Association between metabolic syndrome and periodontitis-the role of lipids, inflammatory cytokines, altered host response, and the microbiome

Short tile: Metabolic syndrome and periodontitis

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Abstract:

Periodontitis has been associated with many systemic diseases and conditions, including metabolic syndrome. Metabolic syndrome is a cluster of conditions that occur concomitantly and together they increase the risk for cardiovascular disease and double the risk for type 2 diabetes. In this review, we will focus on the association between metabolic syndrome and periodontitis; however, we will also include information on diabetes mellitus and cardiovascular disease, since these two conditions are significantly intertwined with metabolic syndrome. In regards to periodontitis and metabolic syndrome, to date, the vast majority of studies point to an association between these two conditions and also demonstrate that periodontitis can contribute to the development of, or can worsen, metabolic syndrome. Evaluating the effect of metabolic syndrome on the salivary microbiome, data presented herein support the hypothesis

that the salivary bacterial profile is altered in metabolic syndrome patients when compared to healthy patients. Considering periodontitis and these three conditions, the vast majority of human and animal studies point to an association between periodontitis and metabolic syndrome, diabetes and cardiovascular disease. Moreover, there is evidence to suggest that metabolic syndrome and diabetes can alter the oral microbiome. However, more studies are needed to fully understand the influence these conditions have on each other.

Introduction

Periodontitis is a "chronic inflammatory disease associated with dysbiotic plaque biofilms and characterized by a progressive destruction of the tooth supporting apparatus" [1]. Periodontitis affects 42.2% of US the population over the age of 30 and 59.8% over the age of 65 [2]. According to the World Health Organization, periodontitis is the major cause of tooth loss in adults [3]. Periodontitis pathogenesis is multifactorial with environmental, microbial, and host involvement affecting disease outcomes. Many systemic conditions have been associated with periodontitis, including diabetes mellitus, cardiovascular disease, and metabolic syndrome [4-11].

Metabolic syndrome is a cluster of conditions that occur concomitantly and together they increase the risk for cardiovascular disease and double the risk for type 2 diabetes [12-15]. Metabolic syndrome affects approximately 34% of the US population [16] and 10% of US adolescents [17]. The prevalence of metabolic syndrome also increases with age and varies with ethnicity and gender [18]. Several definitions of metabolic syndrome exist and differ slightly depending on the issuing agency. The most commonly utilized definition is provided by the National Cholesterol Education Program Adult Treatment Panel III. This definition requires that the individual have at least three of the following risk factors: 1) increased abdominal

circumference, 2) low plasma levels of HDL cholesterol, 3) increased values for plasma triglycerides, 4) elevated blood pressure, and 5) elevated glucose levels [19]. Pre-diabetes is also accepted as part of metabolic syndrome because it is associated with insulin resistance and is highly predictive of new-onset type 2 diabetes [20].

The predominant underlying risk factors for metabolic syndrome appear to be abdominal obesity and insulin resistance. Other associated conditions are physical inactivity, aging, and hormonal imbalance [21]. Among the risk factors, visceral adiposity appears to be a primary trigger for most of the pathways involved in metabolic syndrome [22]. The exact mechanisms behind this systemic response remain unclear, but there is evidence to suggest that the inflammatory state caused by metabolic syndrome is associated with endothelial dysfunction, which might contribute to the increased risk for cardiovascular disease and type 2 diabetes [23-25].

Herein, we will focus on the association between metabolic syndrome and periodontitis; however, we will also include information on diabetes and cardiovascular disease since these two conditions are significantly intertwined with metabolic syndrome.

Association between metabolic syndrome and periodontitis

Much interest has been focused on the connection between periodontitis and metabolic syndrome because both conditions are associated with systemic inflammation and insulin resistance [26, 27] and could potentially influence one another.

To date, there have been several longitudinal and cross-sectional studies as well as a few meta-analyses evaluating the relationship between these two conditions. The vast majority of the data points to an association between metabolic syndrome and periodontitis [10, 11, 28-54] (Table 1). The three meta-analyses, included here, were performed utilizing different parameters, but each of them found an association between metabolic syndrome and periodontitis, with an odds ratio ranging from 1.38 to 1.99 [31, 32, 55]. The meta-analysis by Gobin et al.[55] included 39 studies

and demonstrated an association between periodontitis and metabolic syndrome with a crude odds ratio of 1.99 (95% CI: 1.75-2.25) and an adjusted odds ratio of 1.46 (95% CI: 1.31-1.61). This study also performed a subgroup analysis of different countries. The pooled odds ratio was 1.68 (95% CI:1.41-2) for Japan; 1.75 (95% CI: 1.31-2.34) for the United States, 1.81 (95% CI: 1.35-2.42) for Korea, and 2.29 (95% CI: 1.53-3.41) for China [55]. The meta-analysis conducted by Daudt et al. [32], which included 26 manuscripts with radiographic and clinical examination, also found an association between metabolic syndrome and periodontitis with an odds ratio of 1.38 (95% CI: 1.26-1.51). The authors went on to suggest that patients with metabolic syndrome are 38% more likely to have periodontitis [32]. The systematic review/meta-analysis by Nibali et al. included 20 manuscripts (one longitudinal study) and a total of 36,337 subjects. It concluded that there is a positive association between metabolic syndrome and periodontitis with an odds ratio of 1.71 (95% CI: 1.42-2.03)[31]. A critical review by Watanabe and Cho also concluded that there is a positive association between metabolic syndrome and periodontitis [56]. In addition, several animal studies utilizing different periodontitis models demonstrated that rodents with metabolic syndrome or obesity, due to being fed a high-fat diet, also exhibited exacerbated periodontal bone loss [57-59].

Evaluating the role of metabolic syndrome in periodontitis development and progression, Kaye et al. and Iwasaki et al. performed longitudinal studies and concluded that metabolic syndrome increases the risk of development and progression of periodontitis [35, 42] (Table 1). In fact, Iwasaki et al. concluded that patients with metabolic syndrome were 2.6 times more likely to develop periodontitis. Likewise, the more components of metabolic syndrome an individual exhibited, the more prevalent and extensive the presentation of the periodontitis [35, 38].

Up to now, very little is known about the potential gender predilection in the association between metabolic syndrome and periodontitis and definitive conclusions cannot be made. Nonetheless, among the three relevant studies published, two concluded that there is a stronger association between metabolic syndrome and periodontitis in women [30, 60], while one did not find a relationship between gender, metabolic syndrome, and periodontitis. The authors of the

latter study did comment that their small sample size could have swayed their results [47]. As it relates to age, Minagawa et al. suggested that metabolic syndrome and periodontitis are linked in the elderly population [9], which is consistent with the prevalence of metabolic syndrome and periodontitis increasing with age [61, 62].

Although the vast majority of studies concluded that there is an association between metabolic syndrome and periodontitis, several studies found weak or no associations between these two conditions [34, 45, 51, 63-65] (Table 1). It is worth noting that most of these studies were cross-sectional in nature, with the longitudinal study spanning a period of one year; the study by Nascimento et al. was performed on a relatively young population (31 years of age) [34], whose age bracket has a relatively low prevalence of periodontitis and metabolic syndrome. Additionally, in a three-year longitudinal study, Kobayashi et al. concluded that toothbrushing frequency is inversely related to the incidence of metabolic syndrome [66].

Influence of periodontitis on metabolic syndrome

Studies have suggested that periodontitis can affect systemic conditions [67, 68]. For example, periodontitis elevates the levels of several inflammatory mediators, such as C-reactive protein and interleukin-6 [69, 70]. Moreover, periodontal treatment can decrease circulating levels of inflammatory mediators [71, 72]. Given this information, researchers have sought to evaluate periodontitis' potential to affect metabolic syndrome.

The majority of studies have concluded that periodontitis may contribute to the development or exacerbation of metabolic syndrome [10, 40, 73] (Table 2). Nesbitt et al. performed a cross-sectional study in 190 individuals evaluating periodontitis based on periodontal bone loss and they concluded that periodontitis may contribute to the development of metabolic syndrome [10]. Morita et al. conducted a longitudinal study on 1,023 adults and concluded that deeper periodontal pockets are associated with a positive conversion of one or more metabolic components during a four-year period (odds ratio 1.6; 95% CI: 1.1-2.2) [40]. Moreover, Lopez et

al. suggested that reduction of periodontal inflammation reduces C-reactive protein levels in patients with metabolic syndrome [73].

Periodontal microbiome changes in patients with metabolic syndrome

In recent years, much interest has been given to the microbiome. Metabolic diseases alter the gut microbiome (reviewed by [74]), and it is well known that the oral microbiome varies significantly between healthy and periodontitis patients [75]. Additionally, alterations in the gut microbiome have been linked to obesity and metabolic syndrome [76]. Furthermore, obesity can alter the oral microbiome of individuals with type 2 diabetes [77] and it can reduce microbial diversity in the distal gut [78, 79]. More specifically, individuals with lower microbiome diversity have marked overall adiposity, insulin resistance, and dyslipidemia compared to those with high bacterial richness [79]. Tam et al. evaluated 17 individuals with severe periodontitis and concluded that oral microbial composition varies significantly between obese (BMI $\geq$30) and non-obese individuals with type 2 diabetes. This study implied that obesity was associated with a reduction in species diversity in the oral cavity [77].

