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# Periodontal Disease Is Associated With Brachial Artery Endothelial Dysfunction and Systemic Inflammation

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**Objective**—The purpose of this study was to determine whether periodontal disease is associated with endothelial dysfunction and systemic inflammation. Epidemiological studies suggest that severe periodontal disease is associated with increased cardiovascular disease risk, but the mechanisms remain unknown.

**Methods and Results**—We assessed flow-mediated dilation and nitroglycerin-mediated dilation of the brachial artery using vascular ultrasound in 26 subjects with advanced periodontal disease and 29 control subjects. The groups were matched for age and sex, and patients with hypercholesterolemia, diabetes mellitus, hypertension, and history of cigarette smoking were excluded. We also examined serum levels of C-reactive protein using an established high-sensitivity method. Subjects with advanced periodontal disease had lower flow-mediated dilation compared with control patients ( $7.8 \pm 4.6\%$  versus  $11.7 \pm 5.3\%$ ,  $P=0.005$ ). Nitroglycerin-mediated dilation was equivalent in the two groups. Subjects with advanced periodontitis exhibited higher serum levels of high-sensitivity C-reactive protein compared with healthy controls patients ( $2.3 \pm 2.3$  versus  $1.0 \pm 1.0$  mg/L,  $P=0.03$ ).

**Conclusions**—Subjects with advanced periodontal disease exhibit endothelial dysfunction and evidence of systemic inflammation, possibly placing them at increased risk for cardiovascular disease. (*Arterioscler Thromb Vasc Biol.* 2003; 23:1245-1249.)

**Key Words:** periodontal disease ■ endothelium ■ brachial artery ■ nitroglycerin ■ inflammation

Mild forms of periodontal disease affect most adults, and more severe forms affect 5% to 20% of the population in the United States.<sup>1</sup> The disease is characterized by chronic and progressive bacterial infection of the gums leading to alveolar bone destruction and loss of soft tissue attachment to the teeth. Epidemiological studies suggest a link between severe periodontal disease and atherosclerosis,<sup>2,3</sup> whereas there is no association with milder periodontal disease.<sup>4,5</sup> After adjustment for other risk factors, studies indicate that severe periodontal disease is associated with a 25% to 90% increase in risk for cardiovascular disease.<sup>6,7</sup> Although the mechanisms accounting for such a relationship have not been fully defined, investigators have proposed that periodic transient bacteremia leading to invasion of vascular cells and increased levels of circulating cytokines accelerate the atherogenic process.<sup>8</sup> A pathogenic link between recurrent bacteremia with oral pathogens and acceleration of atherosclerosis is also supported by recent experimental studies.<sup>9</sup>

A likely target for circulating cytokines and oral pathogens is the vascular endothelium, which plays a central role in the regulation of vascular homeostasis.<sup>10</sup> Activation of endothelial cells by inflammatory cytokines promotes a proatherogenic phenotype with increased expression of proinflammatory factors and loss of the antithrombotic, growth-inhibitory,

and vasodilator properties of the endothelium, including a decrease in the biological activity of nitric oxide.<sup>10</sup> These changes occur early in the development of atherosclerosis and likely contribute to the clinical expression of disease. Support for the clinical importance of endothelial dysfunction is provided by recent studies demonstrating increased cardiovascular disease risk in patients with endothelial dysfunction in coronary and peripheral arteries.<sup>11–16</sup>

On the basis of these observations, we hypothesized that severe periodontal disease would be associated with endothelial dysfunction. In the present study, we sought to test this hypothesis using a case-control design and brachial artery flow-mediated dilation as an indicator of endothelial function.

## Methods

### Study Subjects

We recruited otherwise healthy patients with periodontal disease who were referred for care at Boston University, Goldman School of Dental Medicine. Healthy subjects without periodontal disease were recruited by advertisement. We excluded potential subjects if they had a clinical history of cardiovascular disease, diabetes mellitus, hypertension, hypercholesterolemia, cigarette smoking, or other systemic illness or if they were taking any anti-inflammatory, vasoactive, or lipid-lowering medications. Subjects were also excluded if they had received antibiotics within 3 months of study or

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treatment for periodontal disease within 6 months of study. All subjects provided informed consent in accordance with the policies of the Institutional Review Board of Boston Medical Center.

