訳：Treatment of periodontitis and endothelial function

Maurizio S., et al., *N Engl J Med* 356;9. Mar 1, 2007.

歯周病治療と内皮機能

全身性炎症は血管機能を障害すると考えられ、疫学的データより歯周病と心血管症とのつながりが示唆される。

歯周病患者 120 人(重度歯周病で地域治療をうけている 59 人と、集中歯周病治療をうけている 61 人)を選出。FMD(上腕動脈血管径計測)、炎症マーカーと凝固マーカー、内皮活性を、治療前と治療後 1・7・30・60・180 日後に評価した。

治療 24 時間後、集中治療郡で FMD は顕著に低く、CRP・IL-6・内皮活性水溶性マーカーの E-セレクチンと von-Willebrand 因子は顕著に高値であったが、治療 60・180 日後には FMD は改善し、E セレクチンも低下した。改善の進度は歯周病の改善と関係していた。両群に副作用はなくと心血管イベントも起きなかった。

短期集中治療後には全身性炎症と内皮機能障害がみられた。しかし、6 ヶ月の歯周病の治療後には内皮機能の改善がみられた。

訳：Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation.

Salomon Amar, et al., *Arterioscler Thromb Vasc Biol.* 2003;23:1245-1249.

歯周病と上腕動脈内皮機能障害と全身性炎症との関係を検討した。疫学的研究によれば重度の歯周病は心血管病リスクの増加と関係があるといわれるが、そのメカニズムはまだ知られていない。

26人の高度歯周病患者と29人のコントロール群のFMDとNMDをおこない比較検討した(年齢・性差適合。高脂血症・糖尿病・高血圧・喫煙歴のある患者は除く)。血漿CRP濃度も計測。重度歯周病患者群のFMDはコントロール群に比べ低値で、CRPは高値であった。両群にNMDの差はみられなかった。

高度歯周病患者には内皮機能障害と全身性炎症がみられ、それらによる心血管病リスクの増加が危惧される。

歯周治療と内皮機能.

Treatment of Periodontitis and Endothelial Function

ABSTRACT

Systemic inflammation may impair vascular function, and epidemiologic data suggest a possible link between periodontitis and cardiovascular disease.

We randomly assigned 120 patients with severe periodontitis to community-based periodontal care (59 patients) or intensive periodontal treatment (61). Endothelial function, as assessed by measurement of the diameter of the brachial artery during flow (flow-mediated dilatation), and inflammatory biomarkers and markers of coagulation and endothelial activation were evaluated before treatment and 1, 7, 30, 60, and 180 days after treatment.

Twenty-four hours after treatment, flow-mediated dilatation was significantly lower in the intensive-treatment group than in the control-treatment group (absolute difference, 1.4%; 95% confidence interval [CI], 0.5 to 2.3; P=0.002), and levels of C-reactive protein, interleukin-6, and the endothelial-activation markers soluble E-selectin and von Willebrand factor were significantly higher (P<0.05 for all comparisons). However, flow-mediated dilatation was greater and the plasma levels of soluble E-selectin were lower in the intensive-treatment group than in the control-treatment group 60 days after therapy (absolute difference in flow-mediated dilatation, 0.9%; 95% CI, 0.1 to 1.7; P=0.02) and 180 days after therapy (difference, 2.0%; 95% CI, 1.2 to 2.8; P<0.001). The degree of improvement was associated with improvement in measures of periodontal disease (r=0.29 by Spearman rank correlation, P=0.003). There were no serious adverse effects in either of the two groups, and no cardiovascular events occurred.

Intensive periodontal treatment resulted in acute, short-term systemic inflammation and endothelial dysfunction. However, 6 months after therapy, the benefits in oral health were associated with improvement in endothelial function.

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INFLAMMATION PLAYS AN IMPORTANT ROLE in the pathogenesis of atherosclerosis, and low-grade chronic systemic inflammation has been shown to be linked to adverse cardiovascular outcomes.[1] The nature and source of the inflammation, however, remain unclear. Periodontitis, a very common chronic infection of the tissue surrounding the teeth, is associated with elevated levels of C-reactive protein (CRP) and other inflammatory biomarkers.[2-5] Cohort and case–control studies have shown that periodontitis is associated with endothelial dysfunction,[6] atherosclerosis,[7] and an increased risk of myocardial infarction and stroke.[8] In experimental models, periodontal pathogens have been shown to promote platelet aggregation,[9] foam-cell formation,[10] and the development of atheromas.[11,12] Clinical and epidemiologic studies suggesting a link between periodontitis and vascular disease, however, may be confounded by factors such as social status, smoking status, and other classic risk factors for atherosclerosis.[13]

Having shown in previous studies that intensive periodontal therapy results in local (periodontal) and systemic reductions in inflammation,[14-16] we now report the results of a randomized, controlled study conducted to determine the effect of treatment of severe periodontitis on endothelial function. Endothelial dysfunction is thought to represent a common pathway through which a range of risk factors, including inflammation, may influence the long-term process of atherogenesis and acute inflammation may trigger acute cardiovascular events.[17]

METHODS

STUDY DESIGN

We conducted a parallel-group, single-blind, randomized, controlled trial to evaluate the effect of periodontal therapy on endothelial function over a 6-month period (Fig. 1). The primary outcome was the between-group difference in flow-mediated dilatation during the study. Consecutive patients referred to the Eastman Dental Hospital in London for periodontal therapy were invited to participate if they had severe generalized periodontitis (probing pocket depths of >6 mm and marginal alveolar bone loss of >30%) with 50% or more of their teeth affected.[14] Exclusion criteria were the presence of systemic disease (e.g., diabetes mellitus or cardiovascular, kidney, liver, or lung disease), a history or the presence of any other acute or chronic infections, as assessed on clinical examination and routine laboratory testing, and systemic antibiotic treatment within the previous 3 months or any other, regular medication. All patients gave written informed consent. The study was approved by the joint Eastman and University College Hospitals ethics committee.