We also hypothesized that the oral microbiome is altered in metabolic syndrome patients when compared to healthy patients. To test this hypothesis, we performed microbial 16S rDNA profiling of unstimulated saliva from healthy individuals and individuals with metabolic syndrome, with and without periodontitis. The primary objective was to make comparisons between two groups, categorized as metabolic syndrome and systemically healthy (*Two-group analysis: Healthy v. metabolic syndrome)*. The secondary objective was to stratify the metabolic syndrome patients by periodontal health status (*Four-group analysis: Healthy v. Healthy* v. metabolic syndrome HP v. metabolic syndrome periodontitis)*. *N=3 healthy subjects presented with an elevated systolic and/or diastolic blood pressure reading (stage I hypertension values, according to the American Heart Association) and thus were stratified into a Healthy* group.

More specifically, this study was comprised of a total of 22 subjects (12 metabolic syndrome and 10 healthy individuals). The 12 metabolic syndrome patients also had diabetes. Metabolic syndrome subjects were further stratified by periodontal status. Downstream analyses included alpha diversity, Linear discriminant analysis Effect Size (LEfSe), and beta diversity using Principal Coordinate Analysis. Kruskal-Wallis and Linear Discriminant Analysis were used for evaluating statistical significance between healthy and metabolic syndrome microbial communities.

Results

The saliva samples yielded 2,270,978 assigned reads, including 1,155,315 single species, 1,088,960 multispecies, and 24,866 novel species reads. At the species level, 573 total species were represented as operational taxonomic units: 330 single species, 145 multispecies, and 98 novel species. Detailed data can be found on the following site: http://www.homd.org/ftp/publication_data/20170412/qiime_results/cd_mc10/taxa_plots/taxa_summary_plots/bar_charts.html

Evaluating the alpha diversity, the rarefaction plot for the two-group analysis of metabolic syndrome versus Healthy subjects (Figure 1A) and for the four-group analysis of Healthy versus Healthy* versus metabolic syndrome HP versus metabolic syndrome periodontitis using observed operational taxonomic units (Figure 1B) exhibits curves plateauing with the increased sequences sampled, indicative of adequate alpha diversity in terms of species richness. It is clear that the healthy salivary microbiome is more diverse when compared to that found in the metabolic syndrome subjects.

Evaluating beta diversity, three-dimensional Principal Coordinate Analysis plots were generated (Figure 2). The two-group analysis (Figure 2A) suggests that subjects within each group show more relatedness to one another than to a subject of the opposite group. A less organized pattern is demonstrated in the four-group analysis plot and no conclusive result regarding beta diversity can be observed from this plot (Figure 2B).

We next evaluated the phylogenetic relationship of taxa among the groups. A cladogram was generated representing the phylogenetic relationship of taxa associated with the Healthy and metabolic syndrome groups (Figure 3). At the species level, taxa significantly associated with Healthy patients showed relatedness. The cladogram in Figure 3B represents the taxonomic relationship between taxa significantly associated with the four groups analyzed. Phylogenetically-related taxa at the species level are significantly associated with Health (specifically, the taxa stemming from the class Betaproteobacteria from the phylum *Proteobacteria*), Healthy* (including taxa stemming from the phylum SR1, class *Flavobacteria*, and order *Corynebacteriales*), and metabolic syndrome with periodontitis (including taxa stemming from the phylum *Tenericutes* and phylum *Spirochaete*s, as well as the order *Coriobacteriales*).

In summary, the 16S rDNA sequence analyses support the hypothesis that the salivary bacterial profile is altered in metabolic syndrome patients when compared to healthy patients. Despite a small sample size, the healthy group was more diverse than the metabolic syndrome group (Figure 1 and 2). When further stratified, metabolic syndrome HP and metabolic syndrome periodontitis subject groups showed comparatively different microbial profiles from one another and from Healthy subjects (Figure 3). Additionally, the metabolic syndrome periodontitis group showed a large effect size difference and greater abundance of two of the three classic periodontal "red complex" pathogens [80], namely *Tannerella forsythia,* the phylum Spirochaetes, and genus *Treponema.* However, a significant effect size difference was not detected between the groups for *Porphyromonas gingivalis.* Based on our study, additional research with an increased subject population is warranted to further advance these novel findings.

Lessons from animal models: role of dyslipidemia

To date, a few *in vivo* and *in vitro* studies have highlighted the role of dyslipidemia (high-fat diet) in the compounding effect of metabolic syndrome on periodontitis [57-59, 81] (Table 3) and several studies have evaluated the role of impaired glucose in periodontitis; the latter will be discussed in the diabetes section of this manuscript.

Amar et al. demonstrated that mice fed a high-fat diet, but not presenting with diabetes, had 40% more periodontal bone loss and higher titers of *P. gingivalis* compared to the control mice in a *P. gingivalis* bacterial colonization model (silk ligature + *P. gingivalis* oral inoculation). Although this study did not evaluate metabolic syndrome markers, these mice were obese and the study utilized a well-known metabolic syndrome model [57]. Subsequently, utilizing the same metabolic syndrome model, Li et al. demonstrated that mice fed a high-fat diet developed metabolic syndrome, as determined by obesity, hyperinsulinemia, insulin-resistance, and dyslipidemia. In this study, metabolic syndrome led to a significant increase in osteoclastogenesis and periodontal bone loss. Moreover, lipopolysaccharide-induced-periodontitis exacerbated inflammatory cytokine expression (interleukin-6, MCP-1, RANK-L, and MCSF), osteoclastogenesis, and periodontal bone loss [59]. *In vitro* studies utilizing osteoblasts derived from obese mice (New Zealand Obese) demonstrated a decrease in cell proliferation and an increase in osteoblast apoptosis after *P. gingivalis* exposure compared to control mice [82]. Additionally, obese rats (Zucker) with metabolic syndrome had a statistically significant increase in *Aggregatibacter actinomycetemcomitans*-lipopolysaccharide-induced periodontal bone loss, compared to the non-obese, non-metabolic syndrome group. Moreover, statin, a cholesterol-lowering drug often prescribed to individuals with metabolic syndrome [83], alleviated periodontal bone loss in both groups, also pointing to dyslipidemia as a potential exacerbator of periodontal inflammation [84].

To further establish the role of lipids in periodontitis, Li et al. demonstrated that fatty acids (e.g. palmitic acid) amplified the lipopolysaccharide-mediated expression of markers involved in periodontitis, such as interleukin1-α, interleukin1-β, CXCL10, CD86, CSF2, MCP1, TLR2, TNFα, and CD14 *in vitro* [59]. In addition, *in vitro* studies performed in macrophages showed a statistically significant upregulation of CD36, a major fatty acid receptor, upon treatment with

lipopolysaccharide plus palmitate in comparison to those treated with lipopolysaccharide or palmitate alone [81]. Periodontitis and metabolic syndrome, independently increased CD36 levels significantly, and when metabolic syndrome and periodontitis were developed concurrently, there was an additive effect. CD36 expression in periodontal tissues was also positively correlated with osteoclastogenesis.

In summary, human studies and animal models demonstrate an association between metabolic syndrome and periodontitis and metabolic syndrome can alter the oral microbiome and potentiate the deleterious effects of periodontitis.

Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Approximately 415 million people in the world live with diabetes and this number is expected to increase to 642 million by 2040 [85]. In the United States, diabetes is present in 13% of adults. diabetes increases with age, affecting 26.8% of individuals 65 or older [86]. Of all diabetes cases, type 2 diabetes accounts for roughly 90–95% of those with diabetes and encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency [87]. Type 2 diabetes affects more than 380 million people worldwide, representing 8.8% of individuals between the ages of 20-79 [88].

The chronic hyperglycemia caused by type 2 diabetes is associated with long-term damage and disabling and life-threatening health complications, such as cardiovascular disease, neuropathy, and nephropathy [89-92]. Periodontitis is highly likely to develop in individuals with diabetes and constitutes the sixth most frequent complication of diabetes [93]. The reverse is also true, as individuals with periodontitis are more likely to develop diabetes, thus establishing a “two-way” relationship between the two conditions [94, 95]. Since much is known about the two-way relationship between these two conditions, this review will emphasize the role of diabetes on the periodontal microbiome.

Role of type 2 diabetes in periodontitis

The association between diabetes and periodontitis has long been established, with most studies showing that poorly controlled diabetes affects periodontitis development and progression [96-116]. The odds ratio for diabetes patients having periodontitis varies; Emrich et al. reported a 2.81 odds ratio and Tsai et al. reported that the odds ratio in individuals with better controlled diabetes is 1.56 [100, 105]. Nelson et al., having evaluated subjects with type 2 diabetes, concluded that the rate of periodontitis is 2.6 times higher in subjects presenting with type 2 diabetes when compared to those without it [112]. Along those lines, several studies have shown that uncontrolled type 2 diabetes is associated with periodontitis progression; however, controlled type 2 diabetes or altered glycemic levels without diabetes are not associated with periodontitis [98, 109, 117-119]. In addition, the longer duration of diabetes appears to correlate with periodontitis severity [120].

Patients with diabetic retinopathy appear to have more severe periodontitis compared to those without retinopathy [121]. Interestingly, despite the higher prevalence and severity of periodontitis in subjects with diabetes, Newton et al., evaluating 46,132 electronic charts of patients with periodontitis with and without diabetes, concluded that individuals with diabetes had significantly more periodontitis treatment compared to normoglycemic individuals [122].