### Oral and Periodontal Examination

An experienced periodontist assessed each subject for the presence and severity of periodontal disease. This evaluation included measurement of the probing depth and assessment of bleeding on probing, recession, and clinical attachment level. The presence of advanced periodontitis was determined using established criteria,<sup>17,18</sup> which included involvement of at least 6 teeth with pocket depth >5 mm and loss of attachment of  $\geq 3$  mm in 3 aspects of each involved tooth. Control subjects had no clinical signs of periodontal disease and specifically had no tooth pocket depth  $\geq 2$  mm and no attachment loss  $\geq 3$  mm. We excluded subjects with intermediate-severity periodontal disease from the primary analysis.

### Assessment of Vascular Function

Endothelium-dependent flow-mediated dilation and nitroglycerin-mediated dilation of the brachial artery were assessed using established methodology<sup>19</sup> on a separate day within 1 week of the dental examination. Briefly, patients fasted overnight before the study. Blood pressure was measured in the left arm after a 10-minute rest period in a semirecumbent position using an automated monitor (Dinamap XL, Johnson and Johnson Medical). Two-dimensional images and Doppler flow signals from the right brachial artery were digitized at baseline and after 1 minute of hyperemia induced by 5-minute cuff occlusion of the upper arm using a vascular ultrasound system equipped with a 10-MHz vascular probe (Toshiba Medical Inc). After a 10-minute period to reestablish baseline conditions, images were recorded before and 4 minutes after administration of sublingual nitroglycerin (0.4 mg). If the subject had a systolic blood pressure <100 mm Hg, adverse reaction to nitroglycerin, or refused to take nitroglycerin, this portion of the protocol was omitted. Personnel blinded to clinical status measured brachial artery diameters and extent of reactive hyperemia as previously described.<sup>19</sup>

### Biochemical Analyses

A venous blood sample was obtained from the left arm at the time of ultrasound study. Serum total cholesterol, HDL, triglycerides, glucose, and amylase were measured using an automated analyzer (Hitachi model 717, Hitachi Instruments) in the Boston Medical Center Clinical Laboratory. LDL cholesterol was calculated by the Friedewald formula.<sup>20</sup> Serum high-sensitivity C-reactive protein was measured using a nephelometric method on a commercial basis by the Brigham and Women's Hospital Department of Laboratory Medicine as previously described with a limit of detection of 0.17 mg/L.<sup>21</sup>

### Statistical Analysis

The presence of a normal distribution for each variable was tested by the Kolmogorov-Smirnov test, and analysis for non-normal variables was completed after log transformation. On this basis, we examined group differences in brachial artery diameter, flow-mediated dilation, nitroglycerin-mediated dilation, reactive hyperemia, age, systemic blood pressure, serum lipids, and log C-reactive protein using the unpaired *t* test. Categorical parameters were compared using the  $\chi^2$  test or Fisher's exact test, as appropriate. We used the same approach to complete a subgroup analysis after exclusion of consecutive control subjects to match the groups for baseline brachial artery diameter. To additionally examine confounding effects, we performed univariate ANOVA with flow-mediated dilation as the dependent variable, periodontal disease as a fixed factor, and HDL cholesterol, systolic blood pressure, age, and sex as covariates (selection criterion for candidate variables was a univariate  $P < 0.10$ ). In all analyses, statistical significance was assumed when  $P < 0.05$ . Variables are presented as mean  $\pm$  SD in the text and as mean  $\pm$  SEM in the figures.