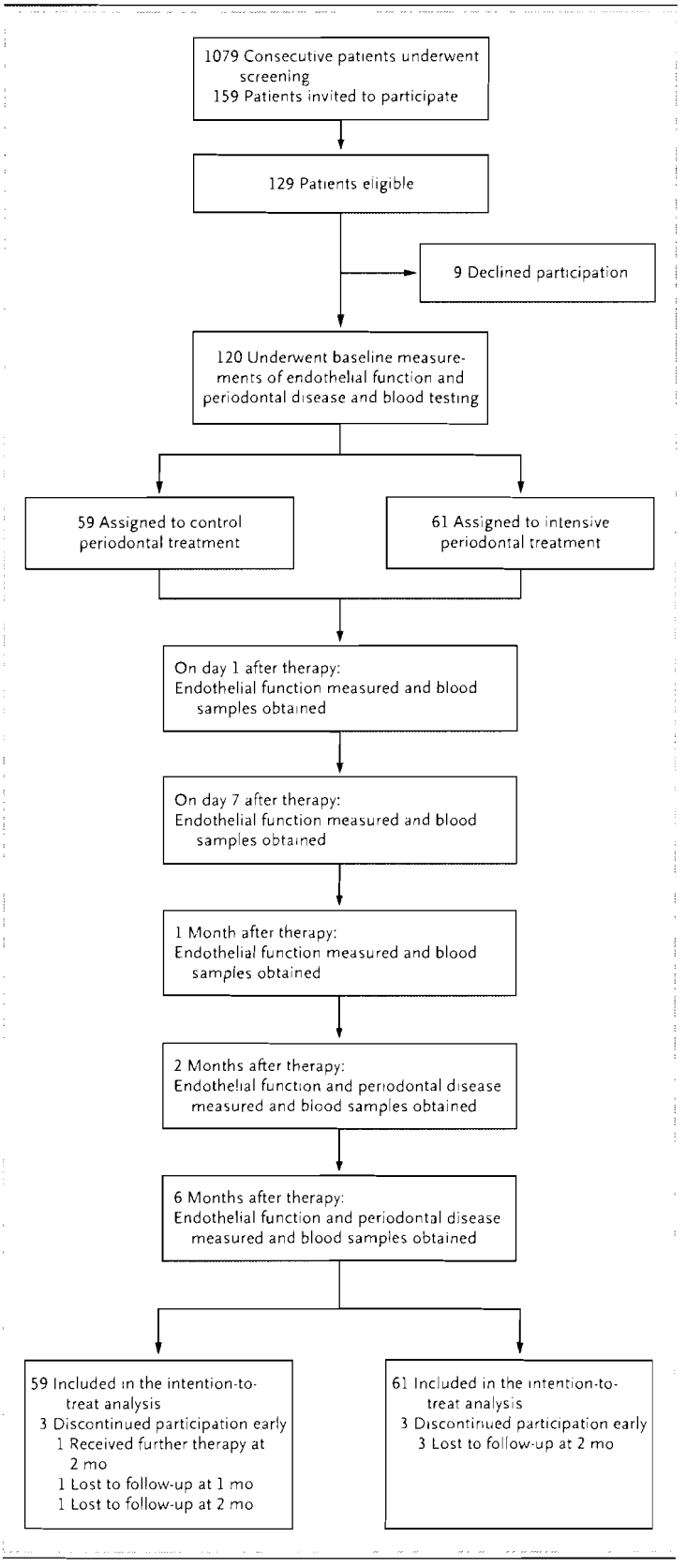
A baseline periodontal examination was performed, and full medical and dental histories were collected by a single examiner. Blood pressure was measured in triplicate (HEM-705CP, Omron), and the average of the readings was recorded. Patients were randomly assigned with the use of a computer-generated table to receive intensive periodontal treatment (the intensive-treatment group) or community-based periodontal care (the control-treatment group). A balanced, permuted-block approach (in blocks of four patients) was used to prepare the randomization tables. To prevent an imbalance between the two groups with respect to smoking status, sex, age, and severity of periodontitis, restricted randomization (minimization)[18] was performed by the study registrar. Treatment assignments were concealed in opaque envelopes and revealed to the therapist only on the day the treatment was administered. Patients underwent dental examinations and vascular studies at 1, 7, 30, 60, and 180 days after the administration of the therapy.