The mechanisms by which diabetes affects periodontitis have been studied in animals and will be described below. However, analyzing periodontal tissues and/or gingival crevicular fluid, it has been noted that interleukin-1β is increased and that diabetes also increases advanced glycation end products and oxidative stress [123-125].

Role of periodontitis in diabetes

Chronic subclinical inflammation plays a role in the pathogenesis of type 2 diabetes [126, 127].

Given that periodontitis leads to subclinical inflammation [128], many studies evaluated the role of periodontitis on glycemic levels, as well as on the incidence of type 2 diabetes and diabetes complications [129-136]. Periodontitis is associated with hemoglobin A1c progression in individuals with diabetes [137]. Additionally, deeper probing depths are more closely associated with increased hemoglobin A1c levels [68, 132, 138, 139]. Even in healthy individuals, periodontitis can worsen glycemic control [140-144].

Regarding the development of type 2 diabetes, most studies have linked periodontitis to a higher chance of developing diabetes [133, 145, 146]. For instance, Winning et al. concluded that the hazard ratio of developing type 2 diabetes in individuals with moderate-severe periodontitis is 1.69 [147]. Another study, which evaluated 22,299 patient charts with a 5.47-year mean follow-up, concluded that patients with periodontitis requiring surgery are at a higher risk of developing type 2 diabetes [133]. Another five-year study, utilizing tooth loosening as a proxy for periodontitis, also identified a correlation between incident diabetes and tooth loosening [148]. On the other hand, some studies were not able to find an association between type 2 diabetes incidence and periodontitis [129, 149]. For instance, Kebede et al. followed 2,047 subjects for a period of 11.1 years; although individuals with incident cases of diabetes tended to have poorer periodontal status, once the data was adjusted for age, gender, and central adiposity, the correlation no longer existed [129]. This contradictory result could have been in part influenced by the population evaluated (Caucasian), a partial periodontal evaluation, examination of hemoglobin A1c at only two data points, and 21% of subjects reporting having had periodontal treatment during the study period.

Severe periodontitis is associated with a higher presence of diabetes-related complications, including retinopathy, foot ulcerations, and renal and cardiovascular complications [134, 135, 138, 150-157]. For example, Saremi et al. evaluated Pima Indians in a long-term study with a median follow-up of 11 years, and concluded that periodontitis is a strong predictor of mortality from ischemic heart disease and diabetic nephropathy [134].

Role of periodontal treatment on glycemic control

Given the role of periodontitis in diabetes, studies have sought to understand whether periodontal treatment would ameliorate glycemic control. Unfortunately, to date, the results are conflicting in this regard [136, 158-165]. These contradictory results could be, at least in part, attributed to differences in the treatment rendered, the duration of the study (1 to 12 months), and because a proxy for periodontitis (dental insurance data) was utilized.

Among the studies, which concluded that periodontal treatment lowered hemoglobin A1c levels [158, 159, 161-163, 166] is a large study by D'Aiuto et al. presenting a 12-month follow-up [158]. In agreement with this study, Spangler et al. evaluated medical records and dental insurance data, as a proxy for periodontitis over a period of five years, and also concluded that patients who received periodontal treatment had slightly lower hemoglobin A1c levels compared to those who did not receive treatment. However, periodontal parameters were not considered in this study, only insurance data [163].

Conversely, several studies did not find a relationship between periodontitis treatment and hemoglobin A1c levels [136, 161, 164, 165]. These findings were likely due to the short-term (3-month) follow-up; this time may not have been sufficient to demonstrate a change in hemoglobin A1c levels.

Given the differences in periodontal treatment in various studies, the inability to restrict patients from obtaining periodontal treatment for an extended period [34, 43], and the short-term follow-up, more studies are needed to determine if periodontal treatment affects glycemic control.

Lessons from animal models: interactions between diabetes and periodontitis

To date, different animal models of type 1 and type 2 diabetes have been utilized with the goal of understanding the mechanisms by which diabetes and periodontitis affect one another. Herein, we will briefly discuss some of these findings.

When evaluating the role of diabetes in periodontitis, studies have shown that diabetes increases periodontal bone loss [167-171], but also that the severity of periodontal breakdown corresponds to the severity of diabetes [172]. Moreover, diabetes without periodontitis leads to periodontal alterations, such as a decrease in bone crest height and an increase in inflammatory cells and osteoclast numbers [173, 174]. The exact mechanisms by which diabetes affects periodontitis are not fully understood; however, it is known that diabetes worsens periodontitis in part by: a) increasing the inflammatory response [167, 171], b) enhancing cell apoptosis [175-177], c) increasing osteoclast formation, d) increasing bone resorption [169, 175], and e) suppressing bone formation [178]. These changes can be partially explained because diabetes: a) increases advanced glycation end products (AGEs) and their respective receptors (RAGE) [4, 179], b) increases TLR2 and TLR4 expression [180, 181], c) alters the RANK-L ratio [182], and d) leads to mitochondrial dysfunction [183, 184], which in turn enhances local and systemic oxidative damage [176].

The reverse is also true; most animal studies have also concluded that periodontitis affects diabetes. For instance, rodents with induced-periodontitis are more glucose intolerant [185], have more severe fasting insulin levels [186], and have increased pancreatic β-cell failure [187]. Periodontitis even alters glucose metabolism in rodents with pre-diabetes [188]. The mechanisms by which periodontitis affects diabetes are not entirely clear but do include increased circulating levels of different cytokines, such as interleukin-1β, TNFα, and adiponectin, as well as increased adipose tissue inflammation [185, 186, 189].

Taken together, animal studies also point to the bi-directional role of diabetes and periodontitis and shed some light on the mechanism by which these conditions lead to a pro-inflammatory

response. However, further studies are needed for a more comprehensive understanding of this relationship.

Type 2 diabetes and the microbiome

Much attention has been given to the ability of chronic conditions to alter the microbiome. For instance, individuals with type 2 diabetes or obesity have a modified gut microbiome (reviewed by [74, 190-192]). Conversely, mice treated with *Enterobacter cloacae* B29, which was isolated from the intestines of obese patients with diabetes, also develop obesity and insulin resistance [193]; showing that microbes can also directly induce diabetes-related symptoms. Furthermore, intervening with the flora or modulating bacterial-mucosal immunity-inflammation may alleviate type 2 diabetes [194]. Given the association between type 2 diabetes, periodontitis, and the changes that have been observed in the gut microbiome in individuals with type 2 diabetes, additional attention has been given to microbiome changes in the periodontium of individuals with type 2 diabetes.

Diabetes affecting the subgingival microbiome

Type 2 diabetes alters the subgingival and salivary bacterial profile of individuals (Table 4) by decreasing diversity and richness [195-200]. This is consistent with data observed within the gut microbiome [74, 190-192, 201]. When evaluating the subgingival microbiome, not only is the diversity decreased in individuals with type 2 diabetes, but when these individuals are further divided by adequate or inadequate glycemic control, there is a notable further decrease in the microbiome diversity in those with inadequate glycemic control (hemoglobin A1c $\geq$8) [202]. In contrast, a study performed by Tam et al. evaluated the salivary microbiome of 17 individuals with periodontitis and type 2 diabetes and was not able to determine statistically if glycemic control can also change the oral microbiota. However, the study noted that in individuals with type 2 diabetes, the microbial composition varied significantly between obese (BMI $\geq$30) and

non-obese individuals with type 2 diabetes, with obesity reducing the diversity of species in the oral cavity [77].

Although the diversity of the subgingival and supragingival microbiome decreases when subjects with type 2 diabetes are compared to normoglycemic individuals, the bacterial shift in individuals with periodontitis is less prominent in type 2 diabetes subjects than in normoglycemic individuals [196, 197, 203].

Not much consensus exists on the specific differences in the microbiome (Table 4). However, individuals with diabetes had higher total taxa of *TM7*, *Aggregatibacte*r, *Neisseria*, *Gemella,* and *Eikenella* [195]. Matsha et al. noted that *Fusobacterium* and *Actinobacteria* are more abundant in subjects with diabetes. Furthermore, in subjects with type 2 diabetes and bleeding on probing, there was an increase in the abundance of *Bacteroidetes* (*P. gingivalis* belongs to this phylum) [198].

Targeted studies utilizing DNA-DNA hybridization technology or polymerase chain reaction assays evaluated the presence of specific bacterial taxa or groups of bacteria and no consensus was reached. Aemaimanan et al. and Babaev et al. reported that individuals with poorly controlled type 2 diabetes have higher levels of red complex bacteria (*P. gingivalis, Treponema denticola,* and *T. forsythia*) [204, 205]. On the other hand, Rodriguez-Hernandez et al. found a decrease in red complex bacteria in Mexican individuals with type 2 diabetes compared to normoglycemic individuals with periodontitis. However, individuals with type 2 diabetes and periodontitis had higher levels of the yellow and orange complexes [206].

There is also a bit of contradiction among different studies when analyzing individual bacterial species. For instance, *P. gingivalis* was increased in individuals with periodontitis and type 2 diabetes [204, 205, 207, 208], but decreased in other individuals [195, 209, 210]. Some studies found that the levels of *T. denticola* were higher in patients with type 2 diabetes compared to

normoglycemic controls [204, 205, 211], while no difference was observed in other studies [207, 209].

