Lipid Peroxidation Is Associated with the Severity of Periodontal Disease and Local Inflammatory Markers in Patients with Type 2 Diabetes

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Context: Periodontitis is the most common lytic disease of bone and is recognized as a common complication of diabetes. Lipid peroxidation (LPO) is increased in diabetes and may be related to modulation of the inflammatory response. LPO levels in patients with diabetes and periodontal disease have not been evaluated.

Objective: The aim of this study was to evaluate the levels of LPO and its correlation with periodontal status and inflammatory cytokines in type 2 diabetic and nondiabetic patients.

Design and Setting: This is a cross-sectional study involving Brazilian patients recruited at the State University of São Paulo.

Patients: The sample comprised 120 patients divided into four groups based upon diabetic and dyslipidemic status: poorly controlled diabetics with dyslipidemia, well-controlled diabetics with dyslipidemia, normoglycemic individuals with dyslipidemia, and healthy individuals.

Main Outcome Measures: Blood analyses were carried out for fasting plasma glucose, glycated hemoglobin, and lipid profile. Periodontal examinations were performed, and gingival crevicular fluid was collected. LPO levels were evaluated by measuring oxidized low-density lipoprotein (ELISA) and malondialdehyde (HPLC). Cytokines were evaluated by the multiplex bead technique.

Results: LPO evaluated by malondialdehyde in plasma and gingival crevicular fluid was significantly increased in diabetes groups. Significant correlations between LPO markers and periodontal parameters indicate a direct relationship between these levels and the severity of inflammation and secretion of inflammatory cytokines, particularly in diabetic patients.

Conclusion: These findings suggest an important association for LPO with the severity of the local inflammatory response to bacteria and the susceptibility to periodontal disease in diabetic patients. (***J Clin Endocrinol Metab* 97: E1353–E1362, 2012)**

Periodontitis is the most common lytic disease of bone and consists of a bacteria-induced inflammatory disorder that involves the structures that support teeth (1, 2). It is a major cause of tooth loss in adults and affects approximately 10–15% of the world's population (3). Periodontitis is initiated by bacteria that induce an inflamma-

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Abbreviations: AGE, Advanced glycation end-products; BMI, body mass index; GCF, gingival crevicular fluid; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPO, lipid peroxidation; MDA, malondialdehyde; ROS, reactive oxygen species.

tory response in the gingiva. It is the bacteria-stimulated inflammation that leads to breakdown of bone that supports the teeth (4–6).

The link between diabetes and oral diseases has not been widely discussed in the medical literature (5). However, it has been reported that periodontitis is one of the first clinical manifestations of diabetes (7) and is recognized as a common complication in diabetic patients (8), particularly in poorly controlled diabetics (9). As an infectious-initiated inflammatory condition, periodontal disease may result in a systemic chronic inflammatory challenge that may have a significant impact on metabolic control in diabetics (10).

Diabetes may contribute to the destructive aspects of the host response that leads to periodontal disease and other inflammatory conditions through the generation of reactive oxygen species (ROS) (11–14). Overproduction of ROS is also thought to contribute to several diabetic complications, including vascular disease (15–17). ROS are unstable, with a short half-life, and thus are difficult to detect (14). Several markers of lipid peroxidation (LPO) have been studied to monitor ROS production. Malondialdehyde (MDA) is one of the most studied end-products of LPO and is often used as a surrogate marker of oxidative stress (18).

This altered oxidative metabolism in diabetes increases LPO; however, there are no studies investigating the link among diabetes, LPO, and inflammation. We therefore hypothesize that diabetes and the associated dyslipidemia characterized by increased LPO can aggravate a microbial-initiated inflammatory process. To this end, we assessed periodontal disease as an indicator of the severity of inflammation in a host-microbe interface and examined the local and systemic levels of LPO in type 2 diabetic patients with dyslipidemia. We also evaluated the correlation of LPO with local signs of inflammation, tissue destruction, and the expression of inflammatory cytokines.

Patients and Methods

Selection criteria and study population

The study was approved by the Ethics in Human Research Committee of the Araraquara School of Dentistry (Universidade Estadual Paulista, Araraquara, Brazil; Protocol number 50/06) and was carried out between May 2009 and November 2010. All volunteers were informed about the aims and methods of this study, and they provided their written consent to participate.

Patients with type 2 diabetes mellitus were recruited from local outpatient diabetic clinics, regional community hospitals, and physicians' offices. Patients without diabetes were consecutively selected from the list of patients who sought treatment at the Araraquara School of Dentistry, Universidade Estadual Paulista, Brazil, at the same time as the type 2 diabetes group.