PERIODONTAL EXAMINATION AND THERAPY

Periodontal data were recorded by a single trained dental examiner, who was unaware of the treatment assignments, at baseline and 2 months and 6 months after administration of the therapy. The data included the periodontal pocket depth and the recession of the gingival margin relative to the cementoenamel junction at six sites per tooth. The presence or absence of supragingival dental plaque and gingival bleeding on probing was also recorded. The averaged whole-mouth number of periodontal lesions (probing depth, >4 mm), the score for full-mouth gingival bleeding on probing (the number of sites with gingival bleeding on probing divided by the total number of sites per mouth, multiplied by 100), and the score for full-mouth plaque (the number of sites with detectable supragingival dental plaque divided by the total number of sites per mouth, multiplied by 100) were calculated for each patient and were compared between the two groups.[14]

Study Design from Screening to Completion of the Trial.

After a baseline visit, eligible patients were randomly assigned to either community-based (control) or intensive periodontal therapy. Patients were reexamined 1 and 7 days and 1, 2, and 6 months after the administration of the therapy. Endothelial function was assessed, blood samples were obtained, and periodontal clinical measurements were performed at the points indicated.



All patients were given instructions in basic oral hygiene. Patients in the control-treatment group underwent a standard cycle of supragingival mechanical scaling and polishing. Patients in the intensive-treatment group underwent the adjunctive full-mouth intensive removal of subgingival dental plaque biofilms with the use of scaling and root planing after the administration of local anesthesia; teeth that could not be saved were extracted, and microspheres of minocycline (Arestin, OraPharma) were delivered locally into the periodontal pockets.[15] Patients in whom periodontal disease progressed received immediate care from a specialist and were withdrawn from the study.

VASCULAR FUNCTION

A range of measures of endothelial function, including vasomotion and circulating markers of coagulation and adhesion status, were studied by members of the study staff who were unaware of the treatment assignments. Endothelium-dependent vasodilatation of the brachial artery (the primary outcome) was assessed by means of ultrasound imaging (Acuson XP 128/10, Siemens) with the use of a 7-MHz linear probe and automated vessel-diameter measurements (Brachial Tools, version 3.2.6, Medical Imaging Applications). The studies were performed by a single examiner who acquired the images of the brachial artery in the morning, while patients were fasting, in a temperature-controlled room after 10 minutes of rest.[19] The brachial artery was imaged above the antecubital fossa continuously for 1 minute at baseline and again after inflation (pressure, 250 mm Hg for 5 minutes) and deflation of a sphygmomanometer cuff placed on the forearm. R-wave–triggered end-diastolic images of the vessel diameter were digitized and recorded at 3-second intervals throughout the procedure and were subsequently analyzed offline with the use of dedicated edge-detection software (Brachial Tools, Medical Imaging Applications). Dilatation was quantified as

the change, expressed as a percentage, from baseline to the peak diameter, between 45 and 75 seconds after release of the blood-pressure cuff (averaged over three frames). After 10 minutes of rest, endothelium-dependent dilatation was measured after sublingual administration of 25 μg of nitroglycerin, according to the same recording protocol.

Doppler-derived flow measurements (performed with the use of a pulse-wave Doppler signal at a 70° angle) were also obtained continuously. The increase in blood flow after release of the blood-pressure cuff was expressed as the reactive-hyperemia ratio (the value for reactive hyperemia divided by the baseline value for blood flow in the forearm). Duplicate analyses were performed by two observers who were unaware of the treatment assignments, the clinical details, and the stage of the experiment. The correlation between the observers was greater than 0.90, with a between-observer coefficient of variation of less than 5% for both vessel diameter and flow-mediated dilatation.

LABORATORY ASSESSMENTS

Serum and plasma samples were obtained from the patients and were immediately processed and stored at −70°C until analysis by laboratory staff who were unaware of the treatment assignments and vascular findings. Full blood counts and measurements of lipid and glucose levels were performed by standard biochemical testing. Serum levels of CRP were measured with an immunoturbidimetric, high-sensitivity assay (Tina-quant CRP immunoturbidimetric assay performed on a Cobas Integra analyzer, Roche Diagnostics). Serum levels of interleukin-6 and soluble E-selectin were measured by enzyme-linked immunosorbent assay (ELISA) (Quantikine HS, R&D Systems), and commercial, high sensitivity ELISAs were used to measure plasma levels of tissue plasminogen activator (t-PA) and plasminogen-activator inhibitor type 1 (PAI-1) (TintElize and Biopool, respectively, Trinity Biotech) and von Willebrand factor (CBA ELISA, Technoclone).

STATISTICAL ANALYSIS

We calculated that a minimum of 120 patients would need to be enrolled to detect a 1% difference in flow-mediated dilatation between the two treatment groups, with a standard deviation of the mean difference of 1.67% at a two-sided alpha level of 5% and 90% power. Data are reported as means and 95% confidence intervals (CIs), unless otherwise specified. All analyses were based on the intention-to-treat principle and were performed by investigators who were unaware of the treatment assignments. We performed a repeated-measures analysis of variance to determine differences in flow-mediated dilatation (the primary outcome) and all secondary and other outcomes between the two groups and over time, using a conservative F-test for the interaction between time and treatment group (SPSS software, version 13). The Greenhouse–Geisser correction for the F test was used to adjust the degrees of freedom for deviation from sphericity (one of the assumptions of repeated-measures analysis of variance [ANOVA]). Logarithmic transformation of the data was performed when appropriate, and post hoc Fisher's paired and unpaired tests of least-significant difference were performed and interpreted with the use of the Bonferroni–Holm adjustment for multiple comparisons.

