

# Gingival Crevicular Fluid Levels of Interleukin-1 $\beta$ and Glycemic Control in Patients With Chronic Periodontitis and Type 2 Diabetes

Steven P. Engebretson,\* Judith Hey-Hadavi,\* Fernando J. Ehrhardt,\* Dan Hsu,\* Romi S. Celenti,\* John T. Grbic,\* and Ira B. Lamster\*

**Background:** Patients with diabetes have increased incidence and severity of periodontal disease not accounted for by differences in the subgingival microbial infection. Poor glycemic control has been consistently associated with periodontal disease severity. Also, recent evidence suggests that hyperglycemia may induce inflammatory cytokine production. Few studies, however, have examined local biochemical measures of periodontal inflammation in patients with type 2 diabetes. The aim of this study was to determine whether glycemic control was related to gingival crevicular fluid (GCF) levels of interleukin-1 $\beta$  (IL-1 $\beta$ ).

**Methods:** GCF samples were collected from 45 patients with type 2 diabetes and untreated chronic periodontitis. Plaque index (PI), bleeding on probing (BOP), probing depth (PD), and attachment level (AL) were recorded at six sites per tooth. IL-1 $\beta$  levels were determined from individual GCF samples by enzyme-linked immunoabsorbent assay (ELISA). Individual site and mean patient values were calculated. Glycated hemoglobin (HbA<sub>1c</sub>) levels were measured from anticoagulated whole blood using an automated affinity chromatography system. Serum glucose was also determined.

**Results:** Clinical periodontal measures (PD, AL, BOP) and measures of glycemic control (HbA<sub>1c</sub>, random glucose) were significantly correlated with GCF IL-1 $\beta$ . Patients with greater than 8% HbA<sub>1c</sub> had significantly higher mean GCF IL-1 $\beta$  levels than patients with less than 8% HbA<sub>1c</sub>. In a multivariate model adjusting for age, gender, PD, AL, BOP, and PI, HbA<sub>1c</sub> and random glucose were independent predictors of high GCF IL-1 $\beta$ .

**Conclusions:** Poor glycemic control is associated with elevated GCF IL-1 $\beta$ . These data are consistent with the hypothesis that hyperglycemia contributes to an heightened inflammatory response, and suggests a mechanism to account for the association between poor glycemic control and periodontal destruction. *J Periodontol 2004;75:1203-1208.*

## KEY WORDS

Diabetes mellitus, non-insulin dependent; gingival crevicular fluid/chemistry; hemoglobin A, glycosylated; interleukin-1; periodontitis/etiology.

Patients with type 2 diabetes have greater incidence and severity of periodontal disease than those without diabetes.<sup>1-4</sup> Hyperglycemia has been thought to play a role in periodontal disease incidence and prevalence.<sup>1,5,6</sup> Patients with relatively good glycemic control are less prone to periodontal destruction in longitudinal studies.<sup>7</sup> Recently an analysis of the National Health and Nutrition Examination Survey (NHANES) III data showed that poor glycemic control in type 2 diabetes patients was associated with greater severity of periodontitis.<sup>8</sup> Early efforts to determine mechanisms that account for increased occurrence of periodontitis in diabetes mellitus focused on differences in the periodontal microflora. One report suggested that hyperglycemia was associated with an altered subgingival microbiota.<sup>9</sup> However, subsequent studies failed to identify differences in specific periodontal pathogens in subjects with diabetes when compared with non-diabetic subjects.<sup>10,11</sup> Studies have since focused on the host response as a means of characterizing the periodontal disease of diabetic individuals. In that regard, IL-1 is biologically active in low concentrations and is a potent bone resorptive cytokine.<sup>12</sup> Elevated levels of IL-1 in the gingival crevicular fluid<sup>13-17</sup> and gingival tissues<sup>18,19</sup> have been associated with chronic periodontitis. Several clinical studies have demonstrated elevated inflammatory mediators,

\* Columbia University School of Dental & Oral Surgery, Division of Periodontics, New York, NY.

including IL-1, in the GCF of patients with type 1 diabetes compared with non-diabetic subjects.<sup>20-23</sup> It is plausible to suggest that elevated IL-1 expression in patients with diabetes may be related to the increased severity of periodontitis observed in these patients. However, there has been only limited investigation of the relationship of glycemic control to the level of inflammatory mediators associated with periodontitis seen in patients with diabetes.