Patients were initially screened by a questionnaire. Medical records and periodontal clinical examinations of all screened patients were assessed. Basic inclusion criteria were age from 35–60 yr, currently not smoking, and at least 15 natural teeth. All individuals were diagnosed with chronic periodontal disease as defined by the American Academy of Periodontology (19), with local signs of inflammation and tissue destruction (presence of deep periodontal pockets ≥ 6 mm) and loss of the connective tissue attachment of gingiva to teeth (clinical attachment loss ≥ 4 mm) in at least four nonadjacent teeth. Exclusion criteria were history of antibiotic therapy in the previous 3 months and/or nonsteroidal antiinflammatory drug therapy in the previous 6 months, pregnancy, any type of hormonal therapy, and/or periodontal treatment or surgery in the preceding 6 months. Particular care was taken to exclude subjects with a history of anemia, diseases known to influence lipoprotein metabolism, such as hypothyroidism and hypopituitarism, and the use of hypolipidemic drugs such as statins or fibrates.

A power analysis determined that 30 patients in each group would be sufficient to detect a 3-U difference in MDA levels, with 90% power and 95% confidence interval. The population was divided into four groups based upon diabetic and dyslipidemic status: poorly controlled diabetics with dyslipidemia (group 1), well-controlled diabetics with dyslipidemia (group 2), normoglycemic individuals with dyslipidemia (group 3), and healthy individuals (group 4) (Table 1). *Post hoc* power calculation ($P = 0.05$) showed that all comparisons had a power higher than 0.8 except for high-sensitivity C-reactive protein (0.56), triglycerides (0.65), and abdominal circumference (0.18).

Clinical record and physical evaluation

The individuals answered a structured questionnaire, and a trained examiner collected information regarding time since the diagnosis of diabetes, use of hypoglycemic medication, and presence of diabetes-associated complications. Subjects completed a physical examination including measurement of abdominal and hip circumference (centimeters), height (meters), weight (kilograms), and body mass index (BMI).

Parameters of metabolic control and lipoprotein profile

All analyses were performed by the same laboratory, and blood samples were collected after a 12-h overnight fast for the evaluation of fasting plasma glucose by modified Bondar and Mead method, glycated hemoglobin (HbA_{1c}) by enzymatic immunoturbidimetry, high-sensitivity C-reactive protein by the nephelomeric method, total cholesterol (TC), triglycerides, and high-density lipoprotein (HDL) by enzymatic methods. Low-density lipoprotein (LDL) was determined by the Friedewald formula. To avoid the inclusion of individuals with transitory dyslipidemia, the cutoff points used were the highest values according to the National Cholesterol Educational Program Adult Treatment III (total cholesterol ≥240 mg/dl, LDL ≥160 mg/dl, HDL ≤40 mg/dl, and triglycerides ≥200 mg/dl). Metabolic control was considered as adequate when HbA_{1c} was less than 8% and as inadequate when HbA_{1c} was 8% or higher. Insulin resistance was also evaluated for insulin levels by the chemiluminescence method (micro-international units per milliliter) for

TABLE 1. Characteristics of the sample: demographic, physical, laboratory, diabetes data, and cytokines expression in GCF

	Group 1 (DM ≥ 8%/ dyslipidemia)	Group 2 (DM < 8%/ dyslipidemia)	Group 3 (without DM/ with dyslipidemia)	Group 4 (without DM/ without dyslipidemia)
Gender (female/male)	18/12	20/10	17/13	19/11
Age (mean ± SD)	48.0 ± 7.6	50.3 ± 6.7	49.0 ± 7.5	45.9 ± 5.9
Ethnicity (White/Brown/Black)	13/10/7	15/11/4	20/8/2	14/11/5
BMI (kg/m^2)	30.5 ± 5.2[a]	31.4 ± 4.1[a]	28.4 ± 3.8	27.3 ± 6.4
Abdominal circumference (cm)	104.3 ± 14.6[a]	109.3 ± 10.8[a]	98.1 ± 9.9	98.2 ± 16.9
Fasting glucose (mg/dl)	226.6 ± 74.2[b,c]	137.5 ± 41.4[c]	90.0 ± 6.4	90.8 ± 7.3
HbA_{1c} (%)	10.4 ± 1.9[d]	6.6 ± 0.9[e,a]	5.4 ± 0.6	5.1 ± 0.6
Insulin (U/liter)	19.7 ± 20.9[a]	21.1 ± 21.5[a]	12.6 ± 8.5	11.1 ± 12.7
HOMA-IR index	9.03 ± 8.11[c]	6.51 ± 4.98[e,a]	2.82 ± 1.90	1.81 ± 1.01
Total cholesterol (mg/dl)	242.7 ± 37.8[f]	243.4 ± 42.9[f]	246.1 ± 42.3[f]	171.6 ± 18.5
HDL cholesterol (mg/dl)	44.8 ± 9.5	46.1 ± 10.5	50.7 ± 11.1	48.4 ± 12.6
LDL cholesterol (mg/dl)	153.4 ± 37.0[f]	147.3 ± 44.3[f]	156.4 ± 44.1[f]	103.8 ± 17.4
Triglycerides (mg/dl)	216.9 ± 94.6[f]	249.8 ± 104.1[f]	194.1 ± 80.6[a]	93.9 ± 35.9
High-sensitivity C-reactive protein	0.5 ± 0.5	0.7 ± 0.6[a]	0.4 ± 0.4	0.4 ± 0.6
Cytokines [median (25–75th percentile)]				
IL-6 (pg/μl)	0.92 (0.35–1.7)[a]	0.45 (0.05–1.0)	0.55 (0.16–0.78)	0.55 (0.14–0.78)
IL-10 (pg/μl)	0.19 (0.10–0.62)[a]	0.14 (0.01–0.23)	0.15 (0.02–0.30)	0.12 (0.01–0.18)
TNF-α (pg/μl)	0.34 (0.14–1.8)[a]	0.28 (0.06–0.65)	0.25 (0.06–0.75)	0.13 (0.05–0.33)
Time since DM onset (yr) (mean ± SD)	6.2 ± 4.2	5.2 ± 6.6		
Presence of diabetes				
Complications (n)	19[g]	12		
One	9	9		
More than one	10	3		
Medication for DM control (n)				
Hypoglycemic	20	15		
Insulin	1	1		
Hypoglycemic/insulin	8	5		
None	1	9		