Secondary outcomes were the between-group differences in the levels of inflammatory markers and markers of coagulation and endothelial activation. Other outcomes included differences in sublingual nitroglycerin-mediated dilatation, routine laboratory measurements, clinical periodontal measurements, and arterial blood pressure. Age, sex, smoking status, race or ethnic group, body-mass index, vessel diameter for flow-mediated dilatation and nitroglycerin-mediated dilatation, and periodontal diagnosis were included in all models as covariates. Race or ethnic group was self-reported. A post hoc correlation analysis (by the Spearman rank-correlation method) was performed to evaluate the relationship between the change in flow-mediated dilatation from baseline to 6 months after therapy and changes in the number of periodontal lesions and gingival bleeding sites from baseline to 6 months. Differences between categorical variables were calculated with the use of the chi-square test. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

During 1 year (September 2003 to September 2004), 159 of the 1079 patients examined met the inclusion criteria for severe generalized periodontal disease. Of these 159 patients, 129 met all the eligibil-

ity criteria, but 9 declined to participate. A total of 120 patients underwent randomization, of whom 114 (56 assigned to the control-treatment group and 58 to the intensive-treatment group) completed the trial, attending all study visits (to the end of the trial, in March 2005). Reasons for withdrawal are shown in Figure 1. One patient in the control-treatment group had clinical signs of periodontal

Baseline Characteristics of the Patients.*

Characteristic	Control-Treatment Group (N=59)	Intensive-Treatment Group (N=61)
Age — yr	47.8±6.3	47.7±7.9
Male sex — no. (%)	30 (51)	30 (49)
Smoking status — no. (%)		
Never smoked	21 (36)	24 (39)
Former smoker	18 (31)	19 (31)
Current smoker	20 (34)	18 (30)
Family history of cardiovascular disease — no. (%)	40 (68)	38 (62)
Race or ethnic group — no. (%)†		
White	41 (69)	41 (68)
Black	6 (10)	8 (13)
Asian	10 (17)	8 (13)
Other	2 (3)	4 (7)
Body-mass index‡	27.3±5.4	27.2±5.0
Blood pressure — mm Hg		
Systolic	124.5±17.4	125.6±15.9
Diastolic	79.2±11.1	80.5±11.4
Brachial-artery diameter — mm	3.6±0.6	3.7±0.8
Reactive hyperemia ratio§	8.9±4.1	8.8±4.2
Flow-mediated dilatation — %	6.5±2.6	7.1±4.2
Nitroglycerin-mediated dilatation — %¶	17.9±6.9	17.9±6.5
CRP — mg/liter	3.8±5.3	2.5±2.7
Interleukin-6 — pg/ml	2.1±3.9	2.4±5.4
Soluble E-selectin — ng/ml	20.3±13.6	19.6±14.0
t-PA — ng/ml	4.5±0.6	3.2±0.4
PAI-1 — ng/ml	21.39±1.8	21.5±1.5
Von Willebrand factor — IU/ml	0.87±0.16	0.90±0.19
Leukocyte count — $\times 10^{-9}$/liter	7.1±2.0	6.4±1.6
Cholesterol — mmol/liter	5.3±1.2	5.3±1.0
High-density lipoprotein	1.5±0.4	1.5±0.4
Low-density lipoprotein	3.2±1.0	3.1±0.9
Glucose — mmol/liter	5.1±0.6	5.1±0.8
Triglycerides — mmol/liter	1.5±1.5	1.4±1.0

* Plus–minus values are means ±SE. To convert values for cholesterol to milligrams per deciliter, divide by 0.02586. To convert values for glucose to milligrams per deciliter, divide by 0.05551. To convert values for triglycerides to milligrams per deciliter, divide by 0.01129. CRP denotes C-reactive protein, t-PA tissue plasminogen activator, and PAI-1 plasminogen-activator inhibitor type 1.

† Race was self-reported.

‡ Body-mass index is defined as the weight in kilograms divided by the square of the height in meters.

§ The reactive hyperemia ratio is the value for reactive hyperemia divided by the baseline value of blood flow in the forearm after release of the blood-pressure cuff.

¶ The total numbers of patients for the analysis of sublingual nitroglycerin–mediated dilatation were 47 in the control-treatment group and 51 in the intensive-treatment group.

disease progression and was withdrawn early. There were no major adverse events in either of the two groups (Table S1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

PATIENT CHARACTERISTICS AND CARDIOVASCULAR RISK FACTORS

The baseline characteristics of the patients in the control-treatment group and the intensive-treatment group were similar (Table 1). There were no significant differences between the two groups in diet, medication regimen, smoking status, lipid levels, body-mass index, and blood glucose levels (Table S2 in the Supplementary Appendix). Systolic blood pressure was significantly higher among patients in the intensive-treatment group 24 hours after the therapy was administered than among those in the control-treatment group (5.0±1.7 mm Hg; 95% CI, 1.7 to 8.4; P=0.004). No other significant differences were subsequently observed.

