), but differences in gingival recession were not significant. On the other hand, hyperglycemics demonstrated a continuous decrease in probing depths and increased recession at 6 mo compared to baseline, with no significant change in attachment levels (Appendix Table 1).

Normoglycemics did not demonstrate significant differences in community membership or structure following nonsurgical periodontal therapy ($P > 0.05$, Adonis test of weighted and unweighted UniFrac distances, Fig. 3A–F). By contrast, hyperglycemics demonstrated a significant clustering away from baseline at 1 and 6 mo following therapy, indicating that recolonization occurred by bacteria belonging to different lineages, as well as in differing abundances (Fig. 3G–L). Not surprisingly, the microbial profiles of normoglycemics and hyperglycemics differed significantly at all time points (Fig. 3M–R). Alpha diversity was not affected by treatment in both hyper- and normoglycemics (Fig. 4A, B); however, significant differences were noted in beta-diversity between the 2 groups (Fig. 4C, D). Following periodontal therapy, several species recolonized at levels significantly lower than baseline in diabetics. Together, these species accounted for 32%, 31%, and 37% of the microbiome at 1, 3, and 6 mo, respectively. In normoglycemics, recolonization following periodontal therapy resulted in lower levels of several rare taxa (accounting for 3%, 7%, and 5% of the microbiota at 1, 3, and 6 mo, respectively) and higher levels of several numerically dominant taxa (accounting for 17%, 19%, and 16% of the microbiota, respectively).

Diabetics responded to periodontal therapy with a decrease in levels of IL-1β and increases in IL-4, IL-10, and INF-γ at 6 mo, while normoglycemic patients demonstrated lower levels of IL-1β at 6 mo (Appendix Table 1, $P < 0.05$, Dunn's method for joint ranking with control). Importantly, following periodontal therapy, no significant differences could be detected between normoglycemics and hyperglycemics in any cytokine at any time point.

We then examined the interaction dynamics between the shifting microbiome and immune mediators using network graph theory. In both normo- and hyperglycemics, periodontal therapy served to dramatically alter network topology (Appendix Fig. 1), progressively increasing the number of host-microbial interactions (as measured by the number of network connections or "edges" between cytokines and bacteria at each postoperative visit when compared to baseline) as well as "rewiring" several interactions. At baseline, more interbacterial than cytokine-microbial connections were evident in both groups (187 interbacterial edges vs. 91 cytokine-bacterial in normoglycemics and 300 vs. 68 in diabetics, Fig. 2). Following periodontal therapy, differential network analysis of normoglycemics demonstrated a doubling in cytokine-bacterial edges at 1 mo and a 10-fold increase at 6 mo (Appendix Fig. 1A–C). In hyperglycemics, there was a doubling at 1 mo but no further