DM, Diabetes mellitus; HOMA-IR, homeostasis model assessment of insulin resistance.

[a] $P < 0.05$ in relation to group 4.

[b] $P < 0.05$ in relation to group 2.

[c] $P < 0.0001$ in relation to groups 3 and 4.

[d] $P < 0.0001$ in relation to the other groups.

[e] $P < 0.05$ in relation to group 3.

[f] $P < 0.0001$ in relation to group 4 (Kruskal-Wallis test; $\alpha = 5\%$).

[g] $P < 0.05$ in relation to group 2 (Mann-Whitney test; $\alpha = 5\%$).

calculation of the homeostasis model assessment of insulin resistance index (20).

Periodontal clinical examination

All patients were subjected to a periodontal clinical examination performed in six sites per tooth by a single trained calibrated periodontist ($\kappa = 0.89$) blinded to allocation of the patients into the groups. Periodontal pocket depth, clinical attachment loss, and bleeding on probing were evaluated with a periodontal probe PCPUNC15-6 (Hu-Friedy, Chicago, IL). Severe periodontal disease was defined as the presence of deep periodontal pockets of at least 6 mm with clinical attachment loss of at least 5 mm and bleeding on probing in at least eight sites distributed in different quadrants of the dentition (21).

Gingival crevicular fluid (GCF) collection

GCF is considered as a serum transudate representative of the inflammatory conditions of the marginal gingival tissues because it contains host- and microbial-derived substances, such as inflammatory cells and serum-derived factors. The potential diagnostic value of the GCF is due to the site-specific nature of the sample, and it can be collected noninvasively, serving as an expedient biological source of patient information.

Specimens were collected under a consistent protocol applied by the same periodontist. In each patient, four different healthy sites (probing depth ≤3 mm, no loss of attachment, and no bleeding on probing) and four periodontally diseased sites (probing depth ≥5 mm, loss of attachment ≥4 mm, and with bleeding on probing) were randomly chosen in different nonadjacent teeth for GCF collection. Collection of GCF was performed as previously described (22) with paper strips (Periopaper; Oraflow Inc., Smithtown, NY), and the volume of GCF was measured with a specific instrument (Periotron; Oraflow). The samples from each patient were pooled in two different tubes, representing healthy and diseased sites and centrifuged at $3000 \times g$ for 5 min, and the supernatants were stored at −80 C until the analysis of MDA and cytokines.