The purpose of this study was to investigate the effects of periodontal disease severity and glycemic control on levels of IL-1 $\beta$  in GCF of patients with type 2 diabetes.

## MATERIALS AND METHODS

### Study Population

The patient population of this study consisted of 45 adult patients recruited during routine medical care visits from an outpatient diabetes center (Naomi Berrie Diabetes Center, Columbia University Health Sciences Center, New York, New York). All subjects were diagnosed with type 2 diabetes at least 6 months previously using standard WHO criteria, and were being treated with stable doses of oral hypoglycemic agents and/or insulin. For entry to the study, patients also required a diagnosis of chronic periodontitis for which non-surgical treatment was indicated. An intraoral radiographic series was used to confirm this diagnosis. Exclusion criteria included pregnancy or lactation, HIV infection, bleeding disorders, or immunosuppressive chemotherapy.

This study was approved by the Institutional Review Board at Columbia University Health Sciences Center, and written informed consent was obtained from all patients.

### GCF Collection

The clinical evaluation was preceded by collection of GCF as previously described<sup>24,25</sup> from the mesio-lingual and mesio-buccal surfaces of the first molar tooth in each quadrant. Where the first molar was absent, the second molar was sampled. If both the first and second molar were missing, the second premolar was sampled. If there were no posterior teeth in a quadrant, no sample was taken from that quadrant. Briefly, teeth were air-dried and isolated with cotton rolls, supragingival plaque was gently removed, and GCF was sampled with precut methylcellulose filter paper strips for 30 seconds. Strips were measured for fluid volume<sup>†</sup> then removed to separate microcentrifuge tubes containing 50  $\mu$ l phosphate buffered saline-Tween 20. The tubes were stored at  $-20^{\circ}$ C until eluted (maximum 48 hours). Following elution, each GCF sample was analyzed separately.

### Clinical Measures

Clinical data included probing depth (PD), attachment level (AL), plaque index (PI), and bleeding on probing (BOP) and were collected at six sites per tooth. PD man-

ually determined was defined as the distance in millimeters from the coronal-most margin of the free gingiva to the most apical penetration of the North Carolina probe. AL was defined as the distance from the cemento-enamel junction to the most apical penetration of the probe. The presence of supragingival plaque was recorded dichotomously during PD measurements. Bleeding on probing within 20 seconds was recorded dichotomously.

### Analysis of IL-1 $\beta$ in GCF

GCF samples were analyzed for IL-1 $\beta$  using a commercially available enzyme-linked immunosorbent assay.<sup>‡</sup> This assay is a sandwich ELISA and was performed according to manufacturer's instructions using human recombinant standards. Results are reported as total amount of IL-1 $\beta$  (in pg  $\pm$  SD) per 30 second sample as described previously<sup>19</sup> and expressed as pg/sample. Where IL-1 levels are compared between patient groups, mean whole mouth values are used.

### HbA1c

Glycated hemoglobin (HbA1c) levels were measured from freshly drawn anticoagulated whole blood using an automated affinity chromatography system.<sup>§</sup> Serum glucose was determined by the glucose oxidase method.<sup>||</sup> Since subjects were seen at various times throughout the day, these are random glucose samples.

### Statistical Analysis

Since GCF IL-1 $\beta$  it is not normally distributed, Spearman correlation was used to calculate correlation coefficients between mean patient GCF IL-1 $\beta$  and PD, AL, BOP, plaque, HbA1c, and serum glucose. In order to compare the GCF IL-1 $\beta$  levels of groups of different glycemic control, we dichotomized subjects based on having an HbA1c value  $>8\%$  or  $\leq 8\%$  and used the Mann-Whitney U test to compare non-normally distributed continuous variables. The association of GCF IL-1 $\beta$  with hyperglycemia was also evaluated by multivariate logistic regression analysis. Adjustments were made for clinical periodontal parameters and other pertinent variables (age, gender, HbA1c). The dependent variable IL-1 $\beta$  was categorized as greater than or equal to the median value ( $\text{Ln}[\text{IL-1}\beta] > 50\%$ ). Adjusted odds ratios and 95% confidence intervals were calculated. All analyses were performed using a statistical software package.<sup>¶</sup>

## RESULTS

### Study Population

The patients were approximately evenly divided between male and female and tended to be older adults with a

<sup>†</sup> Periotron 6000, Interstate Drug Exchange, Amityville, NY.