PERIODONTAL OUTCOMES

Repeated-measures ANOVA of the periodontal outcomes showed a significant interaction between treatment and time (P<0.001). As compared with the control-treatment group, the intensive-treatment group had lower scores for plaque 2 months after therapy (absolute difference, 27%; 95% CI, 21 to 34; P<0.001) and 6 months after therapy (absolute difference, 22%; 95% CI, 15 to 30; P<0.001) (Table 2). Similarly, patients in the intensive-treatment group had lower scores for gingival bleeding than those in the control-treatment group, 2 months after therapy (absolute difference, 39%; 95% CI, 33 to 45; P<0.001) and 6 months after therapy (absolute difference, 39%; 95% CI, 32 to 45; P<0.001). Patients in the intensive-treatment group had fewer periodontal lesions 2 months after therapy (difference between groups, 61; 95% CI, 53 to 69; P<0.001) and 6 months after therapy (difference between groups, 66; 95% CI, 56 to 74; P<0.001) (Table 2). Among patients in the intensive-treatment group, an average of two teeth (95% CI, 0 to 2) were extracted, whereas no extractions were performed in the control-treatment group.

VASCULAR FUNCTION

Repeated-measures analysis of variance of flow-mediated dilatation showed a significant interaction between treatment and time (P<0.001) (Fig. 2). Twenty-four hours after the administration of the assigned therapy, flow-mediated dilatation was lower in the intensive-treatment group than in the control-treatment group (absolute difference, 1.4%; 95% CI, 0.5 to 2.3; P=0.002). However, flow-mediated dilatation was higher in the intensive-treatment group than in the control-treatment group 2 months after therapy (absolute difference, 0.9%; 95% CI, 0.1 to 1.7; P=0.02) and 6 months after therapy (absolute difference, 2.0%; 95% CI, 1.2 to 2.8; P<0.001) (Table S2 in the Supplementary Appendix).

There was a significant effect of time on nitroglycerin-mediated dilatation according to repeated-measures analysis of variance (P=0.008), but there was no interaction between treatment and time (P=0.49), suggesting that the response to nitroglycerin changed in the two groups during the study period (Fig. 2). The differences between

Periodontal Disease at Baseline and 2 Months and 6 Months after Periodontal Therapy.*

Variable	Control-Treatment Group			Intensive-Treatment Group		
	Baseline	2 Months	6 Months	Baseline	2 Months	6 Months
Total no. of teeth	27±3	27±3	27±3	27±3	25±4†‡	25±4†‡
No. of lesions (periodontal pockets)	84±26	81±27	80±31	82±27	20±15†‡	14±12†‡
Sites with detectable plaque (%)§	63±21	42±22†	47±22†	66±20	15±10†‡	25±18†‡
Sites with gingival bleeding (%)¶	68±17	63±19‖	65±20	66±18	24±13†‡	26±16†‡

* Plus–minus values are means ±SD.
† P<0.001 for the comparison with baseline.
‡ P<0.001 for the comparison with the standard-treatment group.
§ Scores for full-mouth gingival bleeding were calculated for each patient as the number of sites with gingival bleeding on probing divided by the total number of sites per mouth, multiplied by 100.
¶ Scores for full-mouth plaque were calculated for each patient as the number of sites with detectable plaque divided by the total number of sites per mouth, multiplied by 100.
‖ P<0.05 for the comparison with baseline.

the two groups in flow-mediated dilatation and sublingual nitroglycerin-mediated dilatation were not correlated with differences in the baseline vessel diameter or the reactive hyperemia ratio (data not shown).