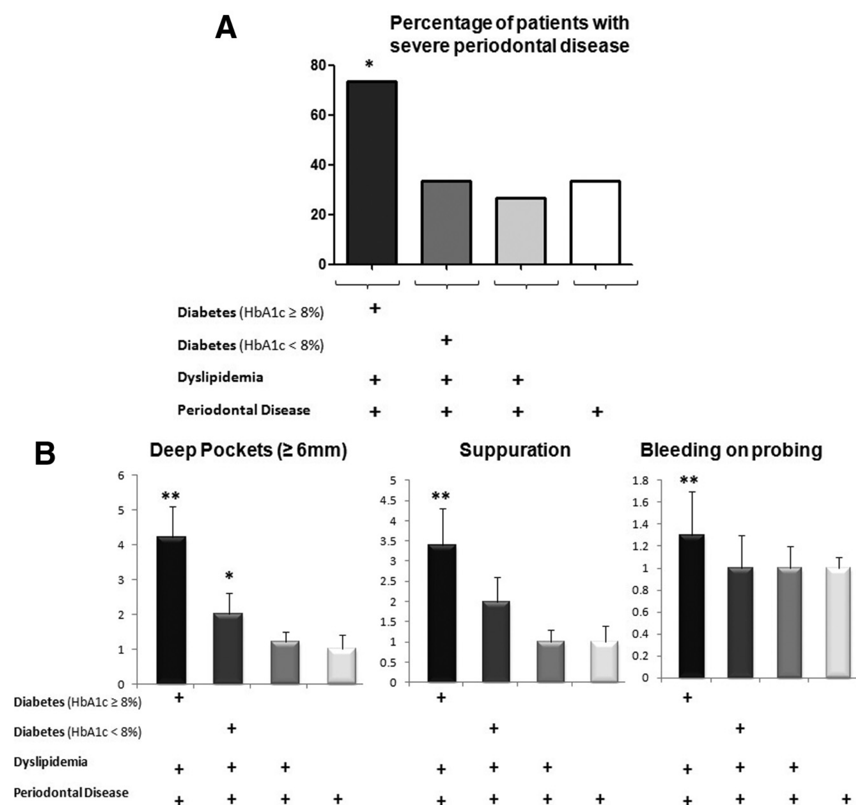

FIG. 1. A, Prevalence of patients with severe periodontal disease. The severity of periodontal disease was assessed by the number of sites with deep periodontal pockets (≥6 mm) and bleeding on probing in at least eight sites. *, $P < 0.05$ in relation to all groups (ANOVA; $\alpha = 5\%$). B, Fold change differences in relative numbers of sites with deep periodontal pockets as defined by depth of at least 6 mm (*left*), suppuration (*middle*), and bleeding on probing (*right*) for all groups. **, $P < 0.01$ in relation to group 4; *, $P < 0.05$ in relation to group 4 (ANOVA; $\alpha = 5\%$).

Statistical analysis

The distribution and normality of the variables were evaluated by the D'Agostino-Pearson test. The differences between the groups for parametric data were evaluated by ANOVA, followed by Bonferroni's test, and nonparametric data were evaluated by the Mann-Whitney *U* test or the Kruskall-Wallis test, followed by Dunn's test. Pearson's or Spearman's partial correlation coefficient was used to investigate the correlations between the variables. To determine the influence of LPO and diabetic status on the severity of periodontal disease and on the expression of local cytokines, separate multivariable logistic and linear regression models were built for each independent variable. Variables found to be associated with the independent and dependent variables, and thus possible confounding factors, were included in the models. Based on previous studies (24, 25), correlation and regression models were adjusted for age, gender, BMI, and triglycerides, considered important as possible confounders in establishing the relationship among the variables of interest. A mediation analysis was performed to assess confounding factors and understand whether one or more variables are mediators to the underlining association between the independent variable and the dependent variable of the interest. The significance level was set at $\alpha = 0.05$. All analyses were carried out with SPSS software, IBM version 19.

Blood collection

Blood samples were collected in tubes with EDTA centrifuged at $3000 \times g$ for 10 min at 4 C. Plasma was obtained and stored at -80 C until the LPO analyses. The assays were performed by professionals blinded to the patients' conditions.

Inflammatory biomarker assays

The concentration of three inflammatory cytokines in GCF, TNF-α, IL-1β, and IL-6, was analyzed by multiplex beads (Bio-Plex system; Bio-Rad Laboratories, Hercules, CA), following the manufacturer's instructions.

LPO assays

Oxidized LDL assay in plasma samples

The levels of oxidized LDL in plasma were analyzed with a commercially available ELISA kit (Mercodia AB, Uppsala, Sweden) following the manufacturer's instructions.

MDA assay

The assessment of MDA in plasma and GCF was determined by HPLC (Shimadzu, Tokyo, Japan) with a reverse-phase HPLC column (C18; 4.6×150 mm) (Phenomenex, Torrance, CA) and compared with MDA standard curves. Plasma and GCF samples were prepared as described previously (23).

Results

Patient screening, selection, and characteristics of the sample

In total, 1728 patients were assessed for eligibility; 537 patients were excluded based on their medical records, 190 because they were using hypolipidemic drugs, 381 after laboratory examinations for anemia, borderline levels of lipid profile, or glycemic levels corresponding to a prediabetic status and particularly during dental evaluation, and 80 declined to participate for personal reasons. Of the remaining 540 patients suitable for study participation, 213 were edentulous or had fewer than 15 teeth, and 207 were excluded after periodontal evaluation for not having chronic periodontitis according to the study criteria. Finally, 120 were identified as meeting the study inclusion criteria; of these, 30 patients were assigned to four different groups.

The general characteristics of the sample are demonstrated in Table 1. There were no age and gender differences between the groups. Diabetics had significantly