<sup>‡</sup> Multikine Kit, Cistrion Biotechnology, Pine Brook, NJ.

<sup>§</sup> BioRad Micromat II, Hercules, CA.

<sup>||</sup> Roche Diagnostics, Basel, Switzerland.

<sup>¶</sup> Statview Version 5.0, SAS Institute, Cary, NC.

**Table 1.**  
**Study Population Characteristics (N = 45)**

	Mean ± SD	Range
Age (years)	54.0 ± 9.8	32-69
Gender (% female)	55%	
Mean PD (mm)	3.35 ± 0.79	2.27-5.31
Sampled site PD (mm)	4.27 ± 0.97	2.63-7.63
Mean AL (mm)	4.05 ± 1.27	2.48-7.62
Plaque (%)	77.2 ± 23	14.6-100
BOP (%)	55.9 ± 27.1	12.8-100
HbA1c (%)	7.82 ± 1.96	4.7-12.0
Years since diagnosis of diabetes	9.08 ± 9.49	0.5-48.0

mean age of 54. Other demographic and clinical parameters (mean ± standard deviation) are provided in Table 1.

### GCF IL-1 $\beta$

A total of 176 GCF samples were collected. Our study design called for the collection of samples from each of four first molar sites. Because of missing teeth, the number of samples collected were: four samples from 42 subjects, three samples from two subjects, and two samples from one subject. GCF levels of IL-1 $\beta$  demonstrated a significant positive correlation with mean probing depth ( $r = 0.613$ ,  $P < 0.0001$ ), mean clinical attachment loss ( $r = 0.587$ ,  $P = 0.0001$ ), percentage of sites exhibiting bleeding on probing (%BOP) ( $r = 0.424$ ,  $P = 0.006$ ), %HbA1c ( $r = 0.371$ ,  $P = 0.01$ ), and random serum glucose ( $r = 0.490$ ,  $P = 0.002$ ), but not percentage of sites exhibiting plaque (%plaque) ( $r = 0.231$ ,  $P = 0.14$ ). We then compared patients according to their relative glycemic control using the Mann-Whitney U test. Table 2 illustrates the results when HbA1c was categorized as  $\leq 8\%$  or  $> 8\%$ . Total amounts of GCF IL-1 $\beta$  were significantly higher in those with greater than 8% HbA1c ( $P = 0.01$ ), while only a trend ( $P = 0.14$ ) was seen for GCF concentrations of GCF IL-1 $\beta$ .

In a multivariate model, using above median GCF IL-1 $\beta$  as the dependent variable, HbA1c remained a significant predictor of GCF IL-1 $\beta$  after adjusting for AL, BOP, PI, age, and gender (adjusted odds ratio [OR], 2.19; 95% confidence interval [CI], 1.24 to 3.87) (Table 3). When probing depth of the sampled site was used as an independent variable in place of AL, HbA1c remained a significant predictor of GCF IL-1 $\beta$  (adjusted OR, 1.79; 95% CI, 1.09 to 2.94). Random serum glucose was substituted for HbA1c in the multivariate

**Table 2.**  
**A Comparison of Median (interquartile range) Patient GCF IL-1 $\beta$  Levels in Total Amount (pg/sample) and Concentration (pg/ $\mu$ l), by Glycemic Control Status**

	GCF IL-1 $\beta$ (pg/sample)	GCF IL-1 $\beta$ (pg/ $\mu$ l)
All subjects	70.2 (33.2-107.2)	67.0 (40.7-93.3)
HbA1c $\leq 8\%$	49.1 (18.3-80)	55.8 (32.3-79.3)
HbA1c $> 8\%$	89.0 (61.7-116.3)*	77.6 (54.7-100.5)

\*  $P = 0.01$ .