**TABLE 1. Clinical Characteristics**

	Control (n=29)	Periodontal Disease (n=26)	<i>P</i>
Age, y	41 $\pm$ 9	42 $\pm$ 10	0.71
Sex, % female	38%	39%	0.97
Fasting glucose, mg/dL	91 $\pm$ 8	91 $\pm$ 8	0.98
Total cholesterol, mg/dL	195 $\pm$ 36	197 $\pm$ 34	0.84
HDL cholesterol, mg/dL	60 $\pm$ 17	52 $\pm$ 12	0.04
Triglycerides, mg/dL	89 $\pm$ 35	111 $\pm$ 81	0.21
LDL cholesterol, mg/dL	117 $\pm$ 32	128 $\pm$ 24	0.21
Systolic blood pressure, mm Hg	116 $\pm$ 14	123 $\pm$ 12	0.08
Diastolic blood pressure, mm Hg	71 $\pm$ 8	72 $\pm$ 11	0.64
Heart rate, bpm	61 $\pm$ 7	63 $\pm$ 8	0.13

## Results

### Clinical Characteristics

A total of 26 otherwise healthy nonsmoking subjects with advanced periodontitis and 29 age- and sex-matched healthy controls were enrolled in the study. Their clinical characteristics are displayed in Table 1. As shown, the two groups were comparable except for lower HDL cholesterol and a trend for higher systolic blood pressure in the patients with periodontal disease.

### Brachial Artery Function

Brachial artery parameters are displayed in Table 2. The two groups had comparable baseline diameter and extent of reactive hyperemia. As shown, brachial artery flow-mediated dilation was significantly lower in subjects with advanced periodontal disease compared with control subjects. When brachial artery flow-mediated dilation was expressed as change in diameter rather than percent change, it was also significantly lower in subjects with advanced periodontal disease. This latter finding suggests that the reduced flow-mediated dilation cannot be attributed to the nonsignificant tendency for larger baseline diameter in subjects with periodontal disease.

To additionally confirm that a difference in baseline diameter does not account for our findings, we examined a subgroup of patients specifically matched for baseline vessel diameter. As shown in Table 3, patients in the two groups remained comparable for age, sex, and other clinical factors. As for the group as a whole, brachial artery flow-mediated dilation was significantly lower in the patients with periodontal disease.

**TABLE 2. Brachial Artery Parameters and C-Reactive Protein**

	Control (n=29)	Periodontal Disease (n=26)	<i>P</i>
Baseline brachial diameter, mm	4.1 $\pm$ 0.9	4.3 $\pm$ 0.7	0.35
Hyperemic flow, mL/min	1150 $\pm$ 620	1240 $\pm$ 540	0.56
Flow-mediated dilation, %	11.7 $\pm$ 5.3	7.8 $\pm$ 4.6	0.005
Flow-mediated dilation, mm	0.45 $\pm$ 0.16	0.31 $\pm$ 0.15	0.003
Nitroglycerin-mediated dilation, %	18.9 $\pm$ 11.0	16.3 $\pm$ 8.3	0.37
C-reactive protein, mg/L	1.0 $\pm$ 1.0	2.3 $\pm$ 2.3	0.03

TABLE 3. Matched for Baseline Brachial Diameter

	Control (n=26)	Periodontal Disease (n=26)	P
Age, y	42±7	42±10	0.83
Sex, % female	29%	39%	0.40
Fasting glucose, mg/dL	91±8	91±8	0.93
Total cholesterol, mg/dL	196±32	197±33	0.87
HDL cholesterol, mg/dL	57±15	52±12	0.15
Triglycerides, mg/dL	93±36	111±81	0.34
LDL cholesterol, mg/dL	120±31	128±24	0.32
Systolic blood pressure, mm Hg	117±14	123±12	0.12
Diastolic blood pressure, mm Hg	71±8	72±11	0.74
Heart rate, bpm	60±8	63±8	0.13
Baseline brachial diameter, mm	4.3±0.8	4.3±0.7	0.94
Hyperemic flow, mL/min	1240±610	1240±540	0.99
Flow-mediated dilation, %	11.1±5.3	7.8±4.6	0.018
Flow-mediated dilation, mm	0.47±0.17	0.31±0.15	0.003
Nitroglycerin-mediated dilation, %	16.6±8.8	16.3±8.3	0.89

When periodontal disease, HDL cholesterol, systolic blood pressure, age, and sex were included in a multivariate model, the independent predictors of impaired flow-mediated dilation were male sex ( $P=0.05$ ) and periodontal disease ( $P=0.01$ ). When baseline brachial diameter was also included in the model, the independent predictors of impaired flow-mediated dilation were larger baseline diameter ( $P=0.004$ ) and periodontal disease ( $P=0.01$ ). When flow-mediated dilation was expressed as absolute change, the only independent predictor was periodontal disease ( $P=0.006$ ).