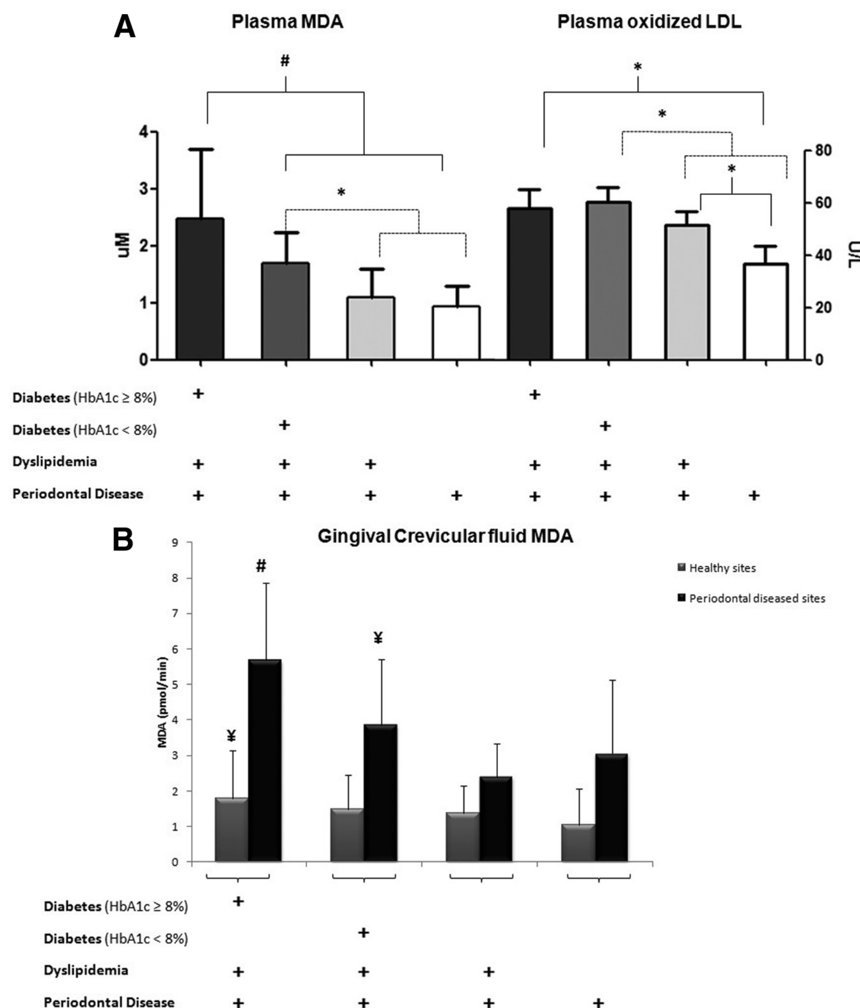

FIG. 2. A, Levels of MDA and oxidized LDL in plasma. #, $P < 0.05$ in relation to all other groups; *, $P < 0.05$ significant difference between the groups (ANOVA; $\alpha = 5\%$). B, Levels of MDA in GCF from healthy and periodontally diseased sites. #, $P < 0.05$ in relation to the other groups; ¥, $P < 0.05$ in relation to the group without diabetes with dyslipidemia and periodontitis (group 3) (ANOVA; $\alpha = 5\%$).

higher values of BMI and abdominal circumference ($P < 0.05$). Glucose metabolism, metabolic control, insulin resistance, lipoprotein profile, and high-sensitivity C-reactive protein for all groups are presented in Table 1. The groups with diabetes had increased levels of fasting glucose, HbA_{1c}, and insulin resistance compared with nondiabetic patients, and groups 1, 2, and 3 all presented dyslipidemia with increased total cholesterol, LDL cholesterol, and triglycerides compared with group 4 (healthy individuals) ($P < 0.05$).

Among diabetic patients, there was no difference in the age of diabetes onset. However, poorly controlled diabetics had more diabetic complications, with the most common complication being retinopathy, followed by nephropathy.

Periodontal evaluation

Periodontal tissue destruction and local inflammation were significantly more severe in diabetics, particularly in group 1 (Fig. 1A and Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org), indicating that poorly controlled diabetes aggravates local host response to microorganisms in the dental biofilm. Relevant clinical periodontal parameters that were significantly different between the groups are illustrated in Fig. 1B in terms of fold change in comparison with group 4 (healthy individuals). Extension of chronic periodontitis was increased 4-fold in poorly controlled diabetics with dyslipidemia and 2-fold in well-controlled diabetics with dyslipidemia. Acute infection, evaluated by the presence of suppuration, and acute inflammation, evaluated by bleeding on probing, were increased 3.5- and 1.3-fold, respectively, in poorly controlled diabetics with dyslipidemia.

Immunological analysis

To assess local inflammation, the expression of TNF-α, IL-6, and IL-10 was measured in GCF. The cytokine expression was normalized to the volume of GCF collected and expressed in concentration (picograms per microliter). All cytokines were increased in the GCF of diabetic patients, being significant in the group with poorly controlled diabetes and dyslipidemia ($P < 0.05$), indicating that diabetes aggravates the local inflammatory response to microorganisms in the dental biofilm (Table 1).

LPO levels

Plasma MDA and oxidized LDL

Plasma MDA levels were slightly increased in dyslipidemic patients, but the presence of diabetes resulted in significantly increased systemic oxidative metabolism ($P < 0.05$) (Fig. 2A). However, the most increased levels of plasma MDA were observed in poorly controlled diabetics with dyslipidemia who had about 2.5 times higher MDA levels when compared with those in the nondiabetic groups and approximately 1.5 times higher MDA levels when compared with well-controlled diabetics with dyslipidemia. Oxidized LDL was increased in all groups with dyslipidemia (groups 1, 2, and 3) (Fig. 2A).