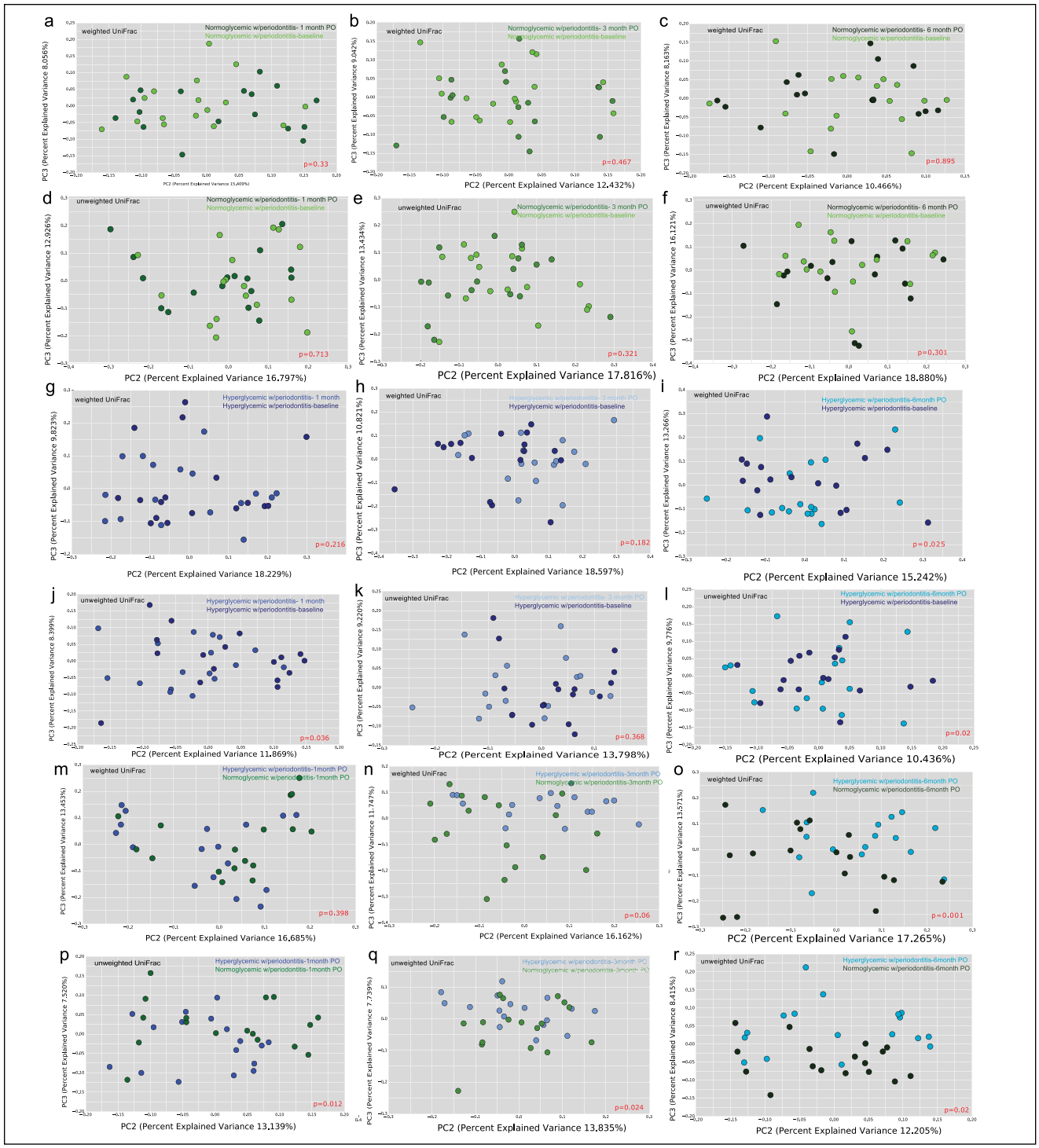

Figure 3. Effect of nonsurgical periodontal therapy on altering community membership and structure in normoglycemics and hyperglycemics. Principal coordinate analysis (PCoA) plot of unweighted and weighted UniFrac distances of the microbiomes at baseline and 1, 3, and 6 mo postoperatively is shown. Normoglycemics did not demonstrate significant differences in community membership or structure following nonsurgical periodontal therapy ($P > 0.05$, Adonis test of weighted and unweighted UniFrac distances, **A–F**). Hyperglycemics demonstrated a significant clustering away from baseline at 1 and 6 mo following therapy, indicating that recolonization occurred by bacteria belonging to different lineages, as well as in differing abundances, creating a significantly different community structure (**G–L**). The microbial profiles of normoglycemics and hyperglycemics differed significantly at all time points (**M–R**).

changes thereafter (Appendix Fig. 1D, E). Interestingly, these shifts accompanied a decrease in the levels of predominant taxa (Fig. 4) and an increasing sparseness in the interbacterial network. In normoglycemics, nodes anchored by IL-4 (D-n score of 0.23, 0.57, and 0.62 at 1, 3, and 6 mo postoperatively, respectively), IL-6 (D-n score of 0.18, 0.73, and 0.58), and