**Table 3.**  
**Multivariate Model Using Median GCF IL-1 $\beta$  (Table 2) as the Dependent Variable (Ln[IL-1]  $> 50\%$ )**

	Coefficient	Odds Ratio	95% CI	P Value
Mean AL (mm)	2.23	4.47	1.21-16.53	0.0246
BOP (%)	-0.997	0.09	0.001-9.65	0.3170
Plaque (%)	0.959	7.6	0.12-471.25	0.3369
HbA1c	2.69	2.19	1.24-3.87	0.0071
Age	0.170	1.008	0.919-1.106	0.8586
Female gender	0.700	1.78	0.352-9.031	0.4846

model and likewise was associated with above median GCF IL-1 $\beta$  (adjusted OR, 1.02; 95% CI, 1.007 to 1.039), but not as strongly as HbA1c.

### DISCUSSION

Our study found that hyperglycemia is independently associated with high GCF levels of IL-1 $\beta$  in patients with type 2 diabetes and chronic periodontitis. Previous studies<sup>14,16,17,19</sup> have shown that GCF IL-1 $\beta$  is strongly related to periodontal clinical parameters, particularly probing depth of the sampled site. Nonetheless, in this study even when including periodontal clinical measures in the multivariate model, hyperglycemia was a significant contributor to GCF IL-1 $\beta$ . Also, glycemia levels were positively associated with GCF IL-1 $\beta$  regardless of whether the glucose measurement reflected the actual blood level at the time of GCF sampling (random serum glucose) or the longer-term measure of glycemic control, HbA1c. While both measures were significant predictors of GCF IL-1 $\beta$ ,

HbA1c was a much stronger predictor of GCF IL-1 $\beta$  than random glucose. These results suggest an altered host response to microbial infection in diabetic subjects mediated, at least in part, by hyperglycemia.

Hyperglycemia has been thought to play a role in periodontal disease incidence and prevalence.<sup>1,5,6</sup> It has been found that patients with relatively good glycemic control are less prone to periodontal destruction in longitudinal studies.<sup>7</sup> Recent analysis of the NHANES III data showed that worse glycemic control in type 2 diabetes patients was associated with more severe periodontitis.<sup>8</sup> The findings of the current study are consistent with these reports.

Our finding of elevated IL-1 $\beta$  in the GCF of patients with type 2 diabetes and poor glycemic control is consistent with a previous study of type 1 diabetes by Salvi et al.<sup>21</sup> In that study, significantly elevated IL-1 $\beta$  and prostaglandin E<sub>2</sub> levels were found in the GCF of patients with diabetes compared with non-diabetic control subjects regardless of periodontal disease severity. However, that study did not find a correlation between GCF IL-1 and HbA1c, which is inconsistent with our study. Our results are also consistent with those of Cutler et al.<sup>23</sup> who showed a trend for increased GCF IL-1 $\beta$  with diminished glycemic control in subjects with diabetes. That study also provided some evidence that systemic levels of triglycerides may be associated with elevated GCF IL-1 $\beta$ . Losche et al.<sup>5</sup> also found elevated circulating lipid levels in subjects with periodontitis, which is consistent with the findings of Cutler et al.<sup>23</sup> Our study did not examine the role of hyperlipidemia in GCF IL-1 $\beta$  levels; hence, we could not evaluate the relative contribution of lipids in our multivariate model. Certainly larger studies are needed to address both the role of hyperlipidemia and hyperglycemia in gingival inflammation.

A hyperresponsive monocytic trait has been proposed by Salvi et al.<sup>21,22</sup> as an explanation for elevated cytokine levels found in type 1 diabetes patients. This hypothesis holds that a heightened inflammatory response, either as a result of gene polymorphism or hyperglycemia, causes a heightened monocyte release of inflammatory mediators. While it is possible that genetic polymorphisms in the production of proinflammatory cytokines contribute to diabetes-related periodontitis, to our knowledge no specific gene or genotype has been tested thus far. Our study supports the concept that hyperglycemia influences elevated inflammatory mediators in GCF and does not rule out the possibility that a hyperresponsive monocyte trait is also present in type 2 diabetes patients.