When type 2 diabetes and periodontitis were evaluated in individuals who smoke, the subgingival microbiome of smokers with type 2 diabetes had lower diversity, higher levels of Gram-negative facultative anaerobes, and lower levels of Gram-negative obligate anaerobes. In addition, the combination of smoking and type 2 diabetes led to synergistic changes in the microbiome [212].

Periodontal treatment and the microbiome

Shi et al. evaluated the subgingival microbiome through metagenomic shotgun sequencing of normoglycemic and type 2 diabetes individuals with periodontitis before and after scaling and root planing. Both groups showed clinical improvement and improvements in the levels of *P. intermedia*, *P. gingivalis* and *T. forsythia*. However, individuals with poor glycemic control showed a reduced shift in the microbiome; reduced shift towards a healthy state [203]. Silva-Boghossian et al. used a targeted approach method (DNA-DNA hybridization) to evaluate 45 species, and observed similar results. Scaling and root planing led to improvement in clinical parameters and a reduction in the pathogenic bacteria in both groups, but the reduction in the type 2 diabetes group was not as significant as what was observed in the normoglycemic group. Regarding Silva-Boghossian et al.'s findings, it is worth noting that the patients with type 2 diabetes had more severe periodontitis compared to the normoglycemic individuals [211]. Therefore, based on the data presented above, it appears that periodontitis treatment leads to a less pathogenic bacterial profile; however, the shift is not as prominent in type 2 diabetes and even less significant in patients with poorer glycemic control.

Type 2 diabetes and the salivary microbiome

Goodson et al. suggested that increased levels of glucose in the saliva may affect the salivary microbiome [213]. To test this hypothesis, several groups evaluated the role of type 2 diabetes

on the salivary microbiome. The salivary and subgingival microbial diversity was decreased in individuals with type 2 diabetes [199]. However, treatment with metformin or metformin in combination with other medications did not rescue the flora. Nonetheless, the supragingival microbiome is different in patients with type 2 diabetes without medications and those being treated with metformin or with a combination of medications [197]. Another study by the same group evaluated the salivary microbiome of normal weight, obese, and obese/type 2 diabetes children (10-19 years of age), noting that there was minimal difference in the alpha diversity among these groups. This study supported a modest link between periodontal inflammation and type 2 diabetes in the pediatric population. The gingival index was higher in individuals with type 2 diabetes but periodontitis was similar among the groups. At the genus level, there was a difference in abundance in eight operational taxonomic units and, after adjusting for the gingival index, there were still five significantly different genus-level operational taxonomic units [214]. While the rate of missing, decayed, and filled teeth was similar between groups, the gingival index was higher in type 2 diabetes. There was no difference in periodontitis, which is not surprising, given that periodontitis is more common in older populations.

Summary of type 2 diabetes, periodontitis, and the microbiome

In summary, type 2 diabetes affects the subgingival and salivary microbiome profile by decreasing diversity and richness. When glycemic control is added to the equation, there is a further decrease in diversity in individuals with inadequate glycemic control. Interestingly, the bacterial shift observed in individuals with periodontitis is less prominent in subjects with type 2 diabetes compared to normoglycemic controls. Moreover, in smokers, the diversity of the microbiome is further reduced. In future studies, it will be interesting to examine the potential for a bidirectional relationship between the periodontal microbiome and diabetes; that is the potential for periodontal microbes to directly induce diabetes-related pathology, since evidence suggests that pathogenic microbes from the gut of obese diabetic patients can directly induce diabetes-related symptoms in rodent models.

Cardiovascular disease

Cardiovascular disease is a group of disorders of the heart and blood vessels that include coronary artery disease, cerebrovascular disease, congestive heart failure, and peripheral vascular disease [215, 216]. In the United States, among individuals >20 years of age, 37.4% of men and 35.9% of women have some form of cardiovascular disease [215]. These conditions can lead to myocardial infarction and stroke and account for one third of all deaths worldwide [217]. Out of these conditions, atherosclerotic cardiovascular disease is the leading cause of vascular disease worldwide [218]. Although genetics plays a role in cardiovascular disease [219], the key risk factors stem from lifestyle, such as dyslipidemia, hypertension, tobacco smoking, and altered glucose metabolism [220]. Unfortunately, these key lifestyle factors are quite common and it is believed that 47% of Americans have at least one of them [221].

Given the prevalence of cardiovascular disease and the need to prevent and treat it, Matilla et al. sought to investigate early on whether dental disease could be correlated with the prevalence of cardiovascular disease, more specifically, with myocardial infarction. This study concluded that dental health (periapical lesions, caries, vertical bone loss, and radiolucency in the furcation) was worse in patients with acute myocardial infarction when compared to healthy individuals [222]. In addition, significant interest has been focused on the potential role of periodontitis in cardiovascular disease. Our review on cardiovascular disease and periodontitis will be succinct, given that, in 2020, a consensus report was published on this topic by the European Federation of Periodontology and the World Heart Federation [223].

Periodontitis and cardiovascular disease markers

Many inflammatory and oxidative stress markers are shared by cardiovascular disease and periodontitis. Therefore, the premise that periodontitis could affect cardiovascular disease is based on the elevated inflammatory serum marker levels in patients with periodontitis compared

to periodontally healthy individuals or patients that have been treated for periodontitis [224-230].

Role of periodontitis in cardiovascular disease

Most clinical studies have shown an association between periodontitis and cardiovascular disease [231-242]. In 2012, a scientific statement was released by the American Heart Association confirming that there was an association between cardiovascular disease and periodontitis, but that a causal relationship could not be determined [243]. Around the same time, a systematic review performed by Dietrich et al. evaluated 12 studies and also concluded that there is an association between periodontitis and atherosclerotic cardiovascular disease. However, this study cautioned that these findings may apply only to certain populations [244]. A large Swedish case-controlled study later evaluated periodontitis and its relation to coronary disease in 805 individuals and concluded that the risk of myocardial infarction significantly increases in patients with periodontitis (odds ratio 1.28, CI 1.03-1.6) even after adjusting for variables, such as smoking habits, diabetes, years of education, and marital status [233]. A more recent study by Sen et al. also concluded that patients with periodontitis have more than double the risk of cardioembolic and thrombotic stroke compared to periodontally healthy individuals [245]. Recently, a review was performed by Herrera et al. [246] with the aim of evaluating the association between periodontitis and cardiovascular disease to assist in the 2020 consensus report, published by the European Federation of Periodontology and the World Heart Federation. They concluded that individuals with periodontitis have a higher prevalence of coronary artery disease and increased risk of myocardial infarction. However, when evaluating the role of periodontitis in a secondary atherosclerotic cardiovascular disease event, there was no consensus [223, 246].

Effect of periodontal treatment on cardiovascular disease

Based on several observational studies and a Cochrane review that evaluated a) self-reported toothbrushing frequency, b) improved oral hygiene, c) dental visit frequency, d) periodontal

treatment, and e) periodontal treatment outcomes, and correlated them to cardiovascular events [247-253], the 2020 consensus report by the World Heart Federation and the European Federation of Periodontology concluded that the progression of atherosclerotic cardiovascular disease may be influenced by successful periodontal treatment, which included oral health instructions and more frequent dental visits, independent of traditional cardiovascular disease risk factor management. However, the consensus report found insufficient evidence to support or refute the potential benefit of periodontitis treatment in preventing or delaying atherosclerotic cardiovascular disease events [223].

Interventional studies have sought to evaluate the effect of periodontal treatment on surrogate markers of cardiovascular disease, such as C-reactive protein, fibrinogen, lipid profiles, white blood cells, and blood pressure [237, 254-258]. In 2012, Bokhari et al. evaluated patients with coronary heart disease and periodontitis and compared the effects of scaling and root planing with no periodontal treatment. The study concluded that scaling and root planing decreases C-reactive protein, fibrinogen, and white blood cell counts [254]. Caula et al. evaluated patients that underwent periodontal treatment who were then followed for a period of six months. This study also concluded that periodontal treatment leads to a reduction in C-reactive protein, in addition to a reduction in triglycerides and erythrocyte sedimentation rates [256]. Houcken et al. recorded a decrease in systolic blood pressure after periodontal treatment [258]. Moreover, data from a systematic review and meta-analysis concluded that periodontal treatment, in addition to reducing serum levels of atherosclerotic cardiovascular disease biomarkers, improves endothelial function [259].

To assess whether the timing of periodontal treatment has systemic effects, Graziani compared scaling and root planing performed within 24 hours versus scaling and root planing performed over a four-week period and evaluated inflammatory markers. The results indicated that there is an increased acute phase response, demonstrated by increased C-reactive protein and interleukin-6, when full mouth scaling and root planing is performed in a 24-hour period. However, these results were transient and both treatment modalities ultimately offered similar

results. Nonetheless, further studies are needed to determine if the elevated acute phase response has any impact on cardiovascular disease risk [257].

The data presented here is in agreement with the 2020 World Heart Federation and European Federation of Periodontology consensus report, which points to evidence that periodontal treatment may reduce low-grade systemic inflammation [223]. It is important to note that a review by Herrera et al. also pointed out that there have been a limited number of studies evaluating the role of periodontal treatment on cardiovascular disease outcomes [246].