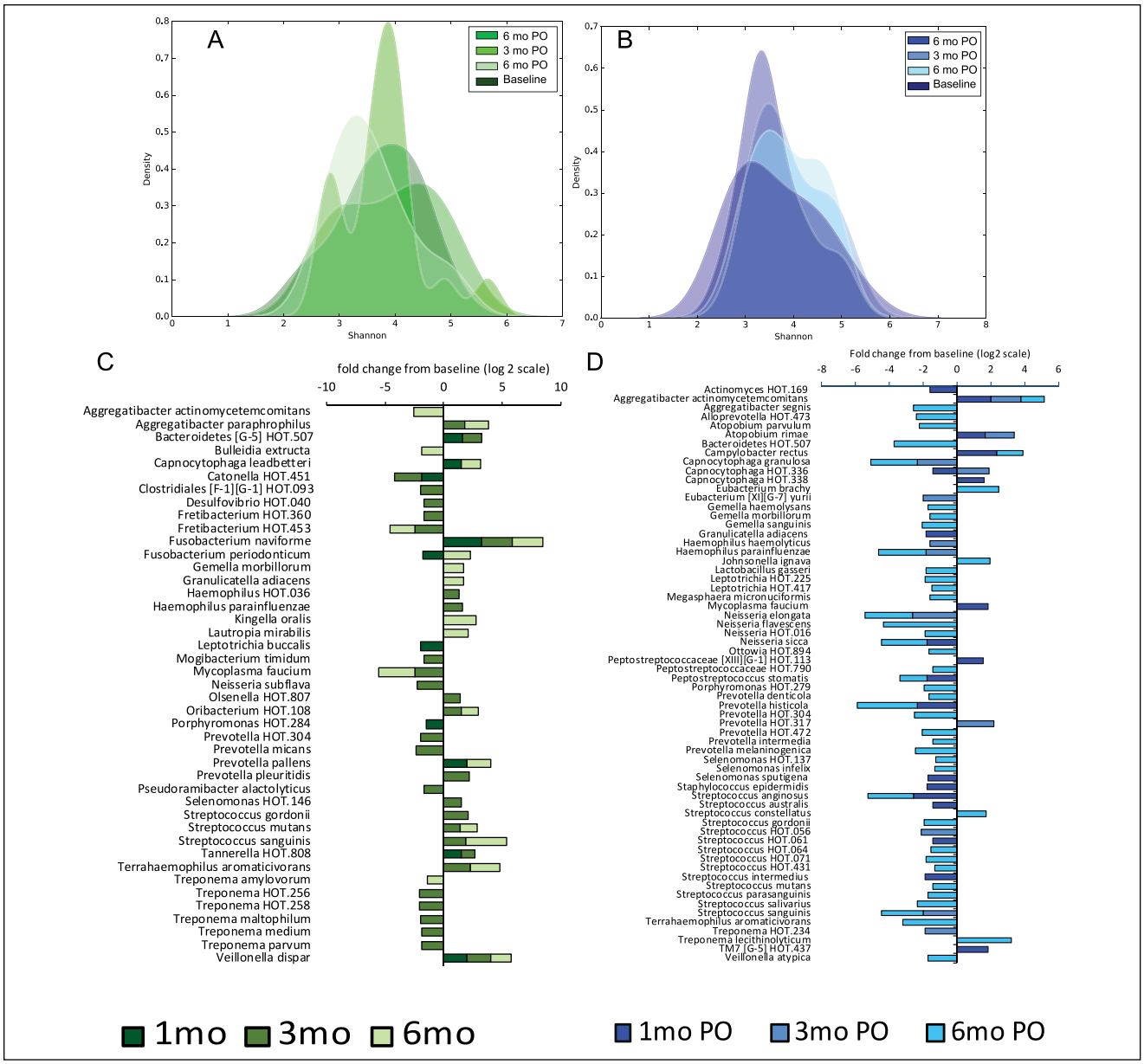

Figure 4. Shifts in microbial community structure and membership following nonsurgical periodontal therapy in normo- and hyperglycemics. (**A**, **B**) Density curves of alpha-diversity (ACE). The peak indicates the median values for each group, and the x-axis shows the data range. (**C**, **D**) Species that increased or decreased in abundances following therapy in normoglycemic and hyperglycemic nonsmokers with periodontitis ($P < 0.05$, FDR-adjusted Wald Test, DE-Seq2). Species are arranged by phylogeny and the fold differences (log2 scale) are shown.

IL-10 (D-n score of 0.09, 0.57, and 0.63) demonstrated progressively greatest rewiring, while in hyperglycemics, IL-1β (D-n score of 0.09, 0.31, and 0.58), IL-6 (D-n score of 0.16, 0.43, and 0.51), INF-γ (D-n score of 0.21, 0.43, and 0.48), and IL-17 (D-n score of 0.12, 0.23, and 0.28) exhibited progressively increasing rewiring, with the addition of new species to the hubs during each observation period. Host-bacterial interactions did not return to those seen in periodontally healthy individuals in either hyperglycemics or normoglycemics. Instead, shift from baseline to 1 mo and the absence of change thereafter suggest that an "alternate stable equilibrium" state was established early in diabetics, while the dynamics still continued to evolve at the end of 6 mo in normoglycemics.

In summary, our data point to an uncoupled host-bacterial interactome in disease and that hyperglycemia poses an additional stressor that further exacerbates this disequilibrium, a finding that is consistent with the damage-response framework of disease. Our data also demonstrate that while periodontal therapy may not consistently alter microbial community structure or significantly lower proinflammatory molecules, it "reboots" the interaction between the host immunoinflammatory system and the newly colonizing microbiome. The

ecosystem does not return to baseline even 6 mo following therapy; however, the data suggest that a state of altered equilibrium might be attained. Again, this rebooting or rewiring appears to be affected by a state of hyperglycemia.

Discussion