An alternative hypothesis to the hyperresponsive monocytic trait theory is that elevated glucose levels may directly or indirectly lead to a heightened inflammatory response. Acute and chronic hyperglycemic models have been studied. Ex vivo stimulation of mono-

cytes of human volunteers with solutions high in glucose resulted in increased tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-6.<sup>26</sup> A recent human experimental study by Esposito et al.<sup>27</sup> demonstrated that circulating levels of TNF- $\alpha$  and IL-6 became elevated acutely in response to a bolus of glucose in healthy and impaired glucose tolerance individuals. These researchers concluded that acute hyperglycemia raised circulating cytokines through an oxidative mechanism, since an infusion of the antioxidant glutathione resulted in no increase of cytokines following a glucose bolus administration. Hence, there is evidence for increased inflammatory mediator activity in the presence of acute hyperglycemia. In our study, random glucose was significantly associated with elevated GCF IL-1 $\beta$ . This finding may indicate that the hyperglycemic excursions in serum glucose frequently encountered by patients with diabetes may contribute acutely to GCF IL-1 $\beta$  levels.

The effects of chronic hyperglycemia on inflammatory mediators are also well documented. Clinical and animal studies have shown that chronic hyperglycemia is associated with increased inflammatory mediator levels.<sup>28-35</sup> A mechanism to account for this finding involves the non-enzymatic glycation of tissue proteins and the production of advanced glycation end products (AGEs).<sup>28</sup> Hyperglycemia results in reversible and irreversible change to the host including formation of AGEs. Receptors for AGEs (RAGE) have been identified. AGE formation and AGE-RAGE interaction at the cell surface of endothelial cells and mononuclear phagocytes have been shown to generate reactive oxygen intermediates<sup>29</sup> and enhance the expression of proinflammatory cytokines<sup>30</sup> through a mechanism involving the nuclear transcription factor kappa B (NF- $\kappa$ B). AGE-RAGE interactions have been shown in vitro to upregulate NF- $\kappa$ B associated gene products<sup>29</sup> which include vascular cell adhesion molecule<sup>31</sup> and proinflammatory cytokines.<sup>32</sup> RAGE is present in the gingival tissues and has been linked to oxidative stress.<sup>33</sup> We reason that if AGE-RAGE interactions are taking place within the gingival tissues, then a heightened cytokine response should differentiate patients with more pronounced hyperglycemia. Since RAGE receptors are present on mononuclear phagocytes,<sup>34</sup> AGE-RAGE interactions in the tissues of diabetic subjects may account for a heightened inflammatory response.<sup>35</sup> Hence, our finding that GCF IL-1 $\beta$  is increased in subjects with worse glycemic control is not unexpected. Our study found that elevated HbA1c was associated with GCF IL-1 $\beta$ , which is consistent with a role for chronic hyperglycemia in the production of gingival inflammation. Since chronic hyperglycemia can lead to the non-enzymatic glycation of tissue proteins, glycation of tissue protein may contribute to pathologic vascular change and subsequent tissue damage.<sup>34</sup> While not an AGE itself, HbA1c is a measure of hemo-



globin glycation, and as such is a measure of chronic hyperglycemia.

Elevated IL-1 $\beta$  represents, in part, a plausible explanation for the increased incidence and severity of periodontal disease in patients with diabetes. The relationship of poor glycemic control to GCF IL-1 $\beta$  requires further study in light of the current findings. Other cytokines are likely to play a role in this regard as well. One limitation of the present study is that only small amounts of GCF can be collected at one visit. The small volume of GCF sample limits the number of inflammatory mediators that can be reliably measured. Clearly there is a need for GCF testing technologies which could measure many inflammatory mediators simultaneously from such very small fluid volumes. Another limitation of this study is that testing was not done for genetic polymorphisms. Future studies should consider genetic variation in IL-1 $\beta$ , and other inflammatory mediator expression.

Our study was designed to sample predetermined anatomic locations within the periodontium rather than the most inflamed or deepest sites. Shallow, medium, and deep probing depths were encountered in this study so that sites with clinically different characteristics could be compared. Sampling only inflamed or deepest sites may give an inaccurate profile of inflammatory mediators since probing depth and clinical inflammation are strongly associated with IL-1 $\beta$ .<sup>17,36</sup> Any description of inflammatory mediators in the GCF from diseased periodontium should, therefore, consider the probing depth of the sampled site.