Periodontal microbiome and cardiovascular disease

Much interest has been placed recently on the microbiome, due to its ability to modulate chronic conditions, including cardiovascular disease [260, 261] . Given that periodontitis is triggered by a dysbiosis of pathogenic bacteria, that certain dental procedures, including toothbrushing and scaling and root planing cause a transient bacteremia, and that periodontitis has been correlated to cardiovascular disease [262-268], researchers sought to determine if periodontopathogens could be found in the cardiovascular system. Indeed, several groups have identified periodontal pathogens, such as *T. denticola, Actinomyces Actinomycetemcomitans, P. gingivalis and T. forsythia,* in cardiovascular tissue specimens (including atherosclerotic plaque, aortic valve, mitral valve, and aortic aneurysm) [269-273]. Although the mechanism by which bacteria enter into circulation is not fully understood, one study suggests that dendritic cells phagocytose and disseminate surviving periodontopathogens to atherosclerotic plaques and that these “primed” dendritic cells may provide key signals for atherogenic conversion [274].

Moreover, while most studies were able to identify pathogenic periodontal bacteria in cardiovascular tissues, the exact role of the bacteria in cardiovascular disease is not fully understood. In an *in vitro* study, Lonn et al. suggested that *P. gingivalis* can modify vascular LDL/VLDL and HDL to an atherogenic form [275]. A clinical study suggested that miR146, a

regulator of innate immune responses, was a key molecule in associating periodontitis and coronary artery syndrome due to its dysregulation by periodontal pathogens [276, 277]. Moreover, a review by Reyes et al. effectively categorized the available research indicating that periodontal bacteria 1) disseminate and reach systemic vascular tissue, 2) are found in the affected tissues, 3) live within the affected site, 4) invade affected cell types *in vitro*, 5) induce atherosclerosis in animal models of disease, and that 6) non-invasive mutants of periodontal bacteria cause significantly reduced pathology *in vitro* and *in vivo,* and 7) periodontal isolates from human atheromas can cause disease in animal models of infection [278].

To summarize the human and animal data regarding the role of periodontal bacteria in cardiovascular disease, there is convincing data that periodontal bacteria can be found in cardiovascular tissues, but the role that bacteria play in these tissues still needs to be further elucidated.

The role of cardiovascular disease in periodontitis

While much emphasis has been placed in determining the potential role of periodontitis in cardiovascular disease, very little has been done to evaluate the role of cardiovascular disease in periodontitis. As a result, the European Federation of Periodontology and World Heart Federation consensus report concluded that, to date, there is little scientific evidence that cardiovascular disease is a risk factor for periodontitis [223].

In conclusion, much is known about the role of periodontitis in cardiovascular disease. However, it is important to note that these two conditions share risk factors, such as smoking, age, socioeconomic conditions, and obesity, that may lead to a possible common pathophysiology for periodontitis and cardiovascular disease [279-281].

Lessons from animal models: periodontitis and cardiovascular disease

In addition to the role of periodontal bacteria in cardiovascular disease, discussed above, animal studies have shed some light on the role of periodontitis in cardiovascular disease. Animal models have demonstrated that periodontitis increases systemic inflammation and cardiovascular disease markers, such as C-reactive protein, interleukin-1 β, interleukin-6, vascular superoxide production, and worsens lipid profile levels (total cholesterol, LDL and triglycerides) [282-284]. Evaluating endothelial changes in a ligature-induced periodontitis model in rats, Brito et al. observed a reduction in endothelium-dependent vasodilatation, which is the hallmark of endothelial dysfunction [284]. Moreover, Kose et al. performed histological analysis of the left ventricular heart tissues of rats and demonstrated that, at an early stage, periodontitis causes degenerative and hypotrophic changes in the heart tissue, and that prolonged systemic inflammatory stress due to periodontitis may enhance the risk of hypertrophic changes [282].

In trying to understand the role of periodontitis in cardiovascular disease, most studies have focused on the role of periodontopathogens in atherosclerosis because of their ability to induce severe oxidative stress, induce an inflammatory response, invade, colonize, and escape immune detection [285-288]. For instance, in response to *P. gingivalis*, endothelial cells undergo oxidative stress and secrete various cytokines, such as TNFα, interleukin-1β, interleukin-6 and IFNγ [286, 289]. Part of the mechanism by which *P. gingivalis* accelerates atherosclerosis involves the NFkB signaling loop [290, 291]. Moreover, periodontal pathogens are able to modulate lipid influx via FABP4 in macrophages, strongly supporting another mechanism by which periodontitis may affect atherosclerotic progression [292].

Conclusion:

In conclusion, metabolic syndrome, diabetes and cardiovascular diseases are associated with periodontitis. Moreover, there is evidence to suggest that metabolic syndrome and diabetes can alter the oral microbiome. However, more studies are needed to fully understand the influence these conditions have on each other.

METHODS

This study was approved by the Institutional Review Board of the University of Michigan Medical School (#HUM000068346.) Twenty-two (N=22) subjects over 18 years of age with a minimum of ten teeth were recruited and informed consent was obtained. Pregnant and lactating women were excluded from the study.

Subject Diagnoses

A single examiner collected clinical data including the subjects' weight, height, waist and hip circumference, blood pressure, and blood glucose levels. Subjects were diagnosed as healthy or metabolic syndrome. A diagnosis of metabolic syndrome was given to subjects who exhibited three or more of the following parameters: abdominal obesity (waist circumference of >102cm in men or >88 cm in women), hyperlipidemia (self-reported), hypertension (>120 diastolic or >80 systolic readings or use of anti-hypertensive medication), or diabetes (self-reported or elevated blood glucose (>120 milligrams/deciliter (mg/dl)) [293]. Patients who did not meet these criteria were considered systemically healthy and were assigned to the healthy group rather than the metabolic syndrome group.

Periodontal Screening and Recording exams were completed for each subject. Subjects with no evident signs of gingivitis or periodontitis, who had probing depths of 1-3 mm, bleeding on probing (BOP) <30%, and no evidence of radiographic bone loss were diagnosed as healthy periodontium (HP). Subjects were diagnosed with periodontitis if they displayed severe gingival inflammation, erythema, possible edema, BOP >30%, radiographic bone loss >15%, and probing depths >5 mm in more than one quadrant.

Data Analysis

Two-group analysis: Healthy v. Metabolic syndrome

A two-group analysis was completed as follows: healthy subjects were compared to subjects with metabolic syndrome. The metabolic syndrome group included those with a healthy periodontium (HP) as well as those with periodontitis. The 12 metabolic syndrome patients had diabetes mellitus. No tobacco smokers were included in the healthy group, but of the metabolic syndrome population, N=5 were current smokers and N=3 were former smokers. Healthy subjects ranged from 20-46 years of age (mean 28.1 years) while metabolic syndrome subjects ranged from 47-78 years of age (mean 64.5 years.)

Four-group analysis: Healthy v. Healthy v. metabolic syndrome HP v. metabolic syndrome periodontitis:* A four-group analysis was conducted on the following groups: 1) Healthy (medically and dentally), 2) Healthy* (subjects who presented with elevated blood pressure readings despite the subject having no self-reported history of hypertension), 3) metabolic syndrome with Healthy periodontium (metabolic syndrome HP), and 4) metabolic syndrome with periodontitis (metabolic syndrome periodontitis). The healthy group consisted of N=7 subjects. N=3 healthy subjects presented with an elevated systolic and/or diastolic blood pressure reading (stage I hypertension values, according to the American Heart Association) and thus were stratified into a Healthy* group. When questioned, all three subjects confirmed that they seek regular, routine medical care with a physician and have not been diagnosed as pre-hypertensive or hypertensive. Metabolic syndrome subjects were stratified into two groups: N=4 metabolic syndrome HP and N=8 metabolic syndrome periodontitis. Of the metabolic syndrome periodontitis group, N=7 (out of eight total) subjects had a history of tobacco use (N=6 current smokers and N=1 former smoker), whereas the metabolic syndrome HP group had only one former tobacco user.

Salivary Analysis

Passive saliva was collected from each subject for five minutes. Samples were subsequently stored in a -80-degree Celsius freezer until they were prepared for analysis. Prior to analysis, samples were thawed and centrifuged. Whole genomic bacterial DNA was extracted from salivary pellets and purified using the MasterPure™ DNA purification kit (Epicentre®, Madison, WI, USA).