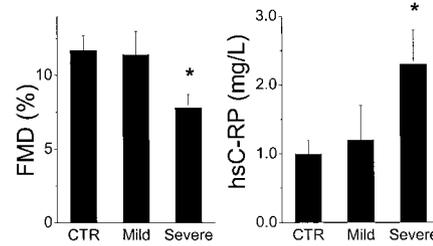
Nitroglycerin was administered to 23 control subjects and 24 subjects with advanced periodontal disease. As shown in Tables 2 and 3, nitroglycerin-mediated dilation was comparable in control subjects and subjects with advanced periodontal disease. This finding provides evidence that the difference between groups reflects a difference in endothelial function rather than a more generalized difference in vascular function.

### C-Reactive Protein

C-reactive protein was measured in 21 control subjects and 17 subjects with advanced periodontal disease. As shown in Table 2, C-reactive protein was higher in patients with advanced periodontal disease compared with control patients. There was a trend for an inverse correlation between flow-mediated dilation and C-reactive protein that did not reach statistical significance ( $r=-0.29$ ,  $P=0.07$ ). There was an inverse correlation between C-reactive protein and nitroglycerin-mediated dilation ( $r=-0.40$ ,  $P=0.02$ ).

### Subjects With Intermediate-Severity Periodontal Disease

During screening for control subjects, we identified 13 subjects with periodontal disease that did not qualify as severe by the criteria detailed in the Methods section. In a post hoc analysis, we compared flow-mediated dilation and



Brachial artery flow-mediated dilation (left) and C-reactive protein (right) were assessed as described in Methods. Patients with mild periodontal disease were similar to control subjects (CTR). However, patients with severe periodontal disease had lower flow-mediated dilation ( $P=0.01$ ) and higher C-reactive protein ( $P=0.03$ ) compared with control subjects.

C-reactive protein in these subjects with the control and severe-disease subjects. As shown in Figure 1, the patients with mild periodontal disease had flow-mediated dilation and C-reactive protein levels that were similar to the control patients.

### Discussion

The present study demonstrated significantly impaired brachial artery flow-mediated dilation in otherwise healthy nonsmoking subjects with advanced periodontal disease compared with age-matched control subjects. The vasodilator response to nitroglycerin and extent of reactive hyperemia were similar in the two groups, suggesting that the findings cannot be attributed to group differences in the function of vascular smooth muscle or the stimulus for vasodilation. Thus, the findings are consistent with an impairment of endothelial vasomotor function in patients with periodontal disease. The degree of impairment in endothelial function observed in patients with periodontal disease (flow-mediated dilation, 7.8%) is comparable with that observed in patients with hypertension<sup>22</sup> and thus is likely to be clinically important. Severe periodontal disease was also associated with increased serum C-reactive protein levels, a finding that is consistent with the hypothesis that endothelial dysfunction in this setting may be attributable to a state of systemic inflammation.

No prior study has specifically examined the relation between periodontal disease and endothelial function in human subjects. However, several experimental studies support the present findings. For example, the oral pathogen *Porphyromonas gingivalis* can infect endothelial cells,<sup>23</sup> and exposure of cultured endothelial cells to *P. gingivalis* is associated with endothelial activation and expression of cell adhesion molecules.<sup>24</sup> The relevance of these changes to cardiovascular disease is supported by the observation that repeated *P. gingivalis* injection is associated with accelerated atherosclerosis in the heterozygous apolipoprotein E-deficient mouse.<sup>9</sup>

Other systemic infectious and inflammatory states are also associated with impaired endothelium-dependent vasodilation in human subjects. For example, Hingorani et al<sup>25</sup> demonstrated a reduction in brachial artery flow-mediated dilation and impairment of endothelium-dependent vasodilation of forearm resistance vessels after *salmonella typhi*

vaccination in healthy subjects. Consistent with the present study, several other studies suggest that elevated levels of C-reactive protein are associated with impaired endothelium-dependent vasodilation in the forearm microcirculation of patients with coronary artery disease.<sup>26,27</sup> Interestingly, there is evidence that endothelial function may improve in parallel with spontaneous reduction in C-reactive protein over time.<sup>26</sup> These associations between inflammation and endothelial dysfunction have been proposed as a potential mechanistic explanation for reported relations between cardiovascular disease and other chronic infections with organisms such as *Chlamydia pneumoniae*, cytomegalovirus, herpes simplex, and *Helicobacter pylori*, although a causative link between these pathogens and atherosclerosis remains unproven.<sup>28–30</sup>