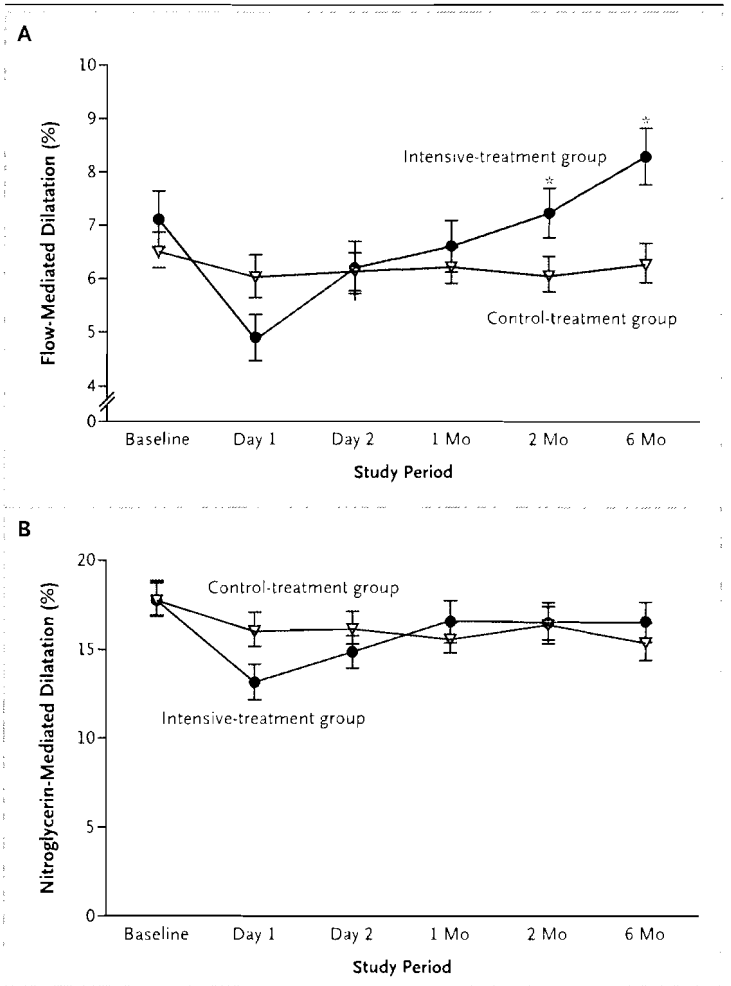
MARKERS OF INFLAMMATION, COAGULATION, AND ADHESION

Repeated-measures analysis of variance showed a significant interaction between treatment and time for most of the secondary biomarkers (neutrophil count, levels of CRP, interleukin-6 [P<0.001], von Willebrand factor [P=0.009], soluble E-selectin [P=0.002], PAI-1 [P=0.04], and t-PA [P=0.87]). Twenty-four hours after therapy, levels of interleukin-6 were higher in the intensive-treatment group than in the control-treatment group (difference, 8.1 pg per milliliter; 95% CI, 6.1 to 10.1; P<0.001), as were the levels of CRP (difference, 13.7 mg per liter; 95% CI, 10.4 to 17.0; P<0.001), PAI-1 (difference, 5.1 ng per milliliter; 95% CI, 1.0 to 9.8; P=0.02), soluble E-selectin (difference, 1.8 ng per milliliter; 95% CI, 1.1 to 2.8; P=0.02), and von Willebrand factor (difference, 1.2 ng per milliliter; 95% CI, 1.1 to 1.3; P=0.001) and the neutrophil count (difference, 1.7×10^9 cells per liter; 95% CI, 1.2 to 2.2; P<0.001) (Fig. 3).

Levels of soluble E-selectin were lower in the intensive-treatment group than in the control-treatment group 2 months after therapy (difference, 2.7 ng per milliliter; 95% CI, 1.4 to 8.6; P=0.02) and 6 months after therapy (difference, 2.8 ng per milliliter; 95% CI, 1.3 to 8.4; P=0.03) (Fig. 3). Between-group differences in serum levels of CRP did not reach statistical significance at 2 months (1.4 mg per liter; 95% CI, −1.0 to 1.8; P=0.09) or at 6 months (1.4 mg per liter; 95% CI, −1.0 to 2.0; P=0.07).

DETERMINANTS OF VASCULAR FUNCTION

There was a significant correlation between the change in flow-mediated dilatation 6 months after periodontal therapy and changes in clinical periodontal outcomes in response to therapy. Improvement in endothelial function was related to a reduction in the number of periodontal lesions (r=0.30 by Spearman rank correlation, P=0.002) and to a reduction in scores for full mouth bleeding (r=0.29 by Spearman rank correlation, P=0.003) (Fig. S1 in the Supplementary Appendix).