DNA was adjusted to a concentration of 20 nanograms/microliter (ng/μl) using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA)

The 16S rDNA next generation sequencing (NGS) was performed using the Human Oral Microbiome Identification system (HOMINGS, The Forsyth Institute, Cambridge, MA, USA) [294, 295]. The laboratory procedures of HOMINGS follows a method modified from a previously published protocol [296]. Polymerase chain reaction (PCR) amplification of deoxyribonucleic acid (DNA) (10-50 ng) was performed using universal primers targeting the V3-V4 region of 16S genes (F341, R806). The products were purified using AMPure purification. Amplicons were pooled in libraries (100 ng) that were gel-purified and quantified by quantitative polymerase chain reaction (qPCR) before being sequenced (MiSeq, Illumina, San Diego, CA, USA). In this study, reads were typically >50,000 per sample. The sequence read pairs were merged to single reads with a script (*join_paired_ends.py*) provided by Quantitative Insights into Microbial Ecology (QIIME) package version 1.91 with default settings. The merged reads were then taxonomically assigned to the species level based on a published algorithm [297] with additional steps to further identify potential novel species. A complete description and the result of the taxonomy assignment is available online at http://www.homd.org/ftp/publication_data/20170412/ . Basic local alignment search tool nucleotide (BLASTN) was used to compare merged sequence reads against a panel of full-length 16S ribosomal ribonucleic acid (rRNA) sequences, consisting of 889 sequences from human oral microbiome database (HOMD) Reference Sequence (RefSeq) V14.5, 495 from HOMD RefSeqExtended V1.1, 3,940 from GreenGeneGold, and 18,166 from National Center for Biotechnology Information (NCBI) 16S rRNA Reference. This combined reference set has a total of 23,490 sequences and represents 13,640 oral and non-oral microbial species. After the taxonomy assignment, species-level operational taxonomic units with at least 10 reads were subject to several downstream bioinformatic analyses, including alpha and beta diversity assessments, provided in QIIME. Samples with <500 read counts were excluded in the QIIME analysis. Species-level taxonomic plots were generated. Alpha and beta diversity measures were calculated for two-group and four-group analysis. Alpha diversity and species richness were evaluated with rarefaction plots using the operational taxonomic units as the metric. Beta

diversity was evaluated with Principal Coordinate Analysis plots, created using generalized UniFrac as the distance measurement based upon the R statistic generated using Analysis of Similarities (ANOSIM).

Additionally, QIIME data was used to employ Galaxy for Linear discriminant analysis Effect Size (LEfSe) for metagenomic analysis. A histogram was generated to visualize the effect size (Linear Discriminant Analysis) difference for two-group and four-group analysis. A cladogram taxonomic tree was generated to evaluate the phylogenetic comparisons for the two-group and four-group analyses at the species level.

Availability of data and material: The datasets generated during and/or analyzed by the current study are available online at: http://www.homd.org/ftp/publication_data/20170412/qiime_results/cd_mc10/taxa_plots/taxa_summary_plots/bar_charts.html

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Figure legends:

Figure 1. Rarefaction plots show distinct levels of alpha diversity between healthy and metabolic Syndrome Subjects. The two-group analysis rarefaction plot (A) represents the rarefaction curves of the Healthy and metabolic syndrome subjects. Both curves show similar species richness and the curves plateau as the number of sequences rise per sample. The four-group analysis rarefaction plot (B) shows similar alpha diversity in terms of species richness and both begin to plateau and the curves of all four groups begin to plateau as the number of sequences rises per sample.

Figure 2. Principal Coordinate Analysis (PCoA) for all groups show the relative relatedness within groups. A 2- dimensional view of the 3-dimensional Principal Coordinate Analysis plot, derived from UniFrac as a distance metric, is shown in A, representing beta diversity for the two-group analysis. It can be appreciated that the two groups appear to be more related within-group than between group as evidenced by clusters. A 2-dimensional view of the 3-dimensional Principal Coordinate Analysis plot, derived from UniFrac as a distance metric, is shown in B, representing beta diversity for the four-group analysis. No conclusive result regarding beta diversity can be observed from these plots.

Figure 3. The cladograms show the phylogenetic relationship and taxonomic groupings of the taxa significantly associated with different subject groups. Fig. 3A shows the phylogenetic relationship and taxonomic groupings of the taxa significantly associated with healthy and metabolic syndrome subjects. Dots of red (Health) and green (metabolic syndrome) represent

significantly associated taxa to the labeled group and the size of the dot corresponds with relative abundance. At the outer edge of the cladogram, a letter is listed to represent this taxon and corresponds with the legend on the right. A strong association between taxa stemming from the phylum Proteobacteria and phylum SR1 and health is shown. Taxonomic relationships are shown in clusters of green for taxa significantly associated with the metabolic syndrome group. Fig. 3B shows the phylogenetic relationship and taxonomic groupings of the taxa significantly associated with Healthy (red), Healthy* (green), metabolic syndrome with Healthy Periodontium (blue) and metabolic syndrome with periodontitis (purple). Colored dots represent significant taxa for the denoted group and the size of the dot corresponds with relative abundance. At the outer edge of the cladogram, letters are used to represent the significant taxa, listed in the legend on the right side of the figure. A strong association is shown between taxa stemming from the Phylum *Tenericutes* and *Spirochaetes* and order *Coriobacteriia* for metabolic syndrome with periodontitis subjects, a strong association is shown between taxa stemming from the phylum SR1 and Healthy* subjects, and taxa associated with the phylum Proteobacteria and Healthy subjects.

Table legends:

Table 1: The role of metabolic syndrome in periodontitis

Abbreviations: Abd: abdominal, ABL: alveolar bone level, BMI: body mass index, BOP: bleeding on probing, BP: blood pressure, CAL: clinical attachment level, CPI: community periodontal index, CRP: C-reactive protein, FPG: fasting plasma glucose, GI: gingival bleeding index, HbA1c: hemoglobin A1c, HDL: high density lipoproteins, LDL: low density lipoproteins, Med: medication, OGTT: oral glucose tolerance test, PD: probing depth, TC: total cholesterol, TG: triglycerides, T2DM: type 2 diabetes mellitus, WC: waist circumference

Table 2: Role of periodontitis in metabolic syndrome

Abbreviations: Abd: abdominal, ABL: alveolar bone level, BMI: body mass index, BP: blood pressure, CAL: clinical attachment level, CPI: community periodontal index, CRP: C-reactive

protein, FPG: fasting plasma glucose, HDL: high density lipoproteins, PD: probing depth, TC: total cholesterol, TG: triglycerides, WC: waist circumference

Table 3: Animal studies of metabolic syndrome and periodontitis

Abbreviations: ABL: alveolar bone level, CPI: community periodontal index, FFA: free fatty acid, FPG: fasting plasma glucose,LPS: lipopolysaccharide, TC: total cholesterol, TG: triglycerides

Table 4: Diabetes mellitus, periodontitis and the microbiome

Abbreviations: BOP: bleeding on probing, CAL: clinical attachment level, CPI: community periodontal index, FPG: fasting plasma glucose, GI: gingival bleeding index, HbA1c: hemoglobin A1c, Med: medication, PD: probing depth, PI: plaque index, S: suppuration

Table 1: The role of metabolic syndrome in periodontitis						
Longitudinal studies						
Authors	**Duration (yrs)**	**Age (yrs)**	**Sample size**	**Metabolic syndrome parameters**	**periodontitis parameters**	**Results**
Morita et al [40]	4	20 to 56	1023	BP, TG, HDL, TC, FPG and BMI	PD (CPI)	Periodontitis was associated with a positive conversion of metabolic syndrome components
Bullon et al [41]	3	20 to 44	188	Pregnancy, weight, BMI, BP, HbA1c, CRP, FPG, TG, TC, LDL and HDL	Plaque, BOP, PD, recession	There is an association between periodontitis and metabolic syndrome
Iwasaki et al [35]	3	≥70	125	Abd obesity, BP, TG, HDL and FPG	CAL	Metabolic syndrome may be a risk factor for periodontitis in older Japanese individuals
Kaye et al [42]	33		760	FPG, BP, TG, WC and HDL	PD, CAL, ABL, tooth mobility	Metabolic syndrome may play a role in the development or worsening of periodontitis
Nascimento et al [34]	8 or 16		539	FPG, HDL, TG, WC and BP	BOP, PD, CAL	Positive association between metabolic syndrome and periodontitis, when the multiple dimensions of both diseases were accounted in latent variables. When metabolic syndrome

						and periodontitis were treated as observed variables, no association was detected
Sakurai et al [43]	2	≥30	390	TG, HDL, BP, FPG and WC	CPI	The prevalence of individuals with more positive metabolic syndrome components was higher in those with persistent/progressive periodontitis than in those with no/improved periodontitis
Tegelberg et al [44]	15		1,964	WC, TG, HDL, BP and FPG	PD and ABL	Metabolic syndrome was associated in an exposure-dependent manner with periodontitis
Adachi et al [45]	1	≥35	136	WC, TG, HDL, BP and FPG	CPI	There were no associations between periodontitis and the development of metabolic syndrome
Cross-sectional/case-control studies						
Authors	**Age of patients**	**Number of patients**	**Metabolic syndrome parameters**	**Perio Parameters**	**Results**	