Patients with diabetes presented significantly higher levels of MDA in the GCF (representative of local LPO)

($P < 0.05$) (Fig. 2B). The mean levels of MDA in the diseased sites of the poorly controlled diabetics were 2.3 times greater than in the normoglycemic with dyslipidemia group ($P < 0.001$), approximately 1.8 times higher than in the healthy group ($P < 0.05$), and 1.6 times higher in the well-controlled diabetics with dyslipidemia ($P < 0.05$) (Fig. 2B). Moreover, GCF from healthy sites in both groups of diabetics presented higher levels of MDA in comparison with GCF from healthy sites in nondiabetics.

Correlations

There was no independent association between lipoprotein profile and a greater severity of periodontal disease in individuals with periodontitis (Table 2). Diabetes was significantly associated with LPO markers, and this was the strongest positive correlation observed (r = 0.65; $P < 0.0001$). Positive associations between systemic levels of LPO and the levels of inflammatory cytokines in the GCF (Table 2) support a role for increased oxidative metabolism in the aggravation of inflammation.

Considering the correlation between systemic levels of LPO, a significant intermediate correlation between MDA in plasma and oxidized LDL (r = 0.460; $P < 0.01$) was observed. In addition, there were significant but weak correlations between plasma and local levels of MDA (r = 0.33; $P < 0.01$) and between plasma oxidized LDL and local levels of MDA (r = 0.32; $P < 0.01$).

TABLE 2. Adjusted correlation between LPO and diabetes status, lipoprotein profile, periodontal parameters, and GCF cytokines

Parameter	Plasma MDA[a]	GCF MDA periodontally diseased sites[a]	Oxidized LDL[a]
Diabetic status			
Fasting glucose (mg/dl)	0.66[c]	0.39[c]	0.35[c]
HbA_{1c}	0.65[c]	0.33[c]	0.43[c]
Lipoprotein profile (mg/dl)			
Total cholesterol	0.24[b]	0.16	0.60[c]
LDL cholesterol	0.23[b]	0.14	0.60[c]
Triglycerides	0.22[b]	−0.03	0.53[c]
Clinical periodontal parameters			
% sites with bleeding on probing	0.30[b]	0.34[b]	0.12
% sites with probing depth ≥ 6 mm	0.25[b]	0.34[b]	0.13
% sites with suppuration	0.32[b]	0.162	0.32[b]
Local inflammatory markers in GCF (pg/μl)			
IL-6	0.30[b]	0.28[b]	0.14
IL-10	0.28[b]	0.29[b]	0.25[b]
TNF-α	0.41[b]	0.30[b]	0.25[b]

Pearson's or Spearman's correlation coefficients are shown (r; α = 5%).

[a] Adjusted for age, gender, and BMI.

[b] $P < 0.05$.

[c] $P < 0.0001$.

Regressions

Regression analyses were performed to obtain insight into the role of diabetes and LPO in local inflammatory responses. LPO markers were directly related to the severity of local inflammation, represented by all clinical measures of periodontal disease (except for bleeding on probing) after adjustment for age, gender, BMI, and triglycerides. Diabetes was also directly related to the severity of local inflammation (Table 3). Table 4 represents the results from a multivariate logistic regression analysis using separate models and taking into account the effects of age, gender, BMI, and triglycerides. There was an approximate 1.81- and 2-fold increase in the odds of having severe bleeding on probing and deep periodontal pockets, respectively, among those patients who have higher plasma levels of MDA. This finding suggests a link between periodontal inflammation/destruction and plasma MDA levels.

A potential insight into this linkage is the demonstration of a positive relationship among periodontal inflammation, diabetes, and LPO by a regression analysis using the levels of inflammatory cytokines in the GCF as the dependent variable (Tables 5 and 6). In particular, plasma levels of MDA were correlated with a more than 2-fold increase in the odds ratio of high IL-6 and TNF-α levels in GCF. Thus, the mediation analysis showed that independent of the effect of the factor fasting glucose (*i.e.* the mediator), the independent association between LPO and periodontal measurement remained statistically significant (Supplemental Table 2).

Discussion

This is the first study to investigate the impact of systemic and local LPO levels on periodontal inflammation and periodontal disease in patients with and without diabetes and dyslipidemia. The results offer new insight into the association of diabetes and dyslipidemia that, by generating LPO products, can increase the severity of a microbial-induced and maintained chronic inflammatory process, represented in this study by a greater severity of periodontal disease.