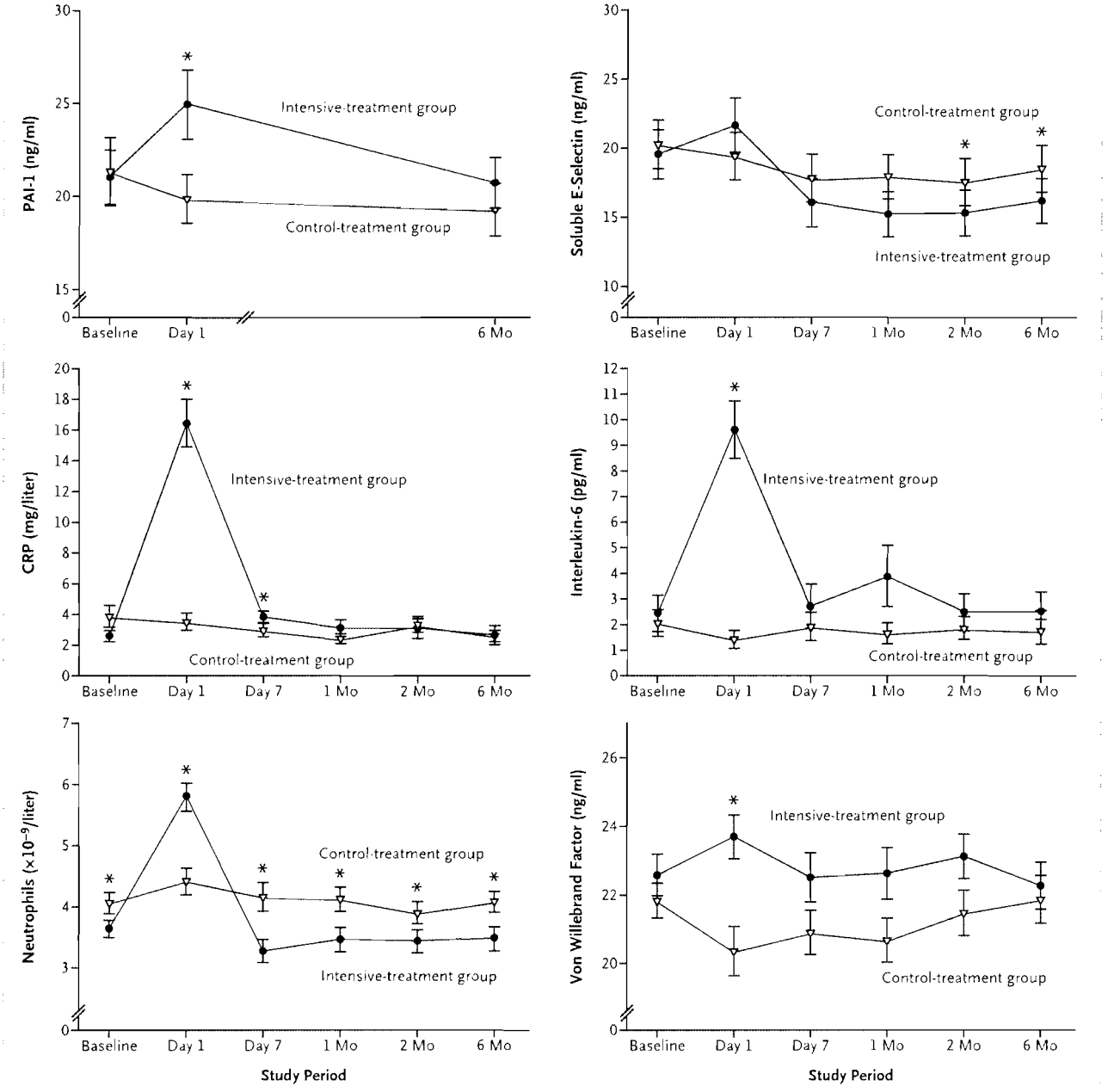


Flow-Mediated Dilatation and Nitroglycerin-Mediated Dilatation during the 6-Month Study Period.

I bars represent SE. Data are for the 61 patients in the intensive-treatment group and the 59 patients in the control-treatment group. Asterisks indicate significant between-group differences (P<0.05). P values were calculated by repeated-measures analysis of variance with the use of the Bonferroni–Holm adjustment for multiple comparisons.

DISCUSSION

This study showed that intensive treatment of periodontitis, a common potential source of low-grade inflammation, results in an improvement in endothelial function. Local intensive mechanical treatment of periodontitis, without the use of systemic drug therapy, resulted in a transient acute systemic inflammatory response and a transient impairment of endothelial function. At 6 months, however, intensive treatment of the periodontitis, as compared with control treatment, was associat-



Circulating Biomarkers in the Two Groups during the 6-Month Study Period.

I bars represent SE. Data are for the 61 patients in the intensive-treatment group and the 59 patients in the control-treatment group. Asterisks indicate significant between-group differences ($P<0.05$). P values were calculated by repeated-measures analysis of variance with the use of the Bonferroni–Holm adjustment for multiple comparisons. PAI-1 denotes plasminogen-activator inhibitor type 1, and CRP C-reactive protein.

ed with reduced indexes of periodontal disease severity and significantly better endothelial function.

Periodontitis is associated with increased levels of markers of inflammation, including CRP, fibrinogen, and cytokines.[2-5,20-22] Emerging evidence suggests that periodontitis may have a role in chronic infection, the associated inflammatory responses in atherosclerosis and its complications, or both, and experimental and clinical studies have indicated a potentially deleterious effect of periodontitis. Periodontal pathogens in vitro can promote platelet aggregation[9] and foam-cell formation.[10] *Porphyromonas gingivalis*, an important periodontal pathogen, increased cholesterol levels and

the formation of atheromas in inoculated apolipoprotein-E knockout mice.[11,12] In clinical studies, the presence of periodontal bacteria[23] and periodontitis[7,23] has been associated with increased thickness of the carotid intima media. Furthermore, case–control and cross-sectional investigations have suggested the possibility of a link to cardiovascular outcomes.[8] Our prospective, randomized study showed that the treatment of periodontitis is associated with alterations in endothelial function.

Endothelial dysfunction occurs early in the pathogenesis of arterial disease, in response to a wide range of risk factors that have been shown to predict cardiovascular events in epidemiologic studies.[24] Furthermore, in the clinical phase of atherosclerosis, endothelial dysfunction is associated with an adverse prognosis.[25,26] We chose flow-mediated dilatation of the brachial artery as our primary end point and as an index of endothelial function. Previous work in our laboratory has led us to adopt a standard protocol for the measurement and analysis of flow-mediated dilatation that minimizes the variability in this end point, and this protocol was used in our trial. Flow-mediated dilatation reflects nitric oxide–mediated vasomotor function in part,[27] and may also provide an index of the effects of nitric oxide on coagulation, cell adhesion, and cell proliferation.[28-30] We have previously shown that the response is reduced after an extrinsic experimental inflammatory stimulus (e.g., vaccination with *Salmonella typhi*)[31] and acute childhood infection.[19]

The mechanism by which periodontitis might affect endothelial function remains uncertain. Periodontitis involves bacterial infection with a range of gram-negative bacteria that invade superficial and deeper gingival tissues, depending on the severity.[32] It is possible, therefore, that these pathogens or their products could affect endothelial function directly, since even brushing the teeth or chewing can result in an ephemeral bacteremia.[33] In cell cultures, *P. gingivalis* has been shown to invade endothelial cells,[34] and periodontal pathogens have been identified in carotid atheromatous plaques in patients undergoing endarterectomy.[35] Alternatively, these pathogens might act as a trigger for a systemic inflammatory response that, in turn, might have detrimental effects on the vascular wall. The emerging evidence that other inflammatory disorders, such as systemic lupus erythematosus[36] and rheumatoid arthritis,[37] are associated with increased cardiovascular risk supports this as a plausible mechanism. In our study, during the acute response to periodontal therapy, there was a broad concordance between markers of inflammation and endothelial function, with a sharp rise in CRP and interleukin-6 levels in association with a substantial impairment in flow-mediated dilatation and increased PAI-1 and soluble E-selectin levels. CRP levels and neutrophil counts were decreased 6 months after the administration of the therapy in both treatment groups, but this effect was not associated with the difference in flow-mediated dilatation between the two groups. These changes may have occurred because CRP and the other markers involved may not adequately reflect the relevant inflammatory pathways or because the long-term effects are independent of a systemic inflammatory response. We studied well-characterized patients with severe periodontal disease who did not have systemic disease but who were at high risk for cardiovascular events. This may explain the relatively high levels of CRP measured at baseline and at the end of the 6-month study period.