Borges et al [65]	30 to 92	1315	BMI, dyslipidemia, BP and FPG	CPI	There were no associations between periodontitis and metabolic syndrome
Shimazaki et al [28]	40 to 79	584	Abd obesity, TG, HDL, BP and FPG	PD and CAL	Metabolic syndrome increases risk of periodontitis
D'Aiuto et al [11]	≥17	13677	WC, TG, HDL, BP, and insulin resistance	BOP, PD	Severe periodontitis is associated with metabolic syndrome in middle-aged individuals
Khader et al [37]	≥25 or above	156	WC, TG, HDL, TC, BP and FPG	PI, GI, PD and CAL	Patients with metabolic syndrome displayed more severe and extensive periodontitis compared to subjects without metabolic syndrome
Li et al [46]	37 to 78	208	Abd obesity, TG, HDL, BP, and FPG/or T2DM	CAL, PD, BOP and PI	Patients with metabolic syndrome had poor periodontal conditions, and periodontitis was associated with metabolic syndrome, independent of other risk factors
Morita et al [29]	24 to 60	2478	WC, TG, HDL, TC, BP and FPG, HbA1c and BMI	CPI	BMI, BP, TG, FPG and HbA1c were significantly elevated in patients with PD of ≥4 mm. The adjusted odds ratio of the presence of periodontitis was 1.8 when the subjects with 2 positive components and without positive component were compared. And the odds ratio was 2.4 when the subjects

					with 3 or 4 positive components and without positive components were compared
Kushiyama et al [47]	40 to 70	1070	Obesity, BP, HDL, TG, and FPG	CPI	The higher the number of metabolic syndrome components the higher the odds ratio of having more severe periodontitis
Andriankaja et al [30]	20 to >90	7431	Abd obesity, BP/or med, TG, HDL and FPG/or med	PD	The association between metabolic syndrome and periodontitis was significant in women. Abdominal obesity appeared to be the contributing metabolic factor for both genders
Benguigui et al [48]	35 to 74	276	WC, TG, HDL, BP and FPG	PI, GI, PD and CAL	There is a relationship between metabolic disturbances and periodontitis, with insulin resistance playing a central role
Han et al [49]	≥18	1046	Abd obesity, TG, HDL, BP and FPG	BOP, PD and calculus	Metabolic syndrome might be associated with periodontitis. The association was confounded by age, gender, and smoking. Metabolic syndrome with high glucose and hypertension showed higher impact on this association
Nesbitt et al [10]	mean: 56.8±12	190	BP, WC, TG and FPG	ABL	Patients with severe periodontitis were approximately 2.5% times more likely to have metabolic syndrome

Timonen et al [63]	30 to 64	2050	Abd obesity Insulin resistance, BP and dyslipidemia	PD	Metabolic syndrome was associated with PD ≥4 mm (adjusted risk ratio 1.19), and with pockets ≥6 mm (adjusted risk ratio 1.5)
Chen et al [36]	>18	253	WC, TG, HDL, TC, BP and FPG or T2DM	PI, GI and PDI	Moderate-severe periodontitis is associated with metabolic syndrome in patients undergoing hemodialysis
Kwon et al [298]	≥19	7178	WC, TG, HDL, BP and FPG	PD	Periodontitis is significantly associated with metabolic syndrome with an odds ratio of 1.55
Fukui et al [299]	34 to 77	6,421	TG, HDL, BP, FPG, HDL, BP and obesity	PD and CAL	Periodontal status, particularly in individuals suspected to have untreated periodontal infection, is significantly associated with metabolic syndrome
Furuta et al [60]	40 to 79	2370	WC, TG, BP HDL and FPG	PD, CAL and BOP	Gender differences appear to exist in the association between periodontitis and metabolic syndrome. Metabolic syndrome might have a stronger association with periodontitis in females compared to males
Sora et al [39]	26 to 87	283	Abd obesity, BP, HDL, TG and FPG (OGTT)	Plaque, PD, CAL and BOP	Metabolic syndrome is associated with the extent of severe periodontitis in this Gullah population with type 2 diabetes

LaMonte et al [64]	50 to 79	657	Abd obesity, BP or med, TG, FPG or med and HDL	ABL, PD and CAL	A consistent association between metabolic syndrome and measures of periodontitis was not seen in this cohort of postmenopausal women
Thanakun et al [33]	35 to 76	125	WC, TG, HDL, BP and FPG	BOP, PD and CAL	Severe periodontitis was associated with metabolic syndrome (odds ratio 3.6) when 4-5 metabolic syndrome components were analyzed the odds ratio increased to 5.49 in this Thai population
Minagawa et al [9]	≥80	234	WC, FPG, BP, and dyslipidemia	PD and CAL	Metabolic syndrome was associated with the presence and severity of periodontitis (crude odds ratio = 2.24)
Chen et al [300]	23 to 58	303	Abd obesity, BP, TG, HDL and FPG	CPI	The prevalence of metabolic syndrome was sufficiently high to be a medical concern, and was associated with periodontitis
Gomes-Filho et al [50]	24 to 89	419	WC, TG, HDL, BP and FPG	PD, CAL and BOP	Periodontitis is associated with metabolic syndrome
Musskopf et al [51]	18 to 81	363	WC, TG, HDL, BP and FPG	PI, GI, PD, CAL and BOP	There is a weak association among metabolic syndrome and periodontitis. The association is observed in the age group between 41 and 60 years.
Jaramillo et al [52]		651	TG, HDL, BP, BMI and glucose tolerance	GI, PI, PD, CAL and BOP	There is a positive association between metabolic syndrome and periodontitis. The adjusted odds ratio is 2.72. Glucose sensitivity is a strongly associated component

Kikui et al [53]	mean: 66.4	1856	BP and/or med, HDL, TGs and/or med, FPG/ and Abd obesity	CPI	Metabolic syndrome and lower HDL cholesterol are associated with periodontitis. Subjects with 2 or more metabolic syndrome components had a significantly higher prevalence of periodontitis
Kim et al [301]	50 to 94	5078	BMI, WC, BP, FPG, HD and TG	PD and CAL	Increasing the severity of periodontitis was associated with the risk of prevalent metabolic syndrome in Korean adults
Pham et al [38]	mean: 57.8 ± 5.7	412	BMI, WC, HDL, BP and FPG	PI, GI, PD, CAL and BOP	More severe and extensive periodontitis was found in metabolic syndrome participants and increased with number of metabolic syndrome components. Participants with higher periodontal parameters had a higher risk of metabolic syndrome
Campos et al [54]		122 with metabolic syndrome and 366 controls	BP, TGs and LDL and/or WC	PI, BOP, PD and CAL	There is an association between metabolic syndrome and periodontitis

Table 2: Effect of periodontitis on metabolic syndrome

Authors	Cross-sectional (CS) or longitudinal (L)	Duration	Age of patients	Number of patients	Metabolic syndrome parameters	periodontitis parameters	Results
Kushiyama et al [47]	CS		40 to 70	1070	Obesity, BP, HDL, TG, and FPG	CPI	The higher the number of metabolic syndrome components, the higher the odds ratio of having more severe periodontitis
Morita et al [40]	L	4	20 to 56, mean: 37.3	1023	BP, TG, HDL, TC, FPG and BMI	CPI	Periodontal pockets were associated with a positive conversion of metabolic-syndrome components
Nesbitt et al [10]	CS		mean: 56.8±12.7	190	BP, WC, TG, and FPG	ABL	Alveolar bone loss is associated with metabolic syndrome

Lopez et al [73]	L	1 year	35 to 65	165	Abd obesity, TG, HDL, BP and FPG	≥4 teeth with ≥4mm and CAL of ≥3mm	Reduction of periodontal inflammation either with scaling and root planing and systemic antibiotics or with plaque control and subgingival scaling reduces CRP levels after 9 months in patients with metabolic syndrome
Kim et al [301]	CS		50 to 94	5078	BMI, WC, BP, FPG, HDL and TG	PD and CAL	Increasing the severity of periodontitis was associated with the risk of prevalent metabolic syndrome in Korean adults

Table 3: Metabolic syndrome - animal data					
Authors	**Duration of the study (weeks)**	**Number of samples**	**Metabolic syndrome parameters**	**Periodontitis parameters**	**Results**
Amar et al [57]	16	N/A	Not evaluated	ABL	Mice with *P. gingivalis*-induced periodontitis and diet-induced obesity had a significantly higher level of alveolar bone loss compared to the lean controls
Watanabe et al [186]	13	28	FPG and fasting insulin levels	ABL	High-fat/periodontitis rats developed more severe insulin resistance compared to High-fat/control, low-fat/periodontitis or low-fat control rats as measured by fasting insulin levels and homeostasis model assessment analysis
Ohnishi et al [302]	20		insulin resistance	ABL	Oxidative stress causes alveolar bone loss in a metabolic syndrome mouse model with type 2 diabetes
Jin et al [58]	4 weeks	44 (22/ group)	FPG, TG, FFA and TC	ABL	Simvastatin inhibited LPS-induced bone loss and periodontal inflammation in rats with metabolic syndrome

Li et al [59]	16 weeks	14	TC, TG, and FFA	ABL	Saturated fatty acid may play a role in metabolic syndrome-associated periodontitis by enhancing LPS-induced inflammatory cytokine expression
Lu, Z et al [81]	16 weeks	28 (14/group)	FPG, TG, FFA, TC and insulin	ABL	CD36 expression is upregulated in mice with periodontitis and metabolic syndrome and is involved in gene expression in macrophages stimulated by palmitate and LPS

Table 4: Diabetes mellitus, periodontitis and the microbiome

Supragingival microbiome

Authors	Cross-sectional or Longitudinal Study	Age of Patients	# of patients	diabetes parameters	Periodontitis parameters	Results
Sbordone et al [303]	Longitudinal (3 yrs)	9 to 17	32	HbA1c	PD, CAL and BOP	There is no significant differences in clinical parameters between type 1 diabetes mellitus and non-diabetes mellitus siblings