Diabetes is associated with greater extension and severity of periodontal disease. The biological mechanisms for this association include persistence of the inflamma-

TABLE 3. Multiple-regression model with the influence of LPO and diabetes status on parameters of severe periodontal disease

	Bleeding on probing (% sites)[a]			Probing depth ≥6 mm (% sites)[a]		
	β	SE	95% CI	β	SE	95% CI
LPO parameter						
Plasma MDA	4.71[b]	1.43	1.88–7.53	5.08[b]	1.19	2.72–7.44
GCF MDA in PD	2.32[b]	0.61	1.11–3.53	2.13[b]	0.52	1.10–3.16
Plasma oxidized LDL	0.23	0.15	−0.07–0.53	0.40[b]	0.13	0.15–0.69
Diabetes status						
Fasting glucose	0.09[c]	0.02	0.05–0.13	0.08[c]	0.02	0.04–0.11
HbA_{1c}	2.52[c]	0.54	1.44–3.59	2.71[c]	0.44	1.84–3.58
HOMA-IR	0.38[b]	0.18	0.02–0.73	0.16	0.16	−0.16–0.47

β, Partial standardized regression coefficient; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; PD, periodontally diseased sites.

[a] Adjusted for age, gender, BMI, and triglycerides.

[b] $P < 0.05$.

[c] $P < 0.0001$.

tory infiltrate (26), impaired wound healing (27, 28), increased periodontal bone loss as result of enhanced resorption and diminished bone formation (29), and increased oxidative metabolism (30, 31). In addition, diabetes is commonly associated with altered lipid metabolism, and diabetes-induced dyslipidemia (32) predisposes the patient to a hyperinflammatory status by modulating the function and activity of macrophages (33).

Our results demonstrate an increased LPO in patients with diabetes, which is particularly strong in patients with elevated HbA_{1c}, total cholesterol, LDL cholesterol, and triglycerides. In addition, there was also a positive correlation between local and systemic markers of LPO and periodontal clinical parameters, indicating that the higher the level of LPO, the more severe the periodontal tissue inflammation. Within this context, it can be suggested that LPO has an important role in the severity of the inflammatory process that was observed by loss of attachment, deep periodontal pockets (≥6 mm), bleeding on probing, and suppuration.

There was no direct association between the severity of inflammatory periodontal disease parameters and the lipid profile, a finding that is supported by two studies (31, 34) but is in contrast to a previous clinical study indicating that dyslipidemia is associated with a worse periodontal status (24). In fact, when diabetes was coupled with dyslipidemia, compared with individuals in the normoglycemic with dyslipidemia group, increased severity of inflammation was observed, represented by greater periodontal tissue damage and local production of inflammatory cytokines, particularly in poorly controlled diabetics. It is relevant to state that it is possible that poor control of diabetes may affect general as well as oral health, which may be aggravated by poor adherence to therapy. Nevertheless, our findings suggest that altered oxidative metabolism in diabetes increases LPO. In this

TABLE 4. Multivariate logistic regression model with the influence of LPO and diabetes status on parameters of severe periodontal disease

	Bleeding on probing (% sites)[a]			Probing depth ≥6 mm (% sites)[a]		
	OR	*P* value	95% CI	OR	*P* value	95% CI
LPO parameter						
Plasma MDA	1.81[b]	0.02	1.12–2.92	2.01[b]	0.004	1.25–3.23
GCF MDA in PD	1.36[b]	0.003	1.11–1.65	1.31[b]	0.009	1.07–1.60
Plasma oxidized LDL	1.03	0.13	0.99–1.08	1.07[b]	0.007	1.02–1.13
Diabetes status						
Fasting glucose	1.01[b]	0.005	1.00–1.02	1.01[b]	0.006	1.0–1.02
HbA_{1c}	1.36[b]	0.002	1.12–1.66	1.46[c]	0.0001	1.21–1.77
HOMA-IR	1.08	0.08	1.00–1.17	1.0	0.88	0.95–1.06

CI, Confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratio; PD, periodontally diseased sites.

[a] Adjusted for age, gender, BMI, and triglycerides.

[b] $P < 0.05$.

[c] $P < 0.0001$.

TABLE 5. Multiple regression model with the influence of LPO and diabetes status on the expression of local cytokines

	IL-6[a]			TNF-α[a]			IL-10[a]		
	β	SE	95% CI	β	SE	95% CI	β	SE	95% CI
LPO parameter									
Plasma MDA	0.32[c]	0.06	0.01–0.34	0.44[c]	0.06	0.2–0.45	0.29[b]	0.17	0.02–0.1
GCF MDA in PD	0.251[b]	0.27	0.20–0.13	0.31[b]	0.03	0.04–0.16	0.25[b]	0.01	0.010–0.04
Plasma oxidized LDL	0.25[b]	0.01	0.01–0.03	0.40[c]	0.007	0.01–0.04	0.31[b]	0.01	0.002–0.01
Diabetes status									
Fasting glucose	0.30[b]	0.13	0.12–0.63	0.37[c]	0.01	0.18–0.5	0.22[b]	0.04	0.01–0.18
HbA_{1c}	0.38[c]	0.13	0.31–0.82	0.30[b]	0.15	0.18–0.75	0.32[b]	0.04	0.07–0.30
HOMA-IR	0.35[c]	0.01	0.01–0.05	0.33[b]	0.15	0.18–0.73	0.33[c]	0.01	0.0–0.01

β, Partial standardized regression coefficient; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; PD, periodontally diseased sites.