For over a century, the central dogma of periodontal disease has been that tissue destruction results from a florid proinflammatory response to a virulent microbiome. Another dogma in microbiology has been that virulence is an intrinsic property of certain organisms and that it bestows upon them the capacity to cause disease. However, protein and gene-based open-ended approaches have enabled the identification of interbacterial as well as host-bacterial interactions on a global scale. This has shifted the paradigm toward viewing disease through a damage-response framework, in which virulence is defined as the "outcome of a host-pathogen interaction" rather than a microbial- or host-driven entity (Casadevall and Pirofski 2003). In line with this paradigm, we combined bacterial abundances and cytokine levels with network graph theory to map shifts in host-bacterial interaction.

Network graph theory is a rapidly evolving analytical framework that was originally developed to study social interactions (Pavlopoulos et al. 2011). It has recently been adapted to study molecular interactions in cells, as well as dynamics within ecosystems (Yoon et al. 2006; Barberan et al. 2012; Greenblum et al. 2012). Within this framework, several topological features can be used to infer community characteristics (Berry and Widder 2014). Network modularity measures the number of connections between coaggregated nodes; the number and nature of submodules provide important biological insights on ecosystem structure and regulation (Charitou et al. 2016). Centrality is a measure of the importance of the node to the alignment of the network. In biological terms, nodes that exhibit high betweenness and edge centrality control the flow of resources to the system and are likely anchors of the community (Berry and Widder 2014). However, analysis of single networks provides only a static perspective of a highly dynamic system, and therefore, we used differential network analysis to interrogate shifts in the cytokine-bacterial interaction following periodontal therapy (Lichtblau et al. 2017). This process uses pairwise subtraction to filter out ubiquitous networks that are common to both states. In doing so, it not only extracts interactions that are unique to each state but also captures those that are too subtle to be visible in a static network.