Silva-Boghossian et al [211]	Longitudinal (3 mos)		40	FPG and HbA1c	PD and CAL, BOP, S and marginal bleeding	After scaling and root planing healthy individuals demonstrated improved periodontal status and reduced levels of putative periodontal pathogens at 3 months compared with those with inadequate metabolic control
Tam et al. [77]	Longitudinal (3mos)	18 to 80	18	HbA1c	PD, CAL, BOP and PI	Differences in microbial composition and diversity between obese and non-obese groups were statistically significant
Shi et al [203]	Longitudinal (4-7 wks)		31	HbA1c	PD, CAL, BOP and GI	In individuals with periodontitis, the shift in the subgingival microbiome from the healthy state was less prominent in type 2 diabetes compared with healthy subjects
Longo et al [202]	Cross-sectional	Adequate GC: 57.9 ± 8.39, Inadequate: 52.55 ± 5.32	21	HbA1c	PD, CAL, BOP, marginal bleeding	The microbiome of individuals with adequate glycemic control presented higher diversity than individuals with inadequate glycemic control. Inadequate glycemic control favored fermenting species. Higher abundances of anginosus group and Streptococcus agalactiae in diabetes may indicate that subgingival sites can be reservoir of potentially invasive pathogens

Rodríguez-Hernández, et al [206]	Cross-sectional	≥18 (non-T2D) and ≥35 (T2D)	178	HbA1c	PD, CAL, BOP, S and gingival inflammation	The microbial profile of individuals with type 2 diabetes was different from non-type 2 individuals' microbiota
Farina et al [196]	Cross-sectional	≥ 40	12	HbA1c/ med	PD and CAL	The presence of type 2 diabetes and/or periodontitis were associated with a subgingival microbiome decrease in richness and diversity. The presence of type 2 diabetes was not associated with significant differences in the relative abundance of 1 or more species in patients either with or without periodontitis
Salivary microbiome						
Sabharwal et al [199]	Cross-sectional	18 to 65	146	HbA1c	PD, BOP and GI	Oral microbial diversity decreased in diabetes and increased with progression of periodontitis compared with periodontally healthy controls
Yang et al [197]	Cross-sectional			FPG	PD, GI, recession and mobility	Salivary microbes were related to drug treatment and certain pathological changes

Matsha et al [198]	ross-sectional	mean: 47.0 ± 13.0	128	FPG and HbA1c	PD, BOP and CPI	Actinobacteria were significantly more abundant in subjects with diabetes, while Proteobacteria were less abundant. In the presence of gingival bleeding and diabetes, as compared with diabetes without gingival bleeding, Actinobacteria were markedly reduced while Bacteroidetes were more abundant. In contrast, no differences in Actinobacteria or Bacteroidetes abundance were observed between diabetes with and without PD ≥4 mm.

Figure 1

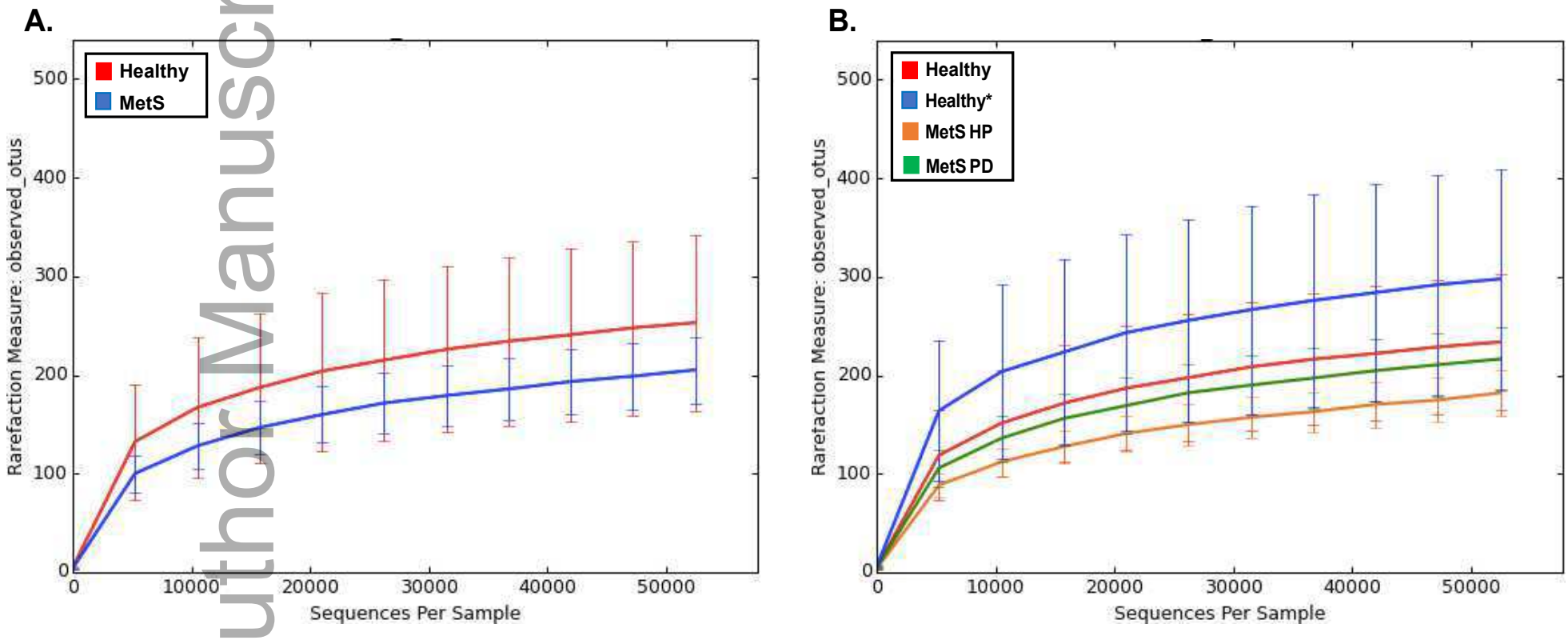

Figure 2

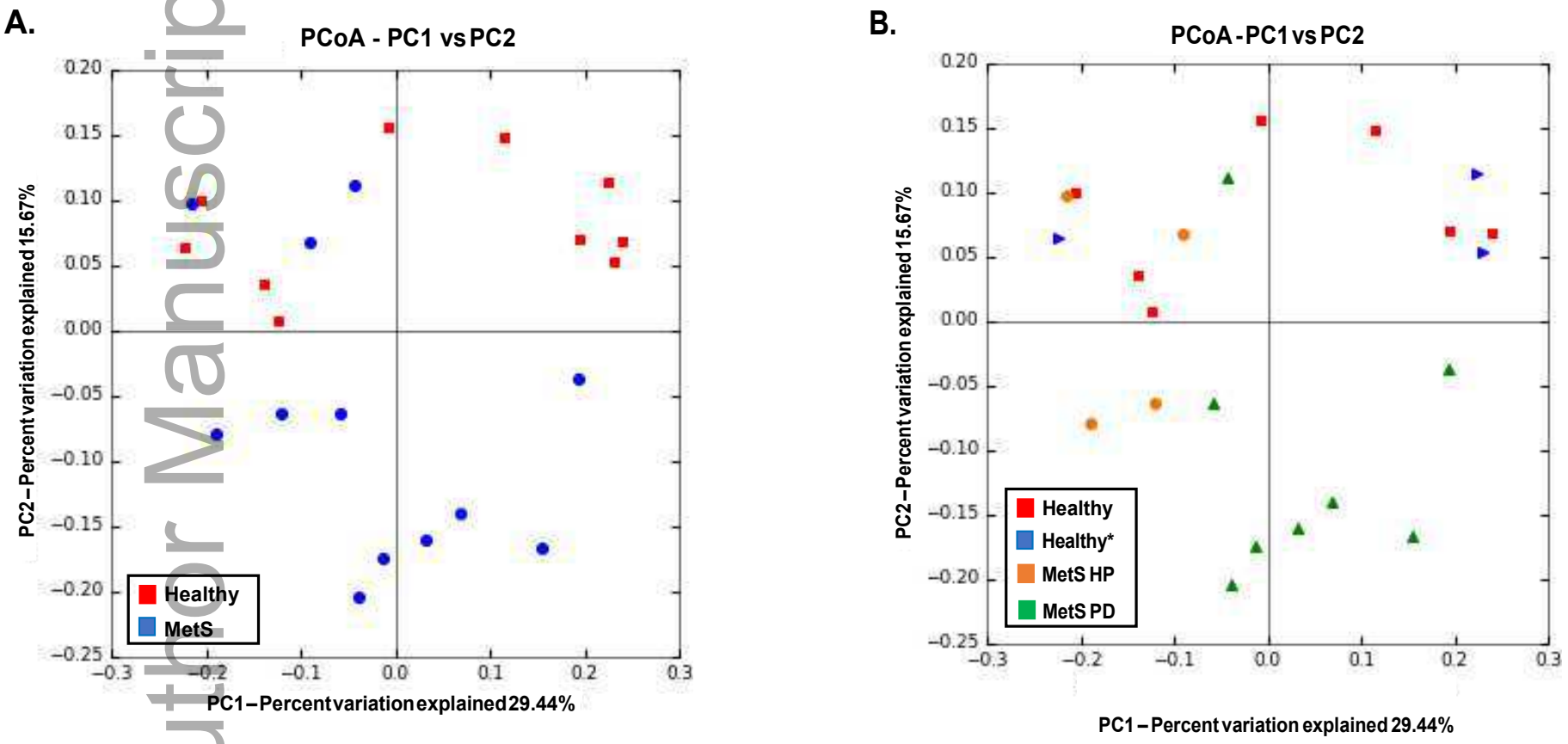

Figure 3