[a] Adjusted for age, gender, BMI, and triglycerides.

[b] $P < 0.05$.

[c] $P < 0.0001$.

sense, dyslipidemia may have a relevant impact on the inflammatory response in the presence of diabetes-increased oxidative metabolism.

LPO can contribute to injury of the host tissue by several mechanisms, including DNA damage, oxidation of enzymes, stimulation of proinflammatory cytokines (15), degenerative changes, and the presence of superoxide anions in areas adjacent to osteoclasts, leading to bone resorption (35). Several sources can generate LPO products, such as lipids in plasma and in cell membranes and the accumulation of advanced glycation end-products (AGE) (36) in various organs, including the gingiva (30). AGE binding with their pattern-recognition receptors can actively participate in inflammation and immune responses, thereby causing the activation of a range of inflammatory and fibrotic pathways, triggering the host response and altering the structure and function of cellular membranes (30, 36), causing the overproduction of ROS (37). In poorly controlled diabetics, there is a nonspecific glycation of lipids and proteins. LDL, for example, can be modified by glycation of the apolipoprotein B-100, resulting in glycated LDL. However, increased levels of glycation of lipids and LPO products are present even in well-controlled diabetics.

Increased oxidative metabolism and accumulation of AGE are involved in the pathogenesis of chronic inflammatory conditions commonly observed in diabetic patients, such as atherosclerosis (15), micro- and macrovascular alterations (16, 17), and periodontitis (11, 13, 14, 38). Some mechanisms can be suggested to elucidate the possible association of LPO with the severity of an inflammatory condition in diabetic and dyslipidemic patients. In the course of an infectious/inflammatory condition, the overproduction of LPO can enhance the inflammatory reaction during the activation of neutrophils and macrophages by pathogens or their products in the tissue (14), releasing proinflammatory cytokines (1). In addition, lipid peroxides can also activate transcription fac-

TABLE 6. Multivariate logistic regression model of the effects of LPO and diabetes status on the expression of local cytokines

	IL-6[a]			TNF-α[a]			IL-10[a]		
	OR	*P* value	95% CI	OR	*P* value	95% CI	OR	*P* value	95% CI
LPO parameter									
Plasma MDA	2.06[b]	0.003	1.28–3.30	2.24[b]	0.002	1.35–3.71	1.17	0.45	0.78–1.77
GCF MDA in PD	1.32[b]	0.004	1.09–1.60	1.21[b]	0.049	1.01–1.48	1.25[b]	0.02	1.04–1.51
Plasma oxidized LDL	1.04	0.06	1.00–1.09	1.10[b]	0.001	1.04–1.17	1.04[b]	0.04	1.01–1.1
Diabetes status									
Fasting glucose	1.01[b]	0.003	1.00–1.02	1.01[b]	0.0001	1.01–1.02	1.01	0.10	0.1–1.0
HbA_{1c}	1.31[b]	0.003	1.09–1.56	1.28[b]	0.005	1.08–1.52	1.11	0.21	0.95–1.3
HOMA-IR	1.07	0.07	1.00–1.16	1.08[b]	0.04	1.04–1.15	1.03	0.22	0.98–1.0

CI, Confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratio; PD, periodontally diseased sites.

[a] Adjusted for age, gender, BMI, and triglycerides.

[b] $P < 0.05$.

[c] $P < 0.0001$.

tors such as nuclear factor-κB (37). nuclear factor-κB can be activated by oxidative stress (37), stimulate the release of proinflammatory and apoptotic mediators, and enhance endothelial expression of vascular cell adhesion molecules (15), resulting in a cascade of cell activation and diffuse inflammation.

Previous studies reported that oxidative stress induced by diabetic status triggers the release of cytokines and enhances their activation (39) and that diabetics, especially those with poor metabolic control, have increased levels of inflammatory cytokines in plasma and in GCF (40). Our findings of significant correlations between plasma levels of MDA and the expression of local (GCF) cytokines, especially TNF-α, have not been reported previously and suggest that systemic and local LPO can increase the expression of inflammatory markers in the inflamed site.

It is important to note that our findings do not address specific mechanisms for the associations observed, and prospective and interventional studies are necessary to confirm a causal relationship. Our results do provide novel data on the association between LPO and the magnitude of a microbial-induced inflammatory process in diabetic and dyslipidemic patients. It is relevant to state that diabetes and periodontal disease have a bidirectional relationship (10, 40), and for this reason, the influence of periodontal disease on diabetes and LPO levels should also be further evaluated.

Thus, the results of our study suggest that LPO is positively correlated with the severity of the inflammatory response. If this association is proved causal by experimental studies, targeting the oxidative metabolism and its downstream effects may be an alternative therapeutic approach to manage diabetes-associated inflammatory complications.

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