There is strong and growing evidence that endothelial dysfunction is relevant to the pathogenesis of cardiovascular disease. Endothelial dysfunction is associated with coronary artery disease and coronary risk factors,<sup>10</sup> and these abnormalities are present before the development of angiographically evident coronary atherosclerosis in patients with coronary risk factors.<sup>31</sup> Interventions known to reduce cardiovascular disease risk, including lipid-lowering therapy, angiotensin-converting enzyme inhibitors, exercise, and smoking cessation, also improve endothelial function.<sup>32</sup> Importantly, the presence of endothelial dysfunction in the coronary and forearm circulation predicts future cardiovascular disease events.<sup>11–16</sup> Thus, the presence of endothelial dysfunction in patients with severe periodontal disease suggests that they may be at higher risk for the future development of cardiovascular disease.

The findings of the present study fit well with prior clinical studies linking periodontal and cardiovascular disease. Severe periodontal disease is associated with more severe atherosclerosis in coronary arteries<sup>3</sup> and carotid arteries.<sup>2</sup> In addition, several studies suggest a greater risk for cardiovascular disease events in these patients. For example, using data drawn from the Normative Aging Study and the Dental Longitudinal Study, Beck et al<sup>7</sup> observed that severe periodontal disease assessed by dental examination was associated with a greater incidence of total and fatal coronary heart disease, with adjusted odds ratios of 1.5 and 1.9, respectively. A study involving participants in the National Health and Nutritional Epidemiological Follow-Up Study also demonstrated a 25% increase in cardiovascular disease risk in patients with severe periodontal disease by examination after adjustment for other risk factors.<sup>6</sup> In contrast, studies from the Health Professions Follow-up Study<sup>4</sup> and the Physicians' Health Study<sup>5</sup> failed to demonstrate this association. The reasons for these apparently conflicting results remain unclear; however, it is notable that the association was stronger and statistically significant when considering patients with severe periodontal disease, as was done in the present study. Another factor may be the use of self-reported periodontal disease, as was done in the Physicians' Health Study, which is likely to be less accurate than formal dental examination. Our findings that only patients with severe periodontal disease have impaired flow-mediated and elevated C-reactive protein levels fit well with these epidemiological studies.

The present study has several limitations. First, endothelial function was examined in the brachial circulation, and extrapolation of the results to the coronary circulation should be done with caution. However, there is growing evidence that studies performed in the peripheral circulation correlate with the coronary circulation<sup>33</sup> and provide prognostic information.<sup>13,14</sup> Furthermore, the noninvasive methodology used in the present study was recently shown to provide prognostic information about cardiovascular disease in high-risk patients.<sup>15,34</sup> Second, the sample size was relatively modest. Despite our attempts to match the groups and control for confounding with a multivariate analysis, the possibility remains that recognized and unrecognized confounding factors account for our observations. Third, the method to assess the severity of periodontal disease primarily relied on loss of periodontal attachment, which might not be the best measure of local inflammation. Despite this limitation, we observed a significant relation between periodontal disease and endothelial dysfunction. Clearly, these results by no means indicate causality. Better evidence for such a relation would be provided by the demonstration that successful treatment of periodontal disease is associated with improved endothelial function.

In summary, the present study demonstrated endothelial vasomotor dysfunction in the conduit brachial artery and elevated serum levels of high-sensitivity C-reactive protein in patients with severe periodontal disease. These findings provide a potential mechanistic explanation for prior epidemiological studies suggesting a link between periodontitis and cardiovascular disease and are consistent with the very strong evidence that chronic inflammatory states are associated with cardiovascular disease. Additional studies will be required to determine whether periodontal disease represents a novel risk factor that might be modifiable with appropriate periodontal intervention.

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