Also, the current finding of elevated GCF IL-1 $\beta$  in diabetic subjects with poor glycemic control raises the possibility of the converse; namely, that local gingival inflammation may adversely influence the glycemic control of patients with diabetes. An excellent review of the complex association between inflammation, hyperlipidemia, and hyperglycemia was recently published.<sup>37</sup> Whether inflammation precedes clinical diabetes is an intriguing proposition. This question has been addressed in at least one large cohort study.<sup>38</sup> Results reported by the Atherosclerosis Risk in Communities Study investigators indicate that baseline levels of inflammatory mediators in serum were significant predictors of incident diabetes at the 7-year follow-up.<sup>38</sup> This association was found even after adjusting for baseline fasting glucose levels. The authors concluded that inflammation may precede development of diabetes. Their data support the hypothesis that host inflammation may contribute to hyperglycemia. It is conceivable that elevated GCF IL-1 $\beta$  may contribute to hyperglycemia. At least one human study has examined the issue of whether chronic periodontitis influences circulating lipid and glucose levels.<sup>5</sup> Losche et al.<sup>5</sup> reported increased levels of serum glucose in non-diabetic subjects with periodontitis compared with age-

and gender-matched non-diabetic subjects without periodontitis. The question of whether gingival inflammation contributes to hyperglycemia in patients with diabetes needs to be addressed in future interventional studies.

In summary, our data indicate a significant increase in GCF inflammatory mediator levels among patients with type 2 diabetes and poor glycemic control. Hyperglycemia may, in part, explain the increased incidence and severity of periodontal disease among type 2 diabetes patients.

#### ACKNOWLEDGMENTS

This study was supported in part by a Columbia University Office of Clinical Trials Pilot Award and NIDCR training grant DE00449, both to Dr. Engebretson.

#### REFERENCES

1. Soskolne WA, Klinger A. The relationship between periodontal diseases and diabetes: An overview. *Ann Periodontol* 2001;6:91-98.
2. Emrich LJ, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. *J Periodontol* 1991;62:123-131.
3. Taylor GW, Loesche WJ, Terpenning MS. Impact of oral diseases on systemic health in the elderly: Diabetes mellitus and aspiration pneumonia. *J Public Health Dent* 2000;60:313-320.
4. Oliver RC, Tervonen T. Periodontitis and tooth loss: Comparing diabetics with the general population. *J Am Dent Assoc* 1993;124:71-76.
5. Losche W, Karapetow F, Pohl A, Pohl C, Kocher T. Plasma lipid and blood glucose levels in patients with destructive periodontal disease. *J Clin Periodontol* 2000;27:537-541.
6. Tervonen T, Knuutila M. Relation of diabetes control to periodontal pocketing and alveolar bone level. *Oral Surg Oral Med Oral Pathol* 1986;61:346-349.
7. Taylor GW, Burt BA, Becker MP, et al. Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *J Periodontol* 1996;67:1085-1093.
8. Tsai C, Hayes C, Taylor GW. Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. *Community Dent Oral Epidemiol* 2002;30:182-192.
9. Zambon JJ, Reynolds H, Fisher JG, Shlossman M, Dunford R, Genco RJ. Microbiological and immunological studies of adult periodontitis in patients with noninsulin-dependent diabetes mellitus. *J Periodontol* 1988;59:23-31.
10. Tervonen T, Oliver RC, Wolff LF, Bereuter J, Anderson L, Aeppli DM. Prevalence of periodontal pathogens with varying metabolic control of diabetes mellitus. *J Clin Periodontol* 1994;21:375-379.
11. Yuan K, Chang CJ, Hsu PC, Sun HS, Tseng CC, Wang JR. Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus and non-diabetes mellitus by polymerase chain reaction. *J Periodontol Res* 2001;36:18-24.
12. Gowen M, Mundy GR. Actions of recombinant interleukin 1, interleukin 2, and interferon-gamma on bone resorption in vitro. *J Immunol* 1986;136:2478-2482.

13. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1 alpha and -1 beta in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. *J Periodontol Res* 1990;25:156-163.
14. Hou LT, Liu CM, Rossomando EF. Crevicular interleukin-1 beta in moderate and severe periodontitis patients and the effect of phase I periodontal treatment. *J Clin Periodontol* 1995;22:162-167.
15. Mathur A, Michalowicz B, Castillo M, Aeppli D. Interleukin-1 alpha, interleukin-8 and interferon-alpha levels in gingival crevicular fluid. *J Periodontol Res* 1996;31:489-495.
16. Figueredo CM, Ribeiro MS, Fischer RG, Gustafsson A. Increased interleukin-1 $\beta$  concentration in gingival crevicular fluid as a characteristic of periodontitis. *J Periodontol* 1999;70:1457-1463.
17. Engebretson SP, Grbic JT, Singer R, Lamster IB. GCF IL-1beta profiles in periodontal disease. *J Clin Periodontol* 2002;29:48-53.
18. Stashenko P, Jandinski JJ, Fujiyoshi P, Rynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. *J Periodontol* 1991;62:504-509.
19. Engebretson SP, Lamster IB, Herrera-Abreu M, et al. The influence of interleukin gene polymorphism on expression of interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$  in periodontal tissue and gingival crevicular fluid. *J Periodontol* 1999;70:567-573.
20. Kurtis B, Develioglu H, Taner IL, Balos K, Tekin IO. IL-6 levels in gingival crevicular fluid (GCF) from patients with non-insulin dependent diabetes mellitus (NIDDM), adult periodontitis and healthy subjects. *J Oral Sci* 1999; 41:163-167.
21. Salvi GE, Yalda B, Collins JG, et al. Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. *J Periodontol* 1997;68:127-135.
22. Salvi GE, Beck JD, Offenbacher S. PGE<sub>2</sub>, IL-1 $\beta$ , and TNF- $\alpha$  responses in diabetics as modifiers of periodontal disease expression. *Ann Periodontol* 1998;3:40-50.
23. Cutler CW, Machen RL, Jotwani R, Iacopino AM. Heightened gingival inflammation and attachment loss in type 2 diabetics with hyperlipidemia. *J Periodontol* 1999;70: 1313-1321.
24. Lamster IB, Oshrain RL, Fiorello LA, Celenti RS, Gordon JM. A comparison of 4 methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. *J Clin Periodontol* 1988;15:347-352.
25. Lamster IB, Oshrain RL, Celenti R, Levine K, Fine JB. Correlation analysis for clinical and gingival crevicular fluid parameters at anatomically related gingival sites. *J Clin Periodontol* 1991;18:272-277.
26. Morohoshi M, Fujisawa K, Uchimura I, Numano F. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996;45:954-959.
27. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* 2002;106:2067-2072.
28. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 1988;318:1315-1321.
29. Yan SD, Schmidt AM, Anderson GM, et al. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 1994;269:9889-9897.
30. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *Diabetes* 2001;50: 2792-2808.
31. Schmidt AM, Crandall J, Hori O, Cao R, Lakatta E. Elevated plasma levels of vascular cell adhesion molecule-1 (VCAM-1) in diabetic patients with microalbuminuria: A marker of vascular dysfunction and progressive vascular disease. *Br J Haematol* 1996;92:747-750.
32. Vlassara H, Brownlee M, Manogue KR, Dinarello CA, Pasagian A. Cachectin/TNF and IL-1 induced by glucose-modified proteins: Role in normal tissue remodeling. *Science* 1988;240:1546-1548.
33. Schmidt AM, Weidman E, Lalla E, et al. Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: A potential mechanism underlying accelerated periodontal disease associated with diabetes. *J Periodontol Res* 1996;31:508-515.
34. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb* 1994;14:1521-1528.
35. Schmidt AM, Hori O, Cao R, et al. RAGE: A novel cellular receptor for advanced glycation end products. *Diabetes* 1996;45(Suppl. 3):S77-S80.
36. Oringer RJ, Al-Shammari KF, Aldredge WA, et al. Effect of locally delivered minocycline microspheres on markers of bone resorption. *J Periodontol* 2002;73:835-842.
37. Fernandez-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* 2003;24:278-301.
38. Duncan BB, Schmidt MI, Offenbacher S, Wu KK, Savage PJ, Heiss G. Factor VIII and other hemostasis variables are related to incident diabetes in adults. The Atherosclerosis Risk in Communities (ARIC) Study. *Diabetes Care* 1999;22:767-772.

Correspondence: Dr. Steven P. Engebretson, School of Dental & Oral Surgery, Columbia University, 630 W. 168th St., PH 7 East 125A, New York, NY 10032. Fax: 212/305-9313; e-mail: spe3@columbia.edu.

Accepted for publication January 8, 2004.