It is likely that periodontitis of the severity seen in the patients in this study affects about 0.5 to 1.0% of the adult population in the United States — about 3 million people in all.[38] An estimated 21 to 80% of U.S. adults have some form of periodontal disease, but it remains uncertain whether those with less severe disease would have improvements in vascular function that would be similar to the improvements in our study population. Further studies are required to determine whether the treatment of severe periodontitis could contribute to the prevention of atherosclerosis and cardiovascular events in adults.

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

Salomon Amar, Noyan Gokce, Sonia Morgan, Mariana Loukideli, Thomas E. Van Dyke and Joseph A. Vita

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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 ($7.8\pm4.6\%$ versus $11.7\pm5.3\%$, $P=0.005$). 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 ± 2.3 versus 1.0 ± 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.[1] 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,[2,3] whereas there is no association with milder periodontal disease.[4,5] 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.[6,7] 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.[8] A pathogenic link between recurrent bacteremia with oral pathogens and acceleration of atherosclerosis is also supported by recent experimental studies.[9]

A likely target for circulating cytokines and oral pathogens is the vascular endothelium, which plays a central role in the regulation of vascular homeostasis.[10] 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.[10] 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.[11-16]

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,[17,18] which included involvement of at least 6 teeth with pocket depth >5 mm and loss of attachment of ≥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 ≥2 mm and no attachment loss ≥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[19] 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.[19]

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.[20] 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.[21]

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 χ^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±SD in the text and as mean±SEM in the figures.

TABLE 1. Clinical Characteristics

	Control (n=29)	Periodontal Disease (n=26)	P
Age, y	41±9	42±10	0.71
Sex, % female	38%	39%	0.97
Fasting glucose, mg/dL	91±8	91±8	0.98
Total cholesterol, mg/dL	195±36	197±34	0.84
HDL cholesterol, mg/dL	60±17	52±12	0.04
Triglycerides, mg/dL	89±35	111±81	0.21
LDL cholesterol, mg/dL	117±32	128±24	0.21
Systolic blood pressure, mm Hg	116±14	123±12	0.08
Diastolic blood pressure, mm Hg	71±8	72±11	0.64
Heart rate, bpm	61±7	63±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)	P
Baseline brachial diameter, mm	4.1±0.9	4.3±0.7	0.35
Hyperemic flow, mL/min	1150±620	1240±540	0.56
Flow-mediated dilation, %	11.7±5.3	7.8±4.6	0.005
Flow-mediated dilation, mm	0.45±0.16	0.31±0.15	0.003
Nitroglycerin-mediated dilation, %	18.9±11.0	16.3±8.3	0.37
C-reactive protein, mg/L	1.0±1.0	2.3±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 ($P=0.006$).

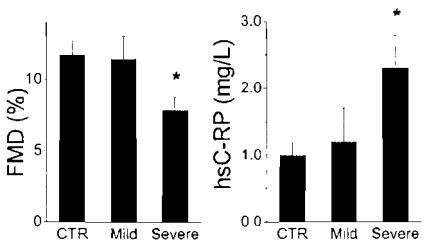
Nitroglycerin was administered to 23 control subjects and 24 subjects with advanced periodontal disease. As shown in Tables 2 and 3, nitroglycerin-mediated dilation was comparable in control subjects and subjects with advanced periodontal disease. This finding provides evidence that the difference between 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 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.



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.

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[22] 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,[23] and exposure of cultured endothelial cells to *P. gingivalis* is associated with endothelial activation and expression of cell adhesion molecules.[24] The relevance of these changes to cardiovascular disease is supported by the observation that repeated *P. gingivalis* injection is associated with accelerated atherogenesis in the heterozygous apolipoprotein E–deficient mouse.[9]

Other systemic infectious and inflammatory states are also associated with impaired endothelium-dependent vasodilation in human subjects. For example, Hingorani et al[25] 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.[26,27] Interestingly, there is evidence that endothelial function may improve in parallel with spontaneous reduction in C-reactive protein over time.[26] 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.[28-30]

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,[10] and these abnormalities are present before the development of angiographically evident coronary atherosclerosis in patients with coronary risk factors.[31] Interventions known to reduce cardiovascular disease risk, including lipid-lowering therapy, angiotensin-converting enzyme inhibitors, exercise, and smoking cessation, also improve endothelial function.[32] Importantly, the presence of endothelial dysfunction in the coronary and forearm circulation predicts future cardiovascular disease events.[11-16] 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[3] and carotid arteries.[2] 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[7] 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.[6] In contrast, studies from the Health Professions Follow-up Study[4] and the Physicians' Health Study[5] 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[33] and provide prognostic information.[13,14] Furthermore, the noninvasive methodology used in the present study was recently shown to provide prognostic information about cardiovascular disease in high-risk patients.[15,34] 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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