No network anchors were identifiable in periodontally healthy controls (since betweenness centrality was homogeneous between species), indicating that this is an ecological niche in equilibrium (Xiao et al. 2017). However, in both normoglycemics and hyperglycemics with periodontitis, small groups of anaerobic bacteria created tightly woven hubs, indicating that they play important roles in controlling the flow of resources in disease. Analysis of predicted functions indicated that fermentation and sulfate reduction might be drivers of these networks in normoglycemics and hyperglycemics, respectively, since they exhibited the greatest betweenness centrality (reflecting the amount of control that these nodes exert over the interactions of other nodes in the network) and the highest degree centrality (an indication that they are the central focal point of the structure) (Yoon et al. 2006). We have previously demonstrated that genes encoding for fermentation, lipopolysaccharide biosynthesis, and type III secretion are enriched in periodontitis (Dabdoub, Ganesan, et al. 2016). The present findings corroborate this earlier observation. Previous investigations have also reported that diabetes creates a dysbiotic microbial community in which sulfate reduction is a predominant shared functionality (Qin et al. 2012) and that increased invasion potential of this dysbiotic community serves to increase glycemic levels (Chassaing et al. 2017). Thus, there appears to be a biological basis for the network hubs observed here.

In both hyperglycemics and normoglycemics with periodontitis, interbacterial networks demonstrated greater modularity and centrality when compared to host-bacterial networks. While one possible explanation is that fewer host mediators than bacterial species were analyzed, we found the exact opposite pattern in healthy controls under the same parameters, suggesting that this is not an artifact. However, further studies using comprehensive proteomics are urgently needed to elucidate the entire spectrum of these interactions.

Within 1 mo of periodontal therapy, the host-bacterial network demonstrated an exponential increase in connections and hubs, even as the beta-diversity decreased significantly. Moreover, the cytokine IL-10 emerged as a highly rewired hub, with a 400-fold increase in connections in normoglycemics and a 20-fold increase in hyperglycemics. Several studies have proposed a central role for IL-10 in controlling periodontal disease progression (Gemmell et al. 2002). While levels of IL-10 did not significantly increase in normoglycemics following therapy, they exhibited the greatest interactions with the newly colonizing bacteria, reinforcing our belief that merely quantifying cytokine concentrations may be too simplistic an approach to identifying biomarkers. Our data suggest that nonsurgical therapy restores clinical health by reinstating a dynamic equilibrium between the human immunoinflammatory system and the resident microbiota, not merely by eliminating "pathogens" or reducing cytokines.

In summary, within the limitations of a small sample size, targeted cytokine analysis, and metataxonomic characterization of the microbiome, the present investigation suggests that a breakdown in host-bacterial mutualism occurs in periodontal disease, with interbacterial interactions rather than host-bacterial interactions being primary determinants of community composition. Hyperglycemia further exacerbates this uncoupled mutualism, and this effect is greater than its effects on microbial composition or tissue inflammation. Nonsurgical periodontal therapy serves to reset the interactome, restoring a role for the host immune system in determining bacterial colonization; however, the system either resets to a state of alternate equilibrium or continues to evolve in its metastable state. Further studies using more comprehensive -omics approaches are needed to unravel

the specifics of these interactions and their implications for resilience and resistance to future disease.

Author Contributions

P.S. Kumar, M.F. Monteiro, R.C.V. Casarin, contributed to conception, design, and data acquisition, drafted and critically revised the manuscript; S.M. Dabdoub, contributed to data acquisition, drafted and critically revised the manuscript; G.L. Miranda, M.Z. Casati, F.R. Cirano, S.P. Pimentel, contributed to design, and data acquisition, critically revised the manuscript; F.V. Ribeiro, contributed to conception